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A Critical Review of Pharmacokinetic Modelling of PFOS and PFOA to Assist in Establishing HGBVs for these Chemicals

Title:

A Critical Review of Pharmacokinetic Modelling of PFOS and PFOA to Assist in Establishing HGBVs for these Chemicals

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Executive Summary

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). Overall it is considered that:

- The US EPA reports provide a good overview of the literature for PFOS and PFOA and, in our view, combined with later papers and reviews, provide the best overall summary of the toxicology of these solutes.
- The US EPA is also to be commended for their physiological pharmacokinetic approach, which we agree, based on our own modelling, provide a more appropriate endpoint for estimation of PFOS and PFOA exposure and likely hazard than conventional methods based on dose only.
- However, in commending the US EPA physiological pharmacokinetic approach, we also point out that their pharmacokinetic modelling has some limitations and uncertainties. In particular, there are three key questions that cannot be answered by the US EPA approach:
  - » Why are there huge discrepancies in half-lives of some PFCs between species?
  - » Why do serum concentrations of PFOA and PFOS appear to rapidly approach steady-state after repeated dosing despite their long half-lives?
  - » Why is there a gender difference in the excretion of PFOA by rats?
- Our analysis suggests that there is substantial secretion of these compounds into bile with effective enterohepatic recirculation, as seen by significant amounts of material detected in faeces many days after a single dose given orally or IV.
- Further, we suggest that the US EPA modelling has ignored saturable uptake by the liver and intestine and efflux by the placenta and the brain.
- Our modelling shows that the serum concentrations of PFOS and PFOA can reach steady state despite a long half-life, contrary to findings by the US EPA modelling.

To assist FSANZ in establishing HBGVs, we developed human equivalent doses (HED) based on average serum concentration prediction, derived from predicted AUC over the duration of dosing using the EPA PK model and parameters. We then showed that using our best parameter estimates and commercial simulation software package, the EPA estimates for the PBPK of a range of studies could be replicated with an error of less than 80%. In the context of the uncertainty factors of 30 fold or so applied to derive the TDI to take into account pharmacodynamic and intra-species differences this uncertainty of 1.5 to 1.8 fold is a very small component of the total uncertainty.

We also developed a fuller model based on our analysis and interpretation of the literature. However, at this time our model is more conceptual than fully described in quantitative terms as the data needed for the latter is lacking.

We comment, in conclusion, that the EPA reports provide an excellent overview of PFOS and PFOA studies carried out to date and that our modelling of endpoints has verified their conclusions, whilst incorporating the same uncertainties. Whilst we have suggested that their modelling and resulting data can be improved, we don't have the data to fully develop and validate an improved model. However, it is clear that their physiological pharmacokinetic modelling and related endpoints for both PFOS and PFOA is much preferred to endpoints based on dosing only – especially noting that the variation in both PFOS and PFOA half-lives between the species cause considerable vagary in scaling up toxicity findings found in animals to man

Contents

1 Background

The Department of Health has contracted Food Standards Australia New Zealand (FSANZ) to provide advice on appropriate health-based guidance values (HBGVs) for perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA). These values are to be used for assessing public health and safety risks arising from consuming food and water contaminated by these chemicals.

PFOS and PFOA have previously been reviewed by a number of international agencies or bodies including US Environmental Protection Agency (EPA), the European Food Safety Authority (EFSA), the Danish Environmental Protection Agency and the US Agency for Toxic Substances and Disease Registry (ATSDR). Despite the data packages largely consisting of the same studies, these agencies have established different HBGVs, with the most significant contributor being the use of pharmacokinetic (PK) modelling. Other key contributors are the availability of quality animal and human toxicity data and the selection of uncertainty factors.

The US EPA published its finding on PFOA and PFOS in two reports; *Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)* (1) and *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)* (2) in May 2016. In September 2016, Professor Mike Roberts was contacted by Nick Fletcher of FSANZ and requested to provide a critical review of the validity of pharmacokinetic modelling applied by the US EPA including a summary of assumptions, limitations and uncertainties, e.g parameters that are not biologically based, other important factors such as gestational, lactational exposures etc. The outcomes of the review were to be contained in a report of publishable quality, to be considered as a part of FSANZ's overall review of the toxicology and health effects of PFOS and PFOA. In addition to the aforementioned US EPA reports, other pivotal international assessments from EFSA, the Danish EPA and the US ATSDR were provided for comparison.

Following discussion and verbal agreement to perform the requested review, a contract was executed on 19 October, 2016. A significant amount of work was required in a short time frame to complete this review. This was mainly performed by Professor Roberts, with assistance in modelling from Dr Xin Liu and document preparation by Dr Jeff Grice. Advice and assistance was sought from other participants where appropriate.

2 Terms of Reference

Professor Roberts ("the Supplier") will provide a report that will contain:

1. A review of the US EPA reports on PFOS and PFOA. Specifically, it must include a critical review of the validity of the PK modelling applied by the US EPA, including a summary of assumptions, limitations and uncertainties.
2. Results of the Supplier's own PK modelling in order for the Authority to establish recommendations for HGBVs.
3. A comprehensive search of the recent literature to ensure that the information is up to date. The search must include a review of reports from the other international agencies and consider the independent review of the interim enHealth guidance values conducted by adjunct Professor, Andrew Bartholomaeus (3).

3 Expected Outcome

Outcome: The Supplier must provide a report to the Authority (FSANZ) which contains a critical evaluation of the pharmacokinetic modelling used by the US EPA, and includes PK models run by the Supplier to assist the Authority to establish appropriate HBGVs. The report must be of publishable quality.

4 Sources of Information

a. Health Effects Support Document for Perfluorooctane Sulfonate (PFOS), EPA Document Number: 822-R-16-002, May 2016 (1)
b. Health Effects Support Document for Perfluorooctanoic Acid (PFOA), EPA Document Number: 822-R-16-003, May 2016 (2)

c. Procedural Review of Health Reference Values Established by enHealth for PFAS (A report by Prof Andrew Bartholomaeus, 30 August 2016) (3).

d. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain. EFSA Question No EFSA-Q-2004-163, Adopted on 21 February 2008 (4)

e. Draft Toxicological Profile for Perfluoroalkyls. US Agency for Toxic Substances and Disease Registry (ATSDR), August 2015 (5).

f. Perfluoroalkylated substances: PFOA, PFOS and PFOSA. Evaluation of health hazards and proposal of a health based quality criterion for drinking water, soil and ground water. The Danish Environmental Protection Agency, Environmental project No. 1665, 2015 (6)

g. A comprehensive search of literature relating to perfluoroalkylated substances, capturing references between 1951 and 2016 (Sources: Google Scholar, Web of Knowledge, Endnote X7.7)

h. Online databases: SciFinder, Chemicalize:
https://scifinder-cas-org.ezproxy.library.uq.edu.au/scifinder/view/scifinder/scifinderExplore.jsf
https://chemicalize.com/#/

5 Critical review of the US EPA reports on PFOS and PFOA, including a critical review of the validity of the PK modelling applied by the US EPA and a summary of assumptions, limitations and uncertainties.

5.1 Introductory Remarks

This review refers to two recent reports released by the United States EPA in May 2016. These are (i) Health Effects Support Document for Perfluorooctane Sulfonate (PFOS) (United States Environmental Protection Agency, Office of Water Mail Code 4304T, EPA 822-R-16-002 (1) and (ii) Health Effects Support Document for Perfluorooctanoic Acid (PFOA) (United States Environmental Protection Agency, Office of Water Mail Code 4304T, EPA 822-R-16-003 (2).

The background of these reports is that there is a requirement by the Administrator of the U.S. Environmental Protection Agency (EPA), under the 1996 Amended US Safe Drinking Water Act (SDWA), to make regulatory determinations on at least five possible contaminants in public water systems that are on the Contaminant Candidate List (CCL) every 5 years. Such an analysis requires both data and a risk analysis that may assist the EPA to best address any issues that may arise. The PFOS and PFOA health assessments were initiated by the Office of Water, Office of Science and Technology in 2009, with the draft Health Effects Support Documents (HESD) for Perfluorooctane Sulfonate Acid (PFOS) and Perfluorooctanoic Acid (PFOA) completed in 2013. After release for public comment in February 2014, an external peer-review panel meeting was held on August 21 and 22, 2014, with the final documents reflecting input from the panel and public comments received. The focus in both of these document is on the toxicokinetics and health effects of each of PFOS and PFOA.

The reports were developed using a range of data including literature identified by EPA and New Jersey Department of Environmental Protection library staff and papers, identified by EPA internal and external peer reviewers, through public comments on the draft assessments and submitted to EPA by the public. The literature included studies on the C-4 to C-12 perfluorocarboxylic acids and C-4, C-6 and C-8 sulfonate compounds that captured a toxicity endpoint or population not examined by studies already included in the draft document, superior study design, providing data that contributes substantially to the weight of evidence for any of the toxicity endpoints and work on mode of action or the quantification approach that are relevant to the study design.

The National Research Council (1983) and EPA's Framework for Human Health Risk Assessment to Inform Decision Making (USEPA 2014a) general guidelines for risk assessment underpinned the hazard identification and dose-response assessment for PFOS and PFOA that was presented and was supported by various other EPA guidelines that were listed.

5.2 Review of the US EPA Reports on PFOS & PFOA

In this review, we have considered PFOS and PFOA together as the two chemicals have similar physicochemical properties and a comparison aids in our review of the EPA documents in terms of defining those findings made in those report that we support as well as those points we feel may be wanting. Tables 1 and 2 (each expressed in two parts) shows the listings of the contents of the PFOS and PFOA reports reviewed in this analysis. It is noted that the two reports have a similar structure of: Executive Summary, Physicochemical Properties, Toxicokinetics, Hazard Identification, Dose-Response Assessment, References and Appendices that facilitates a direct comparison of observations for the two compounds in coming to the conclusions reached here.

Table 1. Contents of EPA Perfluorooctane sulfonate (PFOS) – May 2016 Report (1)

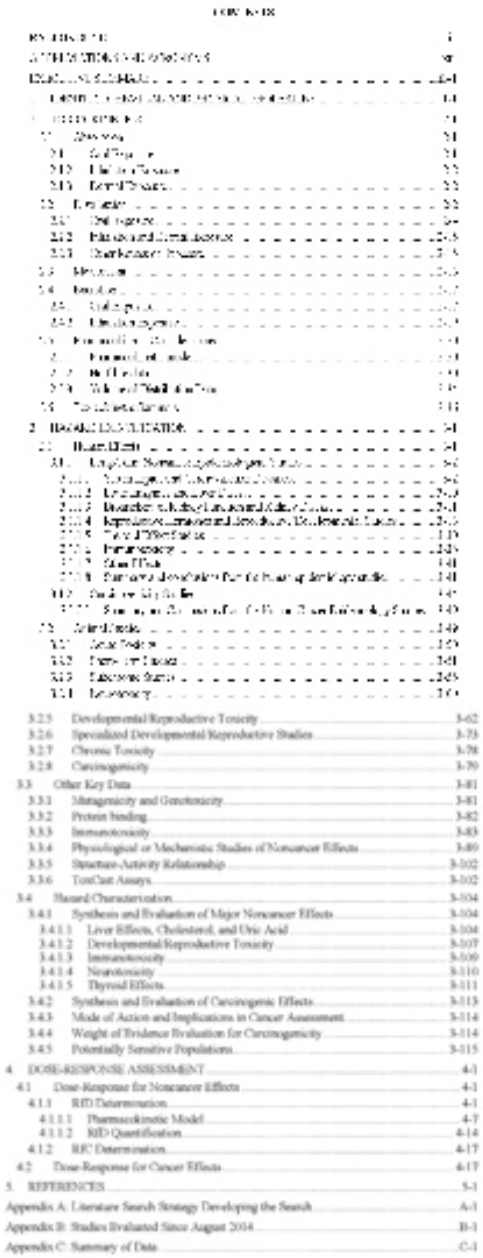
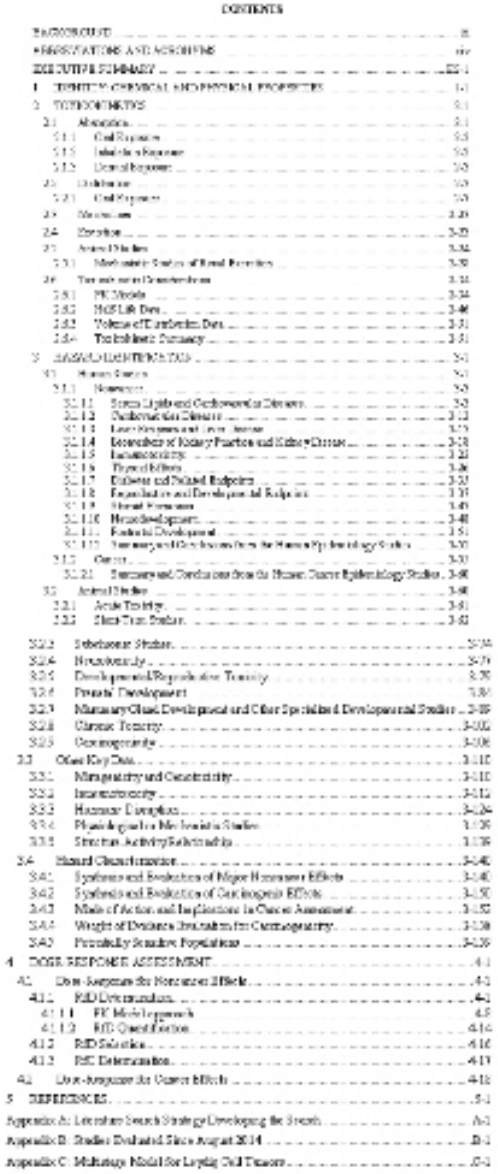


Table 2. Contents of EPA Perfluorooctanoic acid (PFOA) –May 2016 Report (Part 1)



5.2.1 Chemical and physical properties

Historically, electrochemical fluorination and telomerization have been used to prepare PFOA and PFOS. This former production method resulted in a variety of structural isomers, including significant branched chain structures. The latter method results in varying carbon chain lengths.(7, 8) In addition to this variability, the nature of the chemicals themselves has made chemical characterisation difficult to achieve and many properties have not been determined.

PFOS (perfluorooctane sulfonate, (C8HF17O3S, CAS 1763-23-1) is a fluorinated organic compound with an eight-carbon backbone and a sulfonate functional group. The EPA Report has detailed its synthesis and physicochemical properties. Pertinent to our evaluation, it is reported to have a molecular weight of 500.13, a solubility in water of 680 mg/L and organic carbon water partitioning coefficient (Koc) of 2.57. Please note that the Koc is the Organic Carbon Adsorption Coefficient at 25 °C and, for a substance added to a mixture of soil and water, is the ratio of the amount of the substance adsorbed per unit weight of organic carbon in the soil to the concentration of the substance remaining in the water at equilibrium. SciFinder Scholar (Chemical Abstract Service) points out that PFOS is an acid with a predicted pKa value of -3.27±0.50 at 25°, has a log D, the Logarithm of the partition coefficient between octanol and water at a given pH for the mixture of the neutral and ionic forms of a compound, of 1.01 and suggests that it has a solubility in water and various pH buffers of 7.5 g/L – almost 10 times that suggested by EPA in its Table 1.1. What does not come clearly through in this report is that PFOS binds to soil and **PFOS is also surface active, with a critical micelle concentration (CMC) of 1.13 mmol/kg (0.565 g/L) (9)**. This suggests that the aqueous solubility referred to by EPA is actually that for the PFOS monomer in water whereas the ten times larger Scifinder predicted aqueous solubility probably also includes the micelle forms.

As the report points out: “PFOS-related chemicals are used in a variety of products, including surface treatments for soil/stain resistance; surface treatments of textiles, paper, and metals; and in specialized applications such as firefighting foams.” They are resistant to metabolic/ environmental degradation and biotransformation.

PFOA (perfluorooctanoic acid, C8HF15O2, CAS 335-67-1) is a completely fluorinated organic compound with a seven-carbon backbone and a carboxyl functional group. The report describes PFOA’s synthesis and physicochemical properties. PFOA has a molecular weight of 414.064, and a reported solubility in water of 9500 mg/L (9.5 g/L). PFOA is an acid, with a dissociation constant (pKa) which has been the matter of some debate. The value of 2.8 included by the EPA was determined in 1962 (10) and was obtained using a 50% ethanol/water mixture. A more recent determination in 2008 gave a value of 3.8 (also obtained in a mixed solvent) (11), but these values are controversial, as predicted values of -0.5 have been proposed (12, 13). The exact knowledge of the pKa, under normal physiological and in most environmental conditions would enable better prediction of the environmental and physiological effects as it determines the percentage present in the ionised, anion form, and solubility, depending on the pH. PFOA also forms micelles, with the EPA report reporting a critical micelle concentration of 3.6–3.7 g/L for PFOA – almost 10 times that for PFOS. The CMC reported by MacManus (14) was 0.016 M or 6.63 g/L. The online databases SciFinder and Chemicalize point out that PFOA is an acid with a predicted pKa value of 0.5 at 25°, has a predicted log D, the logarithm of the partition coefficient between octanol and water at a given pH for the mixture of the neutral and ionic forms of a compound, of 1.58 (pH 1.7 – 8.0). SciFinder predictions suggest that it has a solubility in water and various pH buffers ranging from sparingly soluble (0.095 g/L) at pH 1 to 13 g/L for pH 6-10 – this is compared to the single EPA value of 9.5 g/L in its Table 1.1 (with no pH stated). Properties such as water solubility would be affected depending on the actual pKa of PFOA. PFOA also can bind to soil, with SciFinder predicted Koc of 18500 at pH 1 (25°C), 13.8 at pH 6 or 13.6 at pH 10 – this is compared to the single value reported in the EPA report as 2.06.

PFOA is widely found in consumer and industrial products as well as in food items, with the EPA Report advising that the major U.S. manufacturers were to have ceased its production by the end of 2015.

5.2.2 Toxicokinetics of PFOS and PFOA

The PFOS report (1) suggests that the data in humans and animals demonstrate ready absorption of PFOS and distribution of the chemical throughout the body by noncovalent binding to serum albumin and other plasma proteins. The PFOA report (2) indicates that PFOA is similar to PFOS in being similarly easily absorbed and distributed throughout the body by noncovalent binding to plasma proteins.

Both PFOS and PFOA have been measured in liver, lung, kidney, and bone in postmortem human tissues and both have a very slow elimination from the human body (in the order of years) with much shorter half-lives (in the order of days) in animals, with the relative half-lives being: monkey > rat > mouse.

The key areas in which we think this EPA report is wanting in terms of how it impacts on the toxicokinetic modelling are as follows:

h.0.0.1 a. Absorption.

Both PFOS and PFOA are likely to be absorbed across the intestine by an anionic transporter process, with published mechanistic evidence available for PFOA but not PFOS. However, the Chang *et al.* (2012) study (15), using a single 14C-PFOS dose of 4.2 mg/kg in solution to 3 male rats found only 3.32% of the total dose in the digestive tract and 3.24% in the faeces at 48 hours after dosing, indicating that this anionic solute is most likely actively taken up across the intestinal wall. Similarly, >90% of PFOA has been shown to be absorbed following oral exposure in rats (16, 17), as described in the EPA report.

Our view differs from the EPA in relation to skin absorption. We suspect that the superb barrier properties of the essentially dead stratum corneum in humans is likely to be a formidable barrier to the ionised PFOS and that it would prevent its percutaneous penetration, irrespective of whether anionic transporters existed in the viable epidermis or not. The same would not necessarily apply to rodent or rabbit skin where there are multitude or hair follicles and a much less well developed and thinner stratum corneum. Indeed, Scott *et al.* (1986) showed that ionised paraquat does pass through animal skin but not through human epidermis (18). PFOA is different in that it is a weak acid and can exist in both the anionic and uncharged forms, with the latter likely to have significant permeation across the human stratum corneum. Consistent with these comments, the skin permeability coefficient for PFOA is almost 100 fold different between rat and human skin (19). However, it is unclear from the EPA report what this actually means from a viewpoint of human exposure. The key missing values are the likely unbound concentration of PFOA in whatever aqueous solution people are exposed to and the pH of those solutions. Whilst mortality has been demonstrated in animals (20), with their several orders of magnitude higher skin permeability, the EPA report is deficient in its estimates of the likely human exposure of real world PFOA solutions. In our view, it is likely to be very low relative to that being seen after oral exposure.

However, we do support the EPA comments made on lung exposure. The lung epithelia is more permeable than the stratum corneum and so, as has been shown by Rusch *et al.* (1979), some absorption by lung inhalation may occur (21). PFOA has also been shown to be taken up by the lung after inhalation exposure as shown by Hinderliter *et al.* (2006) (22).

h.0.0.2 b. Plasma protein binding.

(a) PFOS. It is suggested that PFOS was bound to plasma proteins in various species “at all concentrations with no sign of saturation (99.0–100%). When incubated with separate human-derived plasma protein fractions, PFOS was highly bound (99.8%) to albumin and showed affinity for low density lipoproteins (LDLs, formerly beta-lipoproteins) (95.6%) with some binding to alpha-globulins (59.4%) and gamma-globulins (24.1%).” **(b) PFOA**. PFOA is also bound 97–100% to rat, human, and monkey plasma proteins at concentrations of 1 to 500 ppm, with about 60% bound to albumin. Whilst much is made of PFOS interfering with the binding affinity of liver-fatty acid binding protein (L-FABP) (23), it is not highlighted that PFOS is highly bound to FABP and that FABP can carries the longer chain fatty acids into the nucleus. In particular, an increased L-FABP expression enhances the uptake and targeting of unsaturated as well as saturated LCFAs into nuclei of living cells (24). PFOA binds much less to L-FABP than does PFOS and more than a magnitude less than oleic acid (23, 25). The EPA PFOA report does note that the binding of PFOA with L-FABP within organs “could function to retard distribution to the cytosol, especially at low doses.”

c. Mechanisms of liver toxicity.

Interestingly, PFOS-induced steatosis has been reported to occur in a time- and dose-dependent manner in mature 8-wk old male CD-1 mice administered 0, 1, 5 or 10 mg/kg/day PFOS for 3, 7, 14 or 21 days. The work showed that the gene expression levels of fatty acid translocase (FAT/CD36) and lipoprotein lipase (Lpl) were significantly increased by 10 and/or 5 mg/kg PFOS and that serum levels of very-low d lipoprotein were decreased by 14 days of PFOS exposure, with the implication that PFOS can cause nonalcoholic fatty liver disease (26). We agree with the observation that peroxisome proliferation as a result of binding to and activation of peroxisome proliferator-activated receptor-alpha (PPARα), may be one cause of hepatic lesions in the rat, but that it may not necessarily be the case for PFOS – as we have described above, noting also the strong L-FABP binding of PFOS and, again, that L-FABP is a nuclear transporter for fatty acids. Although peripheral to this report, we note that the EPA report also recognises

that PFOA is not only a PPAR activator but can also activate the CAR, FXR, and PXR and metabolic activities, which are linked to the various nuclear receptors. Recent advances over the last decade have revealed that a number of the effects related to the activation of the nuclear hormone receptors CAR, PXR or PPARα are rodent-specific (27) and not relevant to humans.

h.0.0.3 d. Toxicokinetics.

The EPA reports have provided an extensive review of the known animal (mouse, rat and monkey) toxicokinetics for both PFOS and PFOA. They categorized their analysis by species for nonpregnant animals and then provide distribution data derived from studies during pregnancy and lactation. PFOS appears to have higher fetal serum and brain levels than in the mother based on both experimental data and pharmacokinetic models. It is slowly eliminated in humans with an estimated range of average half-life values between 4.1 to 8.7 years. These are much longer than the half-life values of 121 days, 48 days, and 37 days for the monkey, rat, and mouse, respectively. The long half-lives appear to arise from the processes of enterohepatic recycling and of saturable resorption from the kidney. In comparison, PFOA studies in the monkey where it was shown that on repeated oral dosing, PFOA reaches a steady state concentration in the serum, urine, and faeces within four weeks and is mainly excreted in the urine with an elimination half -life of approximately 20–30 days after either oral or intravenous dosing. PFOA is also rapidly and nearly completely absorbed in the GI tract. It is mainly present in serum/plasma. The volume of distribution is similar across species (~ 0.17 L/kg bw) suggesting extracellular distribution. It is tightly bound to serum protein (mainly albumin, ~90%). PFOA has non-linear kinetics at high doses, which is hypothesized to be due to the saturation of OATs responsible for renal reabsorption in proximal tubules. However, at lower doses closer to those relevant to human environmental exposures, kinetics are consistent with first order processes, and serum levels are proportional to administered dose (28, 29). This non-linearity can affect PFOA distribution; for example, after a single intravenous dose in male rats, a lower proportion of the dose was distributed to the liver (27%) at 17 mg/kg bw compared to 52% at 0.4 mg/kg bw (30).

Urinary excretion is the major route of elimination for PFOA. Biliary and faecal excretion also contributes to the elimination of PFOA, which may be subject to extensive enterohepatic recirculation (30-33). In females, lactation can be a significant route of excretion, as shown in mice (34) and in women (35, 36).

Few data have been gathered on the human tissues to which PFOA is typically distributed. PFOA was detected in approximately one half of the analyzed liver samples (in 6 males and 6 females from Catalonia, Spain; aged 27–79 years), and was significantly higher in males than in females (37), but was below the limits of quantification in livers in cadavers with environmental exposure (38). Neither cerebrospinal fluid (31) nor thyroid (39) have been observed to be relevant partitioning sites for PFOA. PFOA is not metabolized.

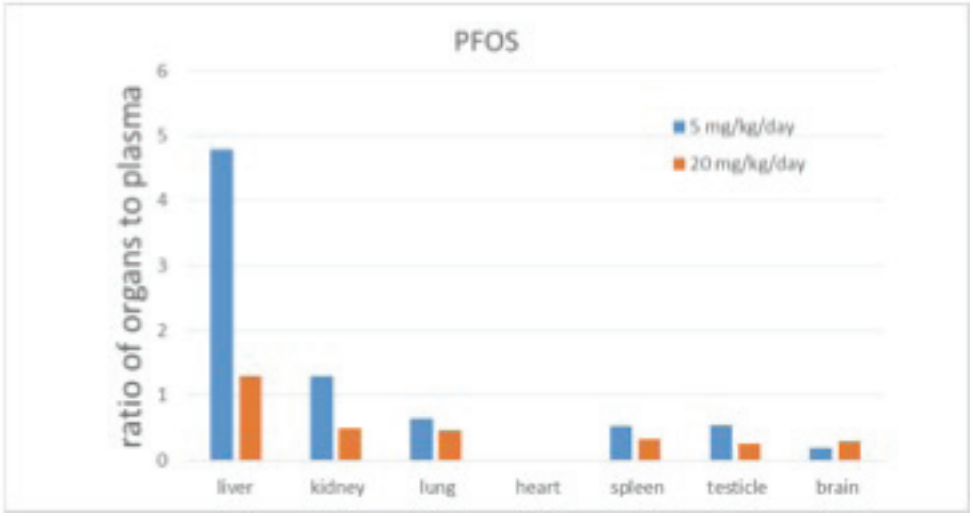
h.0.0.4 e. Ubiquitous nature of PFOS and PFOA uptake and efflux transporters appears not to be recognised in the EPA pharmacokinetic modelling.

It is also suggested that limited data is available on the uptake of PFOS by transporters but it is clearly stated in the PFOA Report that transporters identified for PFOA include organic anion transporters (OATs), organic anion transporting peptides (OATps), multidrug resistance-associated proteins (MRPs), and urate transporters, noting that these transporters respond to PFOA exposure in a dose-related manner. Importantly, the EPA report on PFOA suggested that these "*transporters are critical for gastrointestinal absorption, uptake by the tissues, and excretion via bile and the kidney. These transport systems are located at the membrane surfaces of the intestines, liver, lungs, heart, blood brain barrier, blood placental barrier, blood testes barrier, and mammary glands where they function to protect the organs, tissues, and fetus from foreign compounds.*" Thus, in our view, if saturation of transporters may reduce excretion and increase the exposure of PFOS and PFOA on vital organs such as the brain and the fetus, the impact of PFOS and PFOA exposure on the functioning of and resulting serum levels of both PFOS and PFOA are vital to defining the safe exposure of both in the human population exposed to a variation in systemic doses of PFOS and PFOA and must be taken into account any toxicokinetic model. Table 3, showing data from Cui *et al.* (2009) (40) suggests that there are saturable uptake processes occurring in both the liver and kidney but, importantly, the known OAT efflux transporter from the brain is also being saturated, causing an almost 10 times increase in levels for a 4 fold increase in dose. Harada *et al.* (2007) (31) notes that transporters such as OAT3 might be involved in the efflux of these compounds from the CSF into serum and that the brain levels of PFOA and PFOS may increase more than a corresponding increase in serum concentrations as a result of their saturation. The data of Curran *et al.* (2008)

(EPA Table 2-5) also shows a decrease in the liver/serum ratio for doses in rats above 20mg/kg over 20 days (41). Table 2-8 in the EPA report also shows less than proportional increase in liver and kidney levels with an increase in PFOS dose. The EPA document also recognises the potential upregulation of transporters in quoting Yu *et al.* (2010) (42) on p. 2.4, but do not appear to have included that aspect in their pharmacokinetic modelling. Benskin *et al.* (2009) observed in adult male Sprague-Dawley rats, given a single gavage dose of 0.5 mg PFOA/kg and monitored for 38 days, 91–95% of the daily excreted PFOA was eliminated in the urine after the first 24 hours (43). The half-life for elimination from plasma in male rats was 13.4 days. Cui *et al.* (2010) reported faecal excretion rates of 7.2% and 7.7% for rats in the 5- and 20-mg/kg groups for PFOA, increasing over 28 days to about 25% and 40% for the low- and high- dose groups, respectively (16).We therefore reanalysed a number of the papers referred to in the EPA reports, and others, and report the following figures normalised where possible to serum. Figure 1 shows the Cui *et al.* (2009) data (40). It is evident that the PFOS ratio in the liver and, to a lesser extent in the kidney, is decreased with a 4 times increase in PFOS dose as well as the brain level slightly increasing with dose. A similar phenomena is evident with PFOA for liver and kidney but not for brain.

Table 3. PFOS concentrations in male rat whole blood (µg/g) and various tissues (µg/g) after 28 days. Data from Cui *et al*. (2009) presented in the EPA PFOS report show that a 4 X increase in PFOS dosing led to ~ 4× increase in lung & spleen, 10 ×in brain but only 2× in kidney and liver.

Figure 1. Ratio of organ to serum level with increasing PFOS and PFOA dose; from Cui *et al*. (2009).



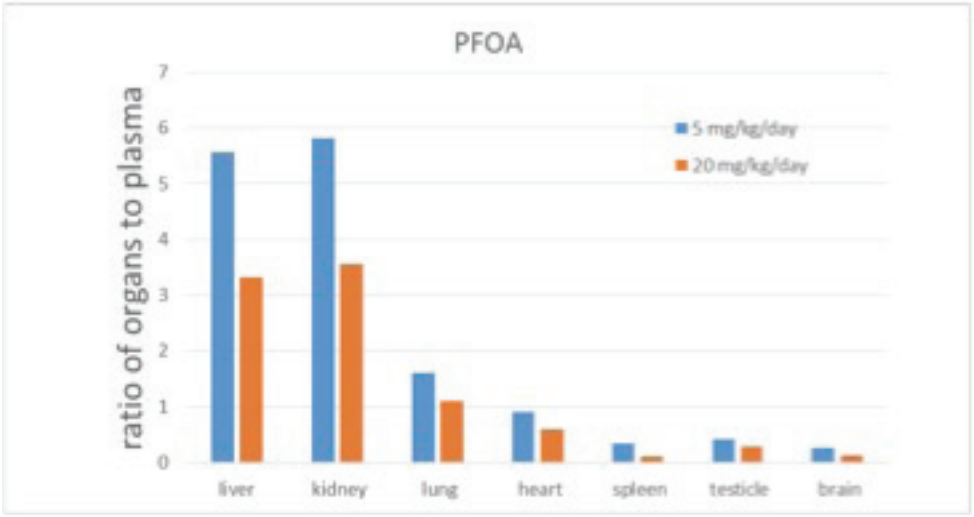
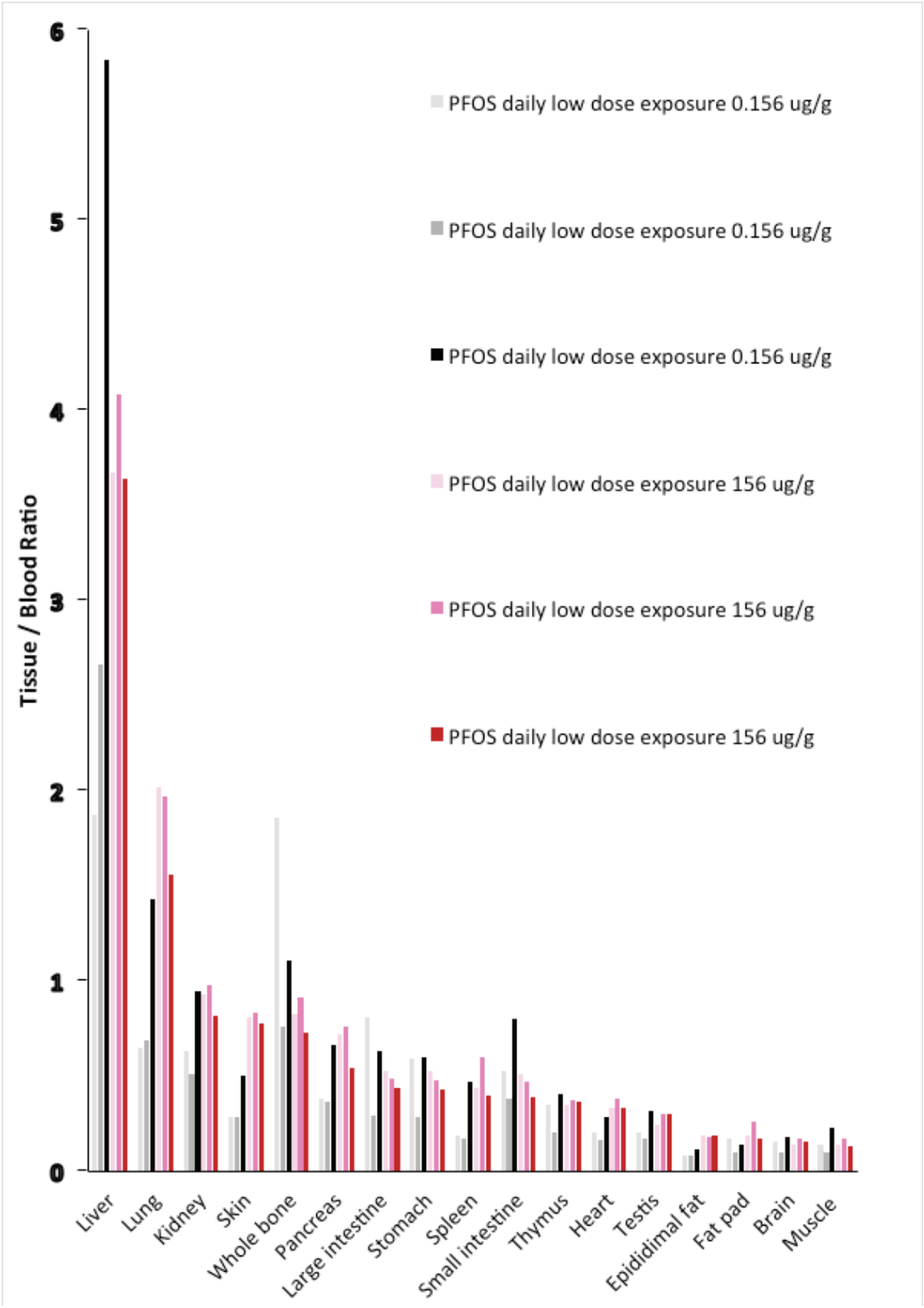


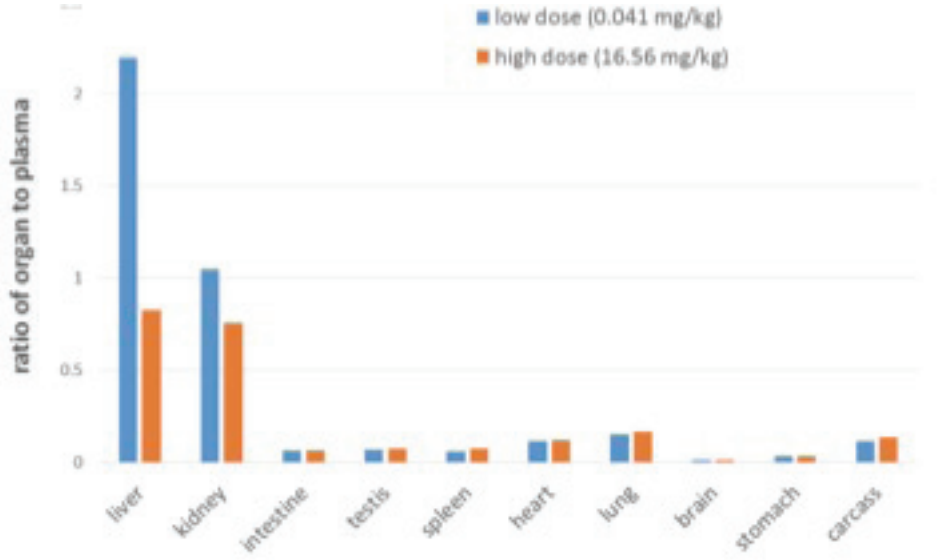
Figure 2 shows the corresponding plot for the tissue distribution (shown as Tissue / Blood ratio) of ^{35}S-labelled PFOS in adult mice after oral exposure to two doses, a low environmentally relevant dose and a high experimental dose, measured on Day 1, Day 3 and Day 5 (44). It is evident that this data is less clear cut but at the longest time (5 days) the liver to blood ratio for the high dose is just under half that for the low dose. Interestingly, the ratio for the kidney and brain appear relatively similar for the two doses.

Figure 2. Effect of dose and time on tissue to blood ratio of 35S-labelled PFOS in adult mice, 1, 3 and 5 days of dosing (grey scale for low, 0.156 µg/g dose exposure & red scale for high, 156 µg/g dose exposure with increasing colour depth with increasing time). [Bogdanska *et al*. (2011) (44)]



The ratios of tissue to serum levels for PFOA in rats, from the study by Kudo *et al.* (2007) (30) are shown in Figure 3 below. Here it is evident that the liver and kidney ratios are reduced at the higher doses but that the other organs are relatively unaffected.

Figure 3. Ratio of tissue to serum levels found for PFOA in rats [Kudo *et al*., (2007)(30)].



Another paper showing very high levels in the liver relative to other organs, including the kidney is the very recent work (post EPA reports) of Kim *et al.* (2016) (45). We suspect that, because PFOS is so highly bound to fatty acid binding protein and as we have shown is a major determinant of hepatic pharmacokinetics of palmitate and its metabolites (46), it could explain the much higher levels being found in the liver compared to other organs. This is not, as postulated by Prof Bartholomaeus, a result of the high uptake into the liver because of its being exposed to all orally absorbed PFOS, as the liver uptake appears similar after both oral and IV dosing (Figure 4).

h.0.0.5 f. Gender effects.

The EPA reports give a considerable emphasis on gender effects in the disposition of PFOA and PFOS. One of the earliest studies to show the differential excretion of PFOA excretion in male and female Holtzman rats was the work of Hanhijarvi *et al.* (1982) (47). They showed that the renal excretion inhibitor, probenecid, markedly reduced PFOA elimination in females but not so much in male rats. Figure 4 is consistent with this finding in that there appear to be no apparent differences between male and female rats in the liver, kidney and other organs for PFOS nor in PFOA for male rats but there are lower levels in the female liver and kidney after oral dosing relative to intravenous dosing (Figure 4). The reason for these differences are not well explained by Kim *et al.* (45) but do appear to be associated with a faster decline of PFOA in female rats compared to PFOA in males and PFOS in both males and females, as illustrated in Figure 5.

Figure 4. Organ levels of PFOA (A) and PFOS (B) and after intravenous and oral dosing of PFOA (1 mg/kg), PFOS (2 mg/kg) in male and female rats [Kim *et al*., (2016)(45)].

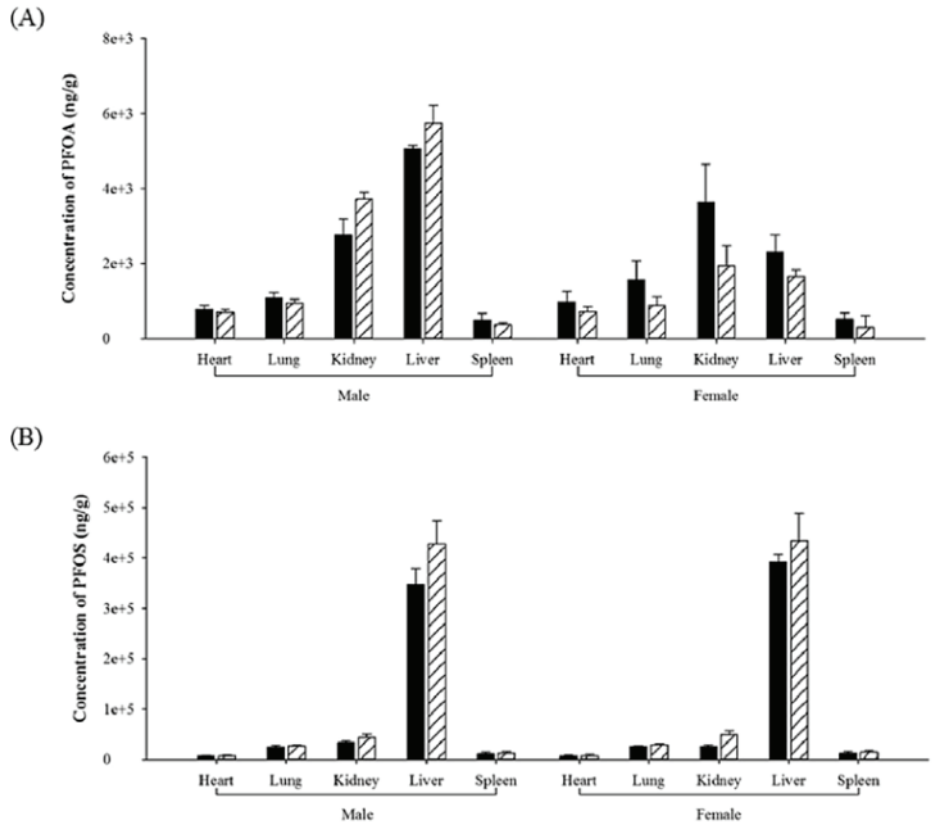
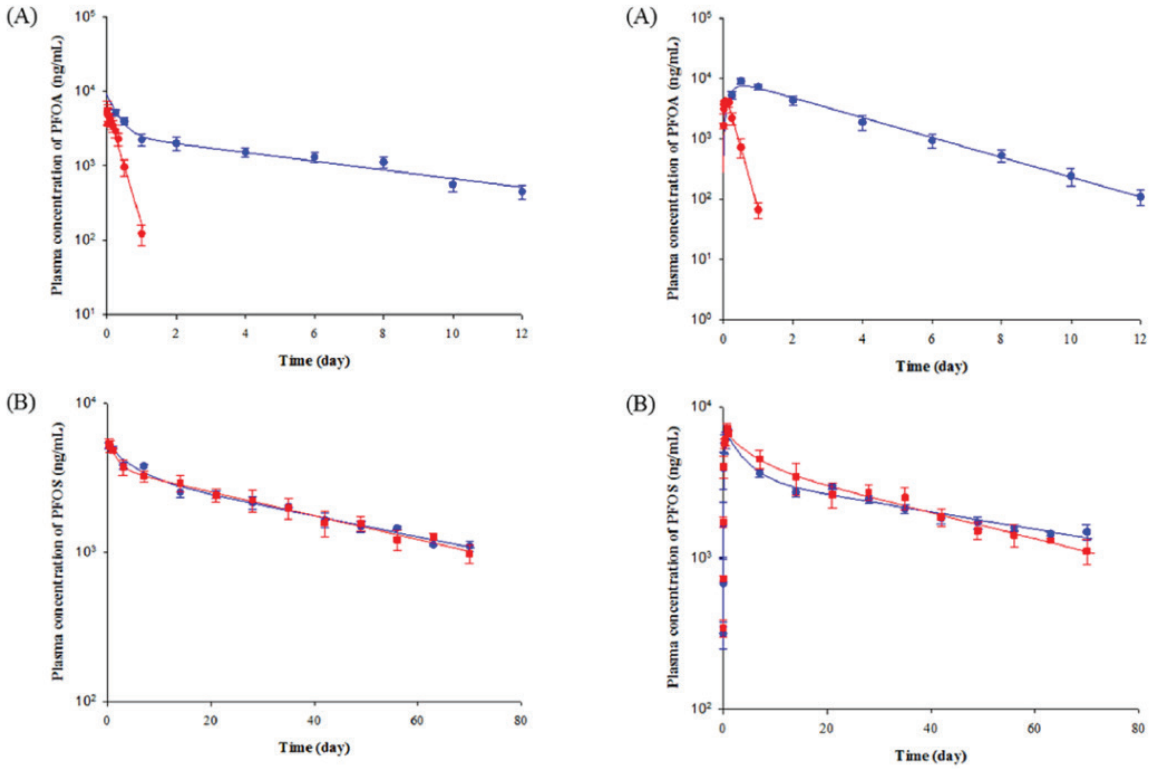
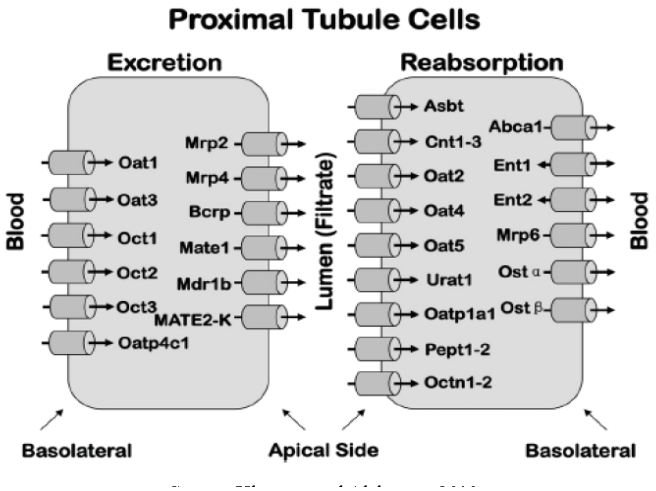


Figure 5. Mean plasma concentration-time profile of (A) PFOA, (B) PFOS after IV (left hand side) and oral (right hand side) administration of PFOA (1 mg/kg), PFOS (2 mg/kg) in male (●) and female (●) rats (mean ± SEM, n = 5) [Kim *et al*. (2016)(45)].



Han *et al.* (2005) (48) concluded that the subcellular distribution of PFOA in the rat liver was gender-dependent because the proportion of PFOA in the liver cytosol of female rats was almost twice that of the male rats and may be due to an unknown liver cytosolic binding protein. This data is consistent with our reported variation in L-FABP expression between the livers of male, female and pregnant rats, noting as well that L-FABP affects fatty acid transport (49). However, it is unclear whether it is in play here because PFOA binds much less to L-FABP than does PFOS and more than an order of magnitude less than oleic acid (23, 25). Importantly, relevant to the next section on enterohepatic recycling, Kim *et al.* (2016) found, as reported earlier by Johnson and Gibson (1979) (17), that about 60% of the dose of PFOA is excreted in urine and faeces of female rats within 24 h after IV or oral administration. Moreover, there was a markedly increased urinary to faecal recovery of PFOA in female rats relative to male rats and to PFOS in both male and female rats (45). These findings are consistent with the assertion by Kim *et al.* (2016) (45) that the gender difference in PFOA pharmacokinetics may be related to differential organic anion transporter expression in male and female rats affecting PFOA excretion via the urine. What is unclear is the potential impact of menstruation as Wong *et al.* (2014) (50) suggested in humans that menstruation could account for about 30% of the PFOS elimination half-life difference between human females and males. This pathway should also apply to PFOA. The EPA also examined a number of studies in which doses of PFOA were varied. For instance, the Rigden *et al.* (2015) (51) study which involved male Sprague-Dawley rats and doses of 0, 10, 33, and 100 mg/kg/day for 3 days and a washout for 3 additional days showed a dose-related increase in urine PFOA concentration and urine PFOA concentration per mg creatinine for the 33- and 100-mg/kg/day groups compared to the 10-mg/kg/day group. The peak in PFOA excretion normalized to creatinine occurred on day 3 after the cessation of dosing. The EPA report concluded that the urine results support the renal resorption hypothesis concept and suggest that there is a threshold limit on urinary resorption. The EPA report on PFOA also recognises the importance of sex hormones in this resorption process and quotes the work of Kudo *et al.* (2002) (52) who showed that male sex hormones appear to decrease, whilst female hormones increase, the renal OATs membranes responsible for this process. We note the final comment of the EPA report on PFOA related to this discussion, states: "*Unfortunately, much work remains to be done to explain the gender differences between male and female rats and to determine whether it is relevant to humans.*"

Figure 6. Location of transporters [Klaassen & Aleksunes (2010)].



However, we suggest that the literature and especially the recent work of Kim *et al.* (2016) (45) sheds some light on this issue. The current EPA model assumes that PFOA is reabsorbed from the kidney filtrate into the kidney. Our observation is that a focus on this mechanism alone is inconsistent with the known literature. For instance, Hanhijarvi *et al.* (1982) (47) markedly reduced female excretion of PFOA in female, but not male Holtzman rats, by administering probenecid with PFOA. This suggests that the females are actively secreting PFOA whereas males are not and raises the question as to whether there should be an active secretory step going from the plasma free fraction compartment to the kidney. In our view, there should be both active renal secretion across the basolateral membrane of the glomerulus as well as tubular reabsorption across the apical tubular cells of the kidney. This model will then be consistent with the EPA reported distribution of OAT transporters (Figure 6) derived from the work of Klaassen & Aleksunes (2010) (53) and females expressing more OAT at both surfaces than males. However, it needs to be emphasised that active tubular reabsorption of water in the urinary filtrate will greatly increase the concentrations of PFOA in that urinary filtrate and so it is this much higher concentration that may impede renal resorption. Indeed, the EPA report did acknowledge that the renal resorption model alone predicted higher levels than were observed in two monkeys - possibly "because the model did not allow for efflux of PFOA into the glomerular filtrate through transporters on the basolateral surface of the tubular cells". Tan's (2008) modification (54) of adding a storage compartment to the Anderson *et al.* (2006) model (55) also did not include this active renal secretion process.

h.0.0.6 g. EPA pharmacokinetic models do not include biliary excretion and enterohepatic recirculation as an excretory process – especially in man.

In our view, a notable omission in the EPA modelling is the recognition of the biliary excretion process. For instance, Yu *et al.* (42) also examined the biliary excretion of PFOS (shown here in Table 4; Table 2 from that paper), but the EPA do not appear to have included that aspect in their modelling.) Table 3 highlights two important points: (a) there is significant excretion of PFOS into bile and (b) the ratio $PFOS_{bile}/PFOS_{serum}$ (the inverse of what is shown in Table 3) decreases with PFOS dose and is consistent with saturation of the PFOS uptake into the liver to be excreted into the bile.

Table 4. Evidence of PFOS biliary excretion in rats in Yu *et al*. (2011) – mean (±SE) PFOS serum and bile concentrations

Table 2 Mean (±SE) PFOS concentrations in serum and bile

PFOS (mg/kg bw)	Serum PFOS (mg/L)	Bile PFOS (mg/L)	$PFOS_{[serum]}/PFOS^{a}_{[bile]}$
0.0	<LOQ[b]	<LOQ	–[c]
0.2	1.09 ± 0.12	1.51 ± 0.42	0.72
1.0	8.20 ± 0.13	3.58 ± 0.66	2.29
3.0	33.5 ± 1.79	6.51 ± 0.67	5.15

[a] Mean serum PFOS concentration to mean bile PFOS concentration

[b] Limit of quantification. The LOQ for PFOS determination was 0.5 μg/L

[c] No ratio can be calculated

This first finding is supported by the work of Harada *et al.* (2007) who obtained serum and bile samples from patients (2 male and 2 female; aged 63–76) undergoing gallstone surgery to determine the bile to serum ratio and biliary resorption rate (31). Harada's Table 4 suggests the biliary excretion dominates over urinary excretion in the overall clearance of both PFOA and PFOS in humans but that urinary excretion for PFOA dominates in rats and is especially high in female rats. Importantly, Harada *et al.* suggests that the reabsorption rates of biliary excreted PFOA and PFOS in humans that were calculated to be 0.89 and 0.97, respectively could contribute to the long half-life in humans.

Table 5. Relative clearance, urinary and biliary excretion and reabsorption for PPOA and PFOS in rats and humans in rats – from Table 4 in the paper of Harada *et al*., 2007.

			Serum half-life (day)	(1) Total clearance (mL/day/kg)	(2) Urinary excretion (mL/day/kg)	(3) Biliary excretion (mL/day/kg)	Reabsorption rate {1 − [(1) − (2)]/(3)}
PFOA	Rat[a]	Male	5.63	50.4	46.1	3.30	–
		Female	0.08	2233	1054	3.52	–
	Human[b]		1387	0.150	0.030	1.06	0.89
PFOS	Human[b]		1971	0.106	0.015	2.98	0.97

[a] Half-lives, total clearance and urinary excretion were reported by Kudo et al. (2002). Biliary excretion was estimated from a report by Vanden Heuvel et al. (1991).

[b] Half-lives in retired workers were reported by Olsen et al. (2005). Urinary excretion was reported by Harada et al. (2005a). Total clearance was calculated based on a volume distribution (V) of 300 mL/kg. The medians of biliary excretion in this study are presented.

Important from a patient treatment perspective is the work of Genuis *et al.* (2010) who showed that ingestion of 4 g/day cholestyramine (a bile acid sequestrant) in three doses for 20 weeks decreased the PFOS serum levels from 23 ng/g serum to 14.4 ng/g serum (56). Cholestyramine also increased both PFOS and PFOA, as well as perfluorohexansulfonate (PFHxF), in stools relative to pretreatment, but more so in females. We have previously reported in an overview of the literature that cholestyramine markedly increased the faecal excretion of the pesticide chlordecone in rats and in humans and that dietary supplementation with 4% cholestyramine enhanced faecal excretion of pentachlorophenol in rhesus monkeys (57). Importantly, cholestyramine is generally regarded as being relatively safe medicine to be administered as a sequestrant over an extended period of time, with an acceptable side-effect profile when used in the medium term. An issue with its long term use is that it may cause a deficiency of fat-soluble nutrients including vitamins A, D, E and K, and coenzyme Q10. A possible way round this is to administer a vitamin supplement.

Fluctuations in a log linear serum concentration – time profiles are often a tell-tale sign of enterohepatic recycling (57). Figure 7 shows two examples of the observed serum concentration – time profiles reported for monkeys by Chang (15). It is evident that fluctuations exist.

Figure 7. Fluctuations in serum level data of Chang *et al*. that may be evidence of enterohepatic recycling.

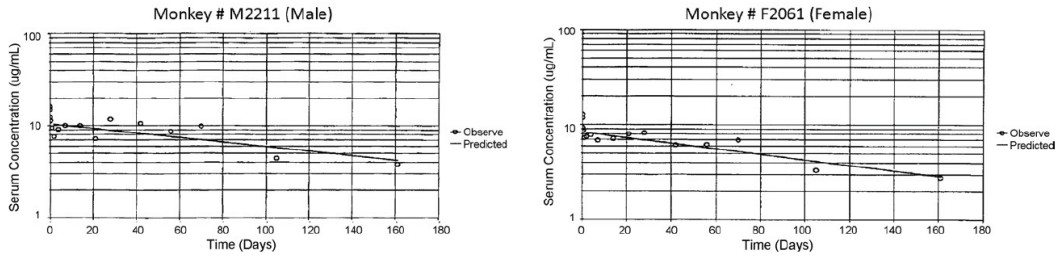


Fig. 11. Serum PFOS concentrations (μg/mL) in male (left column) and female (right column) cynomologus monkeys ($N = 3$/sex) over time after a single IV dose of 2 mg K$^+$PFOS/kg body weight. Error bars represent standard error.

h.0.0.7 h. Saturable active efflux mechanisms from vital organs.

The EPA report further suggests that "PFOS was rarely detected in amniotic fluid unless the serum concentration was ≥ 0.0055 μg/mL" and the CSF to serum ratio of 9.1 x 10^{-3} indicate that PFOS does not easily cross the adult blood-brain barrier. An alternative view is transport does occur but there are effective active efflux mechanisms and, only when these become saturated, PFOS will be seen in significant concentrations in both the brain (as in Table 3) and in the amniotic fluid. The report also comments that both experimental data and pharmacokinetic models show higher levels of PFOS in fetal serum and brain relative to the maternal compartments, suggesting potentially either an imperfect barrier or, possibly more likely, the PFOS efflux transporter expression has yet to mature. Organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs) are involved in the gastrointestinal absorption, uptake by the tissues, and excretion via bile and the kidney of PFOA. They function to protect the lungs, heart, brain, placental barrier, testes, mammary glands and fetus from foreign compounds. Interestingly the ABC transporters are more developed, and at an earlier stage of gestation in rats so that an interspecies variance is likely to be quite significant for these as well.

h.0.0.8 i. As shown in Table 6, there is considerable species variability in elimination half-lives of both PFOS and PFOA.

Estimated half-lives of PFOA in humans range from 2.3 yrs to 8.5 yrs (58-61), while for PFOS, the estimated half-life is 5.4 yrs (60), suggesting that both compounds are not readily eliminated. In contrast, PFOA and PFOS half-lives in monkey (62, 63), rat (43, 45), mouse (15, 28) and rabbit (64) are much shorter, being measured in days or hours. PFOS half-lives are generally longer than PFOA in animals, with monkey showing the longest half-lives of all animals studied 121 days and 21 – 30 days for PFOS and PFOA respectively. However, it needs to be recognised that whilst the half-lives for both PFOS and PFOA have been estimated in animals using washout studies, this approach has not been used to date in humans. The method used was cross-sectional studies not using longitudinal data and, as a result, are in our view of limited validity. In these studies, human elimination half-lives of PFOS and PFOA were determined by fitting the declined serum concentration over 1 – 5 years to a first order model. These estimate depends on the additional assumption that ongoing PFOS or PFOA exposures only contribute negligible amounts to current serum PFOS or PFOA concentrations. If there were substantial PFOS or PFOA exposures during follow-up, the half-lives would be overestimated. In addition, the actual pharmacokinetics of PFOS and PFOA in humans is not likely to be consistent with a one-compartment distribution as evident in monkeys.

Half-life values for serum elimination of PFOA in human populations exposed environmentally through the consumption of PFOA-contaminated drinking water were estimated to be 3.26 years (geometric mean, range= 1.03-14.67 years in Germany) and 2.3 years (95% CI=2.1-2.4 years, Mid-Ohio Valley). An additional cross-sectional study in the Mid-Ohio Valley (with estimated, rather than measured, initial serum concentrations) identified average half-life values of 2.9–8.5 years (with values varying depending on the average serum concentrations in the community and duration since cessation of exposure) (61). Estimates of half-lives in animals, obtained by experimental studies in which the chemicals were administered orally or IV, are much lower, namely 21-30 days, 1.6-8days, 3-16 hrs and 16-22 days for the monkey, male rat, female rat and mouse, respectively.

A point of difference between the analysis we present later and that undertaken by the EPA is that in the EPA Report there is a suggestion that the long half-lives appear to be the result of saturable resorption from the kidney. A key

difficulty and challenge we face in presenting our alternative model later on is that a number of published toxicokinetic models have used saturable resorption as a basis for predicting serum values in animals and humans, including one developed by the U.S. Environmental Protection Agency (EPA) to support this assessment, but have ignored other saturable uptake and secretion process. However, in the absence of quantitative data it is difficult to comment on the magnitude of the likely effect of these saturable processes.

h.0.0.9 j. The mean ± SD volume of distribution (Vd) for PFOS was 202 ± 23 and 274 ± 48 mL/kg, in male and female cynomolgus monkeys, respectively, following a single IV dose of 2 mg/kg (15).

Animals were evaluated up to 23 weeks after dosing, and the resulting volumes of distribution are similar to the 230 mL/kg calibrated from human data by Thompson *et al.* (2010) described above (65). In PFOA studies in cynomolgus monkeys (62), the mean ± SD (Vd) for PFOS was 181 ± 12 and 198 ± 69 mL/kg, in males and females respectively, following a single IV dose of 10 mg/kg

h.0.0.10 k. Toxicity – dose – serum concentrations relationships.

An impressive section in the EPA report is the use of the average serum concentration as a measure of PFOS and PFOA systemic dose exposure and toxicity. Here, the AUC for the LOAEL or NOAEL of each data set (which lasted between 17–182 days) was used to determine an average serum concentration by dividing it by the duration of the study in days. Table 7 (EPA Table 4-6) provides dosing duration and the predicted average serum concentration from each of the modelled studies for PFOS. It is reported that the key internal doses associated with the developmental and liver effect levels (LOAELs) differ by less than an order of magnitude (19.9–157 µg/mL), while the corresponding AUC values (EPA Tables 4-3 through 4-5) differ by more than an order of magnitude (30,100 µg/mL*h–684,000 µg/mL*h). Table 8 (EPA Table 4-6) shows NOAEL and LOAEL based on both daily dose and average serum concentrations for PFOA. The internal doses associated with LOAELs for PFOA differ by less than an order of magnitude (13.1 – 96.2 mg/L), while the corresponding AUCs differ by over two orders of magnitude (5,360 – 38,0,000 mg/L*h).

Table 6. Effect of species and gender on PFOA and PFOS half-lives.

Compound	Species	Gender	Administration route	Dose	Half-life	References
PFOA	Human (retired workers)	24 M, 2 F	Fluorochemical production workers over 5 years	N/A*	3.8 yr	Olsen *et al.* 2007 (66)
	Human (adults)	Combined M, F	Contaminated drinking water	N/A	2.3 yr	Bartell *et al.* 2010 (67)
	Human (adults and children)	Combined M, F	Contaminated drinking water	N/A	3.3 yr	Brede *et al.* 2010 (68)
	Human (adults and children)	Combined M, F	Contaminated drinking water	Highly exposed	2.9 yr	Seals *et al.* 2011 (61)
				Less exposed	8.5 yr	
	Monkey	M	i.v.	10 mg/kg	21 days	Butenhoff *et al.* 2004 (69)
		F			30 days	
	Rabbit	M	Oral gavage	10 mg/kg	5.5 hrs	Hundley *et al.* 2006 (70)
		F			7 hrs	
	Rat	M	N/A	N/A	6-8 days	Kemper. 2003 (71)
		F			3-16 hrs	
	Rat	M	Single dose i.v. or oral	1 mg/kg	1.6-1.8 days	Kim *et al.* 2016 (45)
		F			0.15-0.19 days	
	Mouse	M	Single oral gavage	1 mg/kg or 10 mg/kg	21.7 days	Lou *et al.* 2009 (72)
		F			15.6 days	

Compound	Species	Gender	Administration route	Dose	Half-life	References
PFOS	Human (retired workers)	Combined M, F	Fluorochemical production workers over 5 years	N/A	5.4 yr	Olsen *et al.* 2007 (66)
	Cynomolgus Monkeys	M	i.v.	2mg/kg/day	132 days	Chang *et al.* 2012 (73)
		F			110 days	
	Rat	M	Single dose i.v. or oral	1 mg/kg	26.4-28.7 days	Kim *et al.* 2016 (45)
		F			23.5-24.8 days	
	Rat	M, F	Oral	400 ug/kg	30 -50 days (depending on isomer)	Benskin *et al.* 2009 (43)
	Mouse	M	Oral	1 mg/kg	42.8 days	Chang *et al.* 2012 (73)
		F			37.8 days	

**N/A: no known value*

Table 7. NOAEL and LOAEL, based on dose and serum concentrations for PFOS, as described in EPA's Table 4-6 (1)

Study	Dosing duration days	NOAEL mg/kg/day	NOAEL (Av serum µg/mL)[a]	LOAEL mg/kg/day	LOAEL (Av serum µg/mL)[a]
Seacat et al. 2002 monkey: ↑liver weight + histopathology; ↓body weight; ↓T3; ↑TSH	182	0.15	38 (0.564)	0.75	157 (2.45)
Seacat et al. 2003 male rat: ↑liver weight, centrilobular vacuolization,↑ ALT, ↑BUN	98	0.34	16.5 (0.522)	1.33	64.6 (2.06)
Luebker et al. 2005b: ↓ rat pup body weight[b]	84	0.1	6.26 (0.155)	0.4	25 (0.583)
Luebker et al. 2005a: ↓ rat pup body weight[b]	63	None	None	0.4	19.9 (0.525)
Luebker et al. 2005a rat: ↓ maternal body weight, gestation length and pup survival[b]	63	0.4	19.9 (0.525)	0.8	39.7 (1.09)
Butenhoff et al. 2009 rat developmental neurotoxictiy: ↑increased motor activity↓ habituation	41	0.3	10.4 (0.328)	1.0	34.6 (1.05)
Lau et al. 2003: ↓rat pup survival; ↓maternal and pup body weight	19	1.0	17.5 (0.609)	2.0	35.1 (1.3)

Notes: [a] Average serum concentrations predicted from PK simulations of dose regimens were performed using species-specific parameter distributions. The number in parentheses is the SD.
[b] Multiple effects are included for the Luebker et al. (2005a, 2005b) studies to distinguish between the effects quantified for dose-response.

Table 8. NOAEL and LOAEL, based on dose and serum concentrations for PFOA, as described in EPA's Table 4-6 (2)

Study	Dosing duration days	NOAEL mg/kg/day (AUC mg/L*h)	NOAEL (Av serum mg/L)	LOAEL mg/kg/day (AUC mg/L*h)	LOAEL (Av serum mg/L)
DeWitt et al. 2008: mice; ↓ IgM response to SRBC	15	1.88 (13,800)	38.2 (2.63)	3.75 (22,400)	61.9 (3.58)
Lau et al. 2006: mice reduced pup ossification (m, f), accelerated male puberty	17	None	None	1 (16,400)	38.0 (1.4)
Perkins et al. 2004: rats; ↑liver weight/necrosis	91	0.64 (69,100)	31.6 (0.073)	1.94 (168,000)	77.4 (2.98)
Wolf et al. 2007: mice; GDs 1–17 ↓Pup body weight[a]	17	None	None	3 (33,700)	77.9 (4.3)
Wolf et al. 2007: mice; GDs 7–17 ↓Pup body weight[a]	11	None	None	5 (25,400)	87.9 (4.57)
Butenhoff et al. 2004a: ↓relative body weight/↑ relative kidney weight and ↑kidney:brain weight ratio in F0 and F1 at sacrifice	84	None	None	1 (92,500)	45.9 (1.29)

Notes: Significance $p < 0.05$ or < 0.01
m = male; f = female; SRBC = Sheep Red Blood Cell
[a] serum from pups on PND 22

h.0.0.11 I. RfD calculation.

The derived average concentration (in µg/mL) for the NOAELs and LOAELs in Table 4-6 (EPA report) is scaled using the equation below to predict oral HEDs in mg/kg bw /day for each corresponding serum measurement.

HED = average serum concentration (in µg/mL) × CL

For PFOS, CL = $V_d \times (\ln 2 \div t_{1/2})$ = 0.23 L/kg bw × (0.693 ÷ 1971 days) = 0.000081 L/kg bw/day;

where V_d=0.23 L/kg is from Thompson *et al.* (2010) (65) and $t_{1/2}$=5.4 years (5.4 × 365 = 1971 days) is from Olsen *et al.* (2007) (66).

For PFOA, CL = $Vd \times (\ln 2 \div t_{1/2})$ = 0.17 L/kg bw × (0.693 ÷ 839.5 days) = 0.00014 L/kg bw/day;

where V_d=0.17 L/kg is from Thompson *et al.* (2010) (65) and $t_{1/2}$=2.3 years (2.3 × 365 = 839.5 days) is from Bartell *et al.* (2010) (67)

The resulting HED values are shown in Table 9 and 10 below.

The PK HEDs derived from selected studies were used as POD. An uncertainty factor for intraspecies variability (UFH) of 10 is assigned to account for variability in the responses within the human populations because of both intrinsic (genetic, life stage, health status) and extrinsic (life style) factors that can influence the response to exposure.

An uncertainty factor for interspecies variability (UFA) of three was applied to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). An uncertainty factor for LOAEL to NOAEL extrapolation (UFL) of one to three was applied to all PODs for PFOS. UFLs of one to 10 were applied for PFOA .

Based on the consistency of the response and of the use of the most sensitive endpoint, developmental toxicity, as the critical effect, the RfD of 0.00002 mg/kg/day from Luebker *et al.* (2005) (74) is selected as the RfD for PFOS. This RfD is derived from reduced pup body weight in the two-generation study in rats. The POD for the derivation of the RfD for PFOS is the HED of 0.00051 mg/kg/day that corresponds to a NOAEL that represents approximately 30% of steady-state concentration. An UF of 30 (10 UFH and 3 UFA) was applied to the HED NOAEL to derive an RfD of 0.00002 mg/kg/day. The RfD of 0.00002 mg/kg from Lau *et al.* (2006) (75) is selected for PFOA, which is derived from reduced ossification of the proximal phalanges (forelimb and hindlimb) and accelerated puberty in male pups as the critical effects. The POD for the derivation of the RfD for PFOA is the HED of 0.0053 mg/kg/day that corresponds to a LOAEL that represents approximately 60% of steady-state concentration. An UF of 300 (10 UF_H, 3 UF_A and 10 UF_L) was applied to the HED LOAEL to derive an RfD of 0.00002 mg/kg/day for PFOA.

Table 9. HED derived from the modelled animal average serum concentrations of PFOS

Study	Dosing duration days	NOAEL mg/kg/d	NOAEL Av serum µg/mL	HED mg/kg/d	LOAEL mg/kg/d	LOAEL Av serum µg/mL	HED mg/kg/d
Seacat et al. 2002 monkey: ↑liver weight + histopathology; ↓body weight; ↓T3; ↑TSH	182	0.15	38	0.0031	0.75	157	0.013
Seacat et al. 2003 male rat: ↑liver weight, centrilobular vacuolization,↑ ALT, ↑BUN	98	0.34	16.5	0.0013	1.33	64.6	0.0052
Luebker et al. 2005b rat: ↓pup body weight	84	0.1	6.26	0.00051	0.4	25	0.002
Luebker et al. 2005a rat: ↓ pup body weight	63	None	None	None	0.4	19.9	0.0016
Luebker et al. 2005a rat: ↓maternal body weight, gestation length and pup survival	63	0.4	19.9	0.0016	0.8	39.7	0.0032
Butenhoff et al. 2009 rat developmental neurotoxictiy: ↑motor activity, ↓habituation	41	0.3	10.4	0.00084	1.0	34.6	0.0028
Lau et al. 2003 rat: ↓ pup survival; maternal and pup body weight	19	1.0	17.5	0.0014	2.0	35.1	0.0028

Table 10. HED derived from the modelled animal average serum concentrations of PFOA

Study	Dosing duration days	NOAEL mg/kg/d	NOAEL Av serum mg/L	HED mg/kg/d	LOAEL mg/kg/d	LOAEL (Av serum) mg/L	HED mg/kg/d
DeWitt et al. 2008: mice; ↓ IgM response to SRBC	15	1.88	38.2	0.0053	3.75	61.9	0.0087
Lau et al. 2006: mice reduced pup ossification (m,f), accelerated male puberty	17	None	-	-	1	38.0	0.0053
Perkins et al. 2004: rats; ↑liver weight/necrosis	91	0.64	31.6	0.0044	1.94	77.4	0.0108
Wolf et al. 2007: mice; GDs 1–17 ↓pup body weight	17	None	-	-	3	77.9	0.0109
Wolf, et al. 2007: mice; GDs 7–17 ↓pup body weight[1]	11	None	-	-	5	87.9	0.0123
Butenhoff et al. 2004a: ↓F0 body weight/↑ absolute and relative kidney weight	84	None	-	-	1	45.9	0.0064
Macon et al. (2011) GDs 1–17 ↓mammary gland development[2]	17	-	-	-	0.3	12.4	0.0017

Notes: Significance $p < 0.05$ or < 0.01
m = male; f = female; SRBC = Sheep Red Blood Cell
[1] serum from pups on PND 22
[2] serum from pups on PND 7

6 Analysis of Report by Professor Andrew Bartholomaeus: Procedural Review of Health Reference Values Established by enHealth for PFAS (3)

This independent review sought to examine the interim human health reference values (HRVs) for per- and poly-fluorinated alkyl substances (PFAS) according to the following terms of reference:

1. Approaches and assumptions used by the European Food Safety Authority (EFSA), as outlined in the reports Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts, Scientific Opinion of the Panel on Contaminants in the Food Chain (EFSA, 2008) and Perfluoroalkylated substances in food: occurrence and dietary exposure (EFSA, 2012).
2. Approaches and assumptions used by the United States Environmental Protection Agency (US EPA), as outlined in the 2016 Health Effects Support Document for Perfluorooctane Sulfonate (PFOS) (US EPA, 2016b) and the 2016 Health Effects Support Document for Perfluorooctanoic Acid (PFOA) (US EPA, 2016a).
3. The applicability and relevance of these approaches and assumptions in the Australian context, having regard to existing Australian regulatory science policy as described in such guidance materials as:
    a. Australian Pesticide and Veterinary Medicines Authority (APVMA) Data guidelines (http://apvma.gov.au/registrations-and-permits/data-guidelines) and Application of science to regulatory risk assessment (http://apvma.gov.au/node/15486)
    b. the enHealth Environmental Health Risk Assessment, Guidelines for Assessing Human Health Risks from Environmental Hazards (enHealth, 2012);
    c. the Food Standards Australia New Zealand (FSANZ) Risk Analysisß in Food Regulation publication: (http://www.foodstandards.gov.au/publications/riskanalysisfood regulation/Pages/de fault.aspx (FSANZ)
    d. the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) Handbook for notifiers: https://www.nicnas.gov.au/regulation-andcompljance/nicnas-handbook (NICNAS)
    e. the National Health and Medical Research (NHMRC) Guidelines for Managing Risks in Recreational Water (NHMRC, 2008) and NHMRC Australian Drinking Water Guidelines (NHMRC, 2016).

His report focused on: a) principal sources of variation between the US EPA and EFSA risk assessments of PFAS and the resultant guidance values, and b) the extent to which the different approaches were consistent with that used in Australia and the suitability of the EFSA values selected by enHealth as an interim measure. His report examined the potential sources of strength and weakness in each assessment, including how this work differs in its nature, approach and significance from the more usual regulatory approaches.

Professor Bartholomaeus pointed out that *The Standing Committee on Environmental Health (enHealth) under the guidance of the Australian Health Protection Principal Committee (AHPPC) provides nationally agreed environmental health policy advice, based on the best available evidence and expertise, to the Australian Health Ministers Advisory Council (AHMAC) through the AHPPC. He further notes that: On 15 March 2016, the AHPPC endorsed the enHealth Guidance Statements on Perfluorinated Chemicals (PFCs), which include an undertaking by enHealth to convene an expert group, in early 2016, to provide advice to the AHPPC on the development of an Australian interim HRV for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) for consistent use in the undertaking of human health risk assessments and the management of contaminated sites across Australia*. The various international PFOS and PFOA HRVs from various agencies considered by the workshop vary considerably in their current recommendations on established HRVs (Table 1). We note that Table 1 refers to both the Tolerable Daily Intake (TDI) and Reference Dose (RfD) (both in ng/kg/d). There is also another term that is widely used called the acceptable daily intake (ADI). As clarification, we are aware that the US EPA has replaced both TDI and ADI by the single RfD term, which is defined as an estimate, involving perhaps an order of magnitude in uncertainty, of the daily exposure of a solute in the human population without an appreciable risk of deleterious effects in either normal and sensitive groups during a lifetime.

It is observed that the agencies' recommendations for TDI/RfD for PFOS and PFOA vary by 7.5 times and 75 times, respectively. Importantly, although the EFSA TDI/RfD value for PFOA is almost 8 times that of the next highest value in Table 11, the workshop recognised that a key determinant for human systemic (i.e. internal) exposure after oral

dosing is the duration of that exposure as a consequence of the very long half-life of these solutes in humans, and that reducing HRVs and drinking water guideline values will not significantly affect short term systemic exposure in affected communities, lowering the HRVs established by EFSA would have no short/medium term impact on public health. As a consequence, the enHealth committee agreed to use the *EFSA HRVs as temporary (i.e. interim) values pending the finalisation of the FSANZ review*.

Table 11. Health Reference Values for PFOS and PFOA from International Regulatory Agencies (from Prof Bartholomaeus)

	PFOS			PFOA		
	PoD mg/kg/d	UF	TDI/RfD ng/kg/d	PoD mg/kg/d	UF	TDI/RfD ng/kg/d
EFSA 2008	0.03	200	150	0.3	200	1500
USEPA 2016	0.00051#	30	20	0.0045#	300	20
ATSDR 2015	0.00252#	90	30	0.00154#	90	20
Danish EPA 2015	0.033	1230	30	0.003#	30	100
USEPA DWG 2009	0.03	390	80	0.46	2430	190
Minnesota 2009	0.0025#	30	80	0.0023#	30	77
Germany 2006	0.025	300	100	0.1	1000	100

Based on the human equivalent dose derived from theoretical pharmacokinetic modelling and incorporating a variety of assumptions to compensate for data deficiencies.

Prof Bartholomaeus then summarises the key decisions on Tolerable Daily Intake (TDI) values, interim drinking water guideline values, interim guideline values for surface water, seafood screening guideline values and other aspects. He suggests that because PFAS have an exceptionally long half-life in human blood, the primary determinant of ongoing exposure is the existing blood level and not the daily intake (other than the unlikely scenario of intake of aberrantly high levels of PFAS). **We comment here that it is the initial exposure to the PFAS combined with its long half-life in the body that defines its ongoing exposure and that the existing blood levels are a reflection of that exposure**.

A key issue raised in 4.1.4 is historical reliance on the inherently risk averse or precautionary process for human health risk assessment (HHRA) based on policy, convention and the best available science, including new approaches and refinements in cross species extrapolation. A key challenge is whether reducing uncertainty by using a more physiologically based method is appropriate if it simply replaces one source of uncertainty with another. A key requirement is that the approach is valid and robust. In general, toxicology data obtained in animals or in humans through direct experimental testing or epidemiology studies is based on translating the dose at which the most sensitive toxicological effect is seen in the most sensitive species to the dose Point of Departure (PoD) that defines safe exposure levels for the general human population. Key in this analysis is Hazard Analysis, in which the lowest dose of a solute causing an adverse effect is called the Lowest Observed Adverse Effect Level (LOAEL) and the dose immediately below that dose causing no adverse effects, called No Observed Adverse Effect Level (NOAEL). The use of a pharmacokinetic modelling approach, that seeks to estimate human dosing based on an equivalency in the blood levels in the experimental animal used to characterise a solute's toxicity, to determine the Human Equivalent Dose (HED) is widely used by the US EPA. As it is not widely used by other agencies, it is a key source of variance between PoDs proposed by different agencies.

The requisite human health reference values (HRVs) are then achieved by dividing the PoD by various uncertainty factors (which may take into account variation within pharmacokinetics in humans and potential errors in extrapolating from animals to humans). The resulting HRV is expressed as a tolerable (or acceptable, permissible etc) daily intake value (TDI) (or RfD) in weight units per kg of body weight per day. Prof Bartholomaeus also provided a lucid discussion of how epidemiology studies may underpin HHRAs, including also a consideration of their limitations.

A key component in this report is a consideration of the differences between the EFSA and US EPA assessments. It is the processes used and not the data that underpins the assessments that have led to the differences. Some emphasis is given to hepatic and developmental toxicity, as these most sensitive toxic effects of PFAS in animals dominate the experimental animal data sets used by both US EPA and EFSA. The US EPA has used serum levels for PFOS and PFOA, given in Tables 2 and Table 4, defined from experimental animals by Physiologically Based

Pharmacokinetic Modelling (PBPK) to calculate HED at the NOAEL and LOAEL. **However, we also note that HED for the various studies for PFOS (Table 2) and PFOA (Table 4) vary by about 10 and 7 fold, respectively. Importantly, the EFSA values for NOAEL and LOAEL for the Seacat *et al.* (2002) monkey gavaged data (76) appear somewhat lower than the US EPA data for the same study. Further, contrary to assertions made by Prof Bartholomaeus, notwithstanding the liver and GIT tract exceptions, the concentration of PFAS in target tissues is not necessarily proportional to the serum levels-rather the concentrations in the various organs will be higher or lower depending on the relative uptake and secretion processes in each of the organ. As we discuss later, saturable transport processes can lead to some organs being higher and other lower relative to the increase in serum levels with an increase in PFAS dosing. This limitation also applies to the "dose" experienced by the target tissue (or foetus/neonate) relative to that in the maternal serum. Further, given that the Pharmaceutical Subcommittee of the Therapeutics Goods Administration is consistently involved in reanalysis of company pharmacokinetic analyses, we were somewhat surprised by the statement "The Workshop noted it is difficult for an independent third party to replicate the US EPA PBPK modelling for estimating the average serum concentration in an animal experiment" – especially also as we present such a replication here**.

We also note the important comments that:

1. The US EPA may not have given sufficient weight to evidence supporting the importance of PPAR alpha in mediating developmental effects in rodents, and
2. The US EPA choice of NOAEL is questionable for some studies, especially as the enHealth workshop notes the NOAEL set for the Butenhoff *et al.* study (77) of PFOS 0.3 mg/kg bw/day is based on effects seen only on postnatal day 17 but not on days 13, 21 or 61.
3. The agencies are selective in which studies they use to make their deductions. For instance, the Luebker, *et al.* (2005) study (78), with a PFOS NOAEL of 6.26 µg/ml, is cited by US EPA but, although reviewed by EFSA, not considered by them in selection of a PoD.

Prof Bartholomaeus also presented the key studies used by the US EPA and by EFSA to derive the PoD for PFOA, shown in Tables 4 and 5. It is evident that the HED for the various studies used by the US EPA varied by about 7.2 times and that the EFSA and US EPA NOAEL and LOAEL, without and with serum level adjustment, are similar in terms of order of magnitude. Prof Bartholomaeus concludes that "*As for PFOS the differences between the US EPA and EFSA derived HRVs for PFOA is predominantly dependent on the use of PBPK modelling by the US EPA and the selection of uncertainty factors by each agency, but with the added complexity of the use of BMD modelling by EFSA to identify the dose for the PoD"*.

He goes on to add: "*In considering the utility of one of these models the ATSDR makes the observation that "The human model was calibrated to predict limitation half-times estimated for human populations (e.g. 2.3 or 3.8 years for PFOA, 5.4 years for PFOS). As a result, comparisons made between observed and predicted serum concentrations evaluate whether or not the populations actually exhibit the half-times to which the model was calibrated, and not the validity of the model to predict the internal distribution of PFOA or PFOS. It is not currently possible to assess with confidence whether the human model can accurately predict doses to liver or any other tissues.*"

There are some other comments made by Prof Bartholomaeus, which we endorse or clarify here:

1. "Reaching steady state concentrations (those where intake and elimination are balanced) requires a large proportion of the storage locations to be filled" is not quite correct. To be more precise, steady state concentrations will be achieved for a solute showing linear pharmacokinetics after the solute has reached equilibration distribution in the body at approximately 5 times the elimination half-life of the solute.
2. "For PFCs it is known they are highly bound to serum albumin, they are therefore confined primarily to the extracellular fluid and have limited distribution into other tissues". However, PFOS is also bound to fatty acid binding protein and this protein translocates fatty acids to the nucleus.
3. The comments that the use of HED for liver effects in experimental animals with markedly shorter half-lives than in humans, particularly rats, may greatly overestimate the potential liver exposure, is insightful. We agree that these can lead to issues in dose extrapolation. Whilst we support the generality of the conclusion: "*The US EPA use of HEDs based on PBPK modelling of serum levels of PFAS is not likely to be appropriate*

for liver effects because liver exposure for the same serum PFAS levels will be higher in rats than in humans, at the least during the absorption phase. Actual administered dose in mg/kg bw is likely to be a better basis for determining the PoD for liver effects", we add as a proviso, this can be overcome by specifically modelling liver exposure and note that this has not been done by the US EPA.

4. We also agree that… *"A cross species dose comparison based on serum PFAS rather than oral dose administered to rats, does therefore provide a potentially more robust basis for identifying the PoD provided assumptions used in the PBPK modelling are robust and grounded in adequate data for each species and humans modelled*".
5. Further, we agree that "*a dramatic difference (exists) in pharmacokinetics between humans and all experimental animals"*.
6. We add too that OAT is likely to also be involved in active hepatic uptake as well as in intestinal uptake and in renal tubular reabsorption.

We have not specifically commented on the epidemiological and organ toxicity issues raised in Prof Bartholomaeus's report as we understand that these are outside the focus of our report. Finally, we note the final recommendations made in this report as being:

1. *The adoption of the EFSA health reference values (TDI) and their use to derive Australian drinking water guideline values, as an interim measure pending FSANZ review, can be concluded to be appropriate, to be based on the expert consideration of the strengths and weaknesses of the available risk assessments from international agencies, and to be consistent with current risk assessment practices both in Australia and internationally.*
2. *Consideration should be given to the need for the responsibility for setting HRVs, and particularly for contaminants that are also present in food and water, to be formally supported in future by an existing agency with;*
    a. experience and expertise in setting, and documenting, these values,
    b. appropriate consultation mechanisms in place to ensure the highest possible degree of transparency,
    c. the capacity to provide expert scientific support to expert working groups and decision making committees.
3. *The scientific literature on, and international regulatory assessments of the HRVs for PFAS should be monitored on an ongoing basis by FSANZ in conjunction with enHealth and adjusted up or down as indicated by the emerging data.*
4. *Australia, through FSANZ or another suitable agency, should consider whether there is value in seeking to initiate an international consultative review of HRVs for PFAS through the CODEX/JECFA mechanism to establish consistent international HRVs for these substances.*
5. *As identified in both this review and by the enHealth workshop, pivotal issues that FSANZ should address and consider seeking specialist expert advice on, include;*
    a. *the strengths, weaknesses and validity of the PBPK approach to HED calculation for PFAS,*
    b. *the relative merits of the interpretation of the epidemiology data by the US EPA compared to most other international agencies'*
    c. *the clinical relevance of the observed lower birth weights and elevated cholesterol levels in highly exposed populations,*
    d. *the significance of the recent US National Toxicology Program (NTP) review of the immune toxicity potential of PFAS.*

These recommendations appear to be reasonable.

7 Modelling Approaches Adopted by US EPA

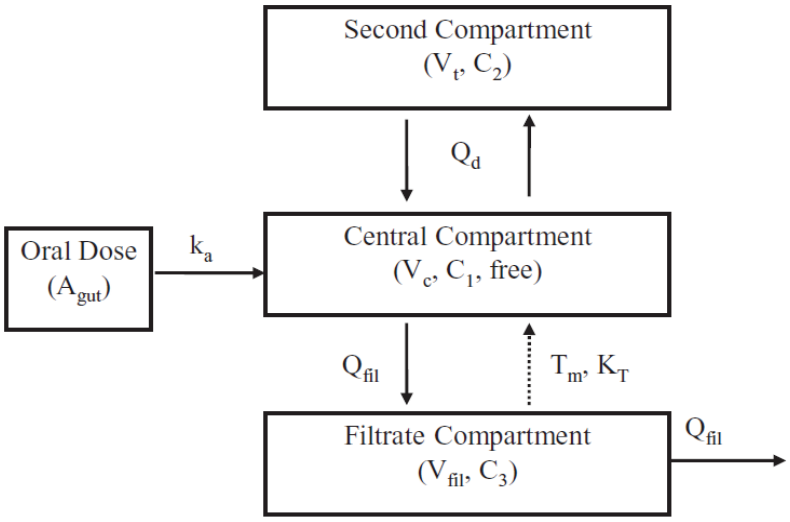
7.1 Summary: Validity of the Modelling Applied by US EPA

- The EPA reports provide a good overview of the literature but their pharmacokinetic modelling has limitations and uncertainties. In particular, there are three key questions that appear not to be answered by the EPA approach:
  a. Why are there huge discrepancies in half-lives of some PFCs between species?
  b. Why do serum concentrations of PFOA and PFOS appear to rapidly approach steady-state after repeated dosing despite their long half-lives?
  c. Why is there a gender difference in the excretion of PFOA by rats?
- Our analysis suggests that there is substantial secretion of these compounds into bile with effective enterohepatic recirculation, as seen by significant amounts of material detected in faeces many days after a single dose given orally or IV.
- Our analysis also suggests that there is an upregulation of active secretory transporters for PFOA at the glomerulus basement membrane and this accounts for the gender differences in the excretion of PFOA.
- Further, we suggest that the EPA modelling has ignored saturable uptake by the liver and intestine and efflux by the placenta and the brain. However, without quantitative data, we are unable to assess what impact these processes have.
- Our modelling shows that the serum concentrations of PFOS and PFOA reach steady state despite a long half-life, contrary to findings by the EPA modelling. This will affect the HED calculations from animal studies where the blood level was taken at sacrifice and back calculated to give an AUC. Exactly how these effects will impact on the PK back calculation in rodents and monkeys is uncertain. However, a saturable processes could lead to a longer time to reach steady state in animal models and lead to a higher AUC estimation leading to a potential overestimate of toxicity in humans on extrapolation.

7.2 Replication and Evaluation of EPA Modelling

The EPA used the 2013 Wambaugh *et al.* model (79) to develop the average serum concentrations at LOAEL or NOAEL level as the point of departure (POD) for RfD derivation, rather than external doses in the studies. The figure below show the diagram of the model:

Figure 8. Model used in EPA analysis [Wambaugh *et al.* (2013)]



Some key assumptions of the model include:

1. First order absorption from gastrointestinal tract after oral exposure
2. Saturable resorption from the kidney filtration with a Michaelis-Menten form
3. Serum carries a significant portion of the total PFOA/PFOS body load - total volume of distribution is not more than 100 times that in the serum.
4. Only free (unbound) compound in blood flow to filtrate and to tissue compartment
5. Not considering inter-subject variability of model parameters- a single numeric value of parameter represents all individuals of the same species, gender, and strain. Body weight, the number of doses, and magnitude of the doses were the only parameters to vary between individuals.

PK data sets used for modelling to determine species specific PK parameters are summarized below in Table 12 for both PFOA and PFOS separately:

Table 12. PFOA and PFOS datasets used in EPA modelling.

PFOA *In Vivo* PK Studies

Study	Subject	Dose Regimen
Butenhoff *et al.* (2004a)	Monkey (M): cynomolgus	7, 10, or 12 weeks (20 mg/kg/day only); 26 weeks (all doses) of daily oral doses
Butenhoff *et al.* (2004b)	Monkey (M/F): cynomolgus	Single iv dose (10 mg/kg)
Kemper (2003)	Rat (M): Sprague Dawley	Single iv dose (1 mg/kg) or oral dose (0.1, 1, 5, and 25 mg/kg)
Kemper (2003)	Rat (F): Sprague Dawley	Single iv dose (1 mg/kg) or oral dose (0.1, 1, 5, and 25 mg/kg)
Lou *et al.* (2009), Christopher Lau, Kaberi Das, Mimi Lin, and John Wambaugh (unpublished)	Mouse (F) CD1	Single oral dose (1, 10, or 60 mg/kg) or 17 day repeated oral dose (20 mg/kg/day) or 29 day repeated oral dose (0.1, 1, or 20 mg/kg/day)
Jamie DeWitt, Robert Luebke, and Carey Copeland (unpublished)	Mouse (F) C57Bl/6	28 days of daily oral doses (0.94, 1.88, 3.75, or 7.5 mg/kg/day)

PFOS *In Vivo* PK Studies

Study	Subject	Dose Regimen
Chang *et al.* (2012)	Monkey (M/F): cynomolgus	Single iv dose (2 mg/kg)
Seacat *et al.* (2002)	Monkey (M/F): cynomolgus	Repeated daily oral dose (0.03, 0.15, or 0.75 mg/kg/day) for 182 days
Chang *et al.* (2012)	Rat (M/F): Sprague Dawley	Single oral or iv dose (2 or 4.2 mg/kg) and oral only at 15 mg/kg
Chang *et al.* (2012)	Mouse (M/F): CD1	Single oral dose (1 or 20 mg/kg)

The PK parameters for different species obtained from EPA modelling are summarized below in Table 13 and 14 for PFOS and PFOA, respectively.

Table 13. Estimated and Assumed PK parameters for PFOS (Wambaugh, *et al*. 2013 (80))

Parameter	Units	CD1 Mouse (F)	CD1 Mouse (M)	Sprague Dawley Rat (F)	Sprague Dawley Rat (M)	Cynomolgus Monkey (M/F)
		Chang *et al.* (2012)	Chang *et al.* (2012)	Chang *et al.* (2012)	Chang *et al.* (2012)	Seacat *et al.* (2002); Chang *et al.* (2012)
BW	kg	0.02	0.02	0.203	0.222	3.42
Cardiac output	L/h/kg0.74	8.68	8.68	12.39	12.39	19.8
ka	L/h	1.66	433.4	4.65	0.836	132
Vcc	L/kg	0.264	0.292	0.535	0.637	0.303
k12	1/h	0.0093	2976	0.0124	0.00524	0.00292
Rv2:v1	-	1.01	1.29	0.957	1.04	1.03
Tmc	mg/h/kg	57.9	11000	1930	1.34e-06	15.5
Kt	mg/L	0.0109	381	9.49	2.45	0.00594
Free	-	0.00963	0.012	0.00807	0.00193	0.0101
Qfilc	-	0.439	27.59	0.0666	0.0122	0.198
Vfilc	L/kg	0.00142	0.51	0.0185	0.000194	0.0534

Table 14. Estimated and Assumed PK parameters for PFOA (Wambaugh, *et al.* 2013 (79))

Parameter	Units	CD1 Mouse (F)	C57Bl/6 Mouse (F)	Sprague Dawley Rat (F)	Sprague Dawley Rat (M)	Cynomolgus Monkey (M/F)
		Lou *et al.* (2009)	Dewitt *et al.* (unpublished)	Kemper (2003)	Kemper (2003)	Butenhoff *et al.* (2004)
BW	kg	0.02	0.02	0.20	0.24	7 (m), 4.5 (f)
Cardiac output	L/h/kg0.74	8.68	8.68	12.39	12.39	19.8
ka	L/h	290	340	1.7	1.1	230
Vcc	L/kg	0.18	0.17	0.14	0.15	0.4
k12	1/h	0.021	0.35	0.098	0.028	0.0011
Rv2:v1	-	1.07	53	9.2	8.4	0.98
Tmc	mg/h/kg	4.91	2.7	1.1	190	3.9
Kt	mg/L	0.037	0.12	1.1	0.092	0.043
Free	-	0.011	0.034	0.086	0.08	0.01
Qfilc	-	0.077	0.017	0.039	0.22	0.15
Vfilc	L/kg	0.00097	0.000076	0.000026	0.0082	0.0021

7.2.1 Replication of the model

To evaluate EPA model, we first write their model in commonly used physiologically-based pharmacokinetic modelling software Berkeley Madonna™. The model diagram is shown in Figure 8. The model assumes that PFOA or PFOS is absorbed from a gut compartment through a first order process with rate constant ka into central compartment. After that, the free fraction of PFOA or PFOS in the central compartment (given by free*C1) distribute to second compartment based on inter-compartmental rate constants (i.e. k_{12} and k_{21}) and is cleared to a filtrate compartment where it is either excreted or resorbed via a saturable process with a Michaelis-Menten form.

The primary and secondary parameters of the model are summarized in tables below:

Table 15. Primary pharmacokinetic parameters used for replication of EPA model.

Parameters	Definition	Unit
BW	Body weight	kg
V_{cc}	Volume of distribution (Central compartment)	L/kg
QC_c	Cardiac output per kg	L/h/kg
Q_{filc}	Renal plasma filtration rate, fraction of cardiac output	-
Tm_c	Transport maximum constant	mg/h/kg
K_t	Transporter affinity constant	mg/L
Free	Fraction of free compound in blood	-
V_{filc}	Volume of renal filtration	L/kg
k_{12}	Transfer rate constant from central to tissue compartment	h-1
$Rv_{2:V1}$	Transfer rate constant from tissue to central compartment	
input	Daily dose	mg/kg/day
k_a	Absorption rate constant	h-1

Table 16. Secondary pharmacokinetic parameters used for replication of EPA model.

Parameters	Definition	Unit
Q_{fil}	$Q_{fil} = Q_{filc} \times QC_c \times BW^{0.74}$ filtration rate for individual animal	L/h
V_c	$V_c = V_{cc} \times$ BW volume of distribution (Central compartment)	L
V_{fil}	$V_{fil} = V_{filc\times}$ BW volume of renal filtration	L
T_m	$Tm = Tm_c \times$ BW transport maximum	mg/h

The differential equations for mass balance used in the model are described below:

$$\frac{dA_{gut}}{dt} = Input - k_a A_{gut} \quad \text{(Gut compartment)}$$

$$\frac{V_c dC_1}{dt} = k_a A_{gut} + k_{21} A_{tissue} - k_{12} V_c C_1 Free - Q_{fil} C_1 Free \quad \text{(Central compartment)}$$

$$\frac{dA_{tissue}}{dt} = k_{12} V_c C_1 Free k_{21} A_{tissue} \quad \text{(Second compartment)}$$

$$\frac{V_{fil} dC_{fil}}{dt} = Q_{fil} C_1 Free - Q_{fil} C_{fil} - \frac{T_m C_{fil}}{K_t + C_{fil}} \quad \text{(Filtrate compartment)}$$

We then re-analysed the same PK data sets used by EPA (Table 12) to derive species specific PK parameters for predicting average serum concentration using Berkeley Madonna™ software. As we did not have access to PFOA PK data from Kemper (2003) (71) and DeWitt *et al.* (unpublished data), these data sets were not included in our repeated analysis.

Our modelling differs with that of EPA in that EPA did not use a commercial software package as we did here but developed their own, in house, software. As stated in their report, "the data were analyzed within a Bayesian framework using Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and genders and to identify serum levels associated with the NOAEL and LOAEL external doses. The model chose vague, bounded prior distributions on the parameters being estimated, allowing them to be significantly informed by the data. The values were assumed to be log-normally distributed, constraining each parameter to a positive value." We also noted that it is stated in a subsequent Wambaugh *et al.* (2013) paper (79): "The Metropolis-Hastings algorithm was used to find the posterior distributions for each parameter such that the predictions of the PK model are consistent with the data and the prior assumptions. A multivariate proposal

distribution for the PK parameters and measurement variances was determined from serval initial runs starting with the Lou *et al.* (2009) CD1 mouse PK values and a diagonal, i.e., uncorrelated, proposal distribution".

As this in house software was not available to us, we replicated the EPA models using a commercial software package that we have found previously to be well suited to this type of analysis. Irrespective of which software is used, we also noted that the Wambaugh *et al.* (2013) paper, reported their estimated parameters as means and 95% credible interval from Bayesian analysis for each parameter. (Credible intervals that are generated by the program R are similar in concept to the more conventional confidence intervals most commonly used in this type of analysis, the difference being that this interval is based on a prior distribution and therefore is fixed whereas the estimated parameter is a random variable, in contrast to confidence intervals where the bounds are random variables and the estimated parameter has a fixed value). We did observe that many of Wambaugh's 95% credible intervals are very wide. However, most importantly, all of our estimates derived using Berkeley Madonna™ software fall within reported 95% credible intervals.

A key limitation in our analysis is that we did not have access to the original PK data and so we could only fit the mean data for each study. It was not clear to us whether the EPA used individual data or mean data in their fitting.

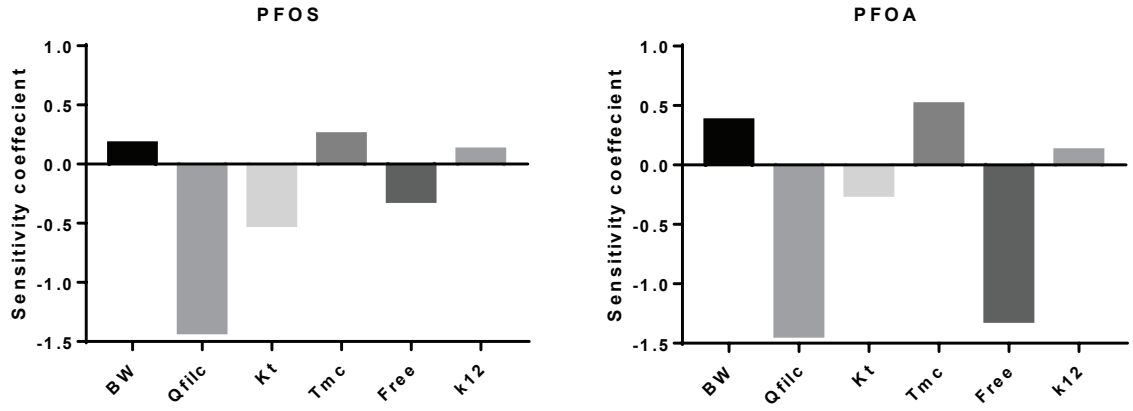
7.2.2 Sensitivity Analysis

A normalized local sensitivity analysis was performed on the EPA model to examine the influence of each model parameter on the model output. Sensitivity coefficients were calculated for the predicted final serum concentration (*C*) with the original parameters (*P*) and for that resulting from a 1% change in each parameter value. The normalized sensitivity coefficients were calculated by the following equation:

$$sensitivity\ coefficient = \frac{dC/C}{dP/P}$$

The normalized sensitivity coefficients for the model parameters for PFOS and PFOA in the monkey with respect to serum PFOS and PFOA concentration are shown in Figure 9. Only parameters with sensitivity coefficients greater than 0.1 are shown.

Figure 9. Sensitivity analysis for PFOS and PFOA.



Sensitivity analysis was performed at 0.03 mg/kg daily doses for PFOS and 3 mg/kg daily doses for PFOA. Both PFOS and PFOA are primarily dependent on the free fraction of compound in the plasma (*Free*) and the parameters governing renal elimination and reabsorption, which are the flow to the filtrate compartment (Q_{filc}), the affinity of transporter (K_t) and maximum transport (T_{mc}).

7.2.3 Reanalysis of PK data sets to obtain pharmacokinetic parameters

The PK parameters obtained from our analysis are summarized in Table 17 and 18 for PFOS and PFOA, respectively. The majority of our estimations differ from PK parameters reported by EPA (Table 13 and 14) by less than an order of magnitude.

Table 17. Summary of estimated and assumed PK parameters for PFOS using EPA Model.

Parameters	Unit	Mouse:CD1 (F)	Mouse: CD1 (M)	Rat: Sprague Dawley (F)	Rat: Sprague Dawley (M)	Monkey: cynomolgus (M/F)
		Chang *et al.* (2012)	Chang *et al.* (2012)	Chang *et al.* (2012)	Chang *et al.* (2012)	Seacat *et al.* (2002)
BW	kg	0.02	0.02	0.203	0.222	3.42
QC_c	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
V_{fillc}	L/kg	0.0039	0.0033	0.011	0.001	0.053
Q_{fillc}		0.5	0.65	0.01	0.015	0.20
V_{cc}	L/kg	0.29	0.28	0.36	0.39	0.30
T_{mC}	mg/h/kg	38.9	28.3	26.9	0.004	15.5
K_T	mg/L	0.1	1.61	9.67	6.47	0.006
Free		0.002	0.003	0.008	0.0033	0.01
k_{12}	h^{-1}	0.003	0.003	0.048	0.0047	0.02
$R_{v2:v1}$		1.37	0.74	0.59	0.79	1.03
k_a	h^{-1}	0.66	6.58	5.41	2.79	132

Table 18. Summary of estimated and assumed PK parameters for PFOA using EPA model.

Parameters	Unit	CD-1 Mouse (F) Lou *et al.* (2009)	Cynomolgus Monkey (M/F) Butenhoff *et al.* (2004)
BW	kg	0.02	7 (m), 4.5 (f)
QC_c	L/h/kg$^{0.74}$	8.68	19.8
V_{fillc}	L/kg	0.00056	0.0026
Q_{fillc}		0.057	0.14
V_{cc}	L/kg	0.13	0.29
T_{mC}	mg/h/kg	5	1.43
K_T	mg/L	0.027	0.036
Free		0.008	0.01
k_{12}	h^{-1}	0.002	0.05
$R_{v2:v1}$		1.39	0.6
k_a	h^{-1}	206	123

The fitting curves of plasma concentration time profiles for each study are shown below in Figure 10 and Figure 11 for PFOS and PFOA respectively.

Figure 10. Plasma concentration-time profiles of PFOS in different species.

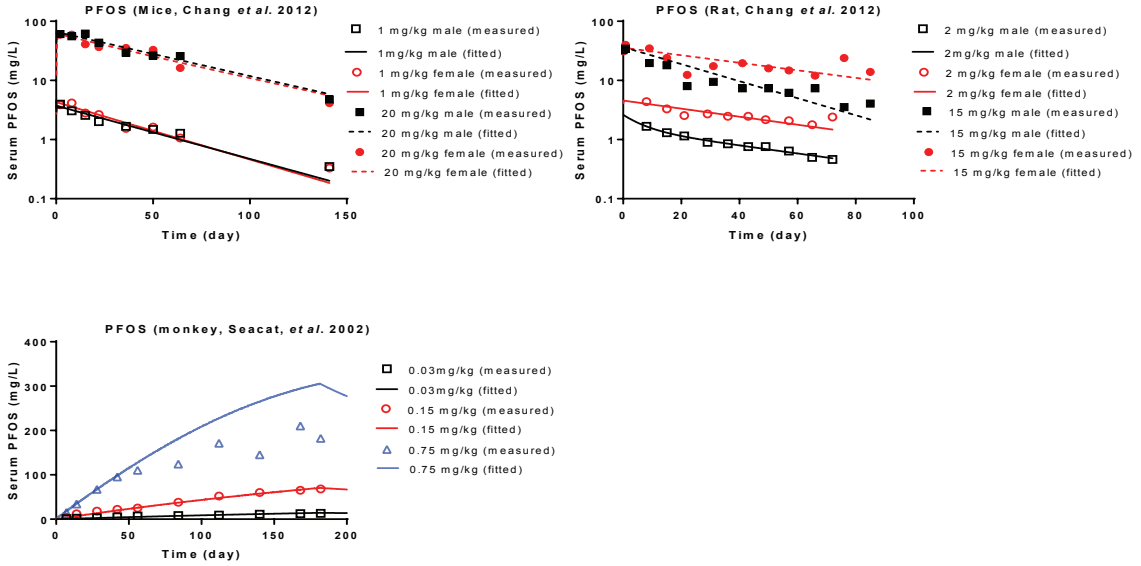
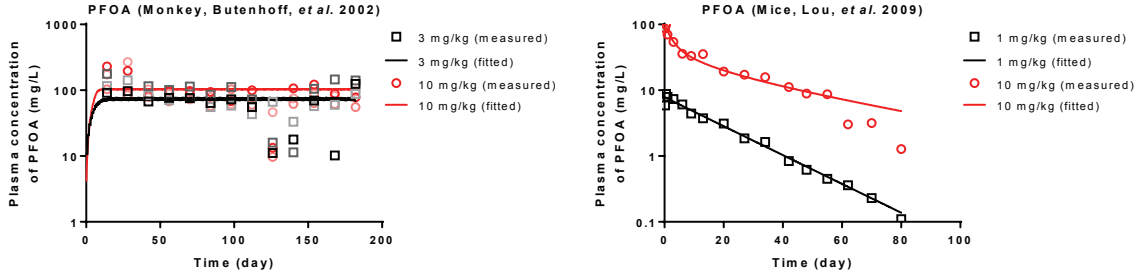


Figure 11. Plasma concentration-time profiles of PFOA in different species.



It is apparent that there is a good correspondence between the model predictions using Berkeley Madonna and the actual data in Figures 10 and 11, with the exception of the PFOS highest dose for the monkey in Figure 10, where there is a slight over estimation.

7.2.4 Prediction of AUC and final serum concentrations for studies with toxicological endpoints

Using PK parameters obtained from our own analysis (Table 17 and 18), we predict AUC and final serum concentrations for some of the studies identified in EPA reports (Table 4-3)(1, 2) and compared those values with EPA's report. The results are summarized in Table 19 and 20 for PFOS and PFOA, respectively. All of our predictions are close to EPA results and the differences are within one fold for both compounds.

Table 19. Comparison of predicted final serum concentration and time integrated serum concentration (AUC) for different treatments of PFOS with EPA results.

Study	Species/ strain	Study dur'n & type	Oral doses mg/ kg/ day	Measured serum conc'n (mg/L)	Species/ strain used	Predicted final serum conc'n (mg/L)			Predicted AUC (mg/L*h)		
						EPA results	Our results	% diff.	EPA results	Our results	% diff.
Lau *et al.* 2003	Female Mouse/ CD-1	GDs1-17 (17 days)	1	NT	Female Mouse/ CD-1	54.8	53.9	-1.64	13500	12352	-8.50
			5	NT		195	251	28.7	57700	59887	3.79
			10	NT		259	415	60.2	88900	111065	24.9
			15	NT		289	462	59.9	106000	145426	37.2
			20	NT		312	477	52.9	118000	165928	40.6
Seacat *et al.* 2002	Monkey/ Cynom-olgus	182 days	0.03	F:13.2 M: 15.8	Monkey/ Cyno-molgus	14.3	14.4	0.70	33800	39960	18.2
			0.15	F:66.8 M: 82.6		68.8	70.4	2.33	166000	197736	19.1
			0.75	F:171 M: 173		225	277	23.1	684000	912576	33.4
Butenhoff *et al.* 2009	Rat/ Sprague-Dawley	Gestation (22 Days)	0.1	1.722	Female Rat/ Sprague-Dawley	3.7	2.08	-43.8	1060	830	-21.7
			0.3	6.245		11.1	6.23	-43.9	3180	2488	-21.8
			1	26.63		37.1	20.7	-44.2	10600	8286	-21.8

NT- not tested.

Table 19 shows that the average difference between the EPA and our results was less than 60% for serum levels and 40% in AUC across all studies.

Table 20. Comparison of predicted final serum concentration and time integrated serum concentration (AUC) for different treatments of PFOA with EPA results.

Study	Species/ strain	Study dur'n and type	Oral doses mg/ kg/ day	Measured serum conc'n (mg/L)	Species/ strain used	Predicted final serum conc'n (mg/L)			Predicted AUC (mg/L*h)		
						EPA results	Our results	% diff.	EPA results	Our results	% diff.
Lau *et al.* 2006	Female Mouse/ CD-1	GDs1-17 (gavage)	1	21.9	Female Mouse/ CD-1	57.6	60.1	4.34	16400	14334	-12.6
			3	40.5		87.2	135	54.8	33600	38916	15.8
			5	71.9		95.2	160	68.1	40700	55428	36.2
			10	116		106	177	67.0	49600	76063	53.4
			20	181		121	187	54.5	61400	94163	53.4
			40	271		148	195	31.8	80100	115859	44.6
Butenhoff *et al.* 2002,2004	Monkey/ Cynom-olgus	26 weeks oral capsule	3	117.9	Monkey/ Cyno-molgus	89.1	68.3	-23.3	380000	313848	-17.4
			10	77.35		121	105	-13.2	553000	439944	-20.4
			20/30	283.2		149	127	-14.8	710000	655335	-7.70

Table 20 shows that the average difference between the EPA and our results was less than 68% for serum levels and 53% in AUC across all studies.

7.3 HED

FSANZ have identified studies amenable for use in derivation of HED for both PFOA and PFOS based on their toxicological analysis.

We have derived HED for those studies based on average serum concentration prediction, which is derived from predicted AUC over the duration of dosing using the EPA PK model and parameters (Table 21). The equation for calculation of HED is shown below:

HED = average serum concentration x CL (in human)

where CL (in human) = 0.000081 L/kg/day for PFOS and CL (in human) = 0.00014 L/kg/day for PFOA (values are obtained from EPA report based on volume of distribution and half-life in human for each compound). The results are summarised in Table 21 and 22 for PFOS and PFOA, respectively.

To further consider the uncertainties in the modelling based on the US EPA PK model and parameters, we have also derived HEDs using the PK parameters from our own analysis (Table 21).

It is noted that the percentage differences between our estimates and those derived by the US EPA were less than 50% or PFOS and 80% for PFOA.

In the context of the uncertainty factors of 30 fold or so applied to derive the TDI to take into account pharmacodynamic and intra-species differences this uncertainty of 1.5 to 1.8 times is quite low

Table 21. HEDs Derived from the Modeled Animal Average Serum Values - PFOS

Study	Dose dur'n (days)	NOAEL (mg/kg/day)	NOAEL (Av Serum Conc. µg/mL)			HED (mg/kg/day)		
			EPA parameter	Our parameter	% change	EPA parameter	Our parameter	% change
Seacat *et al.*, 2002: monkey	182	0.15	38.1	37.9	-0.52	0.0031	0.0031	0
Butenhoff *et al.*, 2012: male rat carcinogenicity	728	0.098	8.65	4.54	-47.5	0.0007	0.0004	-42.8
Butenhoff *et al.*, 2012: female rat carcinogenicity	728	0.12	46	44.6	-3.03	0.0037	0.0036	-2.7
Luebker *et al.*, 2005: female rat	84	0.1	7.14	9.70	35.8	0.0006	0.0008	33.3
Thibodeaux *et al.*, 2003 and Lau *et al.*, 2003: female rat	19	1	15.6	23.9	53.2	0.0013	0.0019	46.2

It is observed that the values for the NOAEL expressed as average serum concentration and HED expressed as mg/kg/day estimated by our approach compared favourably with the EPA for the range of studies shown in table 21. Table 21 shows the percentage differences are less than 55% and 47%, respectively.

Table 22. HEDs Derived from the Modelled Animal Average Serum Values - PFOA

Study	Dosing dur'n (days)	NOAEL (mg/kg/day)	NOAEL (Av Serum Conc. µg/mL)			HED (mg/kg/day)		
			EPA parameter	Our parameter	% change	EPA parameter	Our parameter	% change
Butenhoff *et al.*, 2002: monkey	182	10	101	92.2	-8.71	0.014	0.013	-7.14
Perkins *et al.*, 2004: rat, dec'd weight gain	91	1.94	93.9	-		0.013	-	
Lau *et al.*, 2006: mice, fetotoxicity	17	1	35.1	56.1	59.8	0.0049	0.0078	59.2
Lau *et al.*, 2006: mice, maternal	17	10	197	353	79.2	0.0276	0.0494	79.0

We do not have species specific PK parameters for calculation as no access to PK data.

It is observed that the values for the NOAEL expressed as average serum concentration and HED expressed as mg/kg/day estimated by our approach compared favourably with the EPA for the range of studies shown in Table 21. Table 21 shows the percentage differences are less than 80% for both NOAEL and HED estimation.

7.4 Comparison of HBGVs established by regulatory agencies and advisory bodies

Table 25. Comparison of HBGVs and methodologies used by regulatory agencies and advisory bodies-PFOS

Agency, year	HBGV	Study	PoD	Method for obtaining PoD	UF	Value of HBGV
UKCOT, 2006	Tolerable daily intake1 (provisional)	Seacat *et al.*, 2002 Oral 183 days, Cynomolgus monkeys (↓serum T3 level)	0.03 mg/kg bw/day	NOAEL value	100	300 ng/kg bw/day
EFSA, 2008	Tolerable daily intake[1]	Seacat *et al.*, 2002 Oral 183 days, Cynomolgus monkeys (↓serum T3 and HDL level)	0.03 mg/kg bw/day	NOAEL value	200	150 ng/kg bw/day
Swedish EPA, 2012	Derived no effect level[2] (immunotoxicty)	Peden-Adams *et al.* 2008 Oral 28 days, mice (immunotoxicity)	17.8 ng/ml serum level (0.166 µg/kg bw/day)	NOAEL value	150	0.12 ng/ml serum level
Danish EPA, 2015	Tolerable daily intake[1]	Thomford 2002. 104 week dietary, rat (liver effect)	0.033 mg/kg bw/day	$BMDL_{10}$ value (NOAEL: 0.018 mg/kg bw/day)	1230	30 ng/kg bw/day
ATSDR, 2015	Minimal risk level[3]	Seacat *et al.*, 2002 Oral 183 days, Cynomolgus monkeys (absolute liver weight↑)	2.52×10^{-3} mg/kg bw/day	[a]HED for female NOAEL (0.15 mg/kg/day) serum level 36.4 µg/mL	90	30 ng/kg bw/day
US EPA, 2016	Reference dose[4]	Luebker *et al.*, 2005 Oral 84 days, rat (↓pup body weight)	0.00051 mg/kg bw/day	[b]HED for female rat NOAEL (0.1 mg/kg/day) serum level	30	20 ng/kg bw/day
Canada FPTC, 2016	Tolerable daily intake[1]	Butenhoff *et al.*, 2012 (hepatocellular hypertrophy)	0.021 mg/kg bw/day	NOAEL value	350	60 ng/kg bw/day

[1] A Tolerable Daily Intake is an estimate of the amount of a chemical in food or drinking-water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer (FAO/WHO, 2009).

[2] A Derived No Effect Level is the level of exposure to the substance above which humans should not be exposed (ECHA, 2009).

[3] A Minimal Risk Level is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure (ATSDR, 2015).

[4] A Reference Dose is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US EPA, 2002).

*[a] HED=C_{ss}*k_e*V_d/AF where C_{ss}=36.4 µg/mL; ke=ln2/$t_{1/2}$=0.693/2000days=3.47×10^{-4} day^{-1}; V_d =0.2L/kg; AF(gastrointestinal absorption fraction)=1*

*[b] HED=C_{ss}*CL= C_{ss}*ln2/ t1/2*Vd where C_{ss}=6.26 µg/mL; $t_{1/2}$=1971days; V_d =0.23L/kg*

The NOAEL for different endpoints ranges from 0.018 mg/kg/day identified in rat study based on liver effect to 0.15 mg/kg/day identified in monkey study based on absolute liver weight increase. Different NOAEL is the main source for difference in HBGV from different agency.

It is noted that variation in HBGVs and methodologies used by regulatory agencies and advisory bodies for PFOS is 15 fold, ranging from 20 ng/kg bodyweight/day to 300 ng/kg bodyweight/day.

Table 26 HBGVs established by regulatory agencies and advisory bodies- PFOA

Agency, year	HBGV	Study	PoD	Method for obtaining PoD	UF	Value of HBGV
UKCOT, 2006	Tolerable Daily Intake[1] (provisional)	Palazzolo 1993 and Perkins *et al.* 2004 7 weeks dietary, rats (absolute liver weight↑)	0.3 mg/kg bw/day	$BMDL_{10}$ value	200	1.5 µg/kg bw/day
EFSA, 2008	Tolerable Daily Intake[1]	Palazzolo 1993 and Perkins *et al.* 2004 7 weeks dietary, rats (absolute liver weight↑)	0.3 mg/kg bw/day	$BMDL_{10}$ value	200	1.5 µg/kg bw/day
Swedish EPA, 2012	Derived No Effect Level	Macon *et al.* 2011 Oral 8 days (GD10-17), mice (mammary glanddevelopment)	150 ng per mL serum (LOAEL of 0.01 mg/kg bw/day)	Postnatal day (PND) 7 serum level in female offspring	75	2.0 ng/mL serum
Danish EPA, 2015	Tolerable Daily Intake[1]	Palazzolo 1993 13 weeks dietary, rats (absolute liver weight↑)	0.003 mg/kg bw/day	[a]HED for BMDL10 (0.456 mg/kg)	30	100 ng/kg/day
ATSDR, 2015	Minimal Risk Level[2]	Butenhoff *et al.* 2002 Oral 182 days, monkeys (absolute liver weight↑)	1.54×10^{-3} mg/kg bw/day	[b]HED for BMDL10	90	20 ng/kg/day
US EPA, 2016	Reference Dose[3]	Lau *et al.* 2006 Oral 17 days (GD1-17), mice (↓pup ossification (m,f), ↑male puberty)	0.0053 mg/kg bw/day	[c]HED for LOAEL (1mg/kg bw/day)	300	20 ng/kg/day
Canada FPTC, 2016	Tolerable daily intake[1]	Perkins *et al.* 2014 13-week dietary, rat (liver effect)	0.06 mg/kgbw/day	NOAEL value	2400	25 ng/kg bw/day

PoD = Point of Departure; UF = Uncertainty Factor; HBGV = health-based guidance value

[1] A Tolerable Daily Intake is an estimate of the amount of a substance that can be taken in daily over a lifetime without appreciable health risk.

[2] A Minimal Risk Level is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure.

[3] A reference dose is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

[a] HED-BMDL10=BMDL10/(LOAEL/HED-LOAEL)=0.456 mg/kg bw/day /(0.64 mg/kg bw/day/0.0045 mg/kg bw/day)=0.003 mg/kg bw/day, where HED-LOAEL is from EPA

[b] $HED=C_{ss}*k_e*V_d/AF$ where $C_{ss}=15.53$ µg/mL; $ke=ln2/t_{1/2}=0.693/1400days=4.95\times10^{-4}$ day^{-1}; $V_d=0.2L/kg$; AF(gastrointestinal absorption fraction)=1

[c] $HED=C_{ss}*CL=C_{ss}*ln2/t_{1/2}*V_d$ where $C_{ss}=38$ µg/mL; $t_{1/2}=839.5$ days; $V_d=0.17L/kg$

It is noted that variation in HBGVs and methodologies used by regulatory agencies and advisory bodies for PFOA is quite large - 75 fold, ranging from 20 ng/kg bodyweight/day to 1,500 ng/kg bodyweight/day.

8 Alternative PBPK Models

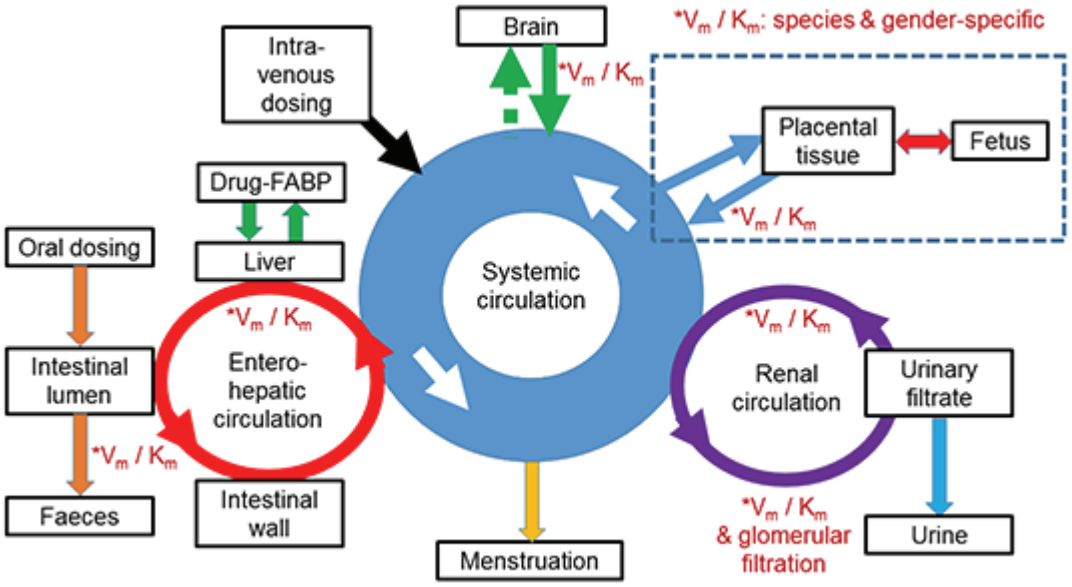
The challenge with all models is to be "as simple as possible to adequately describes the model but no simpler". In our view, the proper description of the toxicokinetics of PFOS and PFOS based on the data we have evaluated requires five circuits:

- Recirculation via the blood to all organs
- Active and passive secretion through the kidney glomerulus followed by active reabsorption into the blood; and
- Active uptake into the intestine (if dosed orally), followed by active uptake into the liver, secretion into the bile and then reabsorption from the intestine in a highly efficient manner –enterohepatic recirculation.
- Uptake into the brain and other vital organs with active efflux into the blood; and
- Uptake into the placenta and active reabsorption into the body.

It is also apparent that there are three main routes for excretion of these solutes from the body:

- Renal excretion
- Biliary excretion into the bile; and
- Associated with menstruation

Figure 12. Processes associated with the absorption, distribution and elimination of PFOS and PFOA



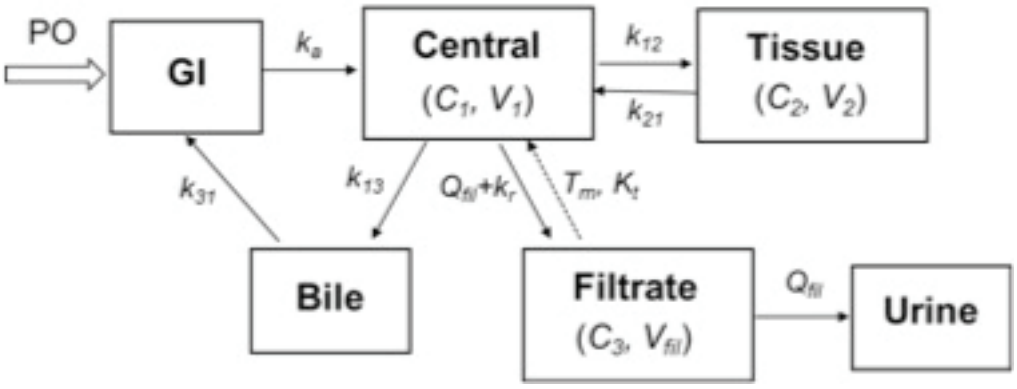
Currently, there is insufficient data to provide parameter estimates for each of the steps in the proposed model. However, the model does allow reconciliation of some findings not presently addressed in the EPA models:

1. Kim *et al.* (2016) (45) showed much faster urinary excretion of PFOA in female rats but not in male rats nor for PFOS in either species after administration of low doses. This is accounted for by a higher expression of transporter enzymes in the glomerular basement membrane of female rats and their much higher renal secretion into the urinary filtrate as suggested by a range of authors. However, we suggest that the differences may not apply to humans as there is no evidence of gender differences in mice, monkeys and humans.
2. The enterohepatic recycling is consistent with the appearance of PFOS and PFOA in the faeces after intravenous dosing and at long times after oral dosing.

3. The saturable hepatic uptake and renal levels is consistent with earlier dose data that showed a reduction in both liver and kidney levels at higher doses of PFOS and PFOA. The saturable resorption of PFOA in the kidney is consistent with the model of Andersen *et al.* (2006) (55). However, it is also emphasised that the available human data suggest low exposure to PFOS and PFOA, so that these effects are more likely in the animal models with the consequence of low organ to blood concentration ratios.
4. The impact of menstruation reported by a number of authors is now recognised.
5. The appearance of PFOS and PFOA in the brain (especially during development and probably during CNS inflammation) is recognised as is the limited capacity of the protective efflux mechanism.
6. It is also recognised that PFOS and PFOA can cross into the placenta but there is also a protective efflux mechanism present. Further, there are marked differences in the development of some of these processes between humans and rats. This may make the rat more sensitive to these toxins but this has not been demonstrated.
7. It is to be recognised that the model presented here is simplistic and that a more comprehensive enterohepatic recirculation model needs to take into account the fluctuations arising from this process, especially after the periodic emptying of the gall bladder in high mammals (57). Consistent with this limitation, we noticed that there is fluctuation in plasma-concentration time profiles of PFOS after iv injection to monkey. It is also reported that 12.6% PFOS was recovered in faeces after a single intravenous injection to monkeys (80), which provides evidence for biliary excretion of this compound. It is possible, but not yet shown, that active efflux secretion across the intestinal wall could add to the recovery in the intestine in addition to that from kidney secretion. We then suspected the presence of enterohepatic recirculation (57) for this compound, which may also be a saturable process involving the same transporters in kidney.

Figure 12 can be simplified by being focused on low doses of PFOA and PFOS so that all saturable processes, with the possible exception of renal resorption, may be regarded as behaving as linear processes at the low doses normally expected for human exposures. A proposed simplified, new model shown in diagram below reflect this enterohepatic recirculation process with most other transport occurring by various linear processes.

Figure 13. Diagram of proposed toxicokinetic model for PFOA and PFOS.



It is also evident that enterohepatic fluctuation (57) is a factor and that a more sophisticated model would be needed to describe this. Further, the renal secretion component of the model does explain the data of Kim *et al.* (2016), in that the modelling is in accord with a higher expression of transporting enzymes that secrete PFOA in female rats as shown in Figure 5.

9 Summary and Conclusion

In summary,

- The EPA reports provide a good overview of the literature for PFOS and PFOA and, in our view, combined with later papers and reviews, provide the best overall summary of the toxicology of these solutes.
- The EPA is also to be commended for their physiological pharmacokinetic approach, which we agree, based on our own modelling, provide a more appropriate endpoint for estimation of PFOS exposure and likely hazard than conventional methods based on dose only.
- However, in commending the EPA physiological pharmacokinetic approach, we also point out that their pharmacokinetic modelling has limitations and uncertainties. In particular, there are three key questions that cannot be answered by the EPA approach:
    a. Why are there huge discrepancies in half-lives of some PFCs between species?
    b. Why do serum concentrations of PFOA and PFOS appear to rapidly approach steady-state after repeated dosing despite their long half-lives?
    c. Why is there a gender difference in the excretion of PFOA by rats?
- Our analysis suggests that there is substantial secretion of these compounds into bile with effective enterohepatic recirculation, as seen by significant amounts of material detected in faeces many days after a single dose given orally or IV.
- Further, we suggest that the EPA modelling has ignored saturable uptake by the liver and intestine and efflux by the placenta and the brain.
- Our modelling shows that the serum concentrations of PFOS and PFOA can reach steady state despite a long half-life, contrary to findings by the EPA modelling.

To assist FSANZ in establishing HBGVs, we established HED based on average serum concentration prediction, derived from predicted AUC over the duration of dosing using the US EPA PK model and parameters. We then showed that using our best parameter estimates and commercial simulation software package, the EPA estimates for the PBPK of a range of studies could be replicated with an error of less than 80% for PFOS and PFOA. In the context of the uncertainty factors of a 30 fold or more applied to derive the TDI to take into account pharmacodynamic and intra-species differences this uncertainty of 1.5 to 1.8 fold is a very small contributor.

We also developed a fuller model based on our analysis and interpretation of the literature. However, we must also add that our model is, at this time, more conceptual than fully described in quantitative terms as the data needed for the latter is lacking.

We comment, in conclusion, that the EPA reports provide an excellent overview of PFOS and PFOA studies carried out to date and that our modelling of endpoints has verified their conclusions, whilst incorporating the same uncertainties. Whilst we have suggested that their modelling and resulting data can be improved, we don't have the data to fully develop and validate an improved model. However, it is clear that their physiological pharmacokinetic modelling and related endpoints for both PFOS and PFOA is much preferred to endpoints based on dosing only – especially noting that the variation in both PFOS and PFOA half-lives between the species cause considerable vagary in scaling up toxicity findings found in animals to man

10 Recommendations

1. We recommend that the EPA physiologically based pharmacokinetic approach to assessing endpoints for PFOS and PFOA safety be preferred over endpoints based on dosing only.
2. There is a need to gather more animal and human data to more fully characterise a physiological pharmacokinetic model and related endpoints for both PFOS and PFOA.

11 References

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