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TOXICOLOGY EXCELLENCE FOR RISK ASSESSMENT

The Safe Dose for Perfluorooctanoate: A North American *Federal* Perspective

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and
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Introduction

- Federal agencies in Mexico, Canada and the United States have assessed the risk to PFAS; the latter two countries have developed extremely different safe dose evaluations for **PFOA**.
- These safe doses are not only disparate but also at enormous odds with other international authorities due in part to differences in:
 - Choice of PFOA's **critical effect***
 - Use of animal-based **PBPK** models in lieu of available human data
 - Different choices of **uncertainty factors** based on data limitations
- New evaluations of **human** information might allay the use of some underlying assumptions and perhaps set the stage for harmonization.

* Critical effect is the first adverse effect or its known and immediate precursor that occurs in the dose scale.

The North American Dilemma: **PFOA** Safe Doses Differ Widely

Agency	ATSDR (2021)	Canada (2018)	EPA (2022) interim	EPA (2023) draft
Study	Koskela et al. (2016)	Perkins et al. (2004)	Grandjean et al., 2012; Budtz-Jorgensen and Grandjean, 2018	Budtz-Jorgensen and Grandjean, 2018; Wilkstrom et al., 2019; and Dong et al., 2019
Critical Effect	Skeletal effects in mice	Rat liver hypertrophy	Human decreased antibodies	Human decreased antibodies & birth weight, increased cholesterol
Human Dose (ug/kg-day)	0.821	0.52	0.000,0149	0.000,275 to 0.000,305
Uncertainty Factor	Human = 10 Animal = 3 LOAEL = 10	Human = 10 Animal = 2.5	Human = 10	Human = 10
“Safe” Dose (ug/kg-day)	0.003	0.02	0.000,0015	0.000,03

~13,000-fold (Canada to EPA interim) or ~700-fold (Canada to EPA draft)

Agency	ATSDR (2021)	
Koskela et al. (2016); (Onishchenko et al. 2011)	Six pregnant C57BL/6 mice exposed to 0.3 mg/kg-day in the diet for gestation days 1-21	
Critical Effect	Skeletal alterations in offspring; neurodevelopmental effects in offspring as adults	
Human Dose (ug/kg-day)	0.821	Effects assumed to be related to AUC; EPA's PBPK model used to determine human dose
Uncertainty Factor	300	Assumed only dose tested was LOAEL (10), human variability (10), mouse to human dynamics (3)
"Safe" Dose (ug/kg-day)	0.003	100-fold lower than ATSDR methyl mercury MRL

ATSDR Principal Study

Koskela A, Finnila MA, Korkalainen M, et al. 2016. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. *Toxicol Appl Pharmacol* 301:14-21.

- Pregnant C57BL/6/Bk1 mice were exposed to PFOA (96% pure) in food at dose levels of 0 mg/kg/day (n=10) or 0.3 mg/kg/day (**n=6**) from GD 1 throughout pregnancy (presumed GD 21).
- It is noted that litter mates of these offspring were examined for neurobehavioral effects in a study conducted by Onishchenko et al. (2011).
- Groups of five female offspring were sacrificed at either 13 or 17 months of age (**statistics** appear to be **offspring-based** and not litter-based).
- The following parameters were used to assess toxicity: body weight and morphometric/biochemical properties in bone (femurs and tibias) of offspring.



ATSDR Pros & Cons

Pros

- Chooses a **critical effect** not directly considered by other authorities
- Results in a **protective** level
- Uses a **PBPK** model to project the equivalent human dose

Cons

- Chosen study has **only one dose** given to **6** pregnant mice
- Likely **pup-based** statistics rather than on pregnant dams; thus, is not usable for risk assessment according to (EPA, 1991)
- Dosimetric adjustment is by AUC; default for developmental toxicity is **Cmax** or peak concentration as per (EPA, 1991)
- Other authorities reject direct risk assessment use of this study

Agency	Health Canada (2018)	
Perkins et al. (2004)	Exposed male Crl:CD rats (n=55 per dose) to 0, 0.06, 0.64, 1.94, and 6.5 mg/kg bw per day in food	
Critical Effect	Male rat hepatocellular hypertrophy	
Human Dose (ug/kg-day)	0.52	Effects assumed to be related to AUC; hybrid PBPK-AKUF model and BMDL ₀₅ used to determine human dose
Uncertainty Factor	25	Human variability (10), mouse to human dynamics (2.5)
“Safe” Dose (ug/kg-day)	0.02	10-fold lower than Canada’s methyl mercury

Health Canada Principal Study

- Perkins et al. (2004) is the most robust study for increased liver weight and hepatocellular hypertrophy exposed male Crl:CD rats (n=55 per dose) to 0, 0.06, 0.64, 1.94, & 6.5 mg/kg bw per day in food.
- Two control groups were included—one group **pair-fed** with the high dose group, and one *ad libitum* feeding group. The rats were divided into sacrifice groups exposed for a duration of 4, 7, or 13 weeks (n=15 per dose per duration), and each dose (and the *ad libitum* controls) had a **recovery group** that was observed for 8 additional weeks after cessation of exposure at week 13 (n=10 per dose).
- Significant dose-related increases in incidence and severity of **hypertrophy** were observed, but effects **did not persist** in recovery groups, and hypertrophy **was not** accompanied by degenerative lesions. **Relative liver weight** was increased at ≥ 0.64 mg/kg bw per day, but effects **did not persist** in recovery groups. Thus, a **NOAEL of 0.06 mg/kg bw** per day for lack of hepatocellular hypertrophy is derived.



Health Canada Pros & Cons

Pros

- Chooses a **critical effect** that is considered relevant by some other authorities
- Results in a **protective** level
- Uses a hybrid **PBPK-AK_{UF}** model to project the equivalent human dose

Cons

- AK_{UF} values are **dependent** on the specific doses...which cannot account for non-linear kinetics
- Other authorities **do not consider** the observed liver hypertrophy and increased relative liver weight to be adverse
- PPAR α activation in rodents is thought to be important in their liver response, but this is **not directly applicable** to humans



Health Canada Current Deliberations

In April 2021, Canada published a [Notice of Intent](#)

- A notice of intent to address the broad class of PFAS was published in the Canada Gazette, Part I: Vol. 155 No. 17 – April 24, 2021.
- The Government of Canada is considering activities that would address PFAS as a class rather than as individual substances or in smaller groups
- The Government of Canada published for public consultation:
 - a draft state of PFAS report in May 2023
 - a draft drinking water objective for PFAS in February 2023

Excerpts from Richard Carrier email to Michael Dourson 10-13-23.



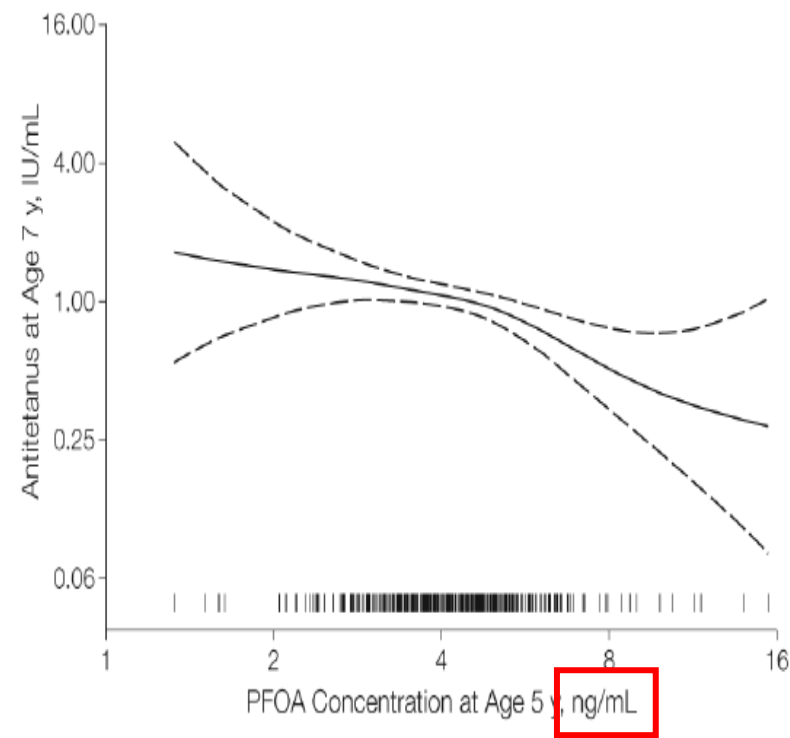
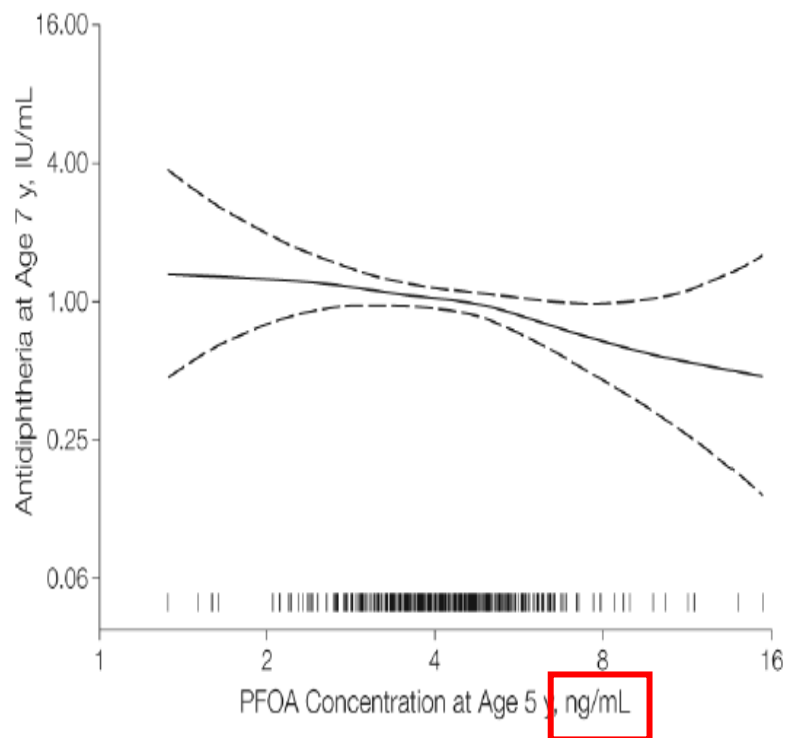
Health Canada Current Deliberations

In April 2021, the Government of Canada published a [Notice of Intent](#)

- As the science on PFAS is rapidly evolving, Health Canada considered the latest scientific information on potential health effects, drinking water treatment, analytical methods and monitoring data to propose an **objective of 30 ppt** of PFAS chemicals in drinking water.
- Once final, this new objective **will replace current guidelines [of 200 ppt]** and remain in place until the reassessment of the PFAS guidelines is completed, and new guidelines are adopted.
- Bottomline is that the current draft drinking water objective is meant to **supersede the 2018** drinking water guideline for both PFOA and PFOS... based on the above considerations, Health Canada does not feel comfortable presenting the science that supported its values back in 2018.
- Excerpts from Richard Carrier email to Michael Dourson 9-5-23.

Agency	US EPA (2022) INTERIM	
Several studies	Human observational studies of Grandjean et al., 2012; Budtx-Jorgensen and Grandjean, 2018	
Critical Effect	Suppression of tetanus vaccine response in 7-year old children	
Human Dose (ug/kg-day)	0.000,0149	Effects are assumed to be related to AUC; a PBPK model used to determine human dose
Uncertainty Factor	10	Human variability
“Safe” Dose (ug/kg-day)	0.000,0015	67,000-fold lower than EPA’s methyl mercury RfD

EPA's Study: Grandjean et al., 2012



Perfluorooctanoic Acid (PFOA) at Age 5 Years and Serum Antibody Concentrations Against Diphtheria and Tetanus at Age 7 Years

The generalized additive models have 3 *df* and were adjusted for age, sex, and vaccine booster type. Dashed lines indicate 95% confidence intervals; vertical bars on the horizontal scale indicate individual observations.

Table 2

Concentrations of PFCs in Serum From Birth Cohort Mothers and Their Children at Age 5 Years and Correlations Pairwise and With the PCB Concentration in Maternal Serum

Compound	PFC Concentration, Geometric Mean (IQR), ng/mL
Maternal PFCs	
PFOS	27.3 (23.2 to 33.1)
PFOA	3.20 (2.56 to 4.01)
PFHxS	4.41 (2.24 to 8.43)
PFNA	0.60 (0.46 to 0.79)
PFDA	0.28 (0.22 to 0.38)
PFCs at age 5 y	
PFOS	16.7 (13.5 to 21.1)
PFOA	4.06 (3.33 to 4.96)
PFHxS	0.63 (0.45 to 0.88)
PFNA	1.00 (0.76 to 1.24)
PFDA	0.28 (0.21 to 0.38)

Grandjean et al., 2012. Serum
Vaccine Antibody Concentrations
in Children Exposed to
Perfluorinated Compounds

A total of 656 consecutive
singleton births were recruited in
the Faroe Islands during
1997-2000, and 587 participated in
follow-up through 2008

Abbreviations: IQR, interquartile range; PCB, polychlorinated biphenyl; PFC, perfluorinated compound; PFDA, perfluorodecanoate; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid.

Heilman et al., 2006. Reduced Antibody Responses to Vaccinations in Children Exposed to Polychlorinated Biphenyls.

Two birth cohorts were formed in the Faroe Islands

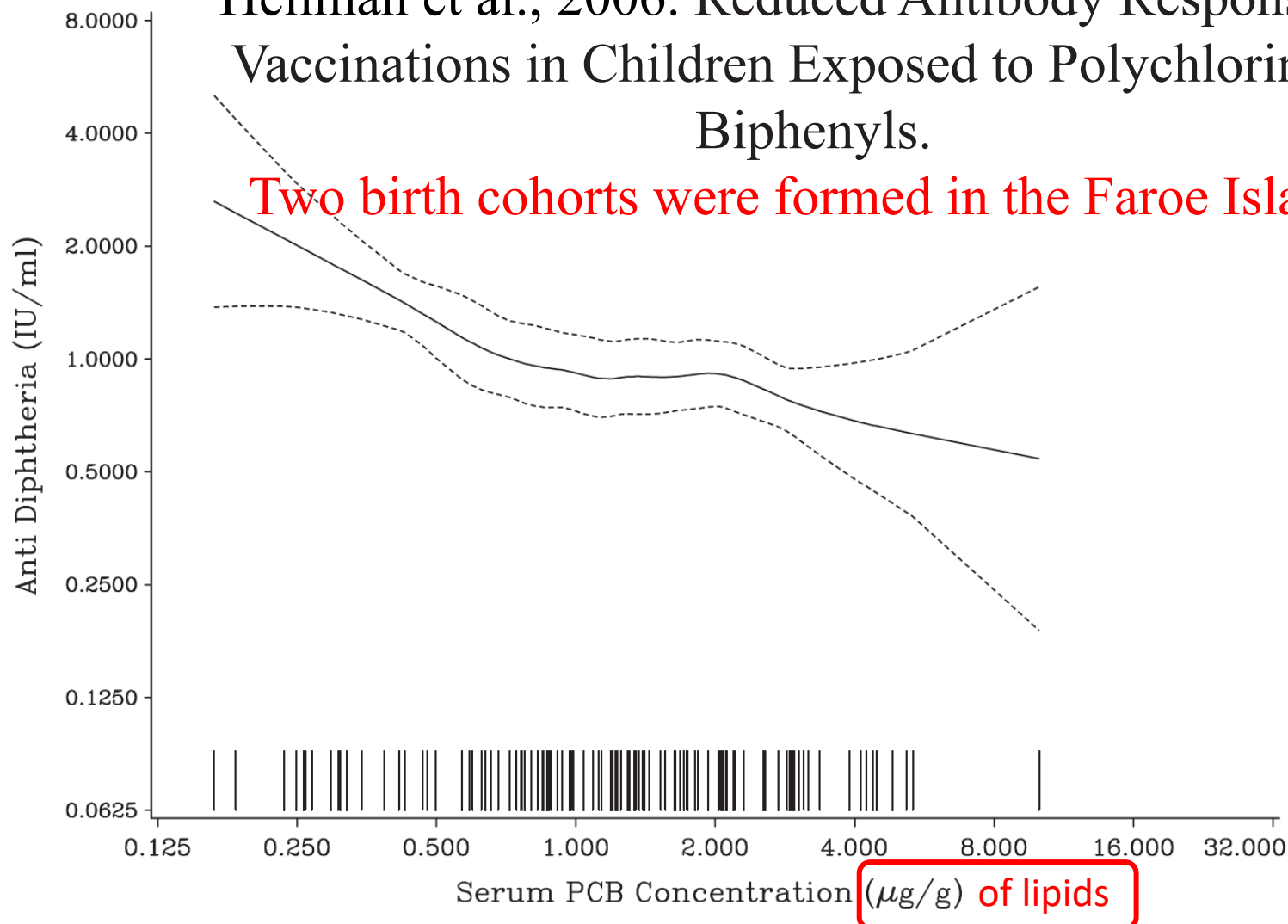


Figure 2....Lower graph, maternal serum concentrations of PCBs plotted against serum diphtheria antibody concentrations, both at 18 months of age. The broken lines indicate the 95% CIs.

Heilman et al., 2006: Faroe Islands and PCBs

- Heilman et al., 2006. Reduced Antibody Responses to Vaccinations in Children Exposed to Polychlorinated Biphenyls.
- Two birth cohorts were formed in the Faroe Islands
- “Taken together, these findings point to the PCB burden in the early postnatal period as the **major determinant** of immunotoxic effects.”
- “The Faroese population is also exposed to other seafood contaminants, and effects of p,p’-DDE and PCB could not be separated due to their close association; other potential confounders appeared to affect the results to a **minimal degree**.”

Heilman et al., 2010. Serum Concentrations of Antibodies Against Vaccine Toxoids in Children Exposed Perinatally to Immunotoxics.

Cohort of 656 singleton births formed in the Faroe Islands

Table 1. Characteristics, with arithmetic mean $\pm$ SD and geometric mean (interquartile range) values, of 587 birth cohort members examined for serum concentrations of specific vaccine antibodies at ages 5 and 7 years.

Variable	<i>n</i>	Result
Maternal age at parturition (years)	587	29.3 $\pm$ 5.1
Previous births (none/one/at least two) (%)	587	27.7/32.8/39.5
Smoking during pregnancy (no/yes) (%)	587	71.4/28.6
Gestational age (weeks)	587	39.6 $\pm$ 1.3
Birth weight (g)	587	3,721 $\pm$ 509
Sex (boys/girls) (%)	587	53.1/46.9
Maternal serum Σ PCB concentration (μ g/g lipid)	427	1.25 (0.83–1.90)
Milk Σ PCB concentration (μ g/g lipid)	521	1.34 (0.85–2.13)
Duration of exclusive breast-feeding (months)	565	4.6 $\pm$ 2.0
Total duration of breast-feeding (months)	569	9.8 $\pm$ 6.6
Serum Σ PCB concentration at age 18 months (μ g/g lipid)	114	1.17 (0.65–2.11)
Serum Σ PCB concentration at age 5 years (μ g/g lipid)	532	1.14 (0.70–1.93)
Serum Σ PCB concentration at age 7 years (μ g/g lipid)	487	0.75 (0.43–1.38)
Age at pre-booster 5-year examination (years)	545	4.95 $\pm$ 0.06
Age at post-booster examination (years)	461	5.20 $\pm$ 0.12
Age at 7-year examination (years)	565	7.53 $\pm$ 0.11

Grandjean et al., 2017. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years.
Of 490 members of a Faroese birth cohort, 275 and 349 participated in clinical examinations and provided blood samples at ages 18 months and 5 years.

Table 1. Characteristics of children who contributed serum antibody concentrations at the two follow-up examinations in Cohort 5.

	Age 18 months (N = 275)	Age 5 years (N = 349)
	<i>n</i> (%)	<i>n</i> (%)
Sex, girls	139 (50.6)	173 (49.6)
	Median (25; 75 percentile)	Median (25; 75 percentile)
Age (months)	18.5 (18.1; 18.9)	60.4 (59.9; 60.9)
PFOS (ng/ml)	7.1 (4.5; 10.0)	4.7 (3.5; 6.3)
PFOA (ng/ml)	2.8 (2.0; 4.5)	2.2 (1.8; 2.8)
PFHxS (ng/ml)	0.2 (0.1; 0.4)	0.3 (0.2; 0.4)
PFNA (ng/ml)	1.0 (0.6; 1.5)	1.1 (0.8; 1.6)
PFDA (ng/ml)	0.3 (0.2; 0.4)	0.3 (0.2; 0.5)
Tetanus (IU/ml)	1.5 (0.5; 3.0)	0.1 (0.1; 0.3)
Diphtheria (IU/ml)	1.6 (0.8; 3.2)	0.1 (0.1; 0.3)
Duration of exclusive breastfeeding (months)	5 (4; 6)	
Duration of mixed breastfeeding (months)	4 (2; 7)	



EPA Pros & Cons

Pros

- Chooses a **critical effect** that is considered relevant by other authorities
- Results in a **protective** level
- Uses a **PBPK** model to project the equivalent human dose

Cons

- Uses human observational cohort **rejected** by other authorities for developing their safe doses
- Study **does not** specify the amounts of PCBs, known immunotoxicants, in the same or related cohorts
- Effects are based on **associations**
- Vaccine effects are **not clinically** relevant

Agency	US EPA (2023) DRAFT	
Several studies	Human observational studies of Budtz-Jorgensen and Grandjean, 2018; Wilkstrom et al., 2019; and Dong et al., 2019)	
Critical Effect	Anti-antibody response; low birth weight; increased cholesterol	
Human Dose (ug/kg-day)	0.000,275 to 0.000,305	Effects are assumed to be related to AUC; a PBPK model was used to determine human dose
Uncertainty Factor	Human = 10	Human variability
“Safe” Dose (ug/kg-day)	0.000,03	~3,000-fold lower than EPA’s methyl mercury RfD



EPA Pros & Cons

Pros

- Chooses **critical effects** that are considered relevant by other authorities
- Results in a **protective** level
- Uses a **PBPK** model to project the equivalent human dose

Cons

- Depends in part on human observational studies for immunotoxicity that have **been rejected** for safe dose development by other authorities
- Assumes that developmental effects are related to AUC which is **not according** to EPA (1991) guidelines
- A clinical PFOA study shows decreases in blood lipids (Convertino et al., 2018) that is **opposite** of EPA's studies



Reasonable Risk Questions

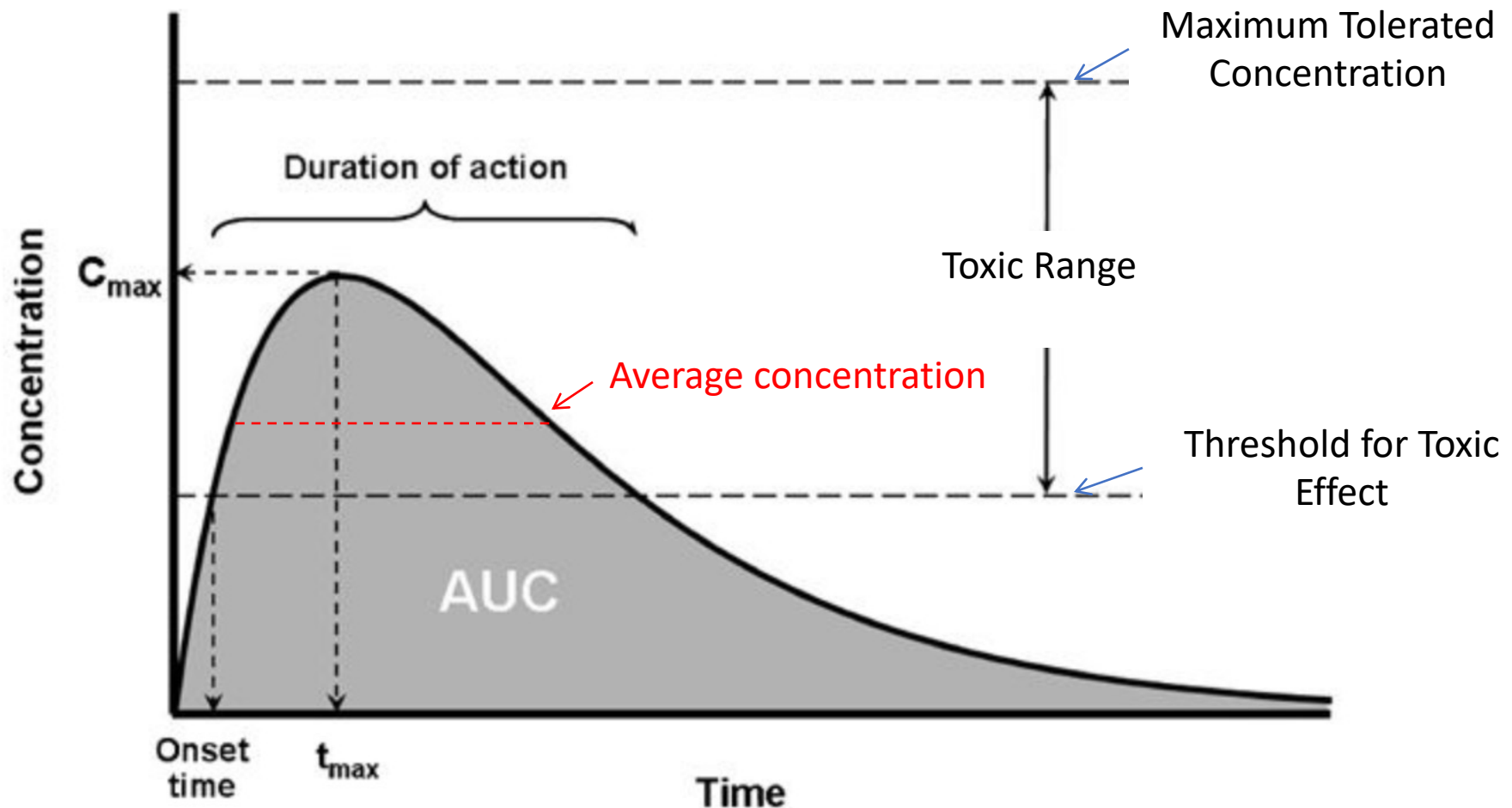
- What is the appropriate **critical effect** for PFOA? Immune? Liver? Neonatal?
- Should we use human population data as the basis of the safe PFOA dose rather than occupational or clinical exposures?
- Since kinetic **differences** among species appear to depend more on **elimination**, and less on absorption, metabolism and distribution:
 - What is the appropriate **dosimetric** measure of PFOA elimination in the chosen animal and humans?
 - Is one human group more **sensitive** than another through reduced elimination?



Appropriate Dosimetry?

Depends on the critical effect:

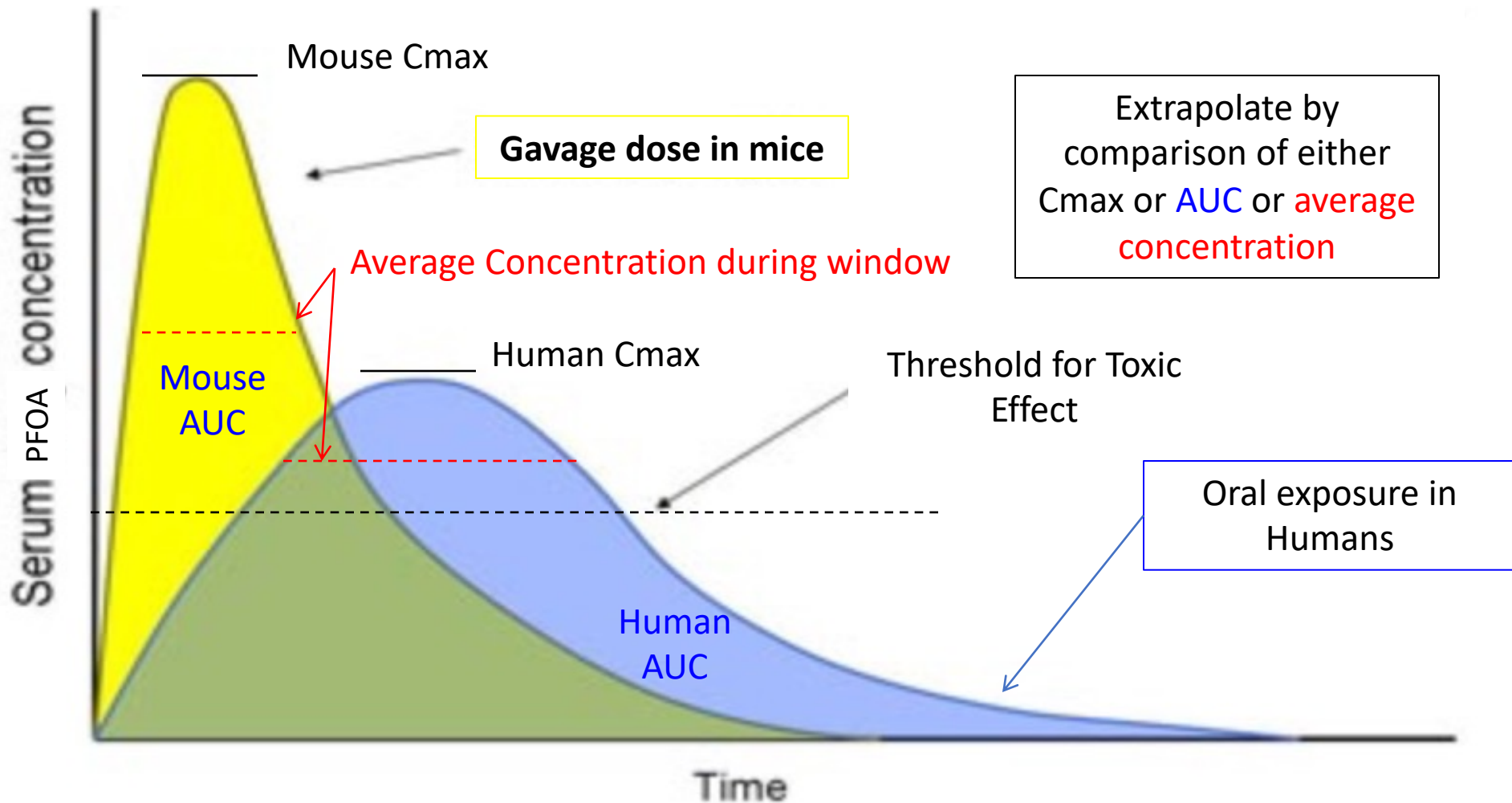
Area under Curve (AUC), Maximum concentration (C_{max}),
Steady State or Average Concentration





Appropriate Dosimetry?

...Depends on the critical effect





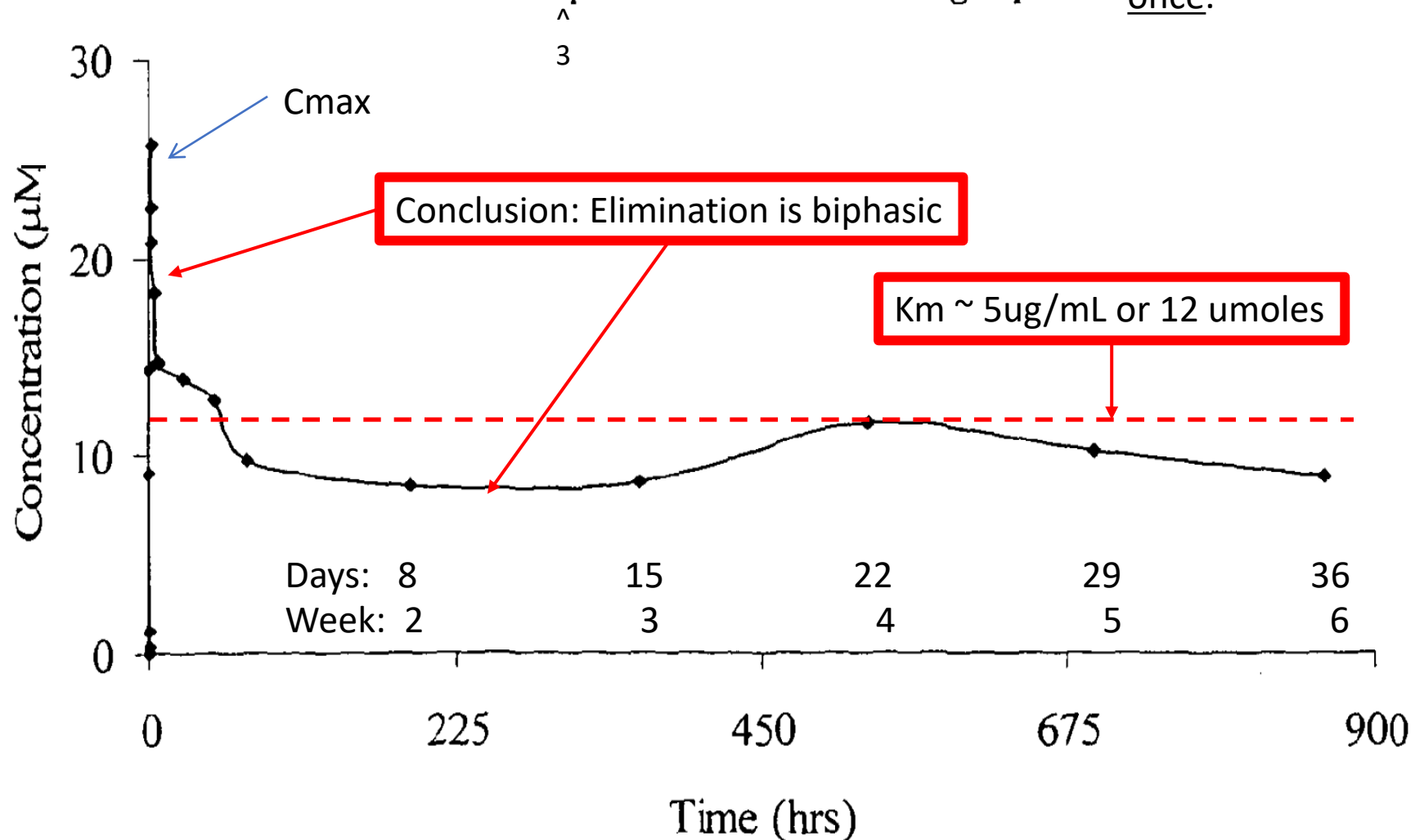
Human Dosimetry

- Elcombe et al. (2013) submitted a US Patent where PFOA was used as a cancer chemotherapeutic agent; recently published in part:
 - Convertino et al., 2018; blood lipids
 - Dourson et al., 2019; data-derived extrapolation factor
 - Dourson and Gadagbui, 2021; PFOA human half-life
- PFOA up to **1200 mg once per week to 43 humans** in various stages of cancer as a phase 1 therapeutic trial
- PFOA blood concentrations were carefully monitored; adequate kidney and liver function was a prerequisite

Figure 14

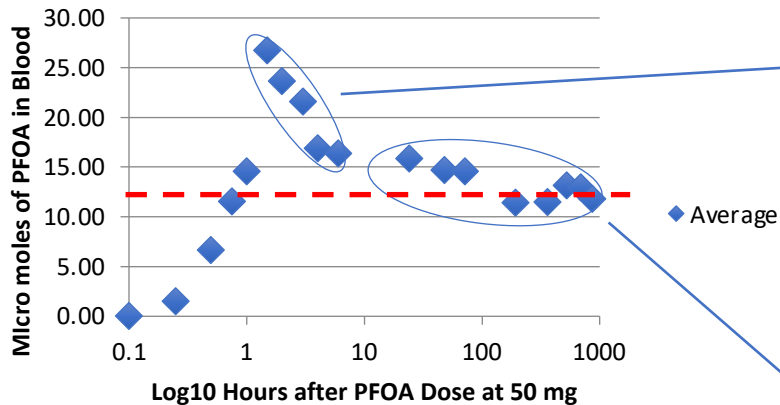
Elcombe et al. (2013)

**Average concentrations of Ammonium Perfluorooctanoate, up to day 37,
measured in patients dosed with 50mg capsules once.**

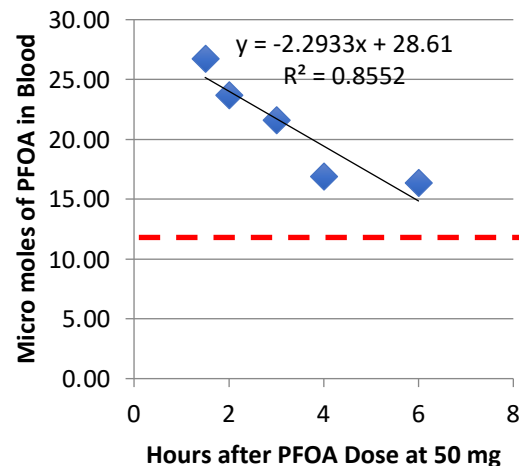


All Times: Patients 1, 2, and 3 given 50 mg of PFOA once (Elcombe et al., 2013)

Km-----

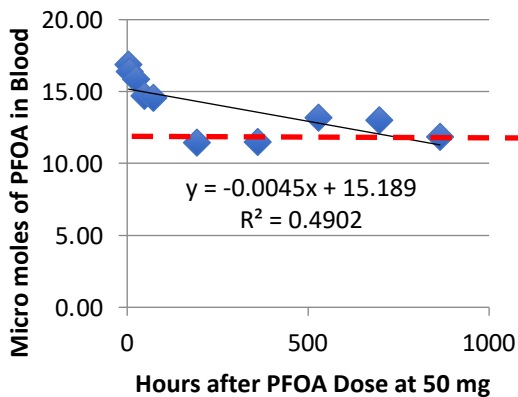


1st Phase Elimination: Patients 1, 2, and 3 given 50 mg of PFOA



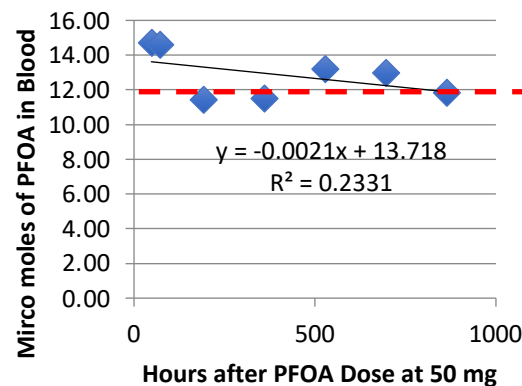
**Half life:
6 hours;**

2nd Phase Elimination: Patients 1, 2, and 3 given 50 mg of PFOA



**Half life:
70 days**

2nd Phase Elimination (alternate): Patients 1, 2, and 3



**Half life:
136 days**



Human Dosimetry

- TERA scientists
- Identified C_{max} and average concentration over time for each patient, compared mouse and human values, and developed a Data-Derived Extrapolation Factor (EPA, 2014)
 - DDEF = 1.3 for C_{max}
 - DDEF = 14 for average concentration during critical time
 - DDEF for AUC was not done since effects did not appear to fit with AUC-based outcome, and AUC is not the default for developmental toxicity EPA (1991). In contrast, both EPA and ATSDR used AUC for these developmental effects.
- Developed rough guess of human half-life at 0.5 to 1.5 years.



EPA (2016) Versus Alternate for PFOA

	<u>EPA (2016)</u>	<u>Alternate</u>
Developmental effects in mice at	1 mg/kg·day	1 mg/kg·day
Toxicokinetic adjustment to human-equivalent dose	÷189, AUC	÷ 14, Avg. window
Human-equivalent dose	0.0053	0.071
Lowest effect level → no effect level uncertainty factor	↓ ÷10	↓ ÷10
	5.3 x 10 ⁻⁴	0.0071
Animal → Human toxicodynamics uncertainty factor	↓ ÷3	↓ ÷3
	1.77 x 10 ⁻⁴	0.0024
Human → Sensitive Human uncertainty factor	↓ ÷10	↓ ÷10
Reference Dose (RfD, mg/kg-day)	1.77 x 10⁻⁵ (2 x 10⁻⁵)	0.00024
Drinking water intake/body weight for lactating women	↓ ÷ 0.054 L/kg·day	↓ ÷ 0.054 L/kg·day
Drinking water equivalent level (DWEL)	0.00037 mg/L	0.0044 mg/L
Relative Source Contribution	↓ X 0.2	↓ X 0.2
Lifetime Health Advisory	70 ng/L (ppt)	880 ng/L (ppt)

ARA PFOA Human Half-life Project

The Conundrum of the PFOA Half-life

2021 International Team (17 participants from 5 countries)

- Jerry Campbell, Ramboll, USA
- Harvey Clewell, Ramboll, USA
- Tony Cox, Cox and Associates, USA
- Michael Dourson, Toxicology Excellence for Risk Assessment, USA
- Shannon Ethridge, International Association of Plumbing, USA
- Norman Forsberg, Arcadis, U.S., Inc., USA
- Bernard Gadagbui, Toxicology Excellence for Risk Assessment, USA
- Ali Hamade, Oregon Health Authority, USA
- Ravi Naidu, CRC CARE, Australia
- Nathan Pechacek, Ecolab, USA
- Tiago Severo Peixe, State University of Londrina, Parana, Brazil
- Robyn Prueitt, Gradient, USA
- Andrew Prussia, Agency for Toxic Substances and Disease Registry, USA
- Mahesh Rachamalla, University of Saskatchewan, Canada
- Lorenz Rhomberg, Gradient, USA
- James Smith, Navy and Marine Corps Public Health Center, USA
- Nitin Verma, Chitkara University, India

Findings from Discussions

Two groups did not identify a single study upon which to base a PFOA half-life estimate, rather identified a subset of studies upon which an estimate may be based. One group identified a single key study.

Geometric mean averaging was considered a superior estimate of central tendency than either arithmetic mean or median approaches

The estimation of the volume of distribution influenced potentially by unmonitored PFOA exposures

Unmonitored PFOA and lack of PFOA specificity (straight chain vs branched isomers) identified as critical issues

Selected 3 studies identified as having the fewest issues

Conclusions from International Collaboration

- The central tendency of the human PFOA half-life is likely less than 2 years
- *Zhang et al., 2013* is the least encumbered study upon which to derive a PFOA half-life
- The single best value appears to be an upper bound geometric mean of **1.3 years** (Zhang et al., 2013)
- Recommendations:
 - More studies of similar design to Zhang et al., 2013
 - Clarification regarding background PFOA exposures of existing studies to enable potential adjustments to PFOA half-life estimates



Risk Characterization Next Steps

- Research
 - **Integrate human clinical and clearance studies** of PFOA into extrapolation from experimental animals, either by PBPK model, DDEF/CSAF, or perhaps a hybrid approach like Health Canada
 - Conduct additional **clearance studies** for PFOA and possibly other PFAS in a normal population of humans
- Assessment/Harmonization
 - First **understand** reasons for the various differences
 - Second, realize that not everyone can be **correct?**
 - Third, senior managers to give assessors **permission** to use the best science to reconsider their positions as appropriate



Summary

- North American PFOA safe doses are sufficiently different that Mexico, Canada and the United States should work together to come up with a single value or perhaps a reasonable range.
- The appropriate dosimeter for interspecies extrapolation depends on the **critical effect**. Dosimeter can be either Cmax, average concentration during a critical time, or AUC, but using AUC for developmental effects is not guideline recommended.
- Newly available human data for PFOA allow a comparison of kinetics between animals and humans and **should be considered** in the development of PFOA safe doses, since this may allow harmonization of disparate risk values.



Funding

- The origin of this work on PFOA came about from briefings of MLD as a senior advisor to the U.S. EPA Administrator in 2017.
- Findings were developed by scientists at TERA, vetted at an Alliance for Risk Assessment (*ARA*) meeting in February, 2019, and confirmed in an international collaboration of 17 scientists.
- 5 manuscripts have been published on this work; one of which won **paper of the year** from the Society of Toxicology's Regulatory and Safety Evaluation Specialty Section
- All efforts have been supported by the Internal Development Reserve funds of TERA.
- Prior work on PFAS safe doses by TERA staff has been supported by West Virginia, Health Canada, and US EPA.



Mission is to support the protection of public health by:

- Developing, reviewing and communicating risk assessment values and analyses;
 - Improving risk methods through research; and
 - Educating risk assessors, managers, and the public on risk assessment issues through collaborations
-
- TERA is a 501c3 environmental science NGO
 - Contact: Michael Dourson: dourson@tera.org