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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 dose metric 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). This research shows the derivation of a DDEF for developmental toxicity for perfluorooctanoate (PFOA), a chemical of current interest. Here, identification of the appropriate dosimetric adjustment from a review of developmental effects identified by EPA (2016) is attempted. Although some of these effects appear to be related to Cmax, most appear to be related to the average concentration or its AUC, but only during the critical period of development for a particular effect. A comparison was made of kinetic data from PFOA exposure in mice with newly available and carefully monitored kinetic data in humans after up to 36 weeks of PFOA exposure in a phase 1 clinical trial by Elcombe et al. (2013). Using the average concentration during the various exposure windows of concern, the DDEF for PFOA was determined to be 1.3 or 14. These values are significantly different than comparable extrapolations by several other authorities based on differences in PFOA half-life among species. Although current population exposures to PFOA are generally much lower than both the experimental animal data and the clinical human study, the development of these DDEFs is consistent with current guidelines of both EPA (2014) and IPCS (2005).

1. 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 Extrapolation Factors (DDEF) or a Physiologically- 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 appropriate and necessary available data include knowledge of kinetic and dynamic differences between the experimental animal of choice and humans. Otherwise, default assumptions that are based on well-established underlying toxicology principles should be used (e.g., Dourson et al., 1996).

The CSAF/DDEF method has been discussed internationally for a number of years, 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 U.S. Environmental Protection Agency, 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 sufficiently general to be used with different chemistries. IPCS (Bhat et al., 2017) recently polled its membership for general use of this method and for 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 critical developmental period. Although thresholds for toxicity are still thought to exist for adverse developmental effects (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.

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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 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 a critical period for developmental toxicity might also be appropriate, 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 [clearance] 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." 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 another measure, such as average exposure concentration, 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.

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

[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 h/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."

2. Methods

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 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?

Specifically, for PFOA, the Texas Commission on Environmental Quality (TCEQ, 2016), EPA (2016), and the Agency for Toxic Substances and Disease Registry (ATSDR, 2018) have followed these questions generally and used developmental toxicity as the critical effect. All three agencies rely on a PBPK model to estimate an appropriate DDEF-surrogate using area under the curve (AUC) as the dose metric, because the large variability in internal concentrations of PFOA among species was considered an important point to be addressed. Other groups such as Health Canada (2018) and the New Jersey Drinking Water Quality Institute (NJDWQI, 2017) focus on liver toxicity as the critical effect, but have also used a PBPK model to estimate an appropriate DDEF-surrogate using AUC as the dose metric.

This series of questions from EPA (2014) was followed using PFOA as an example, but in contrast to these agencies, we have also obtained and analyzed human clinical data from a patent application by Elcombe et al. (2013). In brief, 43 adult humans, both male and female were given weekly oral tablet of PFOA up to 1200 mg for up to 6 weeks as part of a phase 1 clinical trial for cancer chemotherapy. Concentrations of PFOA over time were closely monitored. Adequate kidney and liver function were criteria for acceptance into the trial. Nine individuals continued to receive PFOA after the 6-week trial. This unique data set, not analyzed by any of the various agencies, allows exploration of whether a DDEF can be estimated directly from comparison of mouse and human kinetic data, rather than using a PBPK model with its additional assumptions.

3. Results

Following the EPA (2014) guidance:

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

The identification of the critical effects for PFOA is disparate amongst different authorities as mentioned above. Specifically, TCEQ (2016), EPA (2016), and ATSDR (2018) identify developmental toxicity, although not the same developmental endpoint. Other groups such as Health Canada (2018) and NJDWQI (2017) identify liver toxicity. Other effects, such as immunotoicity and tumorigenicity are also described. Although the resolution of the appropriate critical effect for PFOA is a very important part in its risk assessment, it is not the point of this paper. Rather, the critical effect is assumed to be developmental toxicity as determined by EPA (2016), and then data are analyzed for

Table 1
Summary of Lau et al. (2006) Effects, EPA (2016) LOAEL, and Possible Dose metric.

Effect(s) (from Lau et al., 2006)	LOAEL (mg/kg/day) (from EPA, 2016)	Possible Dose metric: Cmax, average concentration, AUC? (from this research)	Comments (Opinion by authors of this paper)
Increased maternal liver weight	1	Average blood concentration during exposure period	Effect is somewhat 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 evident at birth 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 were not dose-related and no skeletal malformations were noted at exams.
Increased time to birth	10	Average blood concentration during exposure period	Effect was slight (< ½ day) and not dose-related; no dystocia was noted.
Delayed ossification of phalanges	1 or 10	Average blood concentration during exposure period	Effects are not dose-related and may be secondary to maternal effects; usually resolves post-natally.
Reduced ossification of supraoccipital	10	Average blood concentration during exposure period	Effects are not dose-related and usually resolves shortly after birth.
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.
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 toxicity, and usually resolve shortly after birth.
Unossified hyoid	20	Average blood concentration during exposure period	Effects may be due to maternal itoxicity, and usually resolve shortly after birth.
Decrease in live fetuses (# per litter)	20	Average blood concentration during exposure period	
Decrease in fetal body weight	20	Average blood concentration during exposure period	

aAfter gavage dosing of female CD-1 mice for 17 days (GDs 1–17) at doses of 0, 1, 3, 5, 10, 20, or 40 mg/kg/day of PFOA.

judgment of the appropriate dose metric for developing the DDEF.

While there are numerous studies in a variety of animal species, seven studies are highlighted in EPA's risk assessment (EPA, 2016, see Table 4–8). Four of the seven studies are conducted in mice with gavage dosing during pregnancy showing a variety of fetal and maternal effects [Lau et al., 2006; Wolf et al., 2007 (2 studies); Macon et al., 2011]. One of these studies is a 15-day drinking water exposure in mice, but the critical effect was noted after 1 day (DeWitt et al., 2008). Two of these studies (Perkins et al., 2004; Butenhoff et al., 2004) were ~13-week exposures to 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, specifically from the study by Lau et al. (2006), in the development of its safe dose. Thus, this research was conducted using EPA's judgment that the critical effects are the fetal effects from the gavage study of PFOA in mice by Lau et al. (2006).

Table 1 summarizes effect from EPA-chosen study with the intention of judging whether the appropriate dose metric of each effect is Cmax, average concentration, or AUC. 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 that PFOA is not metabolized, or metabolized to a limited extent in mammals (e.g., EPA, 2016; ATSDR, 2018). Thus, PFOA was considered to be the active chemical moiety in this 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 as described by EPA (2016) and others, all of whom have reviewed relevant studies that showed 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 the peroxisome proliferator-activated receptor "alpha" (PPAR-α) dependent and independent mechanisms (NJDWQI, 2017). Among the several developmental effects associated with PFOA exposure in rodents (e.g., Table 1), only the low birth weight received support from human epidemiological studies (European Food and Safety Authority (EFSA), 2018; EPA, 2016). It has been reported that receptor-activated changes in metabolism, hormonal perturbations, and impeded intercellular communication could play a role in the developmental effects of PFOA exposure (EPA, 2016). According to EFSA (2018), the reduced body weight following PFOA exposure in rodents is associated with loss of white adipose tissue, up-regulation of uncoupling protein-1 (UCP-1) and its association with energy expenditure and regulation of food consumption. Developmental effects of PFOA in rodents appear to occur primarily through a PPAR-α dependent mode of action (NJDWQI, 2017; EPA, 2016). PFOA is reported to activate the PPARα receptor in both rodents and humans, but the response is greater in rodents than in humans (EPA, 2016). PPAR-α agonists are known to decrease serum triglyceride levels in rodents and humans (EFSA, 2018). Once PPAR-α is activated, the agonists increase the activity of lipoprotein lipase, resulting in a decrease in triglyceride levels. Activation of PPAR-α leads

to morphological changes in low-density lipoproteins (LDL), from small, dense morphology to large particles that are more rapidly cleared by the liver (EFSA, 2018). The long-chain fatty acids derived from triglycerides are further degraded in the liver via peroxisomal β-oxidation EFSA (2018). Production of high-density lipoprotein is also increased following PPAR-α activation. Per- and polyfluoroalkyl substances (PFAS) (including PFOA) with documented PPAR-α trans-activation may act in a similar way (EFSA, 2018). PFOA has been documented to bind with and activate PPAR-α and developmental exposures to PFOA is known to induce alterations in cholesterol biosynthesis and fatty acid metabolism (Quist et al., 2015). This action of PFOA may be responsible for some of the delays in development. Delayed eye opening, regarded as a sensitive endpoint for PFOA toxicity in mice by EPA (EPA, 2016), and deficits in postnatal weight gain were reported to depend on PPAR-α expression, although other mechanisms may contribute (EFSA, 2008; Abbott et al., 2007). However, other developmental effects such as full litter resorptions or pregnancy loss appear to be independent of PPAR-α expression. There is no MOA evidence for the delayed mammary gland development, another sensitive endpoint for PFOA exposure in mice (EPA, 2016), and because of this NJDWQ (2017)suggests that the effects of PFOA on this endpoint are not relevant to humans. However, NJDWQI (2017) uses a database uncertainty factor, in part, to account for the sensitivity of this endpoint. EFSA (2018) and EPA (2016) have also stated that low glomerular filtration rate (GFR) lowers birth weight in humans. According to EPA (2016), the association reported between PFOA and low birth weight in humans could be attributable to a combination of low GFR and serum PFOA.

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 PPAR-α (Klaunig et al., 2012). According to EPA (2016), PPAR-α 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, NJDWQI (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) (NJDWQI, 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 up-regulates testosterone production in Leydig cells) (NJDWQ, 2017).

Developmental toxicity as the critical effect is the focus of this research for the purpose of developing a DDEF. A reasonable assumption, in fact the default assumption by some agencies, is that these effects are more likely related to Cmax, especially if the critical effects are more related to biomolecular interactions as per IPCS (2005). Indeed, several effects found in Table 1 were judged to be due to Cmax. However, other effects of concern for PFOA, including other developmental effects, may be due to sustained activation of the PPAR-α receptor, and thus might be more associated with average concentration throughout the critical period of development for a particular endpoint, as also described in Table 1. In fact, Cmax, average concentration, and AUC, as well as other possible dose metrics 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 characterized in humans. For example, as discussed more extensively by EPA (2016), PFOA is readily absorbed in humans and animals via all routes of exposure. It is present in most biological fluids (gastric secretions excluded) primarily as the perfluorooctanoate anion. Three transport families, organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs), are reported to play a role in PFOA absorption, distribution, and excretion. These transporters are critical for absorption in the gastrointestinal tract, as well as uptake by the tissues, and excretion via bile and the kidney. The transport systems are located at the membrane surfaces of the several organs and tissues including the intestines, liver, lungs, heart, blood brain barrier, blood placental barrier, blood testes barrier, and mammary glands. The transport proteins function in the uptake of organic anions from gastrointestinal contents and transport of those anions into the portal blood supply, as well as to protect the organs, tissues, and fetus from foreign compounds.

EPA (2016) further state that in both humans and animals, PFOA is distributed throughout the body by noncovalent binding to plasma proteins. Distribution of absorbed PFOA requires vascular transport from the portal of entry to receiving tissues. PFOA accumulates much more in the liver (greater in males and females) than other tissues such as kidneys, lungs, heart, muscles, testes in males and uterus in females. Autopsy examinations revealed that PFOA is accumulated primarily in the bone, lung, liver, and kidney, with levels below detection in brain. PFOA is not metabolized, indicating that the parent compound, not metabolites, is responsible for any effects observed in toxicological studies. Studies in humans indicate that human serum albumin carried the largest portion of the PFOA among the protein components of human plasma. PFOA also shows some affinity for LDLs and limited binding to alpha-globulins and gamma-globulins, alpha-2-macroglobulin and transferrin. Species and gender differences have been reported in the elimination of PFOA, with many of the studies focusing on the role of transporters in the kidney tubules. PFOA is not readily eliminated from humans and other primates. Elimination half-lives differ among the species. Elimination half-lives of 2.3–3.8 years have been reported in the general population and occupationally exposed workers. In animals, half-lives of 21 days (female monkeys), 30 days (male monkeys), 11.5 days (male rats), 3.4 h (female rats), 27.1 days (male mice) and 15.6 days (female mice) have been reported, indicating gender difference between male and female rats but not seen in mice.

Although the reasons for the species and/or gender differences in the half-life is not known, it could be attributed to the differences in renal transport by OATs (Post et al., 2012). OATs transporters, located on both the basolateral (serum interface) and apical surfaces of the brush boarder of the proximal tubule inner surface, are important in the excretion of PFOA. PFOA binding to surfaces of serum proteins (particularly albumin) makes much of it unavailable for removal during glomerular filtration. OATs can function for uptake into the cell across both the basolateral and apical surfaces. Available studies of transporters suggested that female rats are efficient in transporting PFOA across the basolateral and apical membranes of the proximal kidney tubules into the glomerular filtrate, but male rats are not. On the contrary, male rats have a higher rate of resorption than females for the smaller amount they can transport into the glomerular filtrate via a transporter (OATP1a1) in the apical membrane. It has been suggested that this gender difference might be responsible for the inverse relationship observed between the levels of PFOA in female urine and plasma and the plateau of plasma PFOA in male rats compared to their losses via urine (EPA, 2016). It appears that the high expression of OAT involved in urinary elimination is specific to the rat, and neither the mouse nor the human exhibit similar sex-specific differences (Lau et al., 2007). It is not known whether the gender differences between male and female rats is relevant to humans. However, the long half-life of PFOA observed in humans suggests that humans might be more like the male rat than the female rat (EPA, 2016).

Table 2
Estimated Cmax values from Lou et al. (2009, Figure 3) where serum levels are shown from single gavage dose in mice following PFOA exposure.

Dose (mg/kg-day)	Cmax (mg/L per mg/kg)
1	10
10	8.5
60	3.5

Table 3
Estimated Cmax or steady state concentrations in serum of mice after repeat dose gavage of PFOA, from Lou et al. (2009, Figure 7b).

Dose (mg/kg-day)	Cmax or Steady State (mg/L)		
	Day 1	Day 6	Day 17
0.1	0.7	2.0	5.0
1.0	5.0	22	35
5.0	20	60	60

As to the critical effect and choice of species for potential extrapolation to humans, Table 2 is adapted from Lou et al. (2009, Figure 3) and shows the kinetic behavior in serum after a single gavage administration in mice. Cmax values varied with the dose administered by Lou et al. (2009), and were estimated 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.

Table 3, adapted from Lou et al. (2009, Figure 7b), shows the kinetic behavior in serum of mice exposed to PFOA after multiple gavage doses. The 1-day Cmax, 6-day interim, and 17 day steady state values, respectively, were estimated from this Lou et al. (2009) Figure as either 0.7, 3.0 and 5.0 mg/L, after a dose of 0.1 mg/kg-day; as either 5.0, 22 and 35 mg/L after a dose of 1.0 mg/kg-day; and as ether 5.0, 60, and 60 mg/L after a dose of 5.0 mg/kg-day. These apparent steady state values at 17 days imply a half-life in mice of several days.

PFOA is not metabolized, or metabolized to any significant extent in mammals. Thus, PFOA is considered to be the toxic moiety, and the Cmax and steady state values in mice (from Lou et al., 2009) can be compared with available human information to gauge whether derivation of a DDEF is reasonable. Until recently, kinetic data have not been publicly available in humans with which to do this comparison.

- *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?*

To date, few specific kinetic data in humans have 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 other species. 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 in capsules up to 1200 mg once per week for 6 weeks to 43 humans of both sexes in various stages of different cancers in a phase 1 therapeutic trial. Doses and plasma concentrations of PFOA were carefully monitored. Patients with kidney and liver complications were excluded. Summaries of individual weekly Cmax values over time in μM are found in Table 4 for each patient after weekly dose of PFOA. Estimates of average Cmax values over time per dose, rather than in μM, are found in Table 5.

A DDEF could be developed from a comparison of mouse and human data Cmax values after one dose. This DDEF would be 1.3[2] based on an average single dose human Cmax value of 12 mg/L per mg/kg-day from Elcombe et al. (2013), divided by the average murine Cmax value of 9 mg/L per mg/kg-day from Lou et al. (2009).[3] This

Table 4
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).

[2] All DDEFs derived here are given a precision of 2 digits because of uncertainty in the estimated values underlying their development. A precision of 1 digit for these DDEFs might also be appropriate.

[3] Cmax's at doses 1 and 10 mg/kg-day in mice are averaged to roughly match for the full range of estimated human dosing found in Elcombe et al. (2013) of 0.67–1 mg/kg-day.

Table 5
Average Cmax concentrations after each dose in μM per mg/kg-day for six weeks (calculated from Table 4).

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

[a] Values for weeks 2 through 6 are for 1 person.
[b] Doses of 1.8 and 1.9 mg/kg-day were averaged.

calculation is shown in the appendix, Table A.

For critical effects that are Cmax-dependent after only one dose, the DDEF of 1.3 might be an appropriate choice. However, Cmax values are shown to rise in humans after further weekly capsule exposure (Elcombe et al., 2013) and in mice after continued gavage exposure (Lou et al., 2009). Since human exposures to PFOA seldom occur only once, additional analysis is warranted. Specifically, the average human Cmax value after the first 6 weekly doses from Table 5 of 732 μM per mg/kg-day (303 mg/L)[4] was compared with the intermediate value in mice of 22 mg/L after 6 daily doses of 1.0 mg/kg-day shown here in Table 3. A DDEF value based on this ratio is 14 (303 mg/L ÷ 22 mg/L = 14). This comparison seems reasonable because this is where the bulk of the human data lie; a comparison with an intermediate value in mice seems reasonable, because humans were still not at steady state. Other comparisons are possible and could be explored.

In humans, Cmax values have been reported to rise after 6 weeks of continued weekly capsule exposure to also approximate a steady state. Specifically, nine patients in Elcombe et al. (2013, Figure 78) were maintained on capsule dosing beyond six weeks. These patients appeared to reach a steady state at an average value of 1.6-fold higher than their individual 6-week averages, in the range of 12–36 weeks. Appendix Tables B and C show this calculation. Thus, a further possible DDEF value is possible. This one is based on extended human exposure and apparent steady state values at ~480 mg/L (303 mg/L x 1.6 = 480 mg/L) compared with the shorter-term mouse exposure of 17 days, but also steady state value of 35 mg/L from Table 3. This value is also ~14.

Assuming the kinetics in non-pregnant mice are similar to those of pregnant mice, the length of time to reach steady state in mice of 17 days (based on Lou et al., 2009) could be attained during gestation (which in mice is 18 days). Thus, if humans, and specifically pregnant women, are already in steady state, and if the critical effect is one or more developmental toxicities, then a DDEF of 14 could 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 steady state concentrations being compared would apply to any critical period of development. As before, other comparisons are possible and, in this case, should be explored. It is important to note that if a specific type of developmental toxicity is singled-out as the critical effect from Table 1, and, further, if mice and humans are assumed to be in steady state during the appropriate developmental window of this specific effect, then the DDEF would be 14.

Table 6 shows a comparison of these various DDEFs with the mouse and human Cmax and/or steady state or average concentration data compared. Table 7 shows how these DDEFs would affect a health guideline value when compared with default uncertainty factor values.

[4] Average Cmax in humans of 732 μM per mg/kg-day x 414 μg/μmol (the molecular weight of PFOA), divided by 1000 to convert to mg equals 303 mg/L.

4. Discussion

The identification of the critical effects for PFOA is disparate with some groups choosing developmental toxicity (e.g., TCEQ, 2016; EPA, 2016; and ATSDR, 2018) and others choosing liver toxicity (e.g., Health Canada, 2018; NJDWQI, 2017). Still others considers an increase in blood lipids as critical (EFSA, 2018), although this has recently been challenged by Convertino et al. (2018) where blood lipids are seen to decrease with weekly PFOA dosing in the clinical trial of Elcombe et al. (2013). Resolution of the critical effect for PFOA will be an important part of any assessment of this and related chemicals.

In this analysis, it was assumed that the critical effect is developmental toxicity as determined by EPA (2016) and then we analyzed this data set in mice, consistent with EPA (1991) where it 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, perhaps for some effects, including some developmental effects, the MOA for PFOA may be mediated by sustained binding of PFOA with PPAR-α, resulting in continuous disruption of fatty acid metabolism leading to delays in development. The latter mechanism and developmental delay might be more likely associated with average concentration over a critical period of development.

Therefore, the appropriate dosimetric adjustment from a review of effects identified by EPA (2016) was attempted in Table 1 of this text. Some of these effects appear to be related to Cmax, few if any related to AUC, but many of the effects could possibly be attributable to the average exposure concentration during the critical period of development due to the sustained binding of PFOA with PPAR-α. This latter suggestion was made at a review of this research during a recent meeting of the Alliance for Risk Assessment (ARA, 2019).

The kinetic data were then compared 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. The daily doses in humans were adjusted in an effort to approximate the mouse exposure by dividing by an average human 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 using this study might be the observation of effects over several generations. A disadvantage of using White et al. (2011) is that its kinetic information is not as detailed as that found in Lou et al. (2009), making a comparison with the results of the human clinical study more challenging. Another way to utilize these human clinical data is to incorporate them into the existing PBPK models for PFOA by either Loccisano et al. (2011), where information from monkeys is used as a surrogate for missing human information, or by Loccisano et al. (2013), where pregnancy is the key concern as it is in this study, or by Wambaugh et al. (2013), where multiple toxicity and kinetic studies are integrated in a Bayesian PBPK framework to estimate appropriate dose metrics. Roberts et al. (2016) and Pizzurro et al. (2019) also conducted reviews of several of these models and underlying kinetic data that would also benefit from incorporation of these newly available human data.

Although the choice of a specific developmental effect should dictate the appropriate DDEF of either 1.3, 14 or 14 found in Table 6 of this text, a conservative approach would be to assume that at least one or more of the potential critical developmental effects as shown by Lau et al. (2006) and in Table 1 are due to the average concentration during the relevant window of

Table 6
Potential DDEFs based on Cmax ratios or steady state concentration ratios between humans and mice after different exposure durations.

Single Dose Cmax	~6 Week Cmax	12–36 Weeks ~ Steady State*
1.3	14	14

*Based on apparent "steady state" in nine individuals from Elcombe et al. (2013, Figure 78).

Table 7
Impact of derived DDEFs from Table 6 on potential health guidance values.

Animal to Human Factor	Within Human Factor	Composite Factor	Impact on DDEF on Guideline Value
10 (default)	10 (default)	100 (default)	–
3.1 (default)*x **1.3**	10 (default)	40	2.5 fold higher
3.1 (default)*x **14**	10 (default)	430	4.3 fold lower

* Representing the default toxicodynamic part of the experimental animal to human uncertainty (safety/extrapolation) factor in EPA (2014). This value would be 2.5 under in IPCS (2005).

susceptibility for that endpoint. For humans, a conservative assumption would be that one or more of the concordant adverse developmental effects would occur at an average concentration during a comparable period of susceptibility. This conservative choice of DDEF is 14. Furthermore, if mice and humans are assumed to be in steady state during the period of susceptibility for any of the developmental endpoint(s) of concern, which were demonstrated in mice by Lou et al. (2009, Figure 7b) and suggested in humans after presumed continuous exposure (as demonstrated by Elcombe et al. (2014, Figure 78), then the DDEF would still be 14.

Population exposures to PFOA are generally much lower than both the experimental animal data and the clinical human study. Thus, the kinetic comparison and development of the various DDEFs developed here may not be applicable to lower exposure levels in humans. However, and importantly, the development of these DDEFs is consistent with current guidelines[5] of IPCS (2005) and EPA (2014), and the use of any of these values would lead to a different point of departure for the development of the PFOA safe dose by several federal and state authorities.

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 750-fold differences), with several safe doses being 100-fold lower (i.e., more toxic) than other known very toxic substances such as methyl mercury (ITER, 2019). 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 (e.g., EPA, 2016; NJDWQI, 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).

The recent kinetic findings in humans by Elcombe et al. (2013) may alleviate some of this uncertainty in the estimation of a safe dose since they can be compared to experimental data from animal studies, such as conducted here with mice, or incorporated into one or more of the various PBPK models in the future. Limitations may exist in this comparison, however, as the kinetic data in this research are from non-pregnant mice and humans, and in the case of humans, from individuals of both sexes of different ages with advanced disease. Furthermore, PFOA measurements in humans are in plasma and in mice are in serum. However, this human population might be considered a sensitive subpopulation, and if so, a corresponding change in one or more of the usual uncertainty factors might be appropriate.

Estimates of half-life may also be possible from Elcombe et al. (2013, Figure 78), but these estimates appear to be much shorter than literature estimates inferred from chronic exposures of workers and other populations as described by EPA (2016) and others. The variability in estimates might be due to a biphasic elimination evident in the clinical trial where ~5–20 μM appears to be the inflection point in humans (e.g., see Figure 10 of Elcombe et al., 2013), and in mice (Lou et al., 2009) based on potential saturation of resorption of PFOA in the kidney at high doses. Such saturation might not be expected in the general population exposed to much lower doses. Or, this difference might be because the clinical trials are for cancer therapy, and kinetics in humans from these situations may not reflect the average population as mentioned above. Regardless, exploration of these clinical data should provide additional insight to half-life estimates in humans, especially since one or more of the PBPK models already incorporate a biphasic approach (Wambaugh et al., 2013).

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 as described by Health Canada (Meek et al., 1994), IPCS (2005) and EPA (2014).

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-1990s. 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. We use this method here to explore the appropriate dosimetric adjustment when developmental toxicity is the critical effect. We find that in addition to Cmax and AUC, a comparison of the average concentrations during the periods of susceptibility for developmental endpoints is also important.

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Declaration on interest statement

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yrtph.2019.104446.

[5] Either guideline suggests using the kinetics of the experimental animal in the range of the NOAEL/BMD/LOAEL and for humans the lowest available exposure where sufficient data are available. A dose of 1.0 mg/kg-day was chosen in mice from Lau et al. (2006), which is found to be the LOAEL in Table 1 for several (although not all) developmental effects. For humans, because the kinetics for the various doses in Elcombe et al. (2013) appear similar, an average kinetic value from Table 5 is used for the comparison, which also is associated with an average dose of about 1 mg/kg-day. Using a specific lower or higher human dose would change the DDEF of 14 only slightly in either direction (e.g., use of a dose of 0.1 from Table 5 would yield a DDEF of 15).

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