Data Derived Extrapolation Factors for Developmental Toxicity: A Preliminary Research Case Study with Perfluorooctanoate (PFOA)

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Abstract

Guidelines of the United States Environmental Protection Agency (EPA, 1991) and the International Programme on Chemical Safety (IPCS, 2005) suggest two different default positions for dosimetric extrapolation from experimental animals to humans when the dosimetry of the critical effect is not known. The default position of EPA (1991) for developmental toxicity is to use peak concentration (or Cmax) for this dosimetric extrapolation. In contrast, IPCS (2005, page 39) states its default position for dosimetric choice in the absence of data is to use the area under the curve (or AUC). The choice of the appropriate dosimeter is important in the development of either a Chemical Specific Adjustment Factor (CSAF) of IPCS (2005) or a Data Derived Extrapolation Factor (DDEF) of EPA (2014). Our research shows the development of a DDEF for developmental toxicity for perfluorooctanoate (PFOA), a chemical of current interest. Here, we attempted to identify the appropriate dosimetric adjustment from a review of developmental effects identified by EPA (2016). Some of these effects appear to be related to Cmax, few if any related to AUC, and most related to the average concentration during the exposure window of concern for a particular effect. We then compared kinetic data from PFOA exposure during pregnancy in mice with newly available clinical data in humans after up to 25 weeks of PFOA exposure. The resulting DDEF was 14. Although current population exposures to PFOA are generally much lower than both the experimental animal data and the clinical human study, the development of this DDEF is consistent with current guidelines of both EPA (2014) and IPCS (2005).

Introduction

Within the process of non-cancer dose response assessment, such as the development of a Tolerable Daily Intake (TDI) or Reference Dose (RfD), the use of a Chemical Specific Adjustment Factors (CSAF), data-derived uncertainty factors (DDEF) or a Physiological Based Pharmacokinetic (PBPK) model is an important consideration (IPCS, 2005; EPA, 2014). These factors or models are used in the extrapolation of experimental animal results to humans, rather than a default uncertainty factor of 10-fold, when appropriate data are available. The available data include knowledge of kinetic and dynamic differences between the experimental animal of choice and humans, or of default assumptions that are based on established underlying principles.

The CSAF/DDEF method has been discussed internationally for a number of years, arguably starting in the late 1980s with the dosimetric adjustments of inhaled dose for determining Reference Concentrations (RfCs) (Jarabek, 1994). More formal discussions were held by the

IPCS (1994) based on the work of Renwick (1993). Health Canada was the first authority to use CSAF in its deliberative process (Meek et al., 1994), followed by EPA (2004) with its Integrated Risk Information System (IRIS) assessment for the chemical boron. IPCS published its final guidelines in 2005, followed by EPA in 2014. Multiple scientific publications have occurred throughout this process (e.g., Dourson et al., 1998; Zhao et al., 1999; Meek et al, 2001). The CSAF/DDEF method is general enough to be used with different chemistries. IPCS (Bhat et al., 2017) has recently polled its membership for general use of this methods and lessons learned. The results have been generally favorable.

Developmental toxicity is different from many other toxicities of concern from environmental contamination in that it generally develops during a short window of exposure. Although thresholds for toxicity are still thought to exist (Piersma et al., 2011), such exposure suggests a particular approach to the development of DDEFs, for example, the use of peak serum concentration of the chemical of interest (now referred to as Cmax) versus its associated half-life (or area under the curve---AUC) (EPA, 1991). The resulting differences in extrapolation from experimental animals to humans for developmental toxicity based on the choice of Cmax or AUC may be significant.

This research case study will demonstrate the development of a DDEF for developmental toxicity from a chemical of current interest, specifically perfluorooctanoate (PFOA). Our results may also be applicable to other chemicals where the critical effect is also developmental toxicity.

Methods

Guidelines of EPA (1991) and IPCS (2005) suggest two different default positions for dosimetric extrapolation from experimental animals to humans when the dosimetry of the critical effect is not known. The default position of EPA (1991) for developmental toxicity is to use peak concentration (or Cmax) for this dosimetric extrapolation. Specifically, EPA (1991) states "Therefore, it is assumed that, in most cases, a single exposure at any of several developmental stages may be sufficient to produce an adverse developmental effect." [1] EPA goes on to state that it would be inappropriate to use time-weighted averages or adjustment of exposure over a

[1] EPA (1991, page 38) also states that:

> "Second, for developmental toxic effects, a primary assumption is that a single exposure at a critical time in development may produce an adverse developmental effect, i.e., repeated exposure is not a necessary prerequisite for developmental toxicity to be manifested. In most cases, however, the data available for developmental toxicity risk assessment are from studies using exposures over several days of development, and the NOAEL, LOAEL, and/or benchmark dose is most often based on a daily dose, e.g., mg/kg-day. Usually, the daily dose is not adjusted for duration of exposure because appropriate pharmacokinetic data are not available. In cases where such data are available, adjustments may be made to provide an estimate of equal average concentration at the site of action for the human exposure scenario of concern. For example, inhalation studies often use 6 hours/day exposures during development. If the human exposure scenario is continuous and pharmacokinetic data indicate an accumulation with continuous exposure, appropriate adjustments can be made."

different time frame than that actually encountered in developmental toxicity studies, unless data indicated that the critical effect resulted from an accumulation with continuous exposure. However, for continuous human exposure, a time weighted average exposure during the windows of susceptibility might also be appropriate, especially for developmental toxicity, as described in a recent meeting (*ARA*, 2019).

In contrast, IPCS (2005, page 39) states its default position for dosimetric choice in the absence of data is to use the AUC, specifically "In cases where the data are not sufficient to make a clear decision, then the AUC of the parent compound or 1/CL derived from either in vivo or in vitro data should be used; such an approach would be protective, because there is likely to be greater human variability in AUC or 1/CL [clearance] than in Cmax." IPCS (2005) goes on to state that effects resulting from subchronic or chronic exposure would normally be related to the AUC, whereas acute toxicity can be related to either the AUC or the Cmax, especially the latter when a simple bimolecular interaction, such as receptor binding and inhibition of enzymes, produces the effect.

EPA (2014) confirms that the choice of a dose metric associated with the health outcome of interest is most useful when it "describes target tissue exposure in terms of the toxic chemical moiety (parent or metabolite) and is expressed in appropriate time-normalized terms." Moreover, the appropriate dose metric can vary with the mode of action (MOA), duration of exposure, and the adverse effect of concern (EPA, 2006). Selection of an appropriate dose metric, whether it be Cmax, AUC, or something else such as an average exposure during an appropriate window, is based on specific endpoints, including:

- Duration of exposure and effect;
- Identification of the active chemical moiety;
- Selection of the organ or tissue group in which some measure of internal dose is desired;
- Selection of the measure of exposure that best correlates with toxicity.

Based on extensive discussions and scientific debates, both IPCS (2005) and EPA (2014 have established minimum requirements in the review and evaluation of data for the development of CSAFs or DDEFs. Specific EPA (2014) guidance includes a series of questions, specifically:

- What is/are the critical effect(s) and POD being used for this assessment?
- Has the toxicologically active chemical moiety been identified?
- What is the MOA, Adverse Outcome Pathway (AOP), or mechanism for that toxicity? Have the key events been identified and quantified? Do these key events identify important metabolic steps?
- Are the processes of absorption, distribution, metabolism and elimination (i.e., ADME) of the chemical well characterized? If dose-response data are from an animal model, do animals and humans metabolize the chemical(s) in a similar way (qualitatively and quantitatively)?
- Are there data in human populations describing variation in important kinetic parameter values for this chemical(s)? Have sensitive populations and/or life stages been identified? Are the data for these sensitive populations adequate for quantitative analyses?

We follow this series of questions from EPA (2014) in our research using PFOA as an example.

Results

- *What is/are the critical effect(s) and POD being used for this assessment?*

The critical effects for PFOA appear to be related more to developmental toxicity as determined by EPA (2016, Table 4-8). Seven studies are highlighted in EPA's work. Four of them are conducted in mice with gavage dosing during pregnancy showing a variety of fetal and maternal effects. One of these studies is a 15-day drinking water exposure in mice, but critical effect in this study was noted after 1 day. Two of these studies were 13-week exposures of PFOA in rats, but the liver effects at the low doses in these studies may not be adverse according to EPA (2016). Rather, EPA (2016) uses the fetal effects from the mouse studies, and specifically from the study by Lau et al. (2006), in the development of its safe dose. Thus, fetal effects are being used by EPA (2016) as the critical effects from these gavage studies of PFOA in mice, and we developed our research using EPA's judgment.

Tables 1 through 5 summarize the relevant effects from five of these EPA-chosen studies with the intention of judging whether the appropriate dosimeter of each effect is AUC, Cmax, average concentration, or something else. These judgments were then used with appropriate kinetic information to develop a DDEF.

- *Has the toxicologically active chemical moiety been identified?*

It is generally accepted by government and industry experts that PFOA is not metabolized, or metabolized to a limited extent in mammals (EPA,2016, ATSDR, 2018). Thus, we considered PFOA to be the active chemical moiety for our research.

- *What is the MOA, AOP, or mechanism for that toxicity? Have the key events been identified and quantified? Do these key events identify important metabolic steps?*

PFOA exposure resulted in a variety of adverse effects, including hepatotoxicity, developmental toxicity, and immunotoxicity. It is also shown that PFOA induces tumors in the liver, testis and pancreas in chronic studies in the rat. Each of these effects may be evoked by a different process.

For example, Elcombe et al. (2013) considers the MOA to be associated with its ability to mimic fat in the body; specifically PFOA is:

> "a fatty acid mimetic in that it interacts with fatty acid homeostasis and/or a fatty acid mediated pathway. Both CXRl 002 [*note: this is straight-chain PFOA*] and APFO [*note: this*

is ammonium PFOA] isomers and also perfluoroalkyls of different chain lengths possess these properties."

Hepatic and the immune system effects of PFOA may also involve PPAR-alpha dependent and independent mechanisms (New Jersey Department of Water Quality (NJDWQ, 2017). According to NJDWQ (2017), developmental effects of PFOA in rodents appear to occur primarily through PPAR-alpha dependent mechanisms, while some developmental effects such as full litter resorptions appear to be PPAR-alpha independent. There is no MOA evidence for the delayed mammary gland development and NJDWQ (2017) indicated that this suggests that the effects of PFOA on this endpoint are not relevant to humans.

The mode of action for hepatic tumors, Leydig cell tumors, and pancreatic acinar cell adenomas have been attributed to activation of the xenosensor nuclear receptor peroxisome proliferator-activated receptor "alpha" (PPARa) (Klaunig et al., 2012). According to EPA (2016), PPARa agonism appears to be the MOA for testicular tumors and involves inhibition of testosterone biosynthesis and/increase in estradiol as a result of increased activity of aromatase, the cellular enzyme responsible for the metabolic conversion of testosterone to estradiol. In their recent review, NJDWQ (2017) notes that available studies suggest that PFOA causes liver tumors through an estrogenic MOA. For the testicular and pancreatic tumors caused by PFOA in rats, the MOA has not been established.

Other MOAs for PFOA have been suggested. These include effects on intercellular gap junction communication, effects on mitochondria, changes in expression of microRNAs (miRNAs), and effects related to transporter proteins such as organic anion transporters (OATs) and multidrug resistance-associated proteins (MRPs) (NJDWQ, 2017). The MOA proposed for testicular Leydig cell tumors involves inhibition of testosterone biosynthesis and signaling of the hypothalamus to produce gonadotropin releasing hormone (GnRH) (a signaling agent for the pituitary to release luteinizing hormone which upregulates testosterone production in Leydig cells) (NJDWQ, 2017).

For the purposes of developing a DDEF a reasonable assumption is that effects resulting from subchronic or chronic exposure would normally be related to the AUC or perhaps average concentration, especially for chemicals with long half-lives, whereas acute exposure can be related to either AUC, average concentration or Cmax. The latter might be more relevant than either of the former two when a simple bimolecular interaction produces the effect. The use of an average exposure might be a more relevant dosimeter than either AUC or Cmax, if a relevant window of exposure is important.

According to this forgoing discussion, if the critical effects of PFOA are more related to biomolecular interactions, then Cmax might be the more relevant dosimeter, as a few of the developmental effects described in Tables 1-5 appear to indicate. However, other effects of concern for PFOA, including other developmental effects, may be due to sustained activation of the PPAR receptor and might therefore be more associated with average concentration during a relevant window of exposure, as also described in Tables 1-5. In fact, Cmax, average concentration, and AUC, as well as other possible dosimeters should always be considered in any deliberation of CSAF (IPCS, 2005) or DDEF (EPA, 2014).

- *Are the processes of ADME of the chemical well characterized? If dose-response data are from an animal model, do animals and humans metabolize the chemical(s) in a similar way (qualitatively and quantitatively)?*

The ADME has been fairly well characterized in the rat and mouse, less so in other experimental species, and until recently, not well characterized in humans. Figure 1 is adapted from Lou et al. (2009, Figure 3) and shows the kinetic behavior after a single gavage exposure in mice. Cmax values vary with the dose administered by Lou et al. (2009), and are estimated by us as 10 mg/L per mg/kg-day at a dose of 1 mg/kg-day, 8.5 mg/L per mg/kg-day at a dose of 10 mg/kg-day, and 3.5 mg/L per mg/kg-day at a dose of 60 mg/kg-day.

Figure 2, adapted from Lou et al. (2009, Figure 7b), shows the kinetic behavior after multiple gavage doses of PFOA in mice. The 1-day Cmax or 17 day steady state values are estimated by us from this figure as either 0.7 mg/L or 5.0 mg/L after a dose of 0.1 mg/kg-day; as either 5.0 mg/L or 35 mg/L after a dose of 1.0 mg/kg-day; and as ether 5.0 mg/L or 60 mg/L after a dose of 5.0 mg/kg-day. These apparent steady state values at 17 days imply a short half-life in mice of several days.

Since PFOA is not metabolized, or metabolized to any significant extent in mammals, PFOA is considered to be the toxic moiety, and these Cmax and steady state values in mice can be compared with available human information to gauge whether the development of a DDEF is reasonable. Until recently, kinetic data have not been publicly available in humans with which to do this development.

- *Are there data in human populations describing variation in important kinetic parameter values for this chemical(s)? Have sensitive populations and/or life stages been identified? Are the data for these sensitive populations adequate for quantitative analyses?*

Few specific kinetic data in humans have generally been available to compare with experimental animal findings, and groups such as EPA (2016), the Agency for Toxic Substances and Disease Registry (ATSDR, 2018), and Health Canada (2018) have had to rely on assumptions of kinetic findings in closely related species such as monkeys as a surrogates. Fortunately, Elcombe et al. (2013) submitted a US Patent Application where PFOA was used as a cancer chemotherapeutic agent. Findings from this study are freely available and a subset of these data have been recently published as Convertino et al. (2018).

Elcombe et al. (2013) gave PFOA up to 1200 mg once per week to 43 humans in various stages of cancer in a phase 1 therapeutic trial. Doses and blood concentrations of PFOA were carefully monitored. Patients with kidney complications were excluded. Summaries of these findings are found in Table 6 that show the individual weekly Cmax values over time in μM for each patient after his/her weekly dose of PFOA. Estimates of average Cmax values over time per dose, rather than in μM, are found in Table 7.

A DDEF could be developed from a comparison of mouse and human data Cmax values after one dose. This DDEF would be ~1.3 based on an average single dose human Cmax value of 12 mg/L per mg/kg-day from Appendix Table A and an average murine Cmax value of 9 mg/L per mg/kg-day from Lou et al. (2009, Figure 3, doses 1 and 10 mg/kg-day average). This calculation is shown in the appendix.

However, Cmax values are shown to rise to a steady state level in both humans after weekly capsule exposure (Elcombe et al., 2013) and in mice after continued gavage exposure (Lou et al., 2009). So, additional analysis seems warranted. Specifically, the average human Cmax value after 6 doses from Table 7 of 732 μM per mg/kg-day was compared with the "steady state" value of 35 mg/L after 17 doses of 1.0 mg/kg-day in mice from Figure 2. A DDEF value based on this ratio is ~8.7 (i.e., Table 7, 6 weeks, average Cmax in humans of 732 μM per mg/kg-day x 414 μg/μmole (the molecular weight of PFOA), divided by 1000 to convert to mg, and then dividing by 35 mg/L per mg/kg-day found in mice from Figure 2 of this text ~8.5). Other comparisons are possible and could be explored.

In humans, Cmax values have also been seen to rise after 6 weeks of continued gavage exposure to also approximate a steady state. Specifically, nine patients in Elcombe et al. (2013, Figure 78) were maintained on gavage dosing beyond six weeks. This information is shown here as Figure 3. These patients appeared to reach a steady state in the range of about 25 weeks. The average ratio of 6-week Cmax values to these individual patients' apparent "steady state" values is 1.6 (see the Appendix Tables F and G for this calculation). Thus, a further possible DDEF value, one based on extended human exposure and apparent steady state values when compared with the shorter-term mouse exposure of 17 days but also steady state values would be ~14 (i.e., the 6-week DDEF value of 8.7 x 1.6). Fortuitously, the length of time to reach steady state in mice of 17 days is during a large part of the window of fetal development, which is similar to a window of time of 25 weeks in pregnant humans. Thus, if humans, and specifically pregnant humans, are already in steady state, and if the critical effect is developmental toxicity, then a DDEF of 14 can be used to compare the steady state or average levels of PFOA in humans to the steady state or average levels of PFOA in mice, since the appropriate windows of exposure for the critical effect are overlapping. As before, other comparisons are possible and, in this case, should be explored.

Elcombe et al. (2013, Figure 78) might also be useful to gauge the potential half-life of PFOA in humans, at least after high gavage doses. The apparent half-life from these data appears to be 5 weeks, based on apparent time to "steady state" as ~25 weeks (or less).[2] This half-life is dramatically different than other literature values. This difference might be due in part to the suggestion by Lou et al. (2009) that the elimination of PFOA from mice is biphasic, with higher doses being eliminated more quickly due to saturation of resorption in the kidney. After saturation of resorption is alleviated, then the half-life of the remaining PFOA is longer. If such a biphasic elimination is also shown to occur in humans, then the second phase of the half-life would also be correspondingly longer in humans. This appears to be the case from the results of 3 humans in the Elcombe et al. (2013) that were only given one dose of 50 mg in 6 weeks (see Elcombe et al., 2013 Figure 10).

[2] Note: dividing the apparent steady state by 5 half-lives approximates 5 weeks as the half-life.

Table 8 shows a comparison of these various DDEFs with the mouse and human Cmax and/or steady state or average concentration data compared.

Discussion

The critical effect of PFOA appears to be more related to developmental toxicity or other toxicity due to short-term, gavage exposures in mice, consistent with EPA (2016, Table 4-8). Since EPA (1991) states "a primary assumption is that a single exposure at a critical time in development may produce an adverse developmental effect," this suggests that peak concentration (now referred to as Cmax) should be routinely considered in any dosimetric adjustment for developmental toxicity between experimental animals and humans. This suggestion is supported for PFOA in part by a possible MOA as a fatty acid mimic resulting in effects due to simple biomolecular interactions (IPCS, 2005), and in the case of these PFOA studies, the gavage nature of the exposure. However, at least for some effects, including some developmental effects, the MOA for PFOA may be mediated by sustained binding of PFOA with PPAR, resulting in disruption of fatty acid metabolism that leads to a reduced rate of development. Such effects might be more likely associated with average concentration over a given duration of exposure or during a critical window of exposure.

Therefore, we attempted to identify the appropriate dosimetric adjustment from a review of effects identified by EPA (2016) in Tables 1-5 of this text. Some of these effects appear to be related to Cmax, few if any related to AUC, but most of the relationships appear to be due to the average concentration during the exposure window of concern due to this sustained binding of PFOA with PPAR mentioned above. This latter suggestion was made at a review of this research during a recent meeting of the Alliance for Risk Assessment (*ARA*, 2019). We accept this suggestion with enthusiasm.

We then compared kinetic data between mice and humans, specifically the daily gavage dose of PFOA in mice that forms the basis of the critical effect by EPA (2016), and the once per week PFOA exposure in capsules to humans. We adjusted the daily doses in humans to match the mouse exposure by dividing by an average body weight of 75 kg given by Convertino et al. (2018) and a further division by seven days/week. Other ways to harmonize these data are likely possible and should be explored. For example, an assessment might be attempted from the work of White et al. (2011) who administered PFOA by both gavage and drinking water over 2 generations of mice. One advantage of this comparison might be the observation of effects over several generations. A disadvantage of this study is that is that the kinetic information is not as detailed as that found in Lou et al. (2009), making a comparison with the work of the human clinical study more challenging.

Although the choice of specific effect should dictate the appropriate DDEF of either 1.3, 8.7 or 14 found in Table 3 of this text, a conservative approach would be to assume that at least one or more of the potential critical effects are due to the average concentration during a relevant window of exposure within the 17 days of the developmental mouse toxicity study, exemplified by Lau et al. (2006) and Lou et al. (2009). For humans, a conservative assumption would be that one or more of the matching critical effects would occur at an average concentration during a

comparable relevant window of exposure during pregnancy, approximated by the 25 weeks seen in Figure 78 of Elcombe et al. (2013). This apparent steady state of about 25 weeks is fortuitously in the same range as the length of human pregnancy, thus matching the experimental work in mice. Thus, the conservative choice of DDEF is 14.

Population exposures to PFOA are generally much lower than both the experimental animal data and the clinical human study. The kinetic comparison and development of the various DDEFs done here may thus not be applicable to these lower exposure levels in humans. However and importantly, the development of these DDEFs is consistent with current guidelines of IPCS (2005) and EPA (2014), which is to use the kinetics of the experimental animal in the range of the NOAEL/BMD and for humans the lowest available exposure where sufficient data are available. We chose to use a dose of 1.0 mg/kg-day in mice from Figure 2, which is similar to the NOAEL values for several (although not all) developmental effects. For humans, because the kinetics for the various doses appear similar, an average kinetic value from Table 7 is used for the comparison, which also is associated with an average dose of about 1 mg/kg-day.

PFOA is not naturally occurring, so natural background exposures are not expected. However, PFOA and related chemicals are very useful and stable, and as a result have contaminated the environment in many places to a very low level. In some places, the contaminant levels approach the range of safe doses, which of themselves are highly disparate among government agencies (over 100-fold differences). This disparity is because international authorities approach the extrapolation of a safe dose for PFOA and related chemicals in very different manners. For example, authorities in the US tend to focus on experimental animal data and incorporate the differences in half-lives among experimental animals and humans to adjust the safe dose downward (EPA, 2016, NJDWQ, 2017, ATSDR, 2018). Some European authorities focus on human epidemiology studies with an emphasis on longer half-life in humans (European Food Safety Authority, 2018); other European authorities focus on a more traditional approach and are skeptical of the long half-life estimates of others (Committee on Toxicology, 2009). Australian and New Zealand authorities are considering several different approaches (Food Standards Australian New Zealand, 2017; Australian Department of Health, 2017), as is Health Canada (2018). Although the extrapolation of safe doses for PFOA and related chemicals is highly uncertain, the recent kinetic findings in humans by Elcombe et al. (2013) may alleviate some of this uncertainty.

The DDEF/CSAF method explicitly addresses human uncertainty, specifically in the use of data for replacing default uncertainty factors for experimental animals to human extrapolation and from average to sensitive human extrapolation. The DDEF/CSAF method explicitly addresses the calculation of a RfD, RfC, TDI, or similar “safe” dose values. While such values cannot be used to determine risk, or perhaps risk other than zero, they are very useful for identifying ranges of exposures likely to be without the risk of deleterious effects in sensitive subgroups after a lifetime of exposure. Health Canada (Meek et al., 1994), IPCS (2005) and EPA (2014) guidelines go into great detail regarding this.

The DDEF/CSAF method has been used and further developed under the guidance of several authorities and numerous experts. It has been used internationally since the mid-1990’s. Recently, the IPCS (Bhat et al., 2017) has surveyed its membership on the use of this method.

Results of this survey are generally positive as found at: https://www.tandfonline.com/doi/full/10.1080/10408444.2017.1303818.

Finally, estimates of half-life are possible from the human clinical study, as described in Figure 3, but these estimates are much shorter than literature values inferred from chronic exposures of workers and populations. These disparate estimates might be due to a biphasic elimination evident in the clinical trial, but not in the literature, or because the clinical trials are for cancer therapy, and kinetics from these studies may not reflect the average population. Then again, this population might be explored as a possible sensitive subgroup. If this population is judged to be sensitive, then an adjustment to the uncertainty factor for within human variability in kinetics might be appropriate.

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Figure 1. Single dose PFOA exposure adapted from Lou et al., (2009), Figure 3. Estimated Cmax values are shown in the box below this figure.

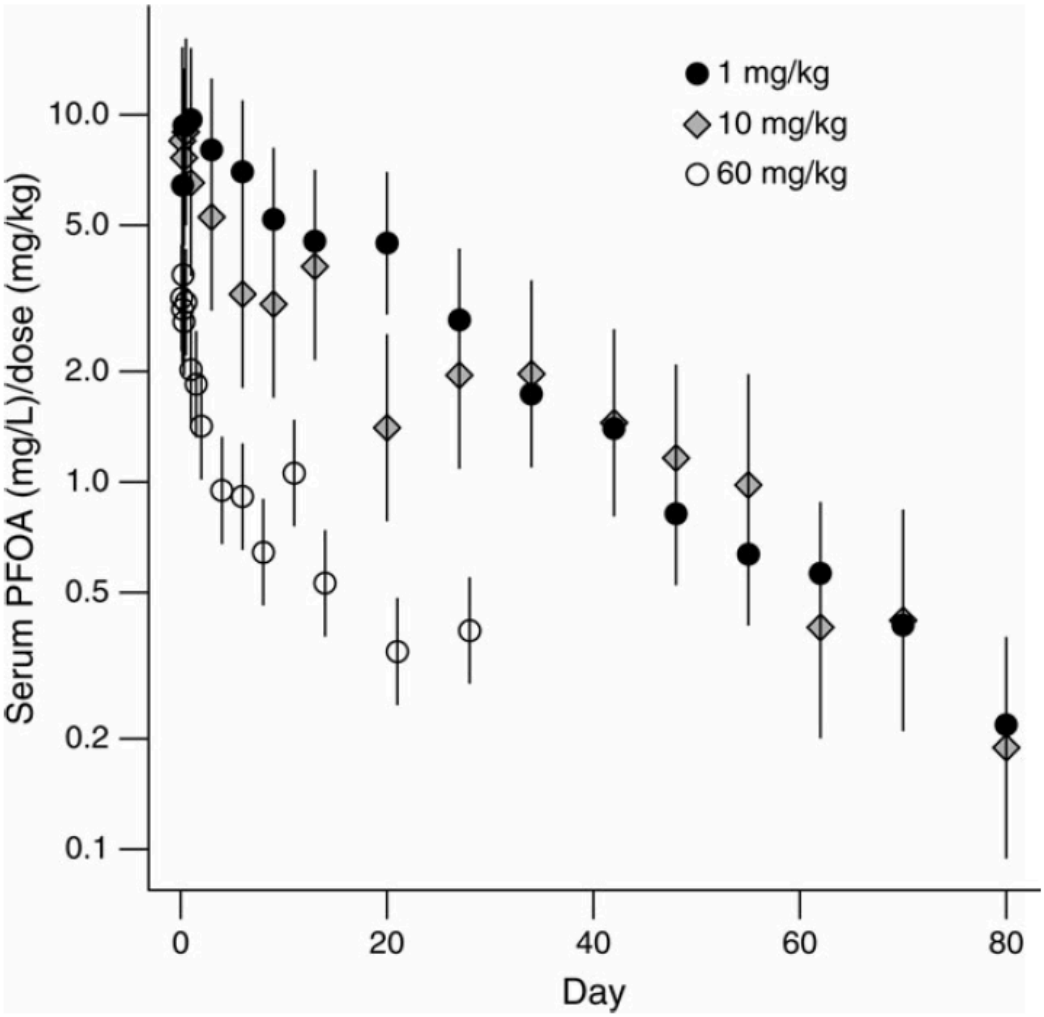

FIG. 3. Serum concentrations scaled by dose for females administered single doses of 1, 10, and 60 mg/kg. Points are means, error bars are 95% confidence intervals for the means. 1 and 10 mg/kg dose groups are largely superimposed and linear in time on this semi-log suggesting linear first-order kinetics at these doses. The 60 mg/kg group has a substantially different shape and time course.

Cmax at 1 mg/kg-day ~10;
Cmax at 10 mg/kg-day ~8.5;
Cmax at 60 mg/kg-day ~3.5;

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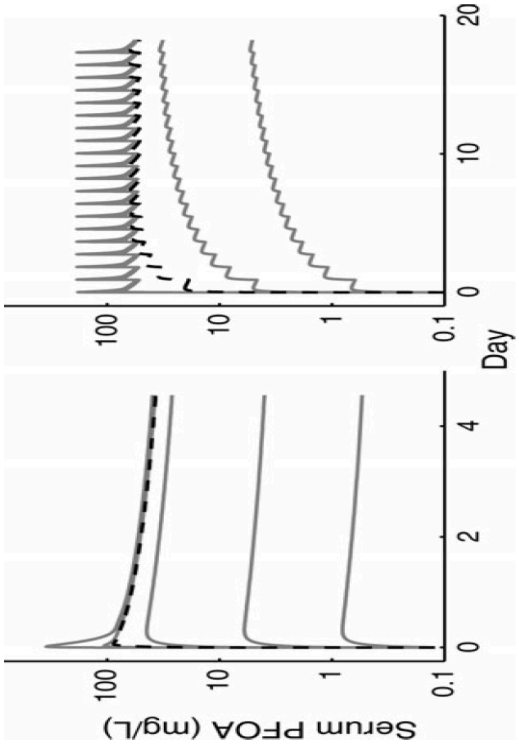
Figure 2. Estimated Cmax or steady state after multiple gavage doses in mice after repeat dose, designated as "bottom" by Lou et al. (2009), but represented by the right panel in this figure. Highest and lowest doses are not shown by Lou et al. (2009) in this "bottom"



This figure can be used to determine Cmax and steady state values after after 1 day and 17 days, respectively.

Dose = 0.1; Cmax is ~0.7 at 1 day and ~5.0 at 17 days
Dose = 1.0; Cmax is ~5.0 at 1 day and ~35 at 17 days
Dose = 5.0; Cmax is ~20 at 1 day and ~60 at 17 days

FIG. 7. Delineation of predictions for the PFOA concentration (mg/l) in the central compartment. For the single dose (top) solid lines depict doses of 0.1, 1, 10, 100, and 1000 mg/kg. The dashed line indicates a dose of 40 mg/kg which is roughly where the onset of nonlinearity occurs. For the repeated dose (bottom) solid lines depict repeated daily doses of 0.001, 0.1, 1, 50, and 500 mg/kg. The dashed line indicates a daily dose of 5 mg/kg.

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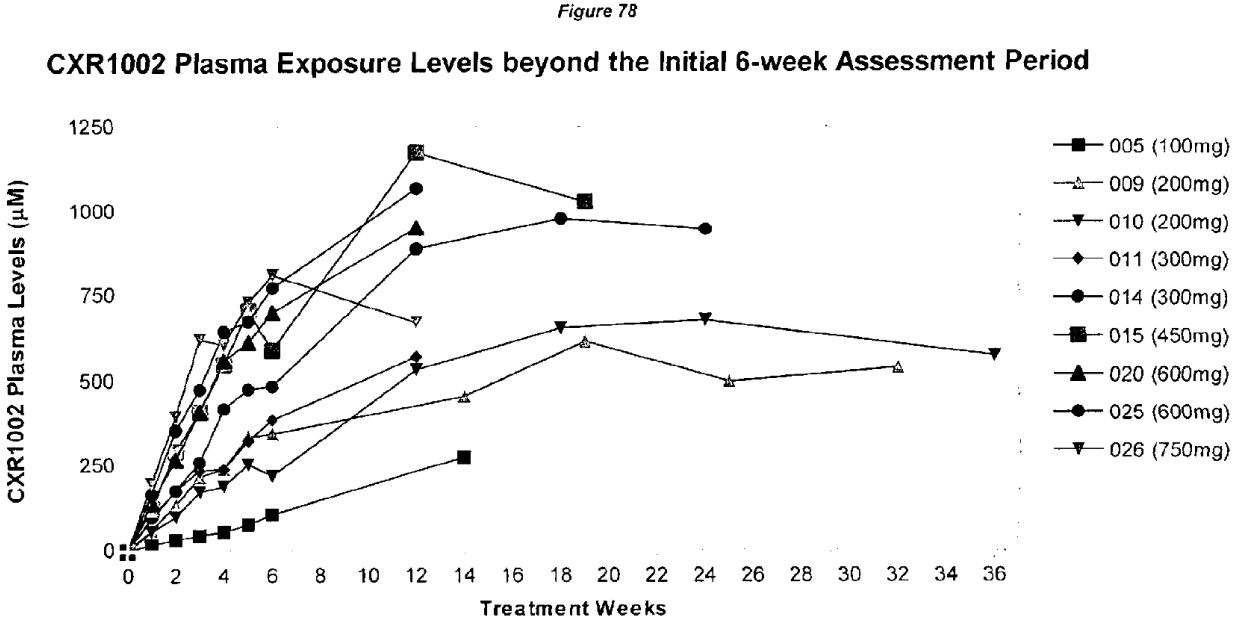
Figure 3. Elcombe et al. (2013) weekly doses in excess of 6 weeks. Information is exactly Figure 78 of their text found on Sheet 71 of 85.



542 **Table 1. Lau et al. (2006) Effects Summary After Gavage Dosing of Female CD-1 mice for**
543 **17 days (GDs 1-17) at Doses of 0, 1, 3, 5, 10, 20, and 40 mg/kg/day of PFOA.**

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax, AUC?	Comments
Increased maternal liver weight	1	Average blood concentration during exposure period	Effect is quasi dose related, but without histopathology is not considered adverse by EPA (2016, page 248) and others.
Accelerated male puberty	1	Average blood concentration during exposure period	
Reduced pup body weight	3	Average blood concentration during exposure period	According to the authors, "Neonatal growth deficits may be related to the nursing dams' capability to lactate, and hence the nutritional status of the suckling pups."
Full litter resorption	5	Cmax	According to the authors "these pregnancy losses probably took place shortly after implantation."
Postnatal survival	5	Average blood concentration during exposure period	Mortality decreases sharply after birth, despite continued PFOA exposure through breast milk, suggesting an in utero cause.
Tail and limb defects	5	Indeterminate	Statistically significant, but effects are not dose related, nor does TERA place confidence in this effect.
Increased time to birth	10	Average blood concentration during exposure period	Effect is not dose related & may be from maternal impact, nor does TERA place confidence in this as an adverse effect.

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax, AUC?	Comments
Ossification of phalanges	1 or 10	Average blood concentration during exposure period	Effects are not dose related and may be due to maternal impacts, nor does TERA place confidence in these as adverse effects.
Microcaedia	10	Indeterminate	Full development of the heart takes 4 days in the mouse (Savolainen et al., 2009). Effects are not dose related and may due to maternal impacts, nor does TERA place confidence in these as adverse effects.
Reduced ossification of supraoccipital	10	Average blood concentration during exposure period	Effects are not dose related and may be due to maternal impacts, nor does TERA place confidence in these as adverse effects.
Maternal weight loss	20	Average blood concentration during exposure period	Effect occurred within 3 days at highest dose of 40 mg/kg-day, within 6 days at 20 mg/kg-day.
Prenatal loss (% per live litter)	20	Indeterminate	
Reduced ossification of calvaria, enlarged fontanel	1 or 20	Average blood concentration during exposure period	Effects are not dose related and may be due to maternal impacts, nor does TERA place confidence in these as adverse effects.
Reduced ossification of supraoccipital	10	Average blood concentration during exposure period	TERA does not place confidence in this as an adverse effect.
Unossified hyoid	20	Average blood concentration during exposure period	Effects may be due to maternal impacts. TERA does not place confidence in these as adverse effects.

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax, AUC?	Comments
Live fetuses (# per litter)	20	Average blood concentration during exposure period	
Fetal body weight	20	Average blood concentration during exposure period	

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546 **Table 2. Wolf et al. (2007) Dose-Related Effects Summary After PFOA Gavage Dosing of**
547 **Female CD-1 mice for 17 days (GDs 1-17) at Doses of 0, 3, 5 mg/kg-day.**

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax or AUC?	Comments
↑ Maternal body weight and body weight gain	3	Average blood concentration during exposure period	The weight gain at 3 mg/kg was greater than that at higher doses. Weight gains are generally not considered adverse.
↑ Absolute and relative maternal liver weight	3	Average blood concentration during exposure period	Increased liver weights are not considered adverse unless accompanied by histopathology.
↑ Absolute and relative male pup liver weight	3	Average blood concentration during exposure period	Increased liver weights are not considered adverse unless accompanied by histopathology.
↓ Female offspring birth weight	3	Average blood concentration during exposure period	Maternal body weight gain influences offspring birth weight.
↑ Relative female pup liver weight	5	Average blood concentration during exposure period	Increased liver weights are not considered adverse unless accompanied by histopathology.

↑ Dams with implants but no live pups	5	Indeterminate	
Delayed eye opening	5	Average blood concentration during exposure period	TERA does not place confidence in this as an adverse effect.
Delayed emergence of body hair	5	Average blood concentration during exposure period	TERA does not place confidence in this as an adverse effect.

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549 **Table 3. Macon et al. (2011) Dose-Related Effects Summary After Gavage Dosing of**
550 **Female CD-1 mice for 17 days (GDs 1-17) at PFOA Doses of 0, 0.3, 1.0, and 3.0 mg/kg/day**

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax or AUC?	Comments
Delayed mammary gland development	0.3	Average blood concentration during exposure period	Comparison of full and half exposure prototcols indicate that late gestational exposure may be more important.

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552 **Table 4. Wolf et al. (2007) Dose-Related Effects Summary After PFOA Restricted Gavage**
553 **Dosing of Female CD-1 mice for 11 days (GDs 7-17) at Doses of 0 and 5 mg/kg/day of**
554 **PFOA**

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax or AUC?	Comments
↑ Maternal body weight gain	5	Average blood concentration during exposure period	Weight gains are generally not considered adverse.
↑ Absolute and relative maternal liver weight	5	Average blood concentration during exposure period	Increased liver weights are not considered adverse unless accompanied by histopathology.

↑ Absolute and relative pup liver weight	5	Average blood concentration during exposure period	Increased liver weights are not considered adverse unless accompanied by histopathology.
↓ Male offspring body weight	5	Average blood concentration during exposure period	
Delayed eye opening	5	Average blood concentration during exposure period	TERA does not place confidence in this as an adverse effect.
Delayed emergence of body hair	5	Average blood concentration during exposure period	TERA does not place confidence in this as an adverse effect.

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557 **Table 5. DeWitt et al. (2008) Dose-Related Effects Summary After PFOA Drinking Water**
558 **Administration of Female C57BL/6N mice for 15 days at PFOA Doses of 0, 0.94, 1.88, 3.75,**
559 **7.5, 15, and 30 mg/kg/day of PFOA**

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Average blood concentration, Cmax or AUC?	Comments
↓ IgM response to SRBC	3.75	Cmax	Occurred on 1 day post-dose.
↓ Absolute and relative spleen weight	3.75	Cmax	Occurred on 1 day post-dose.
↑SRBC-specific IgG	3.75	Indeterminate	Occurred on 15 days post-dose.
↓ Mean body weight	15	Average blood concentration during exposure period	

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562 Table 6: Cmax values after each dose from Elcombe et al. (2013)

Patient	Daily Dose mg/kg-day*	Cmax after each weekly dose in µM					
	week>	1	2	3	4	5	6
1	0.67	25.72	na	na	na	na	na
2	0.67	29.79	na	na	na	na	na
3	0.67	24.64	na	na	na	na	na
4	0.10	19.95	40.37	40.6	52.28	77.49	81.07
	Avg	25	40	41	52	77	81
5	0.19	23.66	50.82	80.2	87.35	100.84	109.1
6	0.19	32.32	47.47	70.55	97	89.54	179.07
7	0.19	30.91	-	55.78	73.03	-	-
	Avg	29	49	69	86	95	144
8	0.38	114.25	171.02	276.84	368.27	426.16	414.33
9	0.38	93.43	170.29	253.19	362.32	471.59	373.31
10	0.38	58.6	119.44	181.86	276.15	256.06	232.44
	Avg	89	154	237	336	385	340
11	0.57	111.65	178.42	237.26	288.21	326.13	386.77
12	0.57	122.9	182.32	240.93	303.06	372.99	-
13	0.57	85.32	-	-	-	-	-
14	0.57	131.24	179.97	297.35	420.49	478.38	562.63
	Avg	113	180	259	337	393	475
15	0.86	231.36	324.96	463.43	578.86	707.8	800.55
16	0.86	164.05	348.41	545.74	721.48	906.59	-
17	0.86	163.18	276.16	341.96	427.08	497.22	525.98
	Avg	186	317	450	576	704	663
18	1.1	338.52	406.73	590.95	-	-	-
20	1.1	413.39	327.38	474.01	562.88	651.85	770.32
21	1.1	203.29	504.5	652.79	734.36	847.13	995.39
22	1.1	198.74	309.8	433.41	595.95	-	-
23	1.1	236.13	400.07	635.73	-	-	-
24	1.1	282.55	488.31	691.46	858.92	813.92	966.13
25**	1.1	230	360	480	640	750	780
	Avg	272	400	565	678	766	878
26	1.4	200.07	397.76	624.63	625.39	732.46	823.68
27	1.4	240.51	410.69	569.22	719.7	811.16	-
28	1.4	206.86	321.26	472.99	654.6	757.67	853.05
	Avg	216	377	556	667	767	838

29	1.8	352.58	606.03	896.3	896.9	971.71	1043.2
30	1.8	332.61	-	-	-	-	-
31	1.8	347.52	554.28	799.77	998.35	1031.14	-
32	1.8	291.69	516.7	-	-	-	-
40	1.9	189.71	367.81	487.42	554.18	697.26	826.44
41	1.9	232.54	412.52	558.23	748.03	802.5	1209.31
42	1.9	358.73	585.96	764.91	1231.51	1281.13	1251.9
	Avg	301	507	701	886	957	1083
33	2.3	441.43	734.84	925.6	1172.58	1231.36	1317.84
34	2.3	559.64	893.14	1115.82	1440.82	1448.79	-
35	2.3	316.74	592.29	704.4	1172.95	-	-
36	2.3	708.42	679.68	968.95	1143.19	-	1293.03
37	2.3	418.44	841.24	1135.41	1393.91	1530.33	-
38	2.3	314.43	538.47	808.36	787.75	931.5	958.1
	Avg	460	713	943	1185	1285	1190

* Doses given in mg/week. Mg/kg-day doses are determined from average body weight of 75 kg as stated by Convertino et al. (2018), and dividing by 7 days/week, except for patients 1, 2, and 3.
na = not applicable since patients 1, 2, and 3 were only given one dose.
**Cmax value approximated from Figure 84 on Sheet 76 of 85 in Elcombe et al. (2013).

570 Table 7. Average Cmax values after each dose in µM per mg/kg-day.

Daily Dose mg/kg-day	**Average Cmax/Concentration after each weekly dose in µM per mg/kg-day**					
week>	1	2	3	4	5	6
0.1[a]	250	404	406	504	775	801
0.19	152	259	353	452	501	758
0.38	234	404	530	883	1012	895
0.57	198	316	454	577	689	833
0.86	217	368	495	670	818	771
1.1	253	362	520	625	700	828
1.4	154	269	397	476	548	599
1.85[b]	163	263	364	474	517	585
2.3	200	310	407	515	559	517
Overall Average >	202	328	436	575	680	732

571 a) Values for weeks 2 through 6 are for 1 person
572 b) Doses of 1.8 and 1.9 mg/kg-day were combined
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Table 8. Potential DDEFs based on Cmax ratios or steady state concentrations between humans and mice at different times.

Single Dose	**~6 Weeks**	**~25 Weeks***
1.3	8.7	14

574 *Based on apparent "steady state" in nine individuals from Figure 3.
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