



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

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9

Abstract

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11 Guidelines of the United States Environmental Protection Agency (EPA, 1991) and the
12 International Programme on Chemical Safety (IPCS, 2005) suggest two different default
13 positions for dosimetric extrapolation from experimental animals to humans when the
14 dosimetry of the critical effect is not known. The default position of EPA (1991) for
15 developmental toxicity is to use peak concentration (or Cmax) for this dosimetric
16 extrapolation. In contrast, IPCS (2005, page 39) states its default position for dosimetric
17 choice in the absence of data is to use the area under the curve (or AUC). The choice of
18 the appropriate dose metric is important in the development of either a Chemical Specific
19 Adjustment Factor (CSAF) of IPCS (2005) or a Data Derived Extrapolation Factor
20 (DDEF) of EPA (2014). This research shows the derivation of a DDEF for
21 developmental toxicity for perfluorooctanoate (PFOA), a chemical of current interest.
22 Here, identification of the appropriate dosimetric adjustment from a review of
23 developmental effects identified by EPA (2016) is attempted. Although some of these

24 effects appear to be related to Cmax, most appear to be related to the average
25 concentration or its AUC, but only during the critical period of development for a
26 particular effect. A comparison was made of kinetic data from PFOA exposure in mice
27 with newly available and carefully monitored kinetic data in humans after up to 36 weeks
28 of PFOA exposure in a phase 1 clinical trial by Elcombe et al. (2013). Using the average
29 concentration during the various exposure windows of concern, the DDEF for PFOA was
30 determined to be 1.3 or 14. These values are significantly different than comparable
31 extrapolations by several other authorities based on differences in PFOA half-life among
32 species. Although current population exposures to PFOA are generally much lower than
33 both the experimental animal data and the clinical human study, the development of these
34 DDEFs is consistent with current guidelines of both EPA (2014) and IPCS (2005).

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Introduction

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40 Within the process of non-cancer dose response assessment, such as the development of a
41 Tolerable Daily Intake (TDI) or Reference Dose (RfD), the use of a Chemical Specific
42 Adjustment Factors (CSAF), Data-derived Extrapolation Factors (DDEF) or a Physiologically-
43 Based Pharmacokinetic (PBPK) model is an important consideration (IPCS, 2005; EPA, 2014).
44 These factors or models are used in the extrapolation of experimental animal results to humans,
45 rather than a default uncertainty factor of 10-fold, when appropriate data are available. The
46 appropriate and necessary available data include knowledge of kinetic and dynamic differences

47 between the experimental animal of choice and humans. Otherwise, default assumptions that are
48 based on well-established underlying toxicology principles should be used (e.g., Dourson et al.,
49 1996).

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51 The CSAF/DDEF method has been discussed internationally for a number of years, starting in
52 the late 1980s with the dosimetric adjustments of inhaled dose for determining Reference
53 Concentrations (RfCs) (Jarabek, 1994). More formal discussions were held by the IPCS (1994)
54 based on the work of Renwick (1993). Health Canada was the first authority to use CSAF in its
55 deliberative process (Meek et al., 1994), followed by EPA (2004) with its Integrated Risk
56 Information System (IRIS) assessment for the chemical boron. IPCS published its final
57 guidelines in 2005, followed by EPA in 2014. Multiple scientific publications have occurred
58 throughout this process (e.g., Dourson et al., 1998; Zhao et al., 1999; Meek et al., 2001). The
59 CSAF/DDEF method is sufficiently general to be used with different chemistries. IPCS (Bhat et
60 al., 2017) recently polled its membership for general use of this method and for lessons learned.
61 The results have been generally favorable.

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63 Developmental toxicity is different from many other toxicities of concern from environmental
64 contamination in that it generally develops during a critical developmental period. Although
65 thresholds for toxicity are still thought to exist for adverse developmental effects (Piersma et al.,
66 2011), such exposure suggests a particular approach to the development of DDEFs, for example,
67 the use of peak serum concentration of the chemical of interest (now referred to as C_{max}) versus
68 its associated half-life (or area under the curve---AUC) (EPA, 1991). The resulting differences

69 in extrapolation from experimental animals to humans for developmental toxicity based on the
70 choice of Cmax or AUC may be significant.

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72 Guidelines of EPA (1991) and IPCS (2005) suggest two different default positions for dosimetric
73 extrapolation from experimental animals to humans when the dosimetry of the critical effect is
74 not known. The default position of EPA (1991) for developmental toxicity is to use peak
75 concentration (or Cmax) for this dosimetric extrapolation. Specifically, EPA (1991) states
76 “Therefore, it is assumed that, in most cases, a single exposure at any of several developmental
77 stages may be sufficient to produce an adverse developmental effect.”¹ EPA goes on to state
78 that it would be inappropriate to use time-weighted averages or adjustment of exposure over a
79 different time frame than that actually encountered in developmental toxicity studies, unless data
80 indicated that the critical effect resulted from an accumulation with continuous exposure.
81 However, for continuous human exposure, a time-weighted average exposure during a critical
82 period for developmental toxicity might also be appropriate, as described in a recent meeting
83 (*ARA*, 2019).

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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.”

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

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95 EPA (2014) confirms that the choice of a dose metric associated with the health outcome of
96 interest is most useful when it “describes target tissue exposure in terms of the toxic chemical
97 moiety (parent or metabolite) and is expressed in appropriate time-normalized terms.”

98 Moreover, the appropriate dose metric can vary with the mode of action (MOA), duration of
99 exposure, and the adverse effect of concern (EPA, 2006). Selection of an appropriate dose
100 metric, whether it be C_{max}, AUC, or another measure, such as average exposure concentration,
101 is based on specific endpoints, including:

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

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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.

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 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)?

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

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135 Specifically, for PFOA, the Texas Commission on Environmental Quality (TCEQ, 2014), EPA
136 (2016), and the Agency for Toxic Substances and Disease Registry (ATSDR, 2018) have
137 followed these questions generally and used developmental toxicity as the critical effect. All
138 three agencies rely on a PBPK model to estimate an appropriate DDEF-surrogate using area
139 under the curve (AUC) as the dose metric, because the large variability in internal concentrations
140 of PFOA among species was considered an important point to be addressed. Other groups such
141 as Health Canada (2018) and the New Jersey Drinking Water Quality Institute (NJDWQI, 2017)
142 focus on liver toxicity as the critical effect, but have also used a PBPK model to estimate an
143 appropriate DDEF-surrogate using AUC as the dose metric.

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145 This series of questions from EPA (2014) was followed using PFOA as an example, but in
146 contrast to these agencies, we have also obtained and analyzed human clinical data from a patent
147 application by Elcombe et al. (2013). In brief, 43 adult humans, both male and female were
148 given weekly oral tablet of PFOA up to 1200 mg for up to 6 weeks as part of a phase 1 clinical
149 trial for stage 4 cancer chemotherapy. Concentrations of PFOA were closely monitored.
150 Adequate kidney function was a criterion for acceptance into the trial. Nine individuals
151 continued to receive PFOA after the 6-week trial. This unique data set, not analyzed by any of
152 the various agencies, allows exploration of whether a DDEF can be estimated directly from

153 comparison of mouse and human kinetic data, rather than using a PBPK model with its
154 additional assumptions.

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157 **Results**

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159 Following the EPA (2014) guidance:

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

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163 The identification of the critical effects for PFOA is disparate amongst different authorities as
164 mentioned above. Specifically, TCEQ (2014), EPA (2016), and ATSDR (2018) identify
165 developmental toxicity, although not the same developmental endpoint. Other groups such as
166 Health Canada (2018) and NJDWQI (2017) identify liver toxicity. Other effects, such as
167 immunotoxicity and tumorigenicity are also described. Although the resolution of the appropriate
168 critical effect for PFOA is a very important part in its risk assessment, it is not the point of this
169 paper. Rather, the critical effect is assumed to be developmental toxicity as determined by EPA
170 (2016), and then data are analyzed for judgment of the appropriate dose metric for developing
171 the DDEF.

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173 While there are numerous studies in a variety of animal species, seven studies are highlighted in
174 EPA's risk assessment (EPA, 2016, see Table 4-8). Four of the seven studies are conducted in
175 mice with gavage dosing during pregnancy showing a variety of fetal and maternal effects [Lau

176 et al., 2006; Wolf et al., 2007 (2 studies); Macon et al., 2011]. One of these studies is a 15-day
177 drinking water exposure in mice, but the critical effect was noted after 1 day (DeWitt et al.,
178 2008). Two of these studies (Perkins et al., 2004; Butenhoff et al., 2004) were ~13-week
179 exposures to PFOA in rats, but the liver effects at the low doses in these studies may not be
180 adverse according to EPA (2016). Rather, EPA (2016) uses the fetal effects from the mouse
181 studies, specifically from the study by Lau et al. (2006), in the development of its safe dose.
182 Thus, this research was conducted using EPA's judgment that the critical effects are the fetal
183 effects from the gavage study of PFOA in mice by Lau et al. (2006).

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185 Table 1 summarizes effect from EPA-chosen study with the intention of judging whether the
186 appropriate dose metric of each effect is Cmax, average concentration, or AUC. These
187 judgments were then used with appropriate kinetic information to develop a DDEF.

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

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191 It is generally accepted that PFOA is not metabolized, or metabolized to a limited extent in
192 mammals (e.g., EPA, 2016; ATSDR, 2018). Thus, PFOA was considered to be the active
193 chemical moiety in this research.

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

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199 PFOA exposure resulted in a variety of adverse effects, including hepatotoxicity, developmental
200 toxicity, and immunotoxicity as described by EPA (2016) and others, all of whom have reviewed
201 relevant studies that showed PFOA induces tumors in the liver, testis and pancreas in chronic
202 studies in the rat. Each of these effects may be evoked by a different process.

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

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207 “a fatty acid mimetic in that it interacts with fatty acid homeostasis and/or a fatty acid
208 mediated pathway. Both CXRI 002 [*note: this is straight-chain PFOA*] and APFO [*note: this*
209 *is ammonium PFOA*] isomers and also perfluoroalkyls of different chain lengths possess
210 these properties.”

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212 Hepatic and the immune system effects of PFOA may also involve the peroxisome proliferator-
213 activated receptor “alpha” (PPAR- α) dependent and independent mechanisms (NJDWQI, 2017).

214 Among the several developmental effects associated with PFOA exposure in rodents (e.g., Table
215 1), only the low birth weight received support from human epidemiological studies (European

216 Food and Safety Authority (EFSA), 2018; EPA, 2016). It has been reported that receptor-
217 activated changes in metabolism, hormonal perturbations, and impeded intercellular

218 communication could play a role in the developmental effects of PFOA exposure (EPA, 2016).

219 According to EFSA (2018), the reduced body weight following PFOA exposure in rodents is
220 associated with loss of white adipose tissue, up-regulation of uncoupling protein-1 (UCP-1) and
221 its association with energy expenditure and regulation of food consumption. Developmental

222 effects of PFOA in rodents appear to occur primarily through a PPAR- α dependent mode of
223 action (NJDWQI, 2017; EPA, 2016). PFOA is reported to activate the PPAR α receptor in both
224 rodents and humans, but the response is greater in rodents than in humans (EPA, 2016). PPAR- α
225 agonists are known to decrease serum triglyceride levels in rodents and humans (EFSA, 2018).
226 Once PPAR- α is activated, the agonists increase the activity of lipoprotein lipase, resulting in a
227 decrease in triglyceride levels. Activation of PPAR- α leads to morphological changes in low-
228 density lipoproteins (LDL), from small, dense morphology to large particles that are more
229 rapidly cleared by the liver (EFSA, 2018). The long-chain fatty acids derived from triglycerides
230 are further degraded in the liver via peroxisomal β -oxidation EFSA (2018). Production of high-
231 density lipoprotein is also increased following PPAR- α activation. Per- and polyfluoroalkyl
232 substances (PFAS) (including PFOA) with documented PPAR- α trans-activation may act in a
233 similar way (EFSA, 2018). PFOA has been documented to bind with and activate PPAR- α and
234 developmental exposures to PFOA is known to induce alterations in cholesterol biosynthesis and
235 fatty acid metabolism (Quist et al., 2015). This action of PFOA may be responsible for some of
236 the delays in development. Delayed eye opening, regarded as a sensitive endpoint for PFOA
237 toxicity in mice by EPA (EPA, 2016), and deficits in postnatal weight gain were reported to
238 depend on PPAR- α expression, although other mechanisms may contribute (EFSA, 2008; Abbot
239 et al., 2007). However, other developmental effects such as full litter resorptions or pregnancy
240 loss appear to be independent of PPAR- α expression. There is no MOA evidence for the delayed
241 mammary gland development, another sensitive endpoint for PFOA exposure in mice (EPA,
242 2016), and NJDWQ (2017) indicated that this suggests that the effects of PFOA on this endpoint
243 are not relevant to humans. However, NJDWQI (2017) uses a database uncertainty factor, in
244 part, to account for the sensitivity of this endpoint. EFSA (2018) and EPA (2016) have also

245 stated that low glomerular filtration rate (GFR) lowers birth weight in humans. According to
246 EPA (2016), the association reported between PFOA and low birth weight in humans could be
247 attributable to a combination of low GFR and serum PFOA.

248
249 The mode of action for hepatic tumors, Leydig cell tumors, and pancreatic acinar cell adenomas
250 have been attributed to activation of the xenosensor nuclear receptor PPAR- α (Klaunig et al.,
251 2012). According to EPA (2016), PPAR- α agonism appears to be the MOA for testicular tumors
252 and involves inhibition of testosterone biosynthesis and increase in estradiol as a result of
253 increased activity of aromatase, the cellular enzyme responsible for the metabolic conversion of
254 testosterone to estradiol. In their recent review, NJDWQI (2017) notes that available studies
255 suggest that PFOA causes liver tumors through an estrogenic MOA. For the testicular and
256 pancreatic tumors caused by PFOA in rats, the MOA has not been established.

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

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267 Developmental toxicity as the critical effect is the focus of this research for the purpose of
268 developing a DDEF. A reasonable assumption, in fact the default assumption by some agencies,
269 is that these effects are more likely related to Cmax, especially if the critical effects are more
270 related to biomolecular interactions as per IPCS (2005). Indeed, several effects found in Table 1
271 were judged to be due to Cmax. However, other effects of concern for PFOA, including other
272 developmental effects, may be due to sustained activation of the PPAR- α receptor, and thus
273 might be more associated with average concentration throughout the critical period of
274 development for a particular endpoint, as also described in Table 1. In fact, Cmax, average
275 concentration, and AUC, as well as other possible dose metrics should always be considered in
276 any deliberation of CSAF (IPCS, 2005) or DDEF (EPA, 2014).

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

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283 The ADME has been fairly well characterized in the rat and mouse, less so in other experimental
284 species, and until recently, not characterized in humans. For example, as discussed more
285 extensively by EPA (2016), PFOA is readily absorbed in humans and animals via all routes of
286 exposure. It is present in most biological fluids (gastric secretions excluded) primarily as the
287 perfluorooctanoate anion. Three transport families, organic anion transporters (OATs), organic
288 anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs),
289 are reported to play a role in PFOA absorption, distribution, and excretion. These transporters are

290 critical for absorption in the gastrointestinal tract, as well as uptake by the tissues, and excretion
291 via bile and the kidney. The transport systems are located at the membrane surfaces of the
292 several organs and tissues including the intestines, liver, lungs, heart, blood brain barrier, blood
293 placental barrier, blood testes barrier, and mammary glands. The transport proteins function in
294 the uptake of organic anions from gastrointestinal contents and transport of those anions into the
295 portal blood supply, as well as to protect the organs, tissues, and fetus from foreign compounds.

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

313 (male mice) and 15.6 days (female mice) have been reported, indicating gender difference
314 between male and female rats but not seen in mice.

315
316 Although the reasons for the species and/or gender differences in the half-life is not known, it
317 could be attributed to the differences in renal transport by OATs (Post et al., 2012). OATs
318 transporters, located on both the basolateral (serum interface) and apical surfaces of the brush
319 boarder of the proximal tubule inner surface, are important in the excretion of PFOA. PFOA
320 binding to surfaces of serum proteins (particularly albumin) makes much of it unavailable for
321 removal during glomerular filtration. OATs can function for uptake into the cell across both the
322 basolateral and apical surfaces. Available studies of transporters suggested that female rats are
323 efficient in transporting PFOA across the basolateral and apical membranes of the proximal
324 kidney tubules into the glomerular filtrate, but male rats are not. On the contrary, male rats have
325 a higher rate of resorption than females for the smaller amount they can transport into the
326 glomerular filtrate via a transporter (OATP1a1) in the apical membrane. It has been suggested
327 that this gender difference might be responsible for the inverse relationship observed between the
328 levels of PFOA in female urine and plasma and the plateau of plasma PFOA in male rats
329 compared to their losses via urine (EPA, 2016). It appears that the high expression of OAT
330 involved in urinary elimination is specific to the rat, and neither the mouse nor the human exhibit
331 similar sex-specific differences (Lau et al., 2007). It is not known whether the gender
332 differences between male and female rats is relevant to humans. However, the long half-life of
333 PFOA observed in humans suggests that humans might be more like the male rat than the female
334 rat (EPA, 2016).

335

336 As to the critical effect and choice of species for potential extrapolation to humans, Table 2 is
337 adapted from Lou et al. (2009, Figure 3) and shows the kinetic behavior in serum after a single
338 gavage administration in mice. Cmax values varied with the dose administered by Lou et al.
339 (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
340 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.

341
342 Table 3, adapted from Lou et al. (2009, Figure 7b), shows the kinetic behavior in serum of mice
343 exposed to PFOA after multiple gavage doses. The 1-day Cmax, 6-day interim, and 17 day
344 steady state values, respectively, were estimated from this Lou et al. (2009) as either 0.7, 3.0 and
345 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-
346 day; and as ether 5.0, 60, and 60 mg/L after a dose of 5.0 mg/kg-day. These apparent steady
347 state values at 17 days imply a half-life in mice of several days.

348
349 PFOA is not metabolized, or metabolized to any significant extent in mammals. PFOA is
350 considered to be the toxic moiety, and the Cmax and steady state values in mice (from Lou et al.,
351 2009) can be compared with available human information to gauge whether derivation of a
352 DDEF is reasonable. Until recently, kinetic data have not been publicly available in humans
353 with which to do this comparison.

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

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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 complications were excluded. Summaries of individual weekly C_{max} values over time in μM are found in Table 4 for each patient after weekly dose of PFOA. Estimates of average C_{max} 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 C_{max} values after one dose. This DDEF would be 1.3^2 based on an average single dose human C_{max} value of 12 mg/L per mg/kg-day from Elcombe et al. (2013), divided by the average murine C_{max} value of 9 mg/L per mg/kg-day from Lou et al. (2009).³ This calculation is shown in the appendix, Table A.

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 C_{max}'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 to 1 mg/kg-day.

379 For critical effects that are C_{max}-dependent after only one dose, the DDEF of 1.3 might be an
380 appropriate choice. However, C_{max} values are shown to rise in humans after further weekly
381 capsule exposure (Elcombe et al., 2013) and in mice after continued gavage exposure (Lou et al.,
382 2009). Since human exposures to PFOA seldom occur only once, additional analysis is
383 warranted. Specifically, the average human C_{max} value after the first 6 weekly doses from
384 Table 5 of 732 μM per mg/kg-day (303 mg/L)⁴ was compared with the intermediate value in
385 mice of 22 mg/L after 6 daily doses of 1.0 mg/kg-day shown here in Table 3. A DDEF value
386 based on this ratio is 14 (303 mg/L ÷ 22 mg/L =14). This comparison seems reasonable because
387 this is where the bulk of the human data lie; a comparison with an intermediate value in mice
388 seems reasonable, because humans were still not at steady state. Other comparisons are possible
389 and could be explored.

390
391 In humans, C_{max} values have been reported to rise after 6 weeks of continued weekly capsule
392 exposure to also approximate a steady state. Specifically, nine patients in Elcombe et al. (2013,
393 Figure 78) were maintained on capsule dosing beyond six weeks. These patients appeared to
394 reach a steady state at an average value of 1.6-fold higher than their individual 6-week averages,
395 in the range of 12 to 36 weeks. Appendix Tables B and C show this calculation. Thus, a further
396 possible DDEF value is possible. This one is based on extended human exposure and apparent
397 steady state values at ~480 mg/L (303 mg/L x 1.6 ~480 mg/L) compared with the shorter-term
398 mouse exposure of 17 days, but also steady state value of 35 mg/L from Table 3. This value is
399 also ~14.

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⁴ Average C_{max} 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 equals 303 mg/L.

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

412
413 Table 6 shows a comparison of these various DDEFs with the mouse and human Cmax and/or
414 steady state or average concentration data compared. Table 7 shows how these DDEFs would
415 affect a health guideline value when compared with default uncertainty factor values.

416

417

418 **Discussion**

419

420 The identification of the critical effects for PFOA is disparate with some groups choosing
421 developmental toxicity (e.g., TCEQ, 2014; EPA, 2016; and ATSDR, 2018) and others choosing
422 liver toxicity (e.g., Health Canada, 2018 and NJDWQI, 2017). Still others considers an increase
423 in blood lipids as critical (EFSA, 2018), although this has recently been challenged by

424 Convertino et al. (2018) where blood lipids are seen to decrease with weekly PFOA dosing in the
425 clinical trial of Elcombe et al. (2013). Resolution of the critical effect for PFOA will be an
426 important part of any assessment of this and related chemicals.

427
428 In this analysis, it was assumed that the critical effect is developmental toxicity as determined by
429 EPA (2016) and then analyzed this data set in mice, consistent with EPA (1991) where it states
430 “a primary assumption is that a single exposure at a critical time in development may produce an
431 adverse developmental effect.” This suggests that peak concentration (now referred to as C_{max})
432 should be routinely considered in any dosimetric adjustment for developmental toxicity between
433 experimental animals and humans. This suggestion is supported for PFOA, in part, by a possible
434 MOA as a fatty acid mimic resulting in effects due to simple biomolecular interactions (IPCS,
435 2005), and, in the case of these PFOA studies, the gavage nature of the exposure. However,
436 perhaps for some effects, including some developmental effects, the MOA for PFOA may be
437 mediated by sustained binding of PFOA with PPAR- α , resulting in continuous disruption of fatty
438 acid metabolism leading to delays in development. The latter mechanism and developmental
439 delay might be more likely associated with average concentration over a critical period of
440 development.

441
442 Therefore, the appropriate dosimetric adjustment from a review of effects identified by EPA
443 (2016) was attempted in Table 1 of this text. Some of these effects appear to be related to C_{max},
444 few if any related to AUC, but many of the effects could possibly be attributable to the average
445 exposure concentration during the critical period of development due to the sustained binding of

446 PFOA with PPAR- α . This latter suggestion was made at a review of this research during a
447 recent meeting of the Alliance for Risk Assessment (ARA, 2019).

448

449 The kinetic data were then compared between mice and humans, specifically the daily gavage
450 dose of PFOA in mice that forms the basis of the critical effect by EPA (2016), and the once per
451 week PFOA exposure in capsules to humans. The daily doses in humans were adjusted in an
452 effort to approximate the mouse exposure by dividing by an average human body weight of 75
453 kg given by Convertino et al. (2018) and a further division by seven days/week. Other ways to
454 harmonize these data are likely possible and should be explored. For example, an assessment
455 might be attempted from the work of White et al. (2011) who administered PFOA by both
456 gavage and drinking water over 2 generations of mice. One advantage of using this study might
457 be the observation of effects over several generations. A disadvantage of using White et al.
458 (2011) is that its kinetic information is not as detailed as that found in Lou et al. (2009), making
459 a comparison with the results of the human clinical study more challenging. Another way to
460 utilize these human clinical data is to incorporate them into the existing PBPK models for PFOA
461 by either Loccisano et al. (2011), where information from monkeys is used as a surrogate for
462 missing human information, or by Loccisano et al. (2013), where pregnancy is the key concern
463 as it is in this study, or by Wambaugh et al. (2013), where multiple toxicity and kinetic studies
464 are integrated in a Bayesian PBPK framework to estimate appropriate dose metrics. Roberts et
465 al. (2016) and Pizzurro et al. (2019) also conducted reviews of several of these models and
466 underlying kinetic data that would also benefit from incorporation of these newly available
467 human data.

468

469 Although the choice of a specific developmental effect should dictate the appropriate DDEF of
470 either 1.3, 14 or 14 found in Table 6 of this text, a conservative approach would be to assume
471 that at least one or more of the potential critical developmental effects as shown by Lau et al.
472 (2006) and in Table 1 are due to the average concentration during the relevant window of
473 susceptibility for that endpoint. For humans, a conservative assumption would be that one or
474 more of the concordant adverse developmental effects would occur at an average concentration
475 during a comparable period of susceptibility. This conservative choice of DDEF is 14.
476 Furthermore, if mice and humans are assumed to be in steady state during the period of
477 susceptibility for any of the developmental endpoint(s) of concern, which were demonstrated in
478 mice by Lou et al. (2009, Figure 7b) and suggested in humans after presumed continuous
479 exposure (as demonstrated by Elcombe et al. (2014, Figure 78), then the DDEF would still be 14.
480
481
482 Population exposures to PFOA are generally much lower than both the experimental animal data
483 and the clinical human study. Thus, the kinetic comparison and development of the various
484 DDEFs developed here may not be applicable to lower exposure levels in humans. However,
485 and importantly, the development of these DDEFs is consistent with current guidelines⁵ of IPCS

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).

486 (2005) and EPA (2014), and the use of any of these values would lead to a different point of
487 departure for the development of the PFOA safe dose by several federal and state authorities.
488
489 PFOA is not naturally occurring, so natural background exposures are not expected. However,
490 PFOA and related chemicals are very useful and stable, and as a result have contaminated the
491 environment in many places to a very low level. In some places, the contaminant levels
492 approach the range of safe doses, which of themselves are highly disparate among government
493 agencies (over 750-fold differences), with several safe doses being 100-fold lower (i.e., more
494 toxic) than other known very toxic substances such as methyl mercury (*ITER*, 2019). This
495 disparity is because international authorities approach the extrapolation of a safe dose for PFOA
496 and related chemicals in very different manners. For example, authorities in the US tend to focus
497 on experimental animal data and incorporate the differences in half-lives among experimental
498 animals and humans to adjust the safe dose downward (EPA, 2016; NJDWQI, 2017; ATSDR,
499 2018). Some European authorities focus on human epidemiology studies with an emphasis on
500 longer half-life in humans (European Food Safety Authority, 2018); other European authorities
501 focus on a more traditional approach and are skeptical of the long half-life estimates of others
502 (Committee on Toxicology, 2009). Australian and New Zealand authorities are considering
503 several different approaches (Food Standards Australian New Zealand, 2017; Australian
504 Department of Health, 2017), as is Health Canada (2018).

505
506 The recent kinetic findings in humans by Elcombe et al. (2013) may alleviate some of this
507 uncertainty in the estimation of a safe dose since they can be compared to experimental data
508 from animal studies, such as conducted here with mice, or incorporated into one or more of the

509 various PBPK models in the future. Limitations may exist in this comparison, however, as the
510 kinetic data in this research are from nonpregnant mice and humans, and in the case of humans,
511 from individuals of both sexes of different ages with advanced disease. Furthermore, PFOA
512 measurements in humans are in plasma and in mice are in serum. However, this human
513 population might be considered a sensitive subpopulation, and if so, a corresponding change in
514 one or more of the usual uncertainty factors might be appropriate

515
516 Estimates of half-life may also be possible from Elcombe et al. (2013, Figure 78), but these
517 estimates appear to be much shorter than literature estimates inferred from chronic exposures of
518 workers and other populations as described by EPA (2016) and others. The variability in
519 estimates might be due to a biphasic elimination evident in the clinical trial where ~5 to 20 μM
520 appears to be the inflection point in humans (e.g., see Figure 10 of Elcombe et al., 2013), and in
521 mice (Lou et al., 2009) based on potential saturation of resorption of PFOA in the kidney at high
522 doses. Such saturation might not be expected in the general population exposed to much lower
523 doses. Or, this difference might be because the clinical trials are for cancer therapy, and kinetics
524 in humans from these situations may not reflect the average population as mentioned above.
525 Regardless, exploration of these clinical data should provide additional insight to half-life
526 estimates in humans, especially since one or more of the PBPK models already incorporate a
527 biphasic approach (Wambaugh et al., 2013).

528
529 The DDEF/CSAF method explicitly addresses human uncertainty, specifically in the use of data
530 for replacing default uncertainty factors for experimental animals to human extrapolation and
531 from average to sensitive human extrapolation. The DDEF/CSAF method explicitly addresses

532 the calculation of a RfD, RfC, TDI, or similar “safe” dose values. While such values cannot be
533 used to determine risk, or perhaps risk other than zero, they are very useful for identifying ranges
534 of exposures likely to be without the risk of deleterious effects in sensitive subgroups after a
535 lifetime of exposure as described by Health Canada (Meek et al., 1994), IPCS (2005) and EPA
536 (2014).

537

538 The DDEF/CSAF method has been used and further developed under the guidance of several
539 authorities and numerous experts. It has been used internationally since the mid-1990s.

540 Recently, the IPCS (Bhat et al., 2017) has surveyed its membership on the use of this method.

541 Results of this survey are generally positive as found at:

542 <https://www.tandfonline.com/doi/full/10.1080/10408444.2017.1303818>. We use this method

543 here to explore the appropriate dosimetric adjustment when developmental toxicity is the critical
544 effect. We find that in addition to Cmax and AUC, a comparison of the average concentrations
545 during the periods of susceptibility for developmental endpoints is also important.

546

547

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550

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References

555 Abbott, B.D., Wolf, C.J., Schmid, J.E., Das, K.P., Zehr, R.D., Helfant, L., Nakayama, S.,
556 Lindstrom, A.B., Strynar, M.J., Lau, C., 2007. Perfluorooctanoic Acid Induced Developmental
557 Toxicity in the Mouse is Dependent on Expression of Peroxisome Proliferator Activated
558 Receptor-alpha. *Toxicol. Sci.* 98, 571-581.

559 ATSDR (Agency for Toxic Substances and Disease Registry), 2018. Toxicological Profile for
560 Perfluoroalkyls Draft for Public Comment. U.S. Department of Health and Human Services.
561 <https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf>

562 ARA (Alliance for Risk Assessment), 2019. Beyond Science and Decisions: From Problem
563 Formulation to Risk Assessment. Final Report of Workshop X held at the Texas Commission on
564 Environmental Quality, Austin, Texas, February 26 & 27, 2019.
565 https://tera.org/Alliance%20for%20Risk/ARA_Dose-Response.htm

566 Australian Department of Health, 2017. Health based guidance values for PFAS. For use in site
567 investigations in Australia. Australian Government Department of Health, Commonwealth of
568 Australia.
569 [https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C9](https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/fs-Health-Based-Guidance-Values.pdf)
570 [00817CDC/\\$File/fs-Health-Based-Guidance-Values.pdf](https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/fs-Health-Based-Guidance-Values.pdf)

571 Bhat, V.S., Meek, M.E., Valcke, M., English, C., Boobis, A., Brown, R., 2017. Evolution of
572 chemical-specific adjustment factors (CSAF) based on recent international experience;
573 increasing utility and facilitating regulatory acceptance. *Crit. Rev. Toxicol.* 47 (9), 733–753.

574 Butenhoff, J.L., G.L. Kennedy, S.R. Frame, J.C. O’Conner, and R.G. York. 2004a. The
575 reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicology*

576 196:95–116. [As cited in EPA, 2016).
577
578 Committee on Toxicity, 2009. Update Statement on the Tolerable Daily Intake for
579 Perfluorooctanoic acid. Committee on Toxicity of Chemicals in Food, Consumer Products and
580 the Environment. United Kingdom.
581 <https://cot.food.gov.uk/sites/default/files/cot/cotstatementpfoa200902.pdf>
582
583 Convertino, M., Church, T.R., Olsen, G.W., Liu, Y., Doyle, E., Elcombe, C.R., Barnett, A.L.,
584 Samuel, L.M., MacPherson, I.R., Evans, T.R., 2018. Stochastic Pharmacokinetic-
585 Pharmacodynamic Modeling for Assessing the Systemic Health Risk of Perfluorooctanoate
586 (PFOA). *Toxicol. Sci.* 163 (1), 293–306.
587
588 DeWitt, J.C., Copeland, C.B., Strynar, M.J., Luebke, R.W., 2008. Perfluorooctanoic acid-
589 induced immunomodulation in adult C57BL/6J or C57BL/6N female mice. *Environ. Health*
590 *Perspect.* 116 (5), 644–650.
591
592 Dourson, M.L., Felton, S.P., Robinson, D., 1996. Evolution of science-based uncertainty factors
593 in noncancer risk assessment. *Regul. Toxicol. Pharmacol.* 24, 108-120.
594
595 Dourson, M.L., Maier, A., Meek, B., Renwick, A., Ohanian, E., Poirier, K., 1998. Re-evaluation
596 of toxicokinetics for data-derived uncertainty factors. *Biol. Trace Elem. Res.* 66 (1–3), 453–463.
597
598 Elcombe, C.R., Wolf, C.R., Westwood, A.L., 2013. US Patent Application Publication, Pub. No.:
599 US 2013/0029928. Available at:
600 <https://patentimages.storage.googleapis.com/24/ee/73/f58267c7d70dde/WO2011101643A1.pdf>.

598 EFSA (European Food and Safety Authority), 2008. Perfluorooctane sulfonate (PFOS),
599 perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants
600 in the Food chain. EFSA J. 653, 1-131.
601 http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/653.pdf
602
603 EFSA (European Food and Safety Authority), 2018. Risk to human health related to the
604 presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Panel on
605 Contaminants in the Food Chain (CONTAM). EFSA J. 16 (12), 5194.
606 <https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5194>
607
608 EPA (United States Environmental Protection Agency), 1991. Guidelines for Developmental
609 Toxicity Risk Assessment. Fed. Reg. 56 (234), 63798–63826.
610 U.S. Environmental Protection Agency, 2004. IRIS Summary for Boron and Compounds.
611 Washington, DC.
612 https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0410_summary.pdf
613
614 EPA (United States Environmental Protection Agency), 2006. Approaches for the Application of
615 Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk
616 Assessment (Final Report). National Center for Environmental Assessment, Washington, DC;
617 EPA/600/R-05/043F. <https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=157668>
618
619 EPA (United States Environmental Protection Agency), 2014. Guidance for Applying
620 Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and

621 Intraspecies Extrapolation. EPA/100/R-14/002F. September.
622 <https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf>
623
624 EPA (United States Environmental Protection Agency), 2016. Health Effects Support Document
625 for Perfluorooctanoic Acid (PFOA). Office of Water (4304T) Health and Ecological Criteria
626 Division Washington, DC 20460. EPA 822-R-16-003. May 2016.
627 https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final-plain.pdf
628
629 Food Standards Australian New Zealand, 2017. Hazard assessment report-perfluorooctane
630 sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS).
631 [http://www.health.gov.au/internet/main/publishing.nsf/content/2200FE086D480353CA2580C90](http://www.health.gov.au/internet/main/publishing.nsf/content/2200FE086D480353CA2580C900817CDC/$File/6.sd1-Hazard-assessment-report.pdf)
632 [0817CDC/\\$File/6.sd1-Hazard-assessment-report.pdf](http://www.health.gov.au/internet/main/publishing.nsf/content/2200FE086D480353CA2580C900817CDC/$File/6.sd1-Hazard-assessment-report.pdf)
633 Health Canada, 2018. Guidelines for Canadian Drinking Water Quality: Guideline Technical
634 Document – Perfluorooctanoic Acid (PFOA). [https://www.canada.ca/en/health-](https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality.html)
635 [canada/services/environmental-workplace-health/reports-publications/water-quality.html](https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality.html).
636 IPCS (International Programme on Chemical Safety), 1994. Assessing human health risks of
637 chemicals: Derivation of guidance values for health-based exposure limits. Environmental
638 Health Criteria No. 170: Assessing human health risks of chemicals. World Health
639 Organization, Geneva. <http://www.inchem.org/documents/ehc/ehc/ehc170.htm>
640 IPCS (International Programme on Chemical Safety), 2005. Chemical-specific adjustment
641 factors for Interspecies differences and human variability: Guidance document for use of data in
642 dose/concentration-response assessment. Geneva Switzerland.

643 https://apps.who.int/iris/bitstream/handle/10665/43294/9241546786_eng.pdf;jsessionid=B768CD63C4
644 [F93BC4F2388483532665D6?sequence=1](https://apps.who.int/iris/bitstream/handle/10665/43294/9241546786_eng.pdf;jsessionid=B768CD63C4)

645 ITER (International Toxicity Estimates for Risk), 2019. Toxnet. National Library of Medicine.
646 See: <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~GVSP91:3>

647 Klaunig, J.E., Hocevar, B.A., Kamendulis, L.M., 2012. Mode of action analysis of
648 perfluorooctanoic acid (PFOA) tumorigenicity and human relevance. *Reprod. Toxicol.* 33, 410–
649 418.

650 Jarabek, A.M., 1994. Inhalation RfC methodology: Dosimetric adjustments and dose-response
651 estimation of noncancer toxicity in the upper respiratory tract. *Inhal. Toxicol.* 6, 301–325.

652 Lau, C., Thibodeaux, J.R., Hanson, R.G., Narotsky, M.G., Rogers, J.M., Lindstrom, A.B.,
653 Strynar, M.J., 2006. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse.
654 *Toxicol. Sci.* 90 (2), 510–518.

655

656 Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl
657 acids: a review of monitoring and toxicological findings. *Toxicol. Sci.* 99 (2):366–394.

658

659 Loccisano A.E., J.L. Campbell Jr., M.E. Andersen, H.J. Clewell III. 2011. Evaluation and
660 prediction of pharmacokinetics of PFOA and PFOS in the monkey and human using a PBPK
661 model. *Reg. Toxicol. Pharmacol.* 59, 157–175.

662

663 Loccisano A.E., Longnecker, M.P., Campbell Jr, J.L., Andersen, M.E., Clewell III, H.J., 2013.
664 Development of PBPK Models for PFOA and PFOS for Human Pregnancy and Lactation Life
665 Stages, *J. Toxicol. Environ. Health, Part A: Current Issues*, 76:1, 25-57.
666

667 Lou, I., Wambaugh, J.F., Lau, C., Hanson, R.G., Lindstrom, A.B., Strynar, M.J., Zehr, R.D.,
668 Setzer, R.W., Barton, H.A., 2009. Modeling single and repeated dose pharmacokinetics of PFOA
669 in mice. *Toxicol. Sci.* 107 (2), 331–341.
670

671 Macon, M.B., Villanueva, L.R., Tatum-Gibbs, K., Zehr, R.D., Strynar, M.J., Stanko, J.P., White,
672 S.S., Helfant, L., Fenton, S.E., 2011. Prenatal perfluorooctanoic acid exposure in CD-1 mice:
673 low-dose developmental effects and internal dosimetry. *Toxicol. Sci.* 122 (1), 134–145.

674 Meek, M.E., Newhook, R., Liteplo, R.G., Armstrong, V.C., 1994. Approach to assessment of
675 risk to human health for priority substances under the Canadian Environmental Protection Act. *J.*
676 *Environ. Sci. Health C.* 12 (2), 105–134.

677 Meek, B., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B., Vu, V., 2001.
678 Guidelines for application of compound specific adjustment factors (CSAF) in
679 dose/concentration response assessment. *Comments Toxicol.* 7 (5-6), 575–590.

680 NJDWQI (New Jersey Drinking Water Quality Institute), 2017. Health-based Maximum
681 Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA).
682 <https://www.nj.gov/dep/watersupply/pdf/pfoa-appendixa.pdf>

683 Perkins, R., J. Butenhoff, G. Kennedy, and M. Palazzolo. 2004. 13-Week dietary toxicity study

684 of ammonium perfluorooctanoate (APFO) in male rats. *Drug & Chemical Toxicology*. 27:361–
685 378.

686 Piersma, A.H., Hernandez L.G., van Benthem J., Muller A.J.J., van Leeuwen R.F.X., Vermeire
687 T.G., van Raaij M.T.M., 2011. Reproductive toxicants have a threshold of adversity. *Crit. Rev.*
688 *Toxicol.* 41 (6), 545–554.

689 Pizzurro, D.M., Seeley, M., Kerper, L.E., Beck, B.D., 2019. Interspecies differences in
690 perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria. *Reg.*
691 *Toxicol. Pharmacol.* 106, 239-250.

692 Post, G.B., Cohn, P.D., Cooper, K.R., 2012. Perfluorooctanoic acid (PFOA), an emerging
693 drinking water contaminant: A critical review of recent literature. *Environ. Res.* 116, 93-117.
694 <https://doi.org/10.1016/j.envres.2012.03.007>

695 Quist, E.M., Filgo, A.J., Cummings, C.A., Kissling, G.E., Hoenerhoff, M.J., Fenton, S.E., 2015.
696 Hepatic mitochondrial alteration in CD-1 mice associated with prenatal exposures to low doses
697 of perfluorooctanoic acid (PFOA). *Toxicol. Pathol.* 43: 546-557. DOI:
698 10.1177/0192623314551841

699 Renwick, A.G., 1993. Data derived safety factors for the evaluation of food additives and
700 environmental contaminants. *Food Addit. Contam.* 10 (3), 275–305.

701 Roberts M., Grice, J., Hungerford N., Liang X., Liu X., 2016. A Critical Review of
702 Pharmacokinetic Modelling of PFOS and PFOA to Assist in Establishing HGBVs for these
703 Chemicals. Food Standards Australia New Zealand. December 7.

704 [https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C9](https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/7.Critical-Review-Pharmacokinetic-Modelling.pdf)
705 [00817CDC/\\$File/7.Critical-Review-Pharmacokinetic-Modelling.pdf](https://www.health.gov.au/internet/main/publishing.nsf/Content/2200FE086D480353CA2580C900817CDC/$File/7.Critical-Review-Pharmacokinetic-Modelling.pdf)

706

707 TCEQ (Texas Commission on Environmental Quality), 2016. Perfluoro Compounds (PFCs)
708 Various CASRN Numbers. January 4.

709 <https://www.tceq.texas.gov/assets/public/implementation/tox/evaluations/pfcs.pdf>.

710

711 Wambaugh, J.F., Setzer, R.W., Pitruzzello, A.M., Liu, J., Reif, D.M., Kleinstreuer, N.C., Ching,
712 N., Wang, Y., Sipes, N., Martin, M., Das, K., DeWitt, J.C., Strynar, M., Judson, R., Houck, K.A.,
713 Lau, C., 2013. Dosimetric Anchoring of In Vivo and In Vitro Studies for Perfluorooctanoate and
714 Perfluorooctanesulfonate. *Toxicol. Sci.* 136 (2), 308–327

715

716 White, S.S., Stanko, J.P., Kato, K., Antonia, M., Calafat, A.M., Hines, E.P., Fenton, S.E., 2011.
717 Gestational and chronic low-dose PFOA exposures and mammary gland growth and
718 differentiation in three generations of CD-1 mice. *Environ. Health Perspect.* 119 (8), 1070–
719 1076.

720

721 Wolf, C.J., Fenton, S.E., Schmid, J.E., Calafat, A.M., Kuklennyik, Z., Bryant, X.A., Thibodeaux,
722 J., Das, K.P., White, S.S., Lau, C.S., Abbott, B.D., 2007. Developmental toxicity of
723 perfluorooctanoic acid in the CD-1 mouse after cross-foster and restricted gestational exposures.
724 *Toxicol Sci.* 95 (2), 462–473.

725 Zhao, Q., Unrine, J., Dourson, M., 1999. Replacing the default values of 10 with data-derived

726 values: a comparison of two different data derived uncertainty factors for boron. Hum Ecol Risk
727 Assess. 5 (5), 973–983.

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735 **Table 1. Summary of Lau et al. (2006)^a Effects, EPA (2016) LOAEL, and Possible Dose**
 736 **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.”

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)
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 <i>in utero</i> 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.

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)
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	Effects may be due to maternal

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)
		concentration during exposure period	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	

737 ^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.

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740 **Table 2. Estimated Cmax values from Lou et al., (2009, Figure 3) where serum levels are**
741 **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

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

Dose (mg/kg-day)	Cmax or Steady State (mg/L per mg/kg)		
	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

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748 **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

749 * Doses given in mg/week. Mg/kg-day doses are determined from average body weight of 75 kg

750 as stated by Convertino et al. (2018), and dividing by 7 days/week, except for patients 1, 2, and

751 3.

752 na = not applicable since patients 1, 2, and 3 were only given one dose.

753 **Cmax value approximated from Figure 84 on Sheet 76 of 85 in Elcombe et al. (2013).

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756 **Table 5. Average Cmax concentrations after each dose in μM per mg/kg-day for six weeks**
 757 **(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
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

758 ^aValues for weeks 2 through 6 are for 1 person

759 ^bDoses of 1.8 and 1.9 mg/kg-day were averaged

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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 to 36 Weeks ~Steady State*
1.3	14	14

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

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764 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

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

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