# Chapter 14
# Dosimetric Anchoring of Toxicological Studies

**John Wambaugh**

**Abstract** Comparing the onset of effects between different toxicological studies is often confounded by pharmacokinetics (PK). Differences between studies can be biological in origin (e.g. species, gender) as well as due to dose regimen (e.g. spacing, magnitude, duration, and route of administration). However, if the pharmacodynamic mechanism underlying the observed toxicological effect is conserved, and some measure of the tissue concentration (i.e., dosimetry) at the site of effect can be determined, then it is expected that this dosimetric anchor should also be conserved across studies. Careful consideration of the PK is required, and mathematical models for PK can address this need. It is relatively easy to extrapolate model predictions if there is a reasonable expectation of linear behavior and conserved PK between test conditions and those to be predicted. For perfluorinated compounds (PFCs), however, we expect PK extrapolation to be much more difficult. Aspects of the distribution, metabolism, and elimination of PFCs have unusual and non-linear features that must be considered. The PK of PFCs is especially unusual in that the half-lives of the longer chain PFCs vary by many orders of magnitude across species, dose regimen, and in some cases, across gender. The empirical saturable renal resorption hypothesis of the Andersen et al. (Toxicol 227(16978759):156–164, 2006) model provides the simplest available non-linear PK model that describes PFCs PK. However, despite the plausible biological mechanism, this model is still empirical, requiring that species-specific parameters are estimated using species-specific PK data. With this model, diverse toxicological studies of PFCs can be shown to be roughly consistent with respect to the internal, dosimetric anchors induced by their various study designs.

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## 14.1 Introduction

If a test compound activates a consistent mode of action across studies, then somewhere between the no observed effect level (NOEL) and the lowest observed effect level (LOEL), the tissue concentrations must be sufficient to perturb that mechanism in a statistically significant number of test animals (Allen et al. 1994). Although more sophisticated analyses (e.g., benchmark dose) are often possible for new studies that provide sufficient detail, meta-analyses comparing across historical literature often have to rely upon the NOEL and LOEL alone (Martin et al. 2009).

Comparing LOELs between studies is often confounded by differences in pharmacokinetics (PK), which can be not only biological in origin (e.g. species, gender) but also due to dose regimen (e.g. spacing, magnitude, duration, and route of administration). However, if the pharmacodynamic mechanism is conserved, and some measure of the tissue concentration (i.e., dosimetry) at the site of toxicological effect can be determined, then it is expected that this *dosimetric anchor* should also be conserved across studies (Rowlands et al. 2014). If the mode of action that is activated in the LOEL dose group is conserved in humans, then the chemical exposures that might cause these effects in humans may be inferred (Boobis 2010).

Careful consideration of the pharmacokinetics (PK) is therefore required in order to link the chemical exposures in the LOEL and NOEL dose groups to the onset of toxicity endpoints (Blaauboer 2010). PK models are needed to make predictions of the tissue concentrations that were caused by a given exposure. It is relatively easy to extrapolate predictions if there is a reasonable expectation of linear behavior and conserved PK between test conditions and those to be predicted. For perfluorinated compounds (PFCs), however, we expect PK extrapolation to be much more difficult. A list of PFCs mentioned in this chapter is provided in Table 14.1.

**Table 14.1** The perfluorinated compounds (PFCs) discussed in this chapter

| PFC abbreviation | Full name | Carbon chain length (linear form) |
|---|---|---|
| PFBS | Perfluorobutanesulfonate | 4 |
| PFHxS | Perfluorohexanesulfonate | 6 |
| PFHpA | Perfluoroheptanoic acid | 7 |
| PFOA | Perfluorooctanoic acid | 8 |
| PFOS | Perfluorooctane sulfonate | 8 |
| PFOSA | Perfluorooctanesulfonamide | 8 |
| PFNA | Perfluorononanoic acid | 9 |
| PFDA | Perfluorodecanoic acid | 10 |

If the study of PK can be considered to be concerned with the absorption, distribution, metabolism, and elimination of xenobiotic compounds by the body, then PFCs may be considered to be unusual with respect to three of those four elements. PK studies in animals have shown that many PFCs are well absorbed (Kudo and Kawashima 2003), but: the distribution of PFCs to tissue is unusual due to both fluorous chemistry (Dobbs and Kimberley 2002) and interactions with numerous transporters in multiple tissues (Kudo and Kawashima 2003); PFCs are extremely metabolically inert (Ylinen et al. 1990); and excretion of some PFCs is complicated by entero-hepatic circulation (Johnson et al. 1984) and potentially by active reabsorption in the kidneys (Andersen et al. 2006).

The PK of PFCs is even more extraordinary in that the half-lives of the longer chain PFCs vary by many orders of magnitude across species, and in some cases, across gender (Lau et al. 2007). Humans typically exhibit the longest half-lives (several years for PFOS and PFOA) (Bartell et al. 2010; Olsen et al. 2007), with monkeys, mice, male rats, and female rats having half-lives of months, weeks, days, and hours respectively (Lau et al. 2007). Such large differences in PK (e.g., female rats excrete PFOA more quickly than males) may result in vast differences in the external dose needed to achieve the same internal dose (Rodriguez et al. 2009; Wambaugh et al. 2013).

Administration of single doses of a PFC to laboratory animals typically produces serum concentration time course curves consistent with a two-compartment distribution (Andersen et al. 2008). However, PFOA is known to have dose-dependent (non-linear) pharmacokinetic properties: though repeated doses rapidly accumulate to a quasi-equilibrium blood concentration, a single dose results in a much longer half-life than would be consistent with the rapid approach to quasi-equilibrium (Andersen et al. 2006; Lou et al. 2009). Given its long half-life, using a linear PK model (e.g. the two-compartment model) to predict exposures resulting from multiple PFOA exposures results in large overestimates of reality (Lou et al. 2009).

The confidence in the PK predictions for PFCs that is needed to provide dosimetric anchoring of in vivo toxicity studies depends on how well one can answer the three big questions of PFC PK from the past 20 years:

1. Why are there huge discrepancies in half-lives of some PFCs between species?
2. Why do serum concentrations of PFOA and PFOS appear to rapidly approach steady-state after repeated dosing despite their long half-lives?
3. Why is there a gender difference in the excretion of PFOA by rats?

To date research have confidently answered the third question (hormone regulated transporter expression), and have reasonable hypotheses for the second (saturable resorption in the kidney proximal tubules), but an answer to the underlying mechanism driving the inter-species question remains elusive, forcing us to rely on empirical approaches that can explain the data we have, but offer little insight into the why or confidence for extrapolation (Andersen et al. 2008; Wambaugh et al. 2013). Crucial challenges remain in understanding the biological processes that drive the time and dose dependent PK phenomena of PFCs (Andersen et al. 2008).

The empirical saturable renal resorption hypothesis of the Andersen et al. (2006) model provides the simplest available PK model with non-linear kinetics. However, despite the plausible biological mechanism, this model is still empirical, requiring that species-specific parameters are estimated using species-specific PK data (Wambaugh et al. 2013). A physiologically-based PK (PBPK) model for PFCs might be preferable because it would allow extrapolation between species, provide better estimates of chemical-specific parameters, and allow estimation of chemical concentration in the specific tissues for which toxicity is observed. However, data for chemical-specific partitioning into most tissues exists only for PFOA. Given the limitations of the available data for estimating parameters, the simpler (Andersen et al. 2006) empirical PK model seems preferable.

## 14.2 Understanding the Non-linear PK of PFCs

The vast differences between species in the elimination half-life of PFCs is the most notable feature of PFCs PK (Lau et al. 2006). The carbon-chain length of PFCs appears to influence the excretion of PFCs, with shorter chain molecules tending to be eliminated more rapidly (Andersen et al. 2008; Ohmori et al. 2003). However, there are exceptions: e.g., the elimination half-life of PFHxS in humans is longer than that of PFOS (Lau et al. 2006). The predominance of excretion of the PFCs PFOA and PFOS is through urine, rather than feces (Cui et al. 2009; Wambaugh et al. 2008). However, administration of cholestyramine to rats increased excretion of both PFOS and PFOA nine times, indicating that there is considerable enterohepatic circulation of these PFCs since cholestyramine is a drug which complexes with anions in the liver to promote biliary excretion (Johnson et al. 1984).

The gender difference in serum half-life of PFCs in rats depends on chain length with larger differences for the longer chain compounds (30 for males vs. 2.5 days for females for PFNA) (Andersen et al. 2008). Estradiol administration to both castrated and non-castrated male rats produced PFOA urine excretion at similar rates to female rats (Ylinen et al. 1990). Castration alone makes clearance in males similar to that of female rats (Kudo and Kawashima 2003). Treatment of castrated males with testosterone reduces clearance to normal male rat levels (Kudo and Kawashima 2003).

Renal clearances of PFOA are significantly smaller than passive elimination by glomerular filtration would predict, indicating a role for reabsorption by transporters in the proximal tubules of the kidney (Harada et al. 2004). The gender differences in the clearance of PFOA may be due to the actions of organic anion transporters in the kidney since several transporter proteins are expressed differentially in male and female adult rats (Buist et al. 2002; Buist and Klaassen 2004; Kudo et al. 2002; Lau et al. 2006). This “saturable resorption process” has been observed for other chemicals (Corley et al. 2005), albeit without gender differences. Both oatp1 and OAT3 mediate the resorption of PFOA in the proximal tubules of rat kidney (Katakura et al. 2007). Oatp1/OATP and OAT3 are both expressed abundantly

in rat, mouse, and human kidneys (Buist et al. 2002; Motohashi et al. 2002; Nakagawa et al. 2008), but their expression is enhanced by the presence of testosterone in rats (Ljubojević et al. 2004). Sex hormone regulated expression of transporters capable of resorbing PFCs in the proximal tubules of the kidney, from which they would otherwise be excreted, provides a plausible mechanism for explaining the gender differences in rat half-lives.

To date, the single biggest advance in the modeling of PFCs PK has been the non-linear model proposed by Andersen et al. (2006). In this model (shown in Fig. 14.2c) it is assumed that PFCs are passively excreted into the proximal tubules of the kidney by glomerular filtration, but that there is a counter process of active transport of the PFCs back from the proximal tubules. This might arise from transporters designed to prevent the excretion of endogenous fatty acids misidentifying PFCs for their non-perfluorinated fatty acid analogs (Andersen et al. 2006). In the event that these transporters are overwhelmed (i.e., saturated) by the concentration of PFC in the proximal tubule filtrate, the remaining PFC in the filtrate is rapidly excreted.

Both linear and branched PFCs have been used in the production of commercial products (Beesoon et al. 2011; Chu and Letcher 2009; Loveless et al. 2006). In rats given equivalent doses, branched, long-chain PFCs resulted in lower serum concentrations than those treated with linear, long-chain PFC (Loveless et al. 2006). Most PK studies focus on linear PFCs. Resorption of PFCs could explain why equivalent doses of linear and branched PFCs produce higher concentration for the linear molecules: if linear PFCs are more similar to endogenous fatty acids, then there may be differing affinities for organic anion transporters (Loveless et al. 2006).

However, if the saturable resorption hypothesis is true, then we must characterize the interactions of PFCs with the endogenous fatty acids that are competing for the same transporters (Andersen et al. 2008). Unfortunately, the difference between the PFOA half-lives in human beings and other animals is not likely to be attributable to differences in the affinities of PFOA for or expression levels of Oatp1/OATP and OAT3 transporters (Nakagawa et al. 2008). However, human Organic Anion Transporter (OAT4) is a transporter of PFOA (Nakagawa et al. 2009), that is only expressed in humans, is an apical type isoform in proximal tubules, and mediates the re-absorption of organic anions (Ekaratanawong et al. 2004; Nakagawa et al. 2009). The uptake of PFOA by OAT4 was greater than that by hOAT1 (Nakagawa et al. 2009). hOAT4 mRNA is abundantly expressed in the placenta as well as in the kidney (Cha et al. 2000; Nakagawa et al. 2009). Thus, OAT4 provides a plausible mechanism of inter-species half-differences in need of further study, but correlation between interspecies expression of OAT4 and half-life of PFCs has not yet been demonstrated.

Although the saturable resorption model of Andersen et al. (2006), does explain the non-linear PK of PFOA and PFOS, there are many other potential non-linearities at play for PFCs PK:

The acid dissociation constants (pKa) of PFOS and PFOA are <1, and for other PFCs they are predicted to be between 0 and 1.5, so it is reasonable to expect that most PFCs are ionized in tissue (Goss 2008; Johnson et al. 1984). The passive

(i.e., not transporter-facilitated) distribution of an arbitrary xenobiotic organic compound into tissue is often understood by studying the partitioning of the ionized and molecular forms of the compound into the aqueous and variously charged lipid phases of the tissue (Peyret and Krishnan 2011; Schmitt 2008). Unfortunately, PFCs confound this approach due to the high self-affinity of perfluorinated chemicals for each other, leading to a "fluorous phase" in addition to the typical aqueous and lipid phases (Dobbs and Kimberley 2002).

Further, at high concentrations, PFCs may even aggregate, further sequestering them from traditional PK interactions. Molecular aggregates (e.g., dimers and trimers) of PFCs have been reported at concentrations as low as the pM range (López-Fontán et al. 2005; Rayne and Forest 2009a). In the mM concentrations range, PFCs can even form large micelles (Rayne and Forest 2009a).

The predicted hydrophobicity (ratio of concentration of in octanol to that in water, or log P) increases with chain length for perfluorinated carboxylic acids: 2.91 (for PFBA), 3.69 (PFPA), 4.50 (PFHxA), 5.36 (PFHpA), 6.26 (PFOA), 7.23 (PFNA), and 8.26 (PFDA) (Rayne and Forest 2009b). The log P for PFOS is 4.67 (Rayne and Forest 2009b). The log P for PFOS or PFOA is roughly two orders of magnitude higher than their non-perfluorinated alkyl counterparts (Jing et al. 2009). These relatively high log P's present something of a paradox, since perfluroalkyl groups on molecules tend to make compounds oleophobic (Jing et al. 2009). However perfluroalkyl groups on molecules also tend to make compounds hydrophobic (Jing et al. 2009), so we can presume that the log P to some extent represents the competition between the fluorous phases and avoidance of both aqueous and lipid phases.

PFCs are highly bound to plasma protein; for example, albumin in plasma has a large capacity for binding PFOA (6–9 binding sites per molecule and mM concentration in plasma) (Han et al. 2003). PFHxS, PFOS, and PFOA are highly bound to human plasma albumin (>99.9 %, 99.8 %, and 99.7 % bound, respectively) (Kerstner-Wood et al. 2003). Plasma protein binding, estimated in vitro, was over 98 % for four PFCs tested in rat (Ohmori et al. 2003). Serum to plasma ratios for PFHxS, PFOS, and PFOA were 1:1 (Ehresman et al. 2007). Whole blood to plasma ratios were roughly 1:2 (Ehresman et al. 2007). PFOA is similarly bound by the serum of female and male rats, indicate that this is not a reason for gender-dependent differences in half-life (Ylinen et al. 1990).

In animals, the liver is a primary organ for distribution (Kemper 2003; Loccisano et al. 2012), at least at low doses (Kudo et al. 2007). PFOS and PFOA liver concentrations are several times higher than serum concentrations, with lesser distribution to the kidneys (Hundley et al. 2006; Johnson and Ober 1980; Lau et al. 2006; Seacat et al. 2002, 2003), however partitioning to liver may be less pronounced in humans (Fàbrega et al. 2014; Pérez et al. 2013). The Kemper (2003) data set for PFOA is perhaps the greatest source of partitioning information, with multiple rat tissues at multiple time points, but unfortunately these studies focused on single doses in the linear PK regime (Wambaugh et al. 2008), and therefore they do not illuminate the non-linearities (Andersen et al. 2006; Lou et al. 2009) or time-dependencies (Harris and Barton 2008; Tan et al. 2008) of PFC PK.

Distribution of PFOA to the liver decreases with increased dose in rat (Kudo et al. 2007), which possibly indicates the saturation of transporters. However, in the liver at least, passive diffusion has been shown to contribute significantly to the overall hepatic uptake (Han et al. 2008). In one analysis of human cadaver livers, the mean liver to serum ratio of PFOS was 1.3:1 (Olsen et al. 2003) which is comparable cynomolgus monkeys (Butenhoff et al. 2004b) but lower than in rat (Fàbrega et al. 2014; Kemper 2003). In the same study, the concentration of PFOSA, PFOA, and PFHxS were below the limit of quantitation in most of the individual liver samples as well as many serum analyses (Olsen et al. 2003). Based on an analysis of multiple human cadaver tissues, there is some evidence that most PFCs in general are found at higher concentrations in human lung tissues than elsewhere in the body, however even in that study PFOS was most concentrated in the liver and PFOA was found to be highest in bone (Pérez et al. 2013).

In summary, although the Andersen et al. (2006) model, derived from saturable resorption hypothesis, provides a good description of the non-linear PK of the PFCs PFOS and PFOA, the non-linear process described by that model could also in part be due to fluorous phase chemistry, plasma protein binding, or hepatic accumulation.

## 14.3 Selecting an Appropriate PK Model for PFCs

Due to the gender and pronounced species differences in elimination of PFCs, comparisons of toxicological effects must use a measure of internal, tissue dose rather than frank administered dose (Lau et al. 2006; Rodriguez et al. 2009; Wambaugh et al. 2013). PK models make predictions of internal, tissue doses; these predictions can be useful for interpolation – e.g., inferring what will happen for a dose between two tested doses – but the primary draw of PK modeling is often extrapolation beyond measured data.

For example, physiologically-based PK (PBPK) models allow extrapolation across physiologies, and therefore species, by separating physiologic PK factors (e.g., cardiac output) from chemical-specific factors that are believed to be independent of physiology (e.g., ratio of tissue concentration to plasma concentration at steady state). Simpler PK models tend to be phenomenological, and are therefore better suited to interpolation, while more complicated models can include biological processes that are understood to be conserved (e.g., between species) and so are suited to extrapolation.

The PK modeling literature for PFCs ranges from empirical one compartment models to PBPK models coupled to empirical excretion models. The general progression of these models is illustrated in Fig. 14.2.

The PFCs studied include PFBA (Chang et al. 2008a), PFBS (Olsen et al. 2009), PFNA (Tatum-Gibbs et al. 2011), PFHxS (Sundström et al. 2012) with most of the literature focusing on PFOS (Andersen et al. 2006; Chang et al. 2012; Harris and Barton 2008; Loccisano et al. 2011, 2012, 2013; Luebker et al. 2005b; Thompson et al. 2010; Trudel et al. 2008) especially PFOA (Andersen et al. 2006; Butenhoff et al. 2004b; Cui et al. 2010; Hinderliter et al. 2005; Hundley et al. 2006; Judson

et al. 2008; Kemper 2003; Lau et al. 2006; Loccisano et al. 2011, 2012, 2013; Lorber and Egeghy 2011; Rodriguez et al. 2009; Tan et al. 2008; Thompson et al. 2010; Trudel et al. 2008; Wambaugh et al. 2008).

The species studied typically include mouse, rat, monkey, and in some observational cases, humans. At this point, no single model yet exists that sufficiently explains the PK of any PFC such that cross-species extrapolation is a matter of simply changing physiological parameters, i.e. even the most elaborate PBPK model for PFOA still requires empirical calibration of the non-linear term in order to describe different species.

Describing the non-linear PK of PFOA and PFOS has been a key focus of many PFC PK efforts. Empirical models, such as the one compartment model in Fig. 14.2a typically allow a crude explanation of the kinetics from a single, low dose, via a constant elimination rate and a volume of distribution describing the empirical relationship between the concentration in a tissue that has been collected experimentally (typically, serum) and the concentration of chemical in the rest of the body. No insight into where in the body the remaining chemical is concentrated is allowed. One compartment models can include an absorption phase during which the concentration of chemical increases, but once the maximum concentration is reached ($C_{max}$) the elimination phase occurs at a fixed clearance flow (CL in L) rate (i.e., CL x $V_d$).

The elimination PK of PFOA and PFNA from serum following a single dose has been shown, however, to have at least two phases – at long times the elimination slows (Kemper 2003; Tatum-Gibbs et al. 2011; Wambaugh et al. 2008). The empirical two compartment model, shown in Fig. 14.2b, predicts this sort of biphasic elimination as the result of exchange between the plasma (or other tissue described by the primary concentration "compartment" $C_1$) and a tissue reservoir of the chemical (the second or deep tissue compartment $C_2$). The two compartment model is still linear; for example, linear models predict that the concentrations from twice the dose will be exactly twice as high at all times.

Figure 14.1 shows results from Lou et al. (2009) in which the PK of PFOA in mice are compared for single doses of 1, 10, and 60 mg/kg, and 2-week regimen of repeated daily 20 mg/kg. The two compartment model predictions (dashed line in Fig. 14.1) demonstrate how a linear model that describes the concentration time-course resulting from the lower, 1 and 10 mg/kg doses does not correctly describe the higher, 60 mg/kg dose. This is because the PK of PFOA become non-linear at higher doses. Further, even a two compartment model calibrated to describe the highest single dose (60 mg/kg, predictions shown by a dotted line in Fig. 14.1), does not correctly extrapolate to predict what happens for repeated doses. This is because at high doses PFOA reaches a steady state much faster than its long half-life would imply. Under linear PK, repeated doses of a chemical with a long half-life (i.e., slow clearance) would take a long time to reach steady state as the concentration gradually builds to a relatively high value (dashed line in Fig. 14.1). As shown by the data points in Fig. 14.1, repeated doses of PFOA rapidly (~2 days) results in a lower, steady state despite a single dose half-life of 3 weeks in mice.

The predictions of the Andersen et al. (2006) saturable resorption model in Fig. 14.1 are shown by a solid line. With this single model, the plasma concentrations resulting

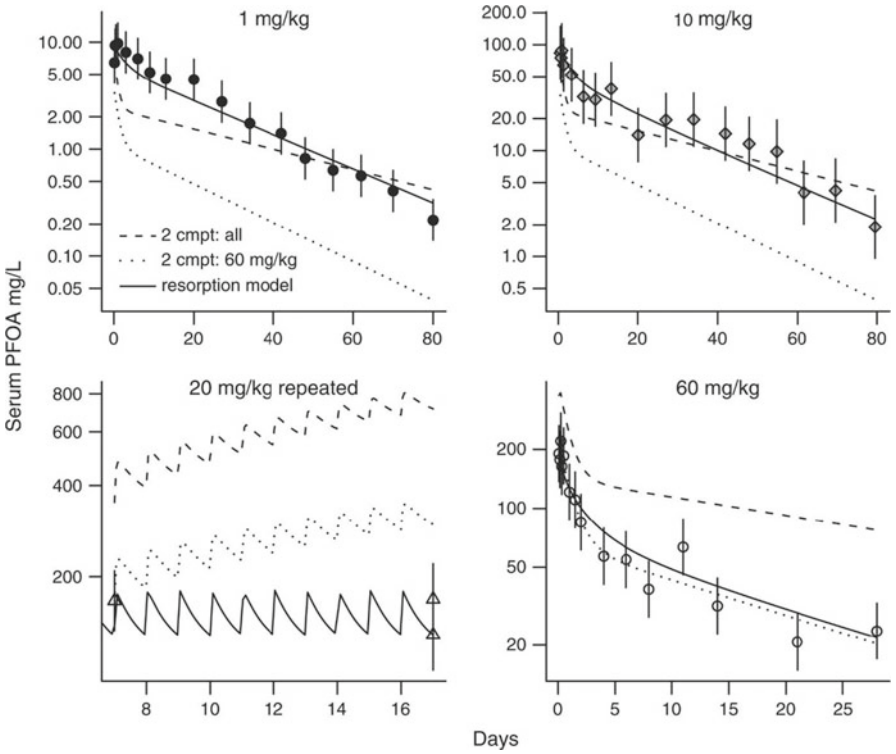


**Fig. 14.1** Comparing predictions for the two-compartment model when fit to all the available data (*dashed line*) with a fit to just the 60 mg/kg data (*dotted line*). Neither model does a good job of describing all of the data, whereas the saturable resorption model (*solid line*) is more consistent between doses (Lou et al. (2009), by permission of Oxford University Press)

from low and high single doses, and from repeated doses, can all be reconciled. Although this model is biologically motivated (i.e., saturable resorption in the proximal tubules is a plausible process) it is still empirical. To date we cannot simply change the parameters describing the filtrate and the transporters involved in order to reconcile the differences in half-lives between species. We can, however, empirically estimate the values of those parameters to make the saturable resorption model fit multiple species (Wambaugh et al. 2013). Because we are making empirical adjustments rather than changing parameters to describe a biological process that is known to be conserved between species, it is prudent to think of the Andersen et al. (2006) model as a model that is generically non-linear, with saturable resorption being the most likely explanation. However, one could hypothesize other non-linear processes that might produce similar results, including saturable plasma protein or other binding, saturable sequestration in the liver, and saturable entero-hepatic recirculation.

The final class of PK models for PFCs, shown in Fig. 14.2d, is a PBPK model. Developed by Loccisano et al. (2011, 2012, 2013), this PBPK model combines partition coefficients estimated from single dose PK studies (Kemper 2003) with the

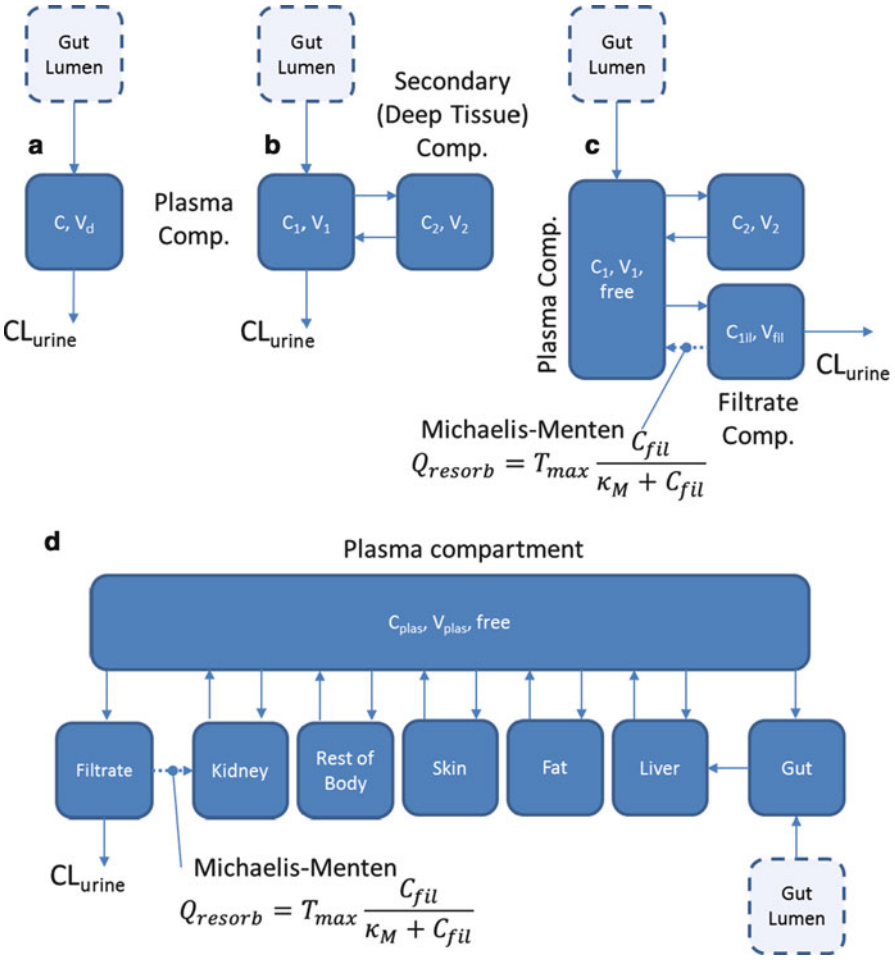


**Fig. 14.2** PK models for PFCs have progressed incrementally from the empirical, linear one compartment (**a**) and two compartment models (**b**) to the empirical, non-linear "saturable resorption" model of Andersen et al. (2006) (**c**) Loccisano et al. 2011, 2012) incorporated the saturable resorption model into a PBPK model (**d**) although the non-linear excretion remained empirical (i.e., must be calibrated to data)

saturable resorption excretion process from the Andersen et al. (2006) model. This PBPK model allows prediction of tissue-specific concentrations, (e.g., liver, fat) for PFOA and PFOS. This PBPK model was extended to a pregnant maternal scenario by Loccisano et al. (2013). Fàbrega et al. (2014) updated this model with partition coefficients derived from human cadavers.

Both the original Andersen et al. (2006) and PBPK models assume a "highly bound" free fraction of 2 % free for both PFOS and PFOA; however, it should be

noted that the measured values of 0.2 % and 0.3 % indicate roughly ten times less PFC available for excretion via glomerular filtration (Kerstner-Wood et al. 2003).

It is important to note the progression of models from Fig. 14.2a–d; even the PBPK model still includes an empirical component with respect to the saturable resorption process. Unfortunately, the parameters describing the saturable resorption process are currently not independent of physiology, cannot be extrapolated, and therefore must be empirically estimated for each new species or physiology. This need for empirical calibration reflects that the current understanding of the non-linear kinetics of PFCs is not yet complete. Any extrapolation with empirical grounding may be fraught with uncertainty.

## 14.4 Dosimetric Anchoring of Animal Studies

Given the dose regimen of a toxicological study, different dose metrics can be predicted using an appropriately parameterized PK models: the time-integrated serum concentration (area under the curve or AUC), average serum concentration, and maximum serum concentration can be predicted for each *in vivo* study. These dosimetric anchors allow comparison across multiple in vivo studies in different species, despite the unusual PK of PFCs. For example, (Rodriguez et al. 2009) determined that, while the administered dose for two PFOA in vivo toxicological studies with similar toxicity endpoints in rats and mice differed by 30-fold (3 mg/kg/day and 0.1 mg/kg/day, respectively) the time-integrated serum concentrations (AUC) values were in fact similar.

There is an abundance of PK data allowing the use of empirically calibrated models for predicting tissue concentrations as the result of exposure to PFOS and PFOA. Wambaugh et al. (2013) collected the results of in vivo toxicity experiments on these PFCs with Tables 14.2 and 14.3 summarizing the study design, LOELs, and where available NOELs, from 10 PFOA studies and 13 PFOS studies. Toxicity endpoints were categorized as liver, thyroid, developmental, reproductive, or immunological (Wambaugh et al. 2013). Dosimetric anchoring via PK modeling demonstrated consistency between these in vivo studies.

In order to facilitate dosimetric anchoring, Wambaugh et al. (2013) used a Bayesian framework to incorporate uneven amounts of PK data from eight in vivo studies that used varying animals and dosing regimens. Model parameter distributions for a consistent PK model were estimated such that a 95 % credible interval for each dose metric could be predicted. The breadth of the credible interval of the predicted dose metrics reflects the uncertainty corresponding to the appropriateness of the PK model used and the available in vivo PK data sets for each species, strain/stock, and gender. Model predictions were assessed by comparing the predicted final serum concentration for each treatment with any measured final serum concentration in the in vivo toxicity experiments, and the predictions were generally similar to the measurements (within a factor of 2) (Wambaugh et al. 2013).

**Table 14.2** PFOA in vivo toxicity studies

| Study | Subject | Dose mg/kg/day | Exposure | NOEL mg/kg/day | LOEL mg/kg/day | Critical effect |
|---|---|---|---|---|---|---|
| | | | | | | **Liver** |
| Butenhoff et al. (2002), (2004b) | Monkey (M) | 3, 10, 30/20 | 26 weeks | NA | 3 | Increased liver weight |
| | Cynomolgus | | Oral capsule | | | |
| Perkins et al. (2004) | Rat (M) | 0.06, 0.64, 1.94, 6.50 | 13 weeks | 0.06 | 0.64 | Increased absolute and relative liver weight, hepatic hypertrophy- reversible following 8 week recovery period |
| | ChR-CD | | Diet | | | |
| Butenhoff et al. (2004a) and York et al. (2010) | Rat (M) | 1, 3, 10, 30 | 6 week pre mating- mating | NA | 1 | Increased absolute and relative liver weight |
| | Sprague-Dawley | | Oral gavage | | | |
| White et al. (2009) and Wolf et al. (2007) | Mouse (F) | 5, 20 | GD7-17 | Maternal: NA | Maternal:5 | Maternal all groups except |
| | CD-1 | | GD10-17 | | | 5(15–17): increased relative liver weight |
| | | | GD13-17 | | | |
| | | | GD15-17 | | | |
| | | | Oral gavage | | | |
| White et al. (2009) and Wolf et al. (2007) | Mouse (F) | 3, 5 | GD1-17 | Maternal: NA | Maternal: 3 | Maternal: increased absolute and relative liver weight |
| | CD-1 | | Oral gavage | | | |
| DeWitt et al. (2008) | Mouse (F) | 3.75, 7.5, 15, 30 | 15 days | NA | 3.75 | Increased relative liver weight |
| | C57BL/6 N | | Drinking water | | | |
| | | | | | | **Developmental** |
| Lau et al. (2006) | Mouse (F) | 1, 3, 5, 10, 20, 40 | GD1-17 | Maternal: NA | Maternal: 1 | Maternal-increased liver weight |
| | CD-1 | | Oral gavage | Developmental: NA | Developmental: 1 | Developmental-accelerated sexual maturity in males |

| | | | | | | |
|---|---|---|---|---|---|---|
| White et al. (2009) and Wolf et al. (2007) | Mouse (F) | 5 (all but GD15-17 group), 20 (GD15-17 group only) | GD7-17 | Maternal: NA | Maternal: 5 | Maternal all groups except |
| | CD-1 | | GD10-17 | Developmental: NA | Developmental: 5 | 5 (15–17): increased relative liver weight |
| | | | GD13-17 | | | 20 GD15-17: decreased pup survival |
| | | | GD15-17 | | | Developmental all groups: increased relative liver weight, delayed mammary gland development at PND29 and PND32 |
| | | | Oral gavage | | | 5(GD7-17, 10–17): delayed eye opening and body hair growth |
| White et al. (2009) and Wolf et al. (2007) | Mouse (F) | 3, 5 | GD1-17 | Maternal: NA | Maternal: 3 | Maternal: increased absolute and relative liver weight |
| | CD-1 | | Oral gavage | Developmental: NA | Developmental: 3 | Developmental 3U + L, 5U, 5U + L: delayed eye opening and hair growth |
| | | | Cross-foster at birth | | | PND 22-all groups: increased relative liver weight |
| | | | | | | PND22-all except 3 L: delayed mammary gland development |
| | | | | | | PND42 all except 3U + L: delayed mammary gland development |
| | | | | | | PND 63 all groups: delayed mammary gland development |

(continued)

**Table 14.2** (continued)

| Study | Subject | Dose mg/kg/day | Exposure | NOEL mg/kg/day | LOEL mg/kg/day | Critical effect |
|---|---|---|---|---|---|---|
| Macon et al. (2011) | Mouse (F) | 0.3, 1, 3 | GD1-17 | NA | 0.3 | All groups: increased offspring relative liver weights |
| | | | Oral gavage | | | |
| | CD-1 | 0.01,0.1, 1.0 | GD10-17 | NA | 0.01 | All groups: stunted mammary epithelial growth |
| | | | Oral gavage | | | |
| | | | | | | 1 mg/kg group only: increased offspring relative liver weights |
| | | | | | | **Immunological** |
| DeWitt et al. (2008) | Mouse (F) | 0, 3.75, 7.5, 15, 30 | 15 days | NA | 3.75 | Reduced SRBC-specific IgM antibody titers |
| | C57BL/6 N | | Drinking water | | | |

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*NA* not applicable/could not be determined, *M* male, *F* female, *GD* gestation day, *LD* lactation day, *PND* post natal day, *U* in utero exposure, *L* lactational exposure, *U+L in utero* and lactational exposure, *SRBC* sheep red blood cells, *IgM* immunoglobulin M

**Table 14.3** PFOS in vivo toxicity studies

| Study | Subject | Dose mg/kg/day | Exposure | NOEL mg/kg/day | LOEL mg/kg/day | Critical effect |
|---|---|---|---|---|---|---|
| | | | | | | **Liver** |
| Curran et al. (2008) | Rat (M) Sprague-Dawley | 0.14, 1.33, 3.21, 6.34 | 28 days, feed | 0.14 | 1.33 | Increased final relative (to BW) liver weight; decreased serum total T4 |
| | 15/group | | | | | |
| Curran et al. (2008) | Rat (F) Sprague-Dawley | 0.15, 1.43, 3.73, 7.58 | 28 days, feed | NA | 0.15 | Increased final relative (to BW) liver weight |
| | 15/group | | | | | |
| Seacat et al. (2003) | Rat (M) Crl:CD(SD) IGS BR | 0.035, 0.14, 0.35, 1.4 | 98 days, feed | 0.14 | 0.35 | Centrilobular hepatic hypertrophy (at 1.4 mg/kg/day increased absolute/relative liver wt and ALT) |
| | 5/group | | | | | |
| Seacat et al. (2003) | Rat (F) Crl:CD(SD) IGS BR | 0.038, 0.15, 0.38, 1.56 | 98 days, feed | 0.38 | 1.56 | Centrilobular hepatic hypertrophy and increased relative liver wt |
| | 5/group | | | | | |
| Seacat et al. (2002) | Monkey (MF) cynomolgus | 0.03, 0.15, 0.75 | 182 days, oral capsule | 0.15 | 0.75 | Increased absolute and relative hepatic wt; centrilobular or diffuse hepatocellular hypertrophy |
| | 6/sex/group | | | | | |
| | | | | | | **Thyroid** |
| Chang et al. (2008b) | Rat (F) Sprague-Dawley | 15 | Single oral dose | NA | 15 | Decreased total T4 at 2, 6 and 24 h |
| | 5–15/group | | | | | Decreased total T3 and rT3 at 24 h |
| | | | | | | Increased free T4 at 2 and 6 h; normal at 24 h |

(continued)

**Table 14.3** (continued)

| Study | Subject | Dose mg/kg/day | Exposure | NOEL mg/kg/day | LOEL mg/kg/day | Critical effect |
|---|---|---|---|---|---|---|
| Curran et al. (2008) | Rat (F) Sprague-Dawley 15/group | 0.15, 1.43, 3.73, 7.58 | 28 days, feed | 0.15 | 1.43 | Decreased total T4 |
| Curran et al. (2008) | Rat (M) Sprague-Dawley 15/group | 0.14, 1.33, 3.21, 6.34 | 28 days, feed | 0.14 | 1.33 | Decreased total T4 |
| | | | | | | **Developmental** |
| Butenhoff et al. (2009) and Chang et al. (2009) | Rat (F) Sprague-Dawley 25/group | 0, 0.1, 0.3, 1.0 | GD 0- PND 20 (41 days), oral gavage | 0.3 | 1.0 | M offspring: decreased habituation response |
| Lau et al. (2003) and Thibodeaux et al. (2003) | Rat (F) Sprague-Dawley 16-25/group | 1, 2, 3, 5, 10 | GDs 2–20 (19 days), oral gavage | 1 | 2 | Decreased pup survival and developmental delays |
| Luebker et al. (2005b) | Rat (F) Crl:CD(SD) IGS BR VAF/+ | 0.1, 0.4, 1.6, 3.2 | 63–76 days (6 weeks prior to mating through gestation and lactation), 2 generations (only 0.1 and 0.4), oral gavage | 0.1 | 0.4 | Developmental delays (eye opening) |
| Lau et al. (2003) | Mouse (F) CD1 | 1, 5, 10, 15, 20 | GD1-18, oral gavage | 5 | 10 | Decreased pup survival |
| | | | | | | **Reproductive** |
| Luebker et al. (2005a) | Rat (F) Crl:CD(SD) IGS BR VAF/+ | 0.1, 0.4, 1.6, 3.2 | 63–76 days, oral gavage | 0.4 | 1.6 | Decreased F1 reproductive outcome |

| | | | | | | |
|---|---|---|---|---|---|---|
| Luebker et al. (2005a) | Rat (F) Crl:CD(SD) IGS BR VAF/+ | 0.4, 0.8, 1.0, 1.2, 1.6, 2.0 | 63–76 days, oral gavage | 1.2 | 1.6 | Decreased viability |
| Chen et al. (2012) | Rat (F) Sprague-Dawley<br>10/group | 0.1, 2 | GD1-2, oral gavage | 0.1 | 2.0 | Histopathological changes to lungs; increased mortality |
| | | | | | | **Immunological** |
| Dong et al. (2009) | Mouse (M) B6C3F1 | 0.0083, 0.083, 0.42, 0.83, 2.08 | 60 days, oral gavage | 0.008 | 0.083 | Increased splenic natural killer cell activity |
| Peden-Adams et al. (2008) | Mouse (M) B6C3F1 | 0.00018, 0.0018 0.0036, 0.018, 0.036, 0.18 | 28 days, oral gavage | 0.00018 | 0.0018 | Suppressed SRBC plaque-forming cell response |
| Peden-Adams et al. (2008) | Mouse (F) B6C3F1 | 0.00018, 0.0018 0.0036, 0.018, 0.036, 0.18 | 28 days, oral gavage | 0.0018 | 0.0036 | Suppressed SRBC plaque-forming cell response |

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*BW* body weight, *T*4 Thyroxine, *T*3 Triiodothyronine, *rT*3 reverse Triiodothyronine, *ALT* Alanine Aminotransferase, *F1* first filial generation, *SRBC* sheep red blood cells

Wambaugh et al. (2013) predicted dose metrics for the LOEL dose group for each endpoint in each study and, where available, the NOEL dose group. For many of the PFOA studies a NOEL group was lacking (i.e. the lowest dose tested showed an effect). The mean and maximum serum concentrations were found to be consistent dose metrics across in vivo studies.

PFOA hepatic effects have the most in vivo studies (Table 14.2). For this combination of chemical and effect, there are ten different in vivo LOELs from six studies (note that Wolf et al. (2007) identified five different LOELs for dosing on different windows of gestational days, e.g. days 7 through 17). For PFOA hepatic effects the outliers with respect to total dose and AUC are from the 180 day monkey study (Butenhoff et al. 2002). Although that study had a LOEL of 3 mg/kg/day, which is superficially similar to the LOELs of the other studies, the total dose of 540 mg/kg is a clear outlier with respect to the other studies.

Figure 14.3 compares the predicted mean serum concentration dose metric corresponding to the LOEL treatment group for each PFOA in vivo study. Where available, the dose metric for the NOEL treatment groups is also shown. If there is no NOEL dose group, all we know is that the effect happened somewhere between zero and the dose metric for the LOEL dose group.

The (Macon et al. 2011) study, which identified developmental effects in the growth of mammary tissue, is the most sensitive PFOA toxicity study considered here, as the predicted average concentration for that gestational day 10–17 LOEL is

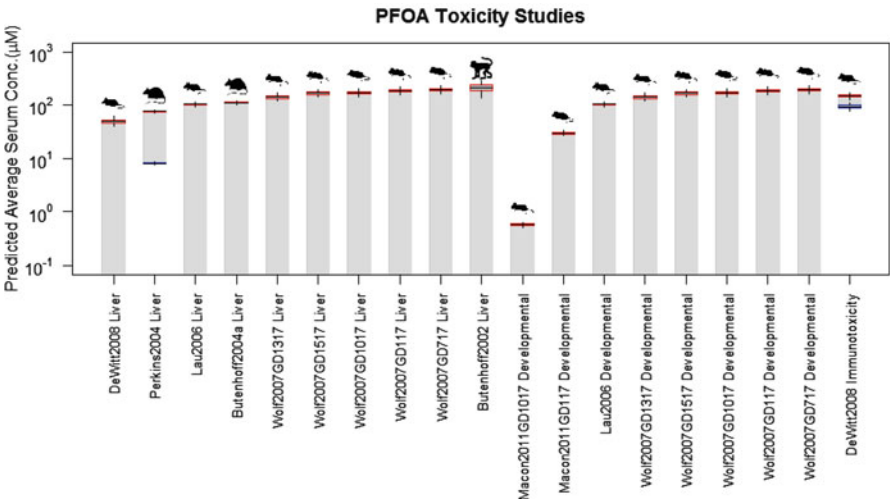


**Fig. 14.3** Average serum concentration during PFOA in vivo toxicity studies (studies grouped by endpoint along y-axis). For each study the box and whisker plots indicate median, mean +- standard deviation, and 95 % credible intervals for LOEL and NOEL (lower of two points when NOEL was observed). Credible intervals are calculated using the distribution of PK model parameters for the Andersen et al. (2006) model, as determined by Wambaugh et al. (2013), for the animal and dose regimen used in each in vivo study

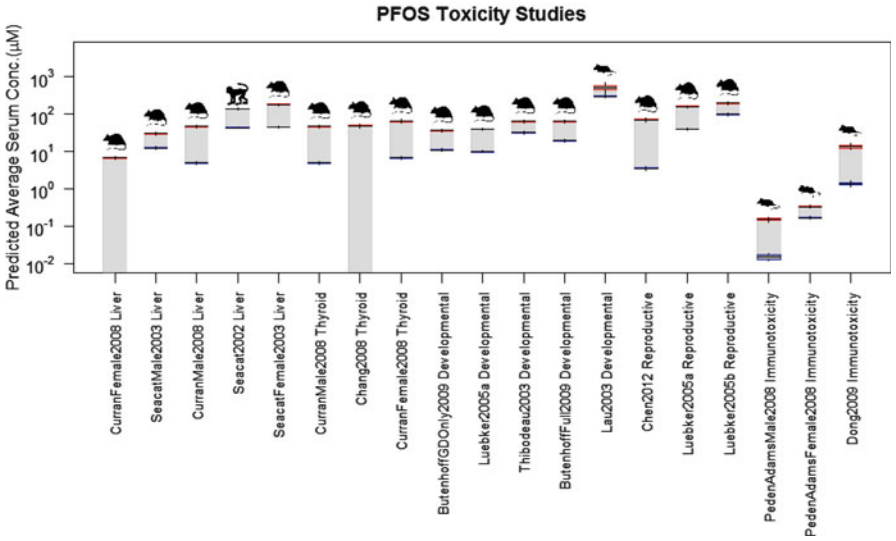


**Fig. 14.4** Average serum concentration during PFOS in vivo toxicity studies (studies grouped by endpoint along y-axis). For each study the box and whisker plots indicate median, mean +- standard deviation, and 95 % credible intervals for LOEL and NOEL (lower of two points when NOEL was observed). Credible intervals are calculated using the distribution of PK model parameters for the Andersen et al. (2006) model, as determined by Wambaugh et al. (2013), for the animal and dose regimen used in each in vivo study

two orders of magnitude lower than most of the other studies, which regardless of species or endpoint, appear roughly consistent in Fig. 14.3a.

In Fig. 14.4 the PFOS in vivo effects have been compared using the predicted mean serum concentration dose metric across studies, species, and genders. Unlike with PFOA, the presence of NOELs for most PFOS studies allows clear argument that the dose metrics are generally consistent. The LOELs and NOELs for the three studies with thyroid effects are entirely consistent, but for each of liver, developmental, reproductive, and immunological effects there is one outlier study (e.g. a study with a NOEL predicted higher than LOELs of the other studies).

The LOELs and NOELs for liver effects are consistent for four studies, but the Curran et al. (2008) female rat LOEL is lower than the NOEL for the other four studies, including the Curran et al. (2008) male rat study. For developmental effects, the LOELs and NOELs are consistent for four studies, but the Lau et al. (2003) mouse study has a NOEL higher than the LOELs of the other studies (which were all rat studies). For the three studies showing reproductive effects, the Chen et al. (2012) LOEL is higher than the NOEL for the Luebker et al. (2005a) study.

Immunological effects for PFOS appear to be much more sensitive than the other endpoints observed. However, there is disagreement between the predicted dose metrics for the Dong et al. (2009) and the Peden-Adams et al. (2008) studies since the Peden-Adams et al. (2008) study identified a LOEL of 0.00018 mg/kg/day for suppressed sheep red blood cell plaque-forming cell response while the Dong et al. (2009) LOEL was 0.008 mg/kg/day for increased splenic natural killer cell activity.

## 14.5 Conclusion

Comparing the onset of in vivo effects across studies requires dosimetric anchoring to a measure of the tissue concentration at the site of toxicological effect (Rowlands et al. 2014). Predicting dose metrics requires PK models, which is challenging for PFCs because, as yet, there is not a model that allows extrapolation between species. Fortunately, there have been several cross species PK studies to collect the necessary data to allow empirical calibration of PK models to specific PFCs and species. For PFOS and PFOA, this sort of data has shown that, despite large differences in half-lives and the administered dose necessary for the onset of toxicological effects, no one species appears to be especially sensitive (Rodriguez et al. 2009; Wambaugh et al. 2013).

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