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TOXICOLOGY EXCELLENCE FOR RISK ASSESSMENT

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

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Why Conduct this Research?

- Many agencies worldwide developed different safe doses for the same chemical
- Safe levels are dependent on the extrapolation of animal data to humans based on differences in the endpoint selected and/or the AK_{AF} factor, the TK portion of the animal to human extrapolation.
- Considered whether data are available to update the TK section of the AK_{AF}
  - EPA, for example, used data from animals to estimate the AK_{AF}
- What the IPCS and EPA recommendations are for developing DDEFs

A Problem…

Agency	UK-COT	Health Canada	US EPA	Australian FASANZ	US ATSDR
Study	Mouse fetal	Rat Systemic	Mouse fetal	Mouse fetal	Mouse fetal
Critical Effect	Liver effects	Rat liver hypertrophy	Decreased pup ossification	Fetal toxicity	Altered pup activity
Human Dose (mg/kg-day)	0.08 (MMDL of 0.3 ÷ 4)	0.00052	0.0053	0.0049	0.000821
Uncertainty Factor	50 (200 ÷ 4)	25	300	30	300
Safe Dose (µg/kg-day)	1.5	0.02	0.02	0.16	0.003

500-fold Difference in Safe Dose

A Potential Solution

- In the development of a Reference Dose (RfD), the use of Data-derived Extrapolation Factors (DDEF) or a Physiologically-Based Pharmacokinetic (PBPK) model is an important consideration (IPCS, 2005; EPA, 2014).

- 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.

- Appropriate and necessary data include knowledge of kinetic and dynamic differences between the experimental animal of choice and humans.

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Requirements for DDEFs Derivation

Both IPCS (2005) and EPA (2014) have established minimum requirements for DDEFs, specifically:

- What is/are the critical effect(s) and the point of departure (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?

Results

What is/are the critical effect(s)?

- The identification of the critical effect for PFOA is disparate. TCEQ (2014), EPA (2016), and ATSDR (2018) identify developmental toxicity. Health Canada (2018) and NJDWQI (2017) identify liver toxicity. Other groups, such as European Food Safety Authority (EFSA, 2018), state lipid changes.

- This research was conducted using EPA's critical effect, specifically, the fetal effects from the study by Lau et al. (2006).

- We summarized effects from Lau et al. (2006) and made judgment regarding the likely dosimeter for each effect

Results

Active chemical form been identified?

- It is generally accepted by government and industry experts that PFOA is not metabolized or metabolized to a limited extent in mammals (EPA, 2016; ATSDR, 2018).

- Thus, PFOA was considered to be the active chemical moiety in this research.

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Results

What is the Mode of Action (MOA)?

PFOA exposure resulted in a variety of adverse effects; each of these effects may be evoked by a different process. In part:

- Elcombe et al. (2013) consider the MOA to be associated with its ability to mimic fat in the body:
  - "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."

- PFOA has been documented to bind with and activate PPAR-α and exposures to PFOA during fetal development is known to induce alterations in cholesterol biosynthesis and/or fatty acid metabolism (Quist et al., 2015). This action of PFOA may be responsible for some of the developmental toxicity.

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Results

ADME of chemical well characterized?

- The ADME has been fairly well characterized in the rat and mouse, less so in other experimental species

- The next two figures are adapted from Lou et al. (2009, Figure 3 and Figure 7b)
  - Figure 1 (Lou et al. Figure 3) shows the kinetic behavior in serum after a single gavage administration of PFOA in mice.
  - Figure 2 (Lou et al. Figure 7b) shows the kinetic behavior in serum of mice exposed to PFOA after multiple gavage doses.

Figure 1. Adapted from Lou et al., (2009, Figure 3.) Serum levels from single gavage dose in mice following PFOA exposure. Estimated Cmax values are shown in the box below this figure.

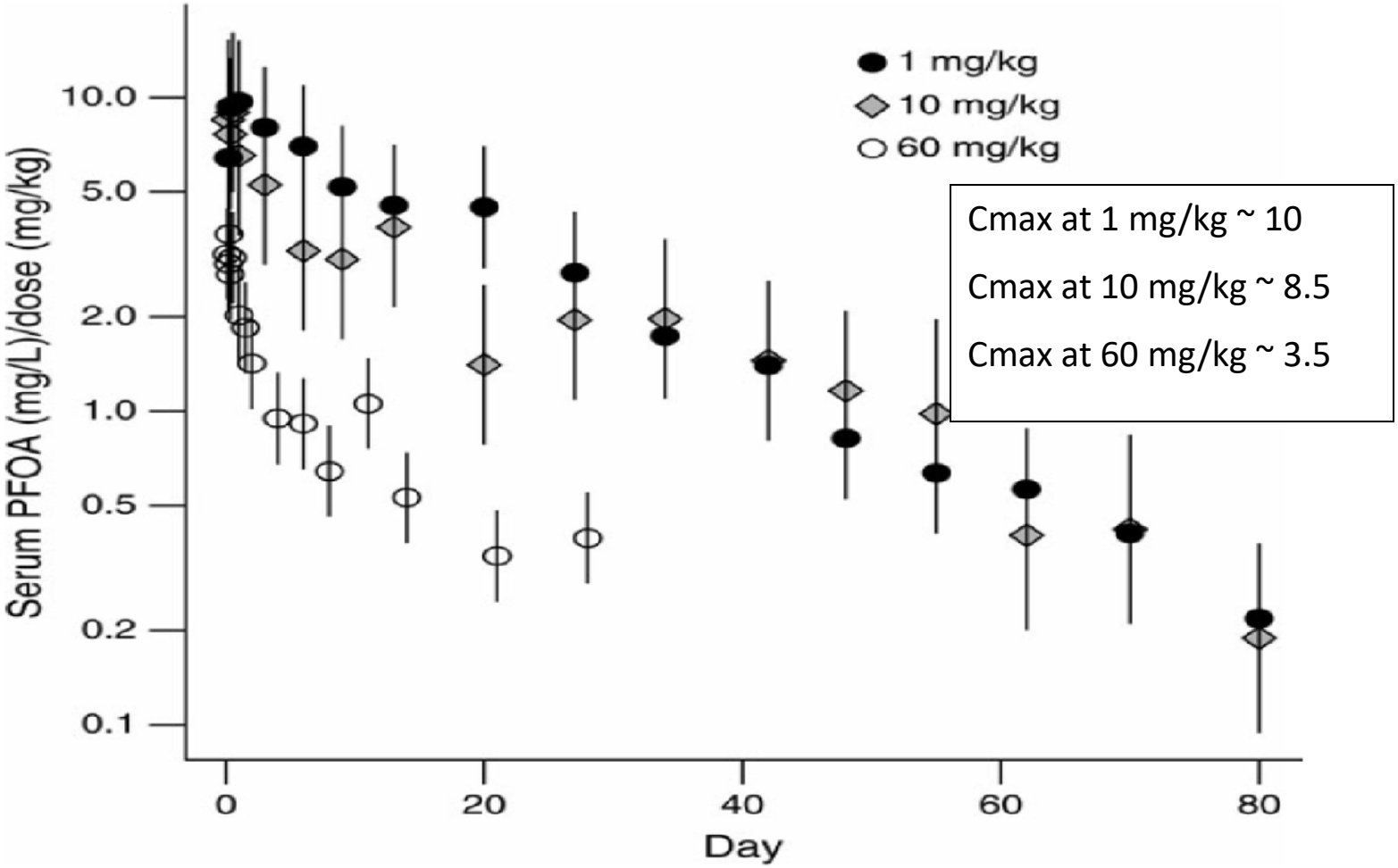

FIG. 3. Serum concentrations scaled by dose for females administered single doses of 1, 10, and 60 mg/kg. Points are means, error bars are 95% confidence intervals for the means. 1 and 10 mg/kg dose groups are largely superimposed and linear in time on this semi-log suggesting linear first-order kinetics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

Figure 2. Estimated Cmax from single doses (left panel) or steady state after repeated 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" or right panel.

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

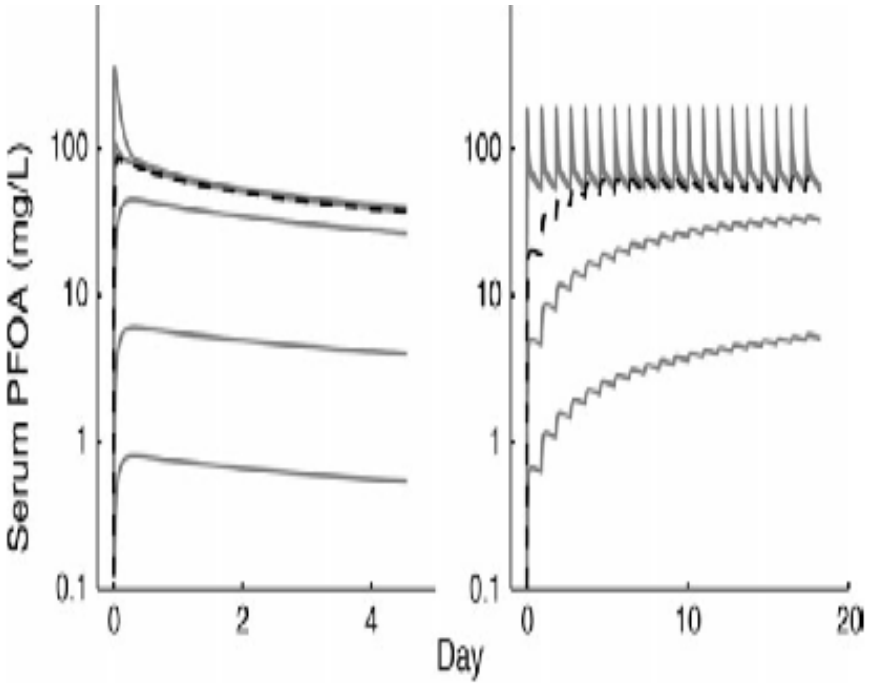

FIG. 7. Delineation of predictions for the PFOA concentration (mg/l) in the central compartment. For the single dose (top) solid lines depict doses of 0.1, 1, 10, 100, and 1000 mg/kg. The dashed line indicates a dose of 40 mg/kg which is roughly where the onset of nonlinearity occurs. For the repeated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

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Results

Kinetic data in human populations?

- To date, few specific kinetic data in humans have been available and we all have had to rely on assumptions of kinetic findings in other species.

- Fortunately, Elcombe et al. (2013) used PFOA as a cancer chemotherapeutic agent. Kinetics well described. Subset of these data published by Convertino et al. (2018).

- Data allowed estimation of a DDEF directly from comparison of mouse and human kinetic data, rather than using a PBPK model with its additional assumptions

Elcombe et al. (2013)

- Submitted a US Patent Application where PFOA was used as a cancer chemotherapeutic agent.
- PFOA up to 1200 mg once per week to 43 patients (24 males and 19 females) with advanced cancer from phase 1 therapeutic trial
  - 9 individuals continued to receive PFOA after the 6-week trial.
- PFOA blood concentrations were carefully monitored.
  - PFOA not metabolized; hence, presumed to reach a steady state level after a number of doses
- Findings were summarized, individual Cmax values identified for each patient after weekly PFOA dose
  - 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 2. Average Cmax concentrations after each dose in µM per mg/kg-day for six weeks (calculated from Elcombe et al. (2013)).

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	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

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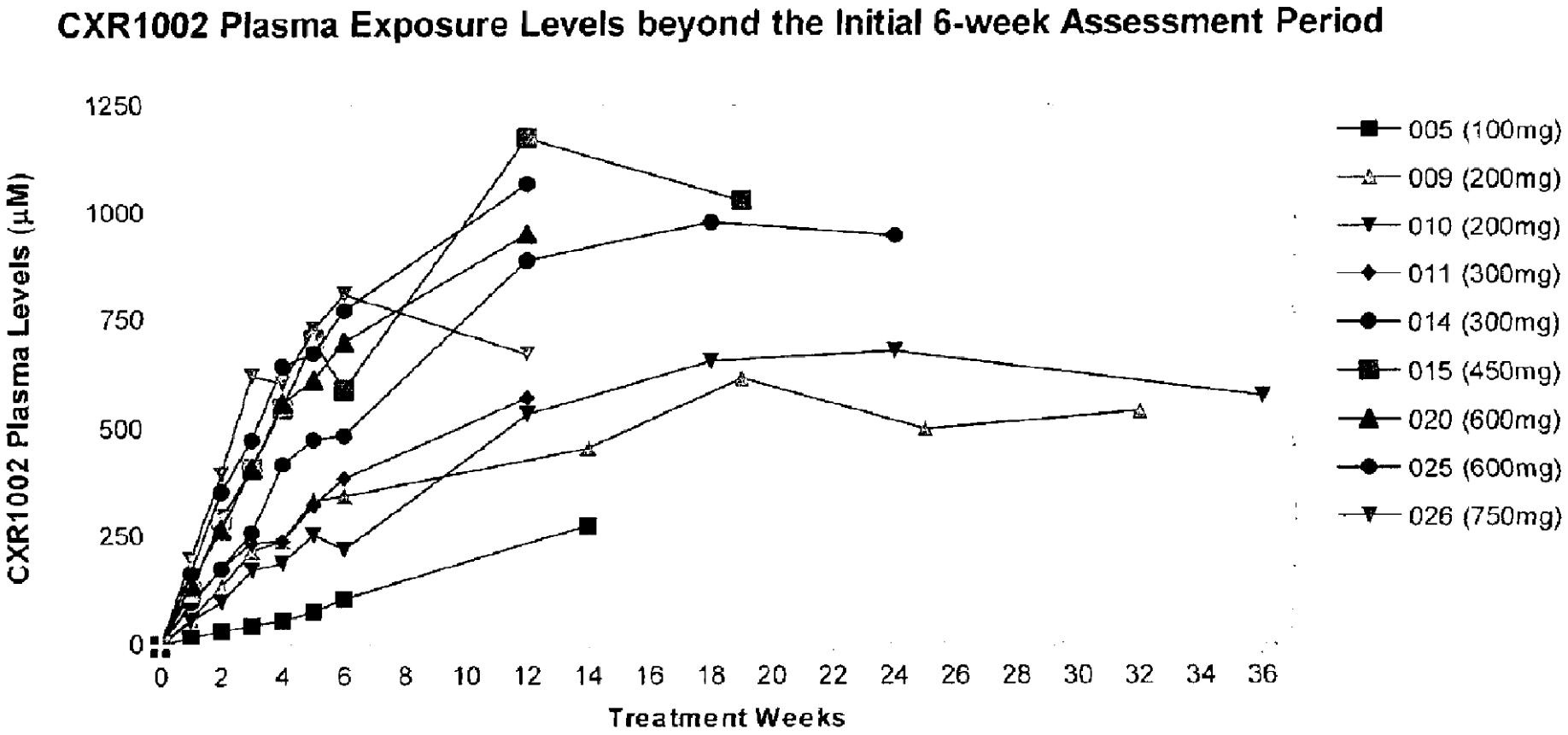
Results
Development of Steady State DDEF

- In humans, Elcombe et al. (2013) reports that Cmax values rise in 9 individuals after 6 weeks of continued weekly capsule exposure to also approximate a steady state.
- This information is shown in the next Figure.
- A DDEF can be based on this 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 the previous Figure.
- This DDEF value is ~14 (i.e., 480 mg/L ÷ 35 mg/L ~14).

*This value is the calculated ratio of Cmax values at 6 weeks in 9 individuals versus their final or plateau values after 6 weeks.

Elcombe et al. (2013) weekly doses in excess of 6 weeks, shown as Figure 78 of their text.

Figure 78



Discussion

- PFOA and related chemicals are very useful and stable, but have contaminated the environment.
- In some places, the contaminant levels approach the range of safe doses, which are highly disparate among governments.
- Kinetic findings in humans by Elcombe et al. (2013) may alleviate some of these differences.
- Limitations exist in this DDEF:
  - Kinetic data are from nonpregnant mice and humans, and
  - In the case of humans, from individuals of both sexes of different ages with advanced disease, however
  - This human population might be considered a sensitive subpopulation.

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Discussion

- We judged that the critical effect is developmental toxicity as determined by EPA (2016).

- Dosimetric adjustment judgments were made in Table 1 (supplemental slide).
  - Some effects appear to be related to Cmax
  - Other effects could be related to AUC or the average concentration during the critical period of development.

- Kinetic data were then compared between mice and humans.

- A conservative choice of DDEF is 14.

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Bottom Line

- Making only one change based on the human clinical study and reliance on EPA methods, the difference between these values is about 13-fold.

- In the EPA (2016) Lifetime HA derivation, the RfD was based on a LOAEL as the point of departure.
  - If EPA were to estimate serum levels of PFOA at the Benchmark Dose (BMD) instead of the LOAEL, this could result in a slightly different RfD and Lifetime HA.

Summary

- The choice of the appropriate dosimeter is important in the development of a Data Derived Extrapolation Factor.
- Comparison was made of kinetic data from PFOA exposure in mice with carefully monitored kinetic data in humans.
- A DDEF for PFOA was estimated to be 14.

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