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

Bernard Gadagbui, MS, PhD, DABT, ERT
Raymond York, PhD., DABT
Chijioke Onyema, MPH
Michael Dourson, PhD, DABT, FATS, FSRA

Problem Formulation

- EPA (1991) and EPA (2014) & IPCS (2005) guidelines:
  - suggest two different default positions for dosimetric extrapolation from experimental animals to humans when the dosimetry of the critical effect is not known
- EPA (1991) default position for developmental toxicity:
  - use peak concentration (or Cmax) for this dosimetric extrapolation
- IPCS (2005) and presumably EPA (2014) default position for developmental toxicity:
  - use Area Under the Curve (AUC)
- Given the discrepancy in the guidelines
  - Could Data-Derived Extrapolation Factors (DDEFs) for developmental toxicity be developed considering Cmax as the dosimeter alongside with AUC?
- Use a preliminary research Case Study to test the impact of using Cmax for developmental toxicity.

EPA (2016) PFOA Assessment

- Critical effects for PFOA related more to developmental toxicity
- Based on 7 studies
  - 4 conducted in mice with gavage dosing during pregnancy showing a variety of fetal and maternal effects
  - 1 conducted in mice with a 15-day drinking water exposure, but critical effect was noted after 1 day
  - 2 conducted in rats for 13-week, but the liver effects at the low doses do not appear to be adverse according to EPA
- Fetal effects used by EPA (2016) as the critical effects from the four gavage studies of PFOA in mice
  - specifically Lau et al. (2006) study to develop the RfD

EPA (2016) PFOA Critical Studies

- TERA scientists reviewed the 5 mouse studies and judged whether the appropriate dosimeter of each effect is:
  - AUC, Cmax, something else, or indeterminate

- Intention was to use these judgments with appropriate kinetic information to contemplate the development of a DDEF

Human Toxicokinetic Data

- Kinetic data for PFOA available in rodents (rats and mice)
  - TERA scientists focused on mouse data as it is the most sensitive-species
    - Single and multiple dosing (e.g., Lou et al. (2009))

- However, little specific kinetic data available in humans until recently (Elcombe et al., 2013).

Elcombe et al. (2013)

- Submitted a US Patent Application where PFOA was used as a cancer chemotherapeutic agent.
- Findings from this study have been recently published in part (Convertino et al., 2018).
- PFOA up to 1200 mg once per week to 43 humans in various stages of cancer as a phase 1 therapeutic trial
- Doses and blood concentrations were carefully monitored.
- TERA scientists summarized the findings, identifying individual Cmax values for each patient after his/her weekly dose of PFOA
  - Estimated average Cmax values per dose and derived a CSAF from comparison of mouse and human Cmax values after a single dose or weekly doses

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

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Cmax or AUC?	Comments
Accelerated male puberty	1	Indeterminate	
Reduced pup body weight	3	Indeterminate	According to the authors, "Neonatal growth deficits may be related to the nursing dams' capability to lactate, and hence the nutritional status of the suckling pups."
Full litter resorption	5	Cmax	According to the authors "these pregnancy losses probably took place shortly after implantation."
Postnatal survival	5	Indeterminate	Mortality decreases sharply after birth, despite continued PFOA exposure through breast milk, suggesting an in utero cause.
Maternal weight loss	20	Indeterminate	Effect occurred within 3 days at highest dose of 40 mg/kg-day, within 6 days at 20 mg/kg-day.
Prenatal loss (% per live litter)	20	Indeterminate	
Live fetuses (# per litter)	20	Indeterminate	

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

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Cmax or AUC?	Comments
↓ Female offspring birth weight	3	Indeterminate	Maternal body weight gain influences offspring birth weight.
↑ Dams with implants but no live pups	5	Indeterminate	

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

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

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

Effect(s)	LOAEL (mg/kg/day)	Dosimeter: Cmax or AUC?	Comments
↓ Male offspring body weight	5	Indeterminate	

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

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

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

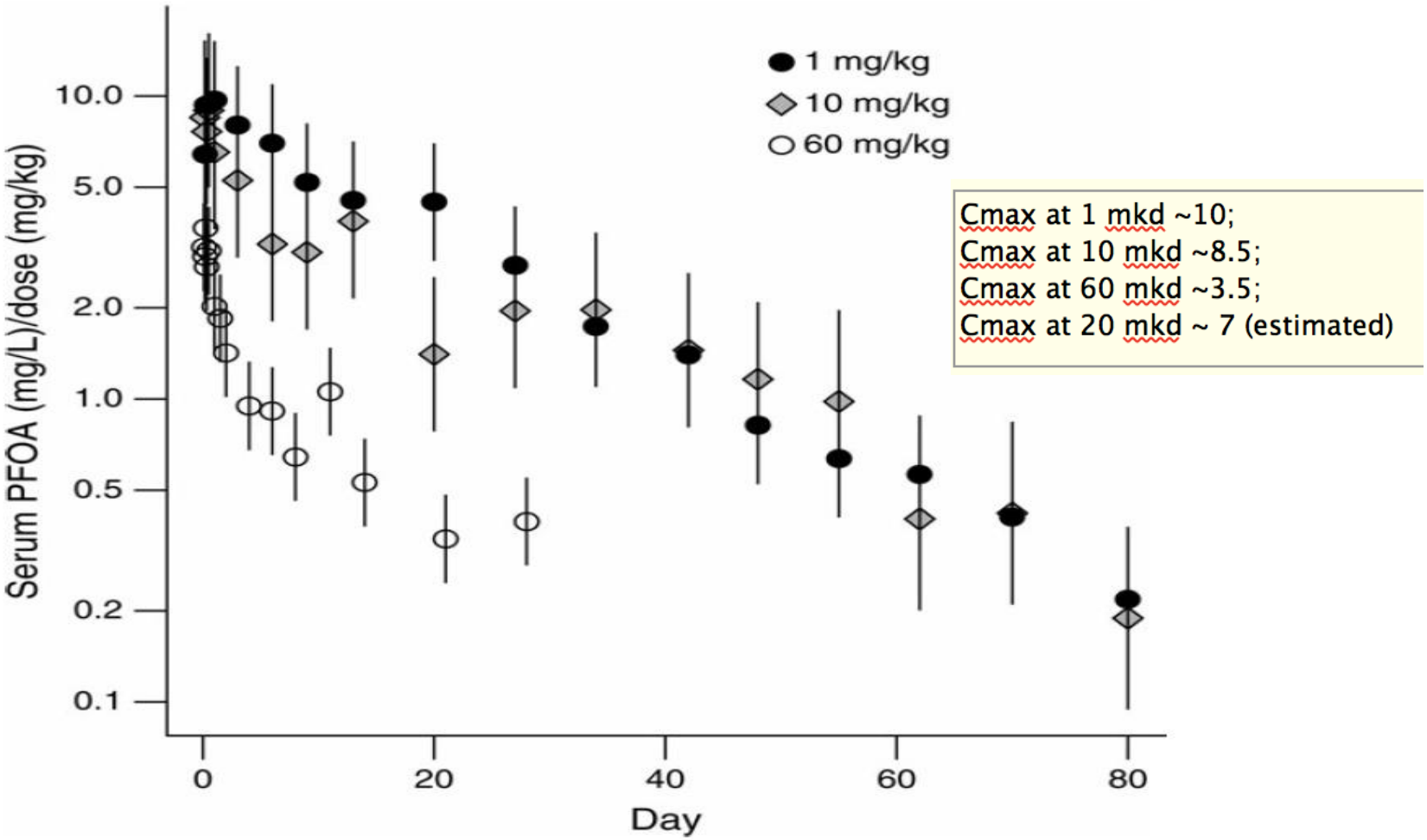


FIG. 3. Serum concentrations scaled by dose for females administered single doses of 1, 10, and 60 mg/kg. Points are means, error bars are 95% confidence intervals for the means. 1 and 10 mg/kg dose groups are largely superimposed and linear in time on this semi-log suggesting linear first-order kinetics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

TERA

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

This figure can be use to determine an adjustment between Cmax after 1 dose and doses after 17 days.

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

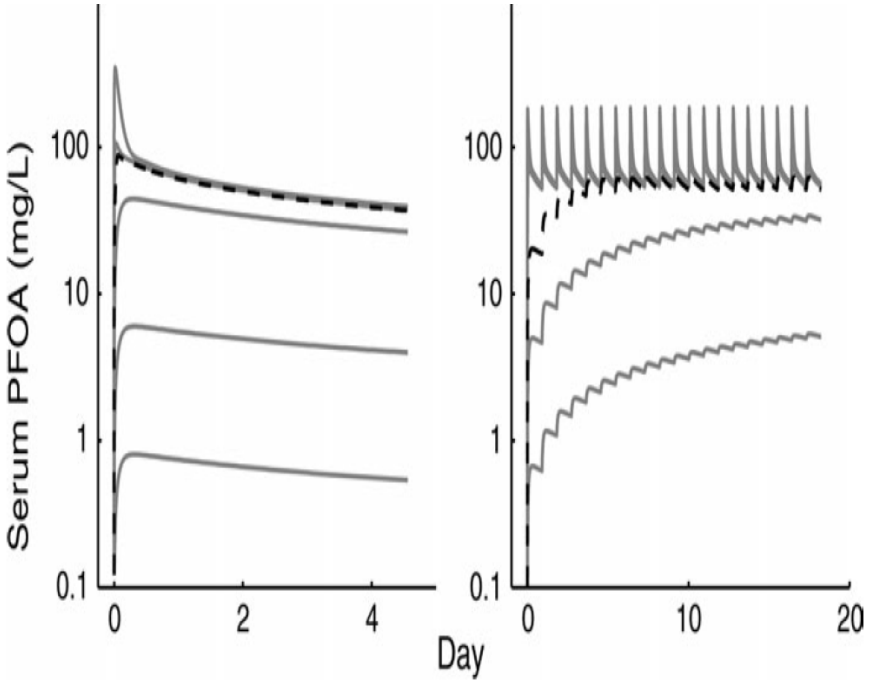


FIG. 7. Delineation of predictions for the PFOA concentration (mg/l) in the central compartment. For the single dose (top) solid lines depict doses of 0.1, 1, 10, 100, and 1000 mg/kg. The dashed line indicates a dose of 40 mg/kg which is roughly where the onset of nonlinearity occurs. For the repeated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

TERA

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

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

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

Data from Elcombe et al. (2013)

TERA

From Table 7, average human Cmax at 6 weeks = 732 µM per mg/kg when
732 µM per mg/kg-day = 732 µmole/L per mg/kg-day
= 732 µmole/L per mg/kg-day x 414 grams/mole (MW)
= 303,048 µg/L per mg/kg
Cmax = 303 mg/L per mg/kg

Elcombe et al. doses	TERA Report Figure 2 (Lou et al., 2009, Figure 7b, mice)		
vvv	Dose (mg/kg-day)	~ 1 day Cmax (mg/L)	~ 17 day (mg/L)
low human dose >>>	0.1	0.7	5
Avg human dose >>>	1	5	35
	5	20	60

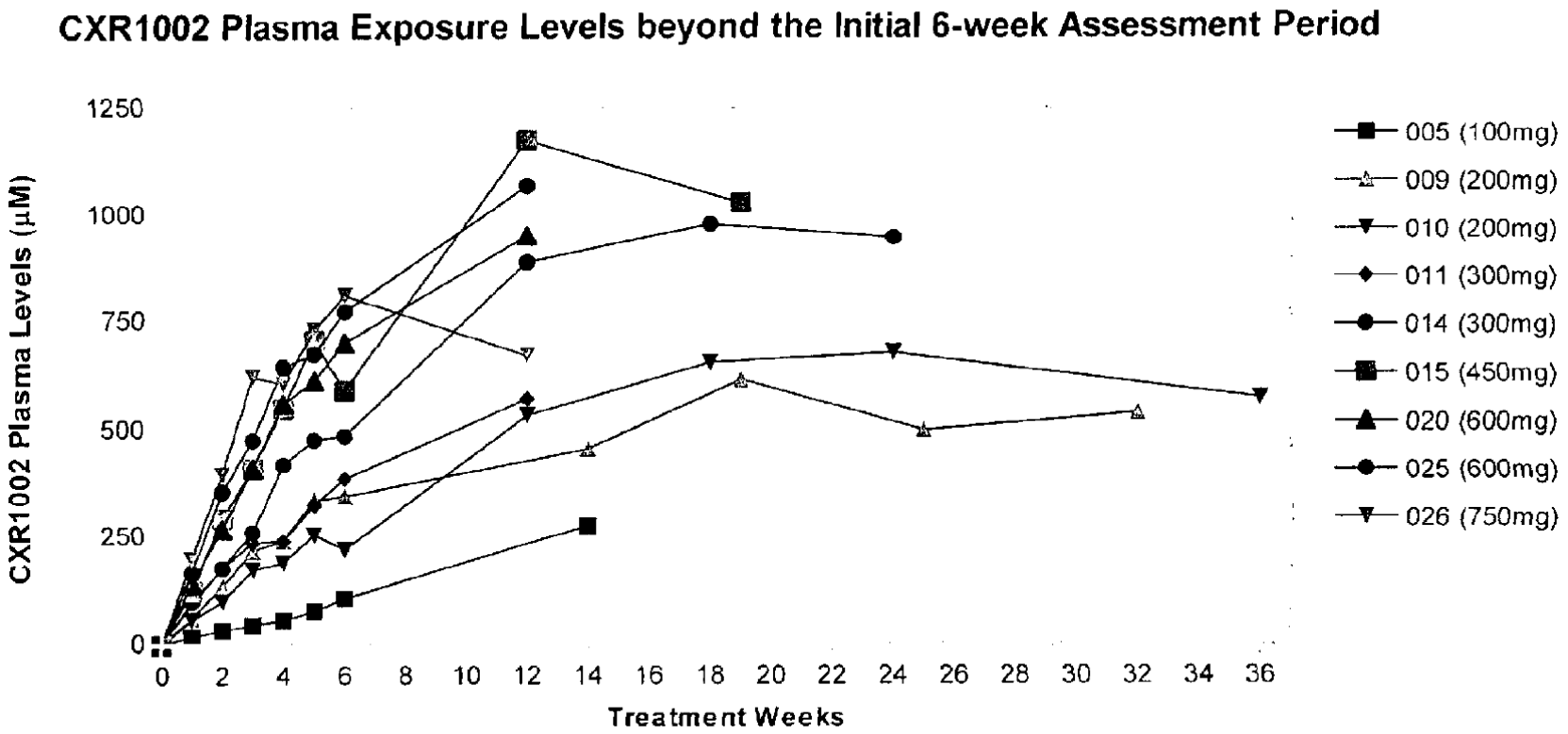
	Cmax in mg/L per mg/kg-day at 0.1 dose	50
	Cmax in mg/L per mg/kg-day at 1.0 dose	35

Human/mouse CSAF with low human dose	=	6.1
Human/mouse CSAF with avg. human dose	=	8.7

TERA

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

Figure 78



Information from Elcombe et al. (2013) Figure 78 on patients beyond 6 weeks of exposure

Patient	Dose	6 week level	Doses/Measurements Past 6 weeks				Average of Past 6 wk	Ratio past/6
			1st	2nd	3rd	4th		
5	100	109	276				276	2.5
9	200	373	471	617	501	540	532	1.4
10	200	232	570	650	660	570	613	2.6
11	300	387	574				574	1.5
14	300	562	890	980	940		937	1.7
15	450	801	1175	1000			1088	1.4
20	600	770	950				950	1.2
25	600	780	1050				1050	1.3
26	750	824	670				670	0.8

Values in yellow from Elcombe et al. (2013) Figure 78; other values from patient tables from this study

Average 1.6

TERA

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

Single Dose	~6 Weeks	~25+ Weeks*
1.3	4.3 to 8.7	7 to 14

?

*Based on apparent “steady state” in nine individuals from Figure 3. Week 25+ range is based on 6 week range multiplied by ratio of 1.6 from 6 week to 25+ week comparison of Cmax values from previous table.

Summary

- The critical effect of PFOA appears to be more related to developmental toxicity or other toxicity due to short-term, gavage exposures in mice, consistent with EPA (2016).

- Some effects of EPA (2016) appear to be related to Cmax, others related to AUC, and many are indeterminable.

- Estimates of Cmax and half-life are possible from the new human study. These estimates should be used with caution, however, since they are from clinical trials for cancer therapy, and kinetics may not reflect an average population.

Summary Continued

- PFOA MOA may be complex, but some effects are likely due to simple biomolecular interactions, since PFOA is chemically inert. If true, these effects would be more likely due to Cmax since "Cmax could be more relevant than AUC when a simple biomolecular interaction produces the effect (IPCS, 2005)."

- EPA (1991) states "a primary assumption is that a single exposure at a critical time in development may produce an adverse developmental effect," suggesting consideration of Cmax, which IPCS (2005) or EPA (2014) also require.

- Bottom line: We agree with EPA & IPCS. PFOA evaluations should consider Cmax. New human data should help.

Charge Questions

1. The judgement of Cmax or AUC is possible for several of the effects listed in the mouse developmental studies, but many of these judgments are indeterminate.
   - Do you agree with the judgment of Cmax or AUC for the listed effects?
   - Does it seem reasonable to consider the default of Cmax for these indeterminate judgments as per EPA (1991)?
   - Is some other dosimeter, like AUC, more reasonable?

2. The kinetic comparisons between the mouse and human are based on daily gavage dose in mice and weekly capsule exposure in humans, which have been converted to daily doses in humans by dividing by 7 days/week.
   - Does this conversion make sense?
   - Should another conversion be used?

Charge Questions cont'd

3. Do you agree with the estimated DDEFs?
   - Are other ways possible to improve the derivation of these DDEFs?

4. The apparent half life estimated from a small number of humans based on the data from Elcombe et al. (2013) appears to be much shorter than literature values would indicate.
   - Would these patients be expected to have a different half-life than the average or normal population?
     - If so, in which direction would the half-live be expected to change?

Funding

- The origin of this work came about from briefings of MLD as a U.S. Environmental Protection Agency senior advisor in 2017.

- Afterwards, scientists with Toxicology Excellence for Risk Assessment (TERA), confirmed these discussions of EPA staff, and submitted comments to the Agency for Toxic Substances and Disease Registry (ATSDR) regarding its PFAS kinetics.

- The submission of comments to ATSDR and development and presentation of this research case study has been supported by the Internal Development Reserve funds of TERA.

TERA