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Practical Guidance on the Development of Data Derive Extrapolation Factors (DDEFs) for Developmental Toxicity: A Preliminary Case Study with PFOA

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Research Case Study:

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1. Provide a few sentences summarizing the method illustrated by the case study.

Within the process of non-cancer dose response assessment, such as the development of a Reference Dose (RfD), the use of a data-derived uncertainty factor (DDEF) or a Physiological Based Pharmacokinetic (PBPK) model is an important consideration (IPCS, 2005; EPA, 2014). This factor or model is used in the extrapolation of experimental animal results to humans, rather than the use of 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.

Developmental toxicity is different from many other toxicities of concern from environmental contamination in that it generally develops during a short window of exposure. 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 large.

This case study will demonstrate the use of DDEF for developmental toxicity from a chemical of current interest, specifically PFOA. The results will also be likely applicable to other chemicals where the critical effect is also developmental toxicity.

2. Describe the problem formulation(s) the case study is designed to address. How is the method described in the case useful for addressing the problem formulation?

Guidelines of EPA (1991) and EPA (2014) & 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, whereas the default position for IPCS (2005) and EPA (2014) is to use Area Under the Curve (AUC).

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Specifically, EPA (1991, page 33) states that:

> "Extrapolation of toxicity data between species can be aided considerably by the availability of data on the pharmacokinetics of a particular agent in the species tested and, when available, in humans. Information on absorption, half-life, steady-state and/or peak plasma concentrations [*i.e., Cmax*], placental metabolism and transfer, excretion in breast milk, comparative metabolism, and concentrations of the parent compound and metabolites may be useful in predicting risk for developmental toxicity."

EPA (1991, page 38) goes on to state 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 hr/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."

EPA (1991, pages 45 & 46) is also seen to state:

> "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. Most of the data available for risk assessment involve exposures over several days of development. Thus, human exposure estimates used to calculate margins of exposure (MOE, see following section) or to compare with the RfDDT or RfCDT are usually based on a daily dose that is not adjusted for duration or pattern of exposure. For example, it would be inappropriate in developmental toxicity risk assessments to use time-weighted averages or adjustment of exposure over a different time frame than that actually encountered (such as the adjustment of a 6-hr inhalation exposure to account for a 24-hr exposure scenario), unless pharmacokinetic data were available to indicate an accumulation with continuous exposure. In the case of intermittent exposures, examination of the peak exposure(s) [i.e., Cmax], as well as the average exposure over the time period of exposure, would be important."

In contrast, the International Programme on Chemical Safety (2005, page 39) states its default position for dosimetric choice in the absence of data, specifically that:

- "A reasonable assumption is that effects resulting from subchronic or chronic exposure would normally be related to the AUC, especially for chemicals with long half-lives, whereas acute toxicity can be related to either the AUC or the Cmax. Cmax

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could be more relevant than AUC when a simple bimolecular interaction produces the effect. Examples include acute pharmacological effect as a consequence of receptor binding and inhibition of enzymes, such as the inhibition of cholinesterase by carbamates (JMPR, 2002, 2005), and the reaction can be described by a direct-effect model.

- 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 than in Cmax."

In a somewhat similar vein, EPA (2014, pages 22 and 23) state that:

> "Dose metric is a measure of the internal dose of a chemical agent. 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. The choice of the dose metric is an important component in TK extrapolations. This choice depends on whether toxicity is best ascribed to a transient tissue exposure or a cumulative dose to the target tissue. For a given chemical, the appropriate dose metric will also be determined by, and can vary with, the MOA, duration of exposure, and the adverse effect of concern (U.S. EPA, 2006). Selection of an appropriate dose metric based on specific endpoints involves several elements 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.

3. Comment on whether the method is general enough to be used directly, or if it can be extrapolated, for application to other chemicals and/or problem formulations. Please explain why or why not.

The CSAF/DDEF method is general enough to be used with multiple 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.

4. Discuss the overall strengths and weaknesses of the method.

This method has been discussed internationally for a number of years, arguably starting in 1987 with the publications of Jarabek and colleagues for 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),

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followed by EPA in 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).

5. Outline the minimum data requirements and describe the types of data sets that are needed.

Minimum requirements have been established by both IPCS (2005) and EPA (2014). Some important questions from EPA (2014, page 22) to develop a CSAF or DDEF for TK include:

- 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, 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 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) below.

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

The critical effects for perfluoroctanoate (PFOA) appear to be related more to developmental toxicity as determined by EPA (2016, Table 4-8, page 4-13). Seven studies are highlighted in this table. 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, but critical effect in this study was noted after 1 day. Two of these studies were 13-week studies in rats, but the liver effects at the low doses in these studies do not appear to be adverse according to EPA (2016) page 244 where it states:

> According to Hall et al. (2012), increases in liver weight can be considered adverse when accompanied by cellular necrosis, inflammation, fibrosis of the liver, and/or macrovesicular steatosis. There was some evidence of hepatic necrosis in the studies of Perkins et al. (2004) and in the male F1 generation adult rats from the Butenhoff et al. study (2004a), but the incidences were not statistically significant or described in detail. To the extent that adverse lesions reflect sensitivity in the animals impacted, they are used in the assessment to reflect that the liver hypertrophy and increased liver weight are adverse in individual animals where they are accompanied by necrosis.

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It is not abundantly clear from this description whether EPA considers these liver effects to be adverse or not. However, EPA (2016) goes on to use the fetal effects from the mouse studies, and specifically from the study by Lau et al. (2006), in the development of their safe dose. Thus, fetal effects are being used by EPA (2016) as the critical effects from these four gavage studies of PFOA in mice.

Tables 1 through 5 of this research case study summarize the relevant effects from five of these studies with the intention of judging whether the appropriate dosimeter of each effect is AUC, Cmax, something else, or indeterminate. These judgments will then be used with appropriate kinetic information to contemplate the development of a DDEF.

Table 1. Lau et al. (2006) Effects Summary After Gavage Dosing of Female CD-1 mice for 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: Cmax or AUC?	Comments
Increased maternal liver weight	1	AUC	Effect is quasi dose related, but without histopathology is not considered adverse by EPA (2016, page 248) and others.
Accelerated male puberty	1	Indeterminate	
Reduced pup body weight	3	Indeterminate	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	Indeterminate	Mortality decreases sharply after birth, despite continued PFOA exposure through breast milk, suggesting an in utero cause.
Tail and limb defects	5	Cmax	Statistically significant, but effects are not dose related, nor does TERA place confidence in this effect.
Increased time to birth	10	Indeterminate	Effect is not dose related & may be from maternal impact, nor does TERA place confidence in this as an adverse effect.

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Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Cmax or AUC?	Comments
Ossification of phalanges	1 or 10	Indeterminate	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	Cmax	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	Indeterminate	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	Cmax	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	Cmax	TERA does not place confidence in this as an adverse effect.
Unossified hyoid	20	Cmax	Effects may be due to maternal impacts. TERA does not place confidence in these as adverse effects.
Live fetuses (# per litter)	20	Indeterminate	
Fetal body weight	20	Indeterminate	

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Table 2. Wolf et al. (2007) Dose-Related Effects Summary After PFOA Gavage Dosing of 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: Cmax or AUC?	Comments
↑ Maternal body weight and body weight gain	3	AUC	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	AUC	Increased liver weights are not considered adverse unless accompanied by histopathology.
↑ Absolute and relative male pup liver weight	3	Indeterminate	Increased liver weights are not considered adverse unless accompanied by histopathology.
↓ Female offspring birth weight	3	Indeterminate	Maternal body weight gain influences offspring birth weight.
↑ Relative female pup liver weight	5	Indeterminate	Increased liver weights are not considered adverse unless accompanied by histopathology.
↑ Dams with implants but no live pups	5	Indeterminate	
Delayed eye opening	5	Indeterminate	TERA does not place confidence in this as an adverse effect.
Delayed emergence of body hair	5	Indeterminate	TERA does not place confidence in this as an adverse effect.

Table 3. Macon et al. (2011) Dose-Related Effects Summary After Gavage Dosing of 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: Cmax or AUC?	Comments
Delayed mammary gland development	0.3	Cmax	Comparison of full and half exposure prototcols indicate that late gestational exposure may be more important.

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

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Cmax or AUC?	Comments
↑ Maternal body weight gain	5	AUC	Weight gains are generally not considered adverse.
↑ Absolute and relative maternal liver weight	5	AUC	Increased liver weights are not considered adverse unless accompanied by histopathology.
↑ Absolute and relative pup liver weight	5	Indeterminate	Increased liver weights are not considered adverse unless accompanied by histopathology.
↓ Male offspring body weight	5	Indeterminate	
Delayed eye opening	5	Indeterminate	TERA does not place confidence in this as an adverse effect.
Delayed emergence of body hair	5	Indeterminate	TERA does not place confidence in this as an adverse effect.

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

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: 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	Indeterminate	

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5.2 Has the toxicologically active chemical moiety been identified?

Yes, PFOA is not generally metabolized, or metabolized to a limited extent in mammals. It is considered to be the active chemical moiety.

5.3 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.

The MOA for PFOA appears to be complex, but based on Elcomb et al. (2013, page 1) likely includes:

> "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. This has been demonstrated in Vanden Heuvel et al. (1996) where it was shown that different nuclear hormone receptors were activated by PFOA and how this compared to natural fatty acid activation of the same receptors. Wolf et al. (2008) showed a dose response of various chain length perfluoroalkyls against PAR alpha (FIG. 3 of Wolf et al. (2008)) in a transiently transfected COS-1 cell model to compare the C4 to C9 chain lengths. It has now been shown that APFO and the CXRl 002 isomer has additional mechanisms of action accounting for some of its anti-tumour effects."

Hepatic and the immune system effects of PFOA 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 reproductive effects such as full litter resorptions appear to be PPAR-alpha independent. There is no mode of action 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 these 3 tumors – hepatic, Leydig cell and pancreatic acinar cell adenomas have been attributed to activation of the xenosensor nuclear receptor peroxisome proliferatoractivated receptor a (PPARa) (Klaunig et al., 2012). According to EPA (2016), PPAR alpha agonism appears to be the mode of action for testicular tumors; 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 mode of action. For the testicular and pancreatic tumors caused by PFOA in rats, the mode of action has not been established.

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Other modes of action 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 or CSAF a reasonable assumption is that effects resulting from subchronic or chronic exposure would normally be related to the AUC, especially for chemicals with long half-lives, whereas acute toxicity can be related to either the AUC or the Cmax. Furthermore, Cmax could be more relevant than AUC when a simple bimolecular interaction produces the effect.

According to these statements, if the critical effects of PFOA are more related to biomolecular interactions, then Cmax might be the more relevant dosimeter. The use of Cmax would also be consistent with several of the developmental effects described in Tables 1-5 that appear to be related to Cmax, regardless of the default dosimeter evoked. However, AUC, or at least changes in Cmax over time should also be considered in any deliberation of DDEF.

5.4 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 taken directly 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 here 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, 3.5 mg/L per mg/kg-day at a dose of 60 mg/kg-day, and ~7 mg/L per mg/kg-day at an untested dose of 20 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 from this figure here as 0.7 mg/L or 5.0 mg/L after a dose of 0.1 mg/kg-day, 5.0 mg/L or 35 mg/L after a dose of 1.0 mg/kg-day, and 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 the PFOA is not expected to be metabolized, or metabolized to any significant extent in mammals, PFOA is considered to be the toxic moiety. Thus, these Cmax and steady state values in mice can be compared with available human information to gauge whether the development of DDEFs or CSAFs are reasonable. Until recently, such data have not been publicly available in humans.

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Figure 1. Single dose PFOA exposure adapted from Lou et al., (2009), Figure 3. Estimated Cmax values are shown below (mkd = mg/kg-day)

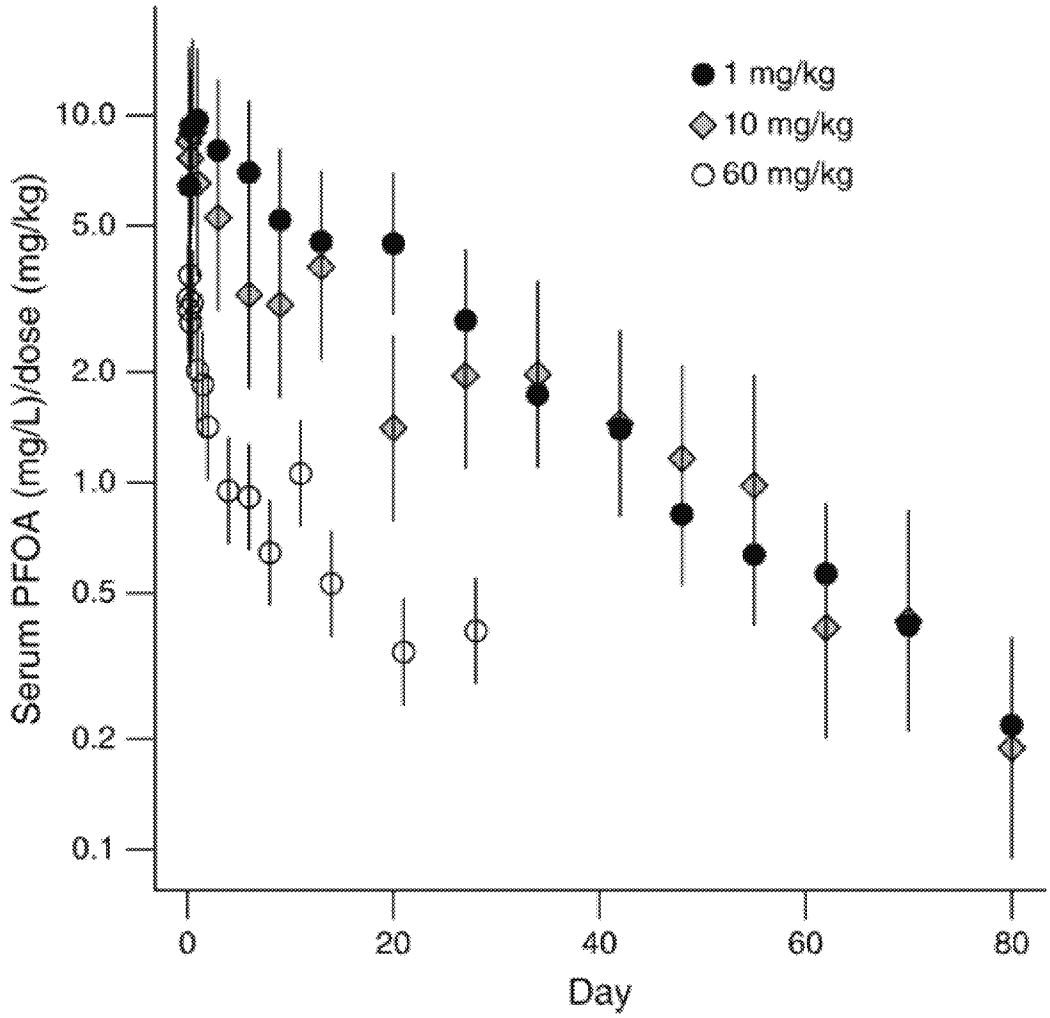

FIG. 3. Serum concentrations scaled by dose for females administered single doses of 1, 10, and 60 mg/kg. Points are means, error bars are 95% confidence intervals for the means. 1 and 10 mg/kg dose groups are largely superimposed and linear in time on this semi-log suggesting linear first-order kinetics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

Cmax at 1 mkd ~10;
Cmax at 10 mkd ~8.5;
Cmax at 60 mkd ~3.5;
Cmax at 20 mkd ~ 7 (estimated)

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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"

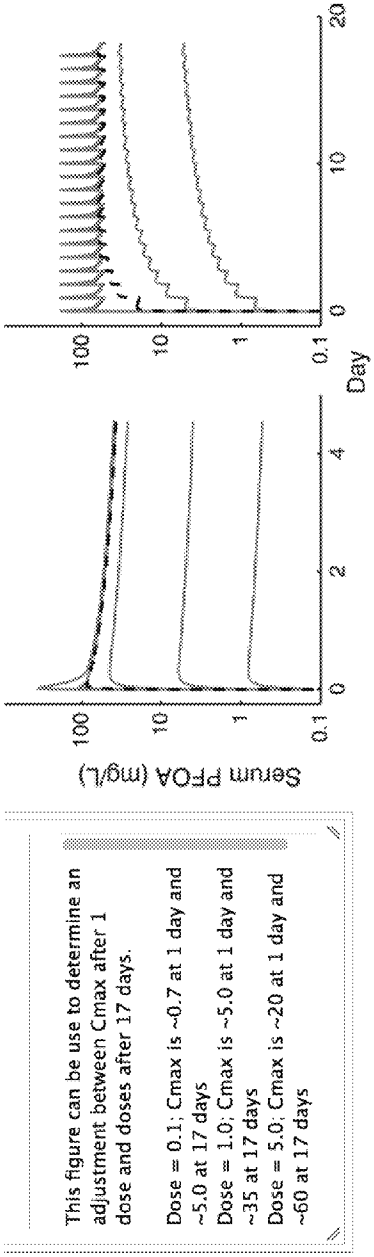

FIG. 7. Delineation of predictions for the PFOA concentration (mg/l) in the central compartment. For the single dose (top) solid lines depict doses of 0.1, 1, 10, 100, and 1000 mg/kg. The dashed line indicates a dose of 40 mg/kg which is roughly where the onset of nonlinearity occurs. For the repeated 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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5.5 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?

Until recently, little specific kinetic data in humans have been available. However, Elcombe et al. (2013) submitted a US Patent Application where PFOA was used as a cancer chemotherapeutic agent. Findings from this study have been recently published in part 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 as a phase 1 therapeutic trial. Doses and blood concentrations were carefully monitored. Summaries of these findings are found in Table 6 that show the individual Cmax values in µM for each patient after his/her weekly dose of PFOA. Estimates of average Cmax values per dose from these data 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 Elcombe et al. (2013) and an average murine Cmax value of 9.0 mg/L per mg/kg-day from Lou et al. (2009, Figure 3). This calculation is shown in the appendix. Other comparisons are possible and could be explored.

However, Cmax values are known to rise in both humans after weekly capsule exposure (Elcombe et al., 2013) and in mice after continued gavage exposure (Lou et al., 2009) due to slower excretion of PFOA when compared with other species, such as rats. So, an additional analysis was conducted here. 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/umole (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 ~8.7). Other comparisons are possible and could be explored.

However, Cmax values have been seen to rise in humans after 6 weeks of continued gavage exposure. Specifically, nine patients in Elcombe et al. (2013, Figure 78) were maintained on gavage dosing beyond six weeks. This is shown here as Figure 3. These patients appeared to reach a steady state in the range of about 25 weeks or less. The average ratio of 6-week Cmax values to these individual patients' apparent "steady state" values is 1.6 (see the appendix for this calculation). Thus, a further possible DDEF value, one based on extended human exposure when compared with the shorter-term mouse exposure of 17 days would be ~14 (i.e., 8.7 x 1.6). 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). (note: dividing the apparent steady state by 5 half-lives approximates 5 weeks as the half-

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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

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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).

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

Daily Dose mg/kg-day	**Average Cmax after each weekly dose in µM per mg/kg-day**					
week>	1	2	3	4	5	6
0.1	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*	163	263	364	474	517	585
2.3	200	310	407	515	559	517
Overall Average >	202	328	436	575	680	732

- Doses of 1.8 and 1.9 mg/kg-day were combined

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life). 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.

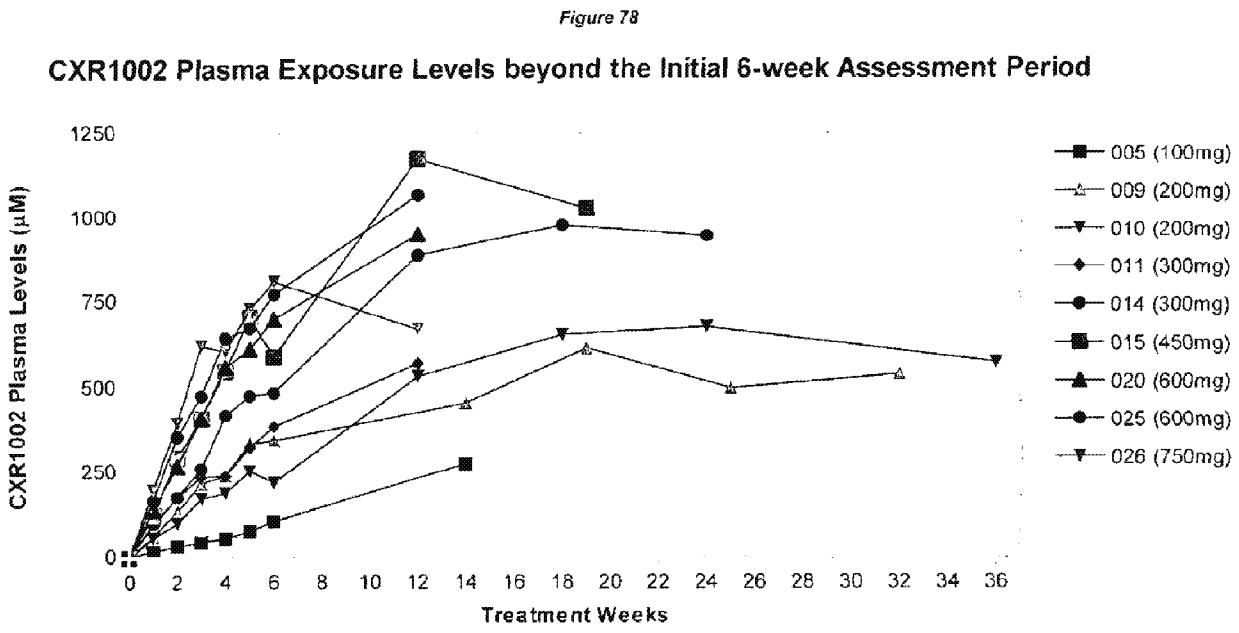
Table 8 shows a comparison of these various DDEFs with the mouse and human Cmax and/or "steady state" data compared.

Table 8. Potential DDEFs based on Cmax ratios between humans and mice at different times.

Single Dose	~6 Weeks	~25 Weeks*
1.3	8.5	14

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

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.



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5.6 **Summary**

- 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). Furthermore, 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, based in part on the toxic moiety, the MOA, and in the case of PFOA, the gavage nature of the exposure. Consideration of Cmax as the appropriate dosimeter is also a requirement for any type toxicity for either IPCS (2005) or EPA (2014) guidelines.

- Identification of the appropriate dosimetric adjustment has been attempted from a review of effects identified by EPA (2016) in Tables 1-5 of this text. Some of these effects appear to be more related to Cmax, some more related to AUC, and the relationships of others are indeterminable.

- The MOA of PFOA is complex and likely relates to many events. Some of these effects may be related to simple biomolecular interactions, especially since the parent chemical is chemically inert and resembles fatty acids naturally occurring in the body. If true, these effects would be more likely associated with the dosimeter Cmax as per IPCS (2005) who state: "Cmax could be more relevant than AUC when a simple bimolecular interaction produces the effect."

- Estimates of Cmax and half-life are possible from the new human study, as described in Table 6 and Figure 3. These estimates should be used with caution, however, since they are from humans in clinical trials for cancer therapy, and kinetics from these studies may not reflect the average population. Then again, this population should be explored as a possible sensitive subgroup. If this population is judged to be sensitive, then an adjustment to any uncertainty factor used for within human variability might be appropriate.

Does your research case study:

A. Describe the dose-response relationship in the dose range relevant to human exposure?

The specific data being compared is daily gavage dose of PFOA in mice that forms the basis of the critical effect by EPA (2016) and others, and once per week PFOA exposure in capsules to humans. The daily doses have been adjusted for humans to match the mouse exposure by dividing by an average body weight of 75 kg (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.

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 in this research case study with the experimental and clinical data may not be

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applicable to these lower exposure levels in humans, but this comparison is consistent with current guidelines of IPCS (2005) and EPA (2014).

B. Address human variability and sensitive populations?

The DDEF/CSAF method explicitly addresses human variability. Health Canada (Meek et al., 1994), IPCS (2005) and EPA (2014) guidelines go into great detail regarding this.

C. Address background exposures or responses?

PFOA is not a naturally occurring chemical, so naturally occurring 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 (over 100-fold differences) among different government authorities.

D. Address incorporation of existing biological understanding of the likely mode of action?

The MOA for PFOA and related chemicals is likely to be complex but at least some understanding of it as a fat-mimic in the body has enabled it to be used as a cancer chemotherapeutic agent.

E. Address other extrapolations, if relevant – insufficient data, including duration extrapolations, interspecies extrapolation?

International authorities approach the extrapolation of a safe dose for PFOA and related chemicals in very different manners. Authorities in the US, for example, tend to focus on experimental animal data and the disparity in half-lives among experimental animals and humans and adjust the safe dose downward by sometimes over 100-fold. Some European authorities focus on human epidemiology studies with an emphasis on longer half-life in humans; other European authorities focus on a more traditional approach and are skeptical of the long half-life estimates of others. Australian authorities are considering several different approaches.

The extrapolation of safe doses for PFOA and related chemicals is highly uncertain, but recent kinetic findings in humans may alleviate some of this uncertainty.

F. Address 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. Health Canada (Meek et al., 1994), IPCS (2005) and EPA (2014) guidelines go into great detail regarding this.

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G. Allow the calculation of risk (probability of response for the endpoint of interest) in the exposed human population?

The DDEF/CSAF method explicitly addresses the calculation of a Reference Dose (RfD), Reference Concentration (RfC), Tolerable Daily Intake (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.

H. Work practically? If the method still requires development, how close is it to practical implementation?

The DDEF/CSAF method has been used and further developed, arguably, since 1987, 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 found at: https://www.tandfonline.com/doi/full/10.1080/10408444.2017.1303818.

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