NTP
National Toxicology Program

Rapid Evaluation and Assessment of Chemical Toxicity (REACT): Per- and Polyfluoroalkyl Substances (PFAS)

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NTP Board of Scientific Counselors
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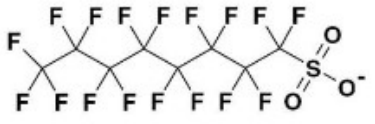
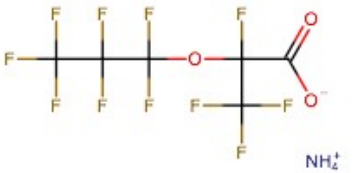
PFAS Background

- Diverse group of compounds
- Used in carpeting, apparels, upholstery, food paper wrappings, and fire-fighting foams
- Persistent and bioaccumulative
- Long chain perfluorinated chemicals are well studied; their use is in decline
- Shorter and branched chain compounds increasing in production and use; less well studied

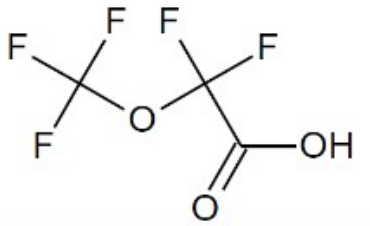


PFOS

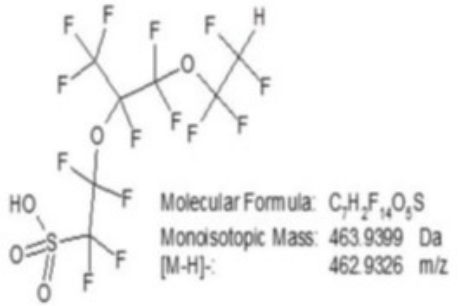
PFOA



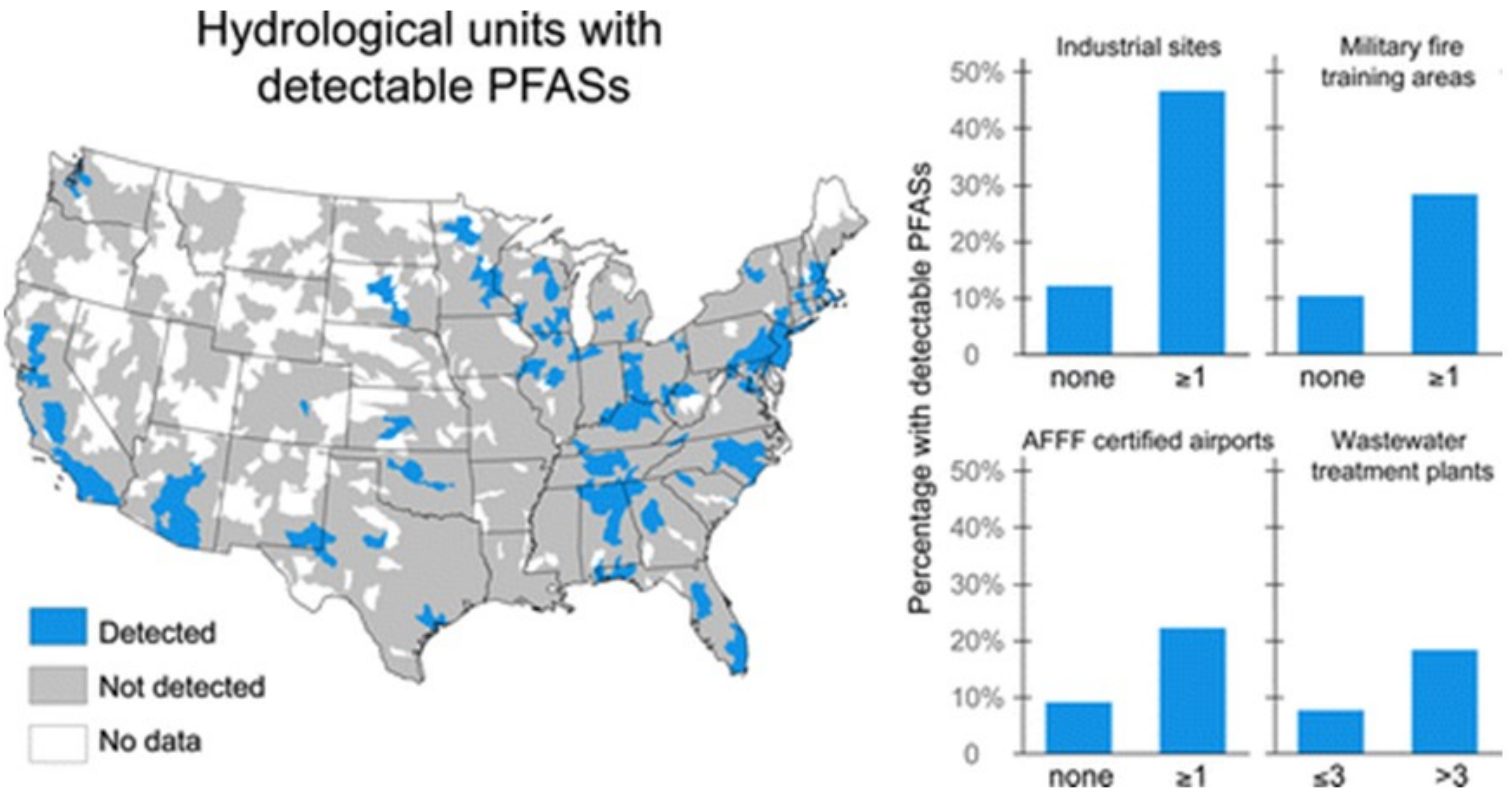
GenX



PFMOAA



Widespread Contamination to PFAS in US Watersheds



Hu et al., ES&T letters 2016 81% assoc with manufacturing site

Ongoing PFAS NTP Studies

- PFOA Chronic bioassay: Male and female rats. Exposure included a perinatal (GD 6 – PND 21) and non-perinatal component to determine if early life exposure alters response.
  - Pathology tables expected to be posted early 2018 and NTP Technical Report peer reviewed in late 2018
- 28-day toxicity studies: Male and Female Rats
  - 7 PFASs evaluated: PFBS, PFHxS, PFOS, PFHxA, PFOA, PFNA, and PFDA
    - Tables expected to posted early 2018 and Toxicity Reports to follow
- Toxicokinetic studies in male and female rats:
  - Evaluated PFBS, PFHxS, PFOS, PFHxA, PFOA, PFDA, and 8:2 fluorotelomer
- Immunotoxicity assessment:
  - PFDA evaluation in female rats and mice (manuscript submitted)
- Published in vitro studies:
  - In vitro mitochondrial toxicity evaluation of 16 PFASs using rat liver: Wallace *et al*.. *Toxicology Letters* 2013; 222(3)
  - In vitro assessment of immunotoxicity of 5 PFASs: Corsini *et al*. *Toxicology and Applied Pharmacology* 2012; 258(2)
  - In vitro assessment of immunotoxicity of PFOA and PFOS: Corsini *et al*. *Toxicology and Applied Pharmacology* 2011; 250(2)
  - In vitro neurotoxicity evaluation of 4 PFASs using PC12 cells: Slotkin *et al*. *Environmental Health Perspectives* 2008; 116(6)

PFOS and PFOA Alternatives of Interest

- Total number of PFAS >1500 chemicals.
  - Includes products, impurities and degradates.
- Significant Regulatory and Public Health Interest
  - USEPA: Several hundred of interest narrowing down to between 75-150.
  - FDA: Interested in PFAS used in packaging
  - DOD: Aqueous Fire Fighting Foams (AFFF).
  - ATSDR, CPSC, State public health agencies.
  - Federal Information Exchange on PFAS (Feb 2018)
    - National Science and Technology Council, Committee on Environment
    - EPA, DOD, NIH (co-chairs)

Challenges

- Nominations more complex.
  - Class nominations:
    - PFAS
    - Flame Retardants
    - Ionic Liquids
    - PAHs
- Expectations have changed
  - Impatience at pace of traditional NTP hazard assessment studies
  - Communication is now instantaneous (email, texts, etc.)
- Challenge for high throughput screening.
  - You can't just turn on the robot and get the data.

Problem Formulation and Approach

- What are the types of biological activity and toxicological information that NTP can develop in a *responsive timeframe* on these classes of chemicals?
  - How can this information be used to make public health decisions?
- What are the appropriate tools to bring to this problem?
- How do we organize this information to provide useful products?
- How do we report this biological activity/toxicological information in a timely manner?

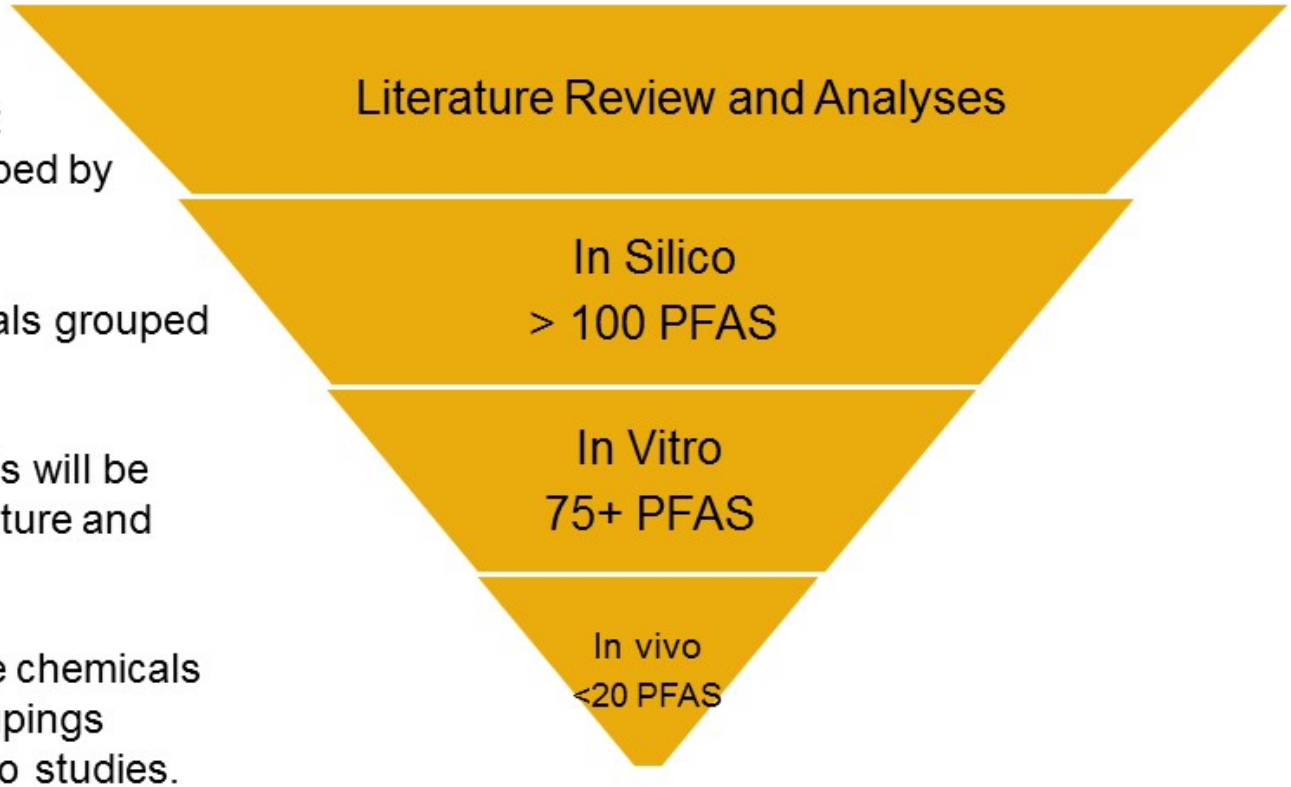
Screening and Testing Prioritization

Literature review: Chemicals Grouped by knowledge

In Silico: chemicals grouped by structure.

In vitro: chemicals will be grouped by structure and biology.

In vivo: prototype chemicals from in vitro groupings move on to in vivo studies.



PFAS Assessement is Based on Read Across

- Read Across
  - When the already available data of a data-rich substance (the source) is used for a data-poor substance (the target), which is considered similar enough to the source substance to use the same data as a basis for the safety assessment.
- Sufficient Similarity –
  - Use structure and in vitro data to group chemicals
  - NTP has developed statistical methods for Sufficient Similarity in our Gingko Biloba studies.
- Use the PFAS from the NTP 28 day studies as anchor chemicals for read across.
- Likely need to run other PFAS as anchors.

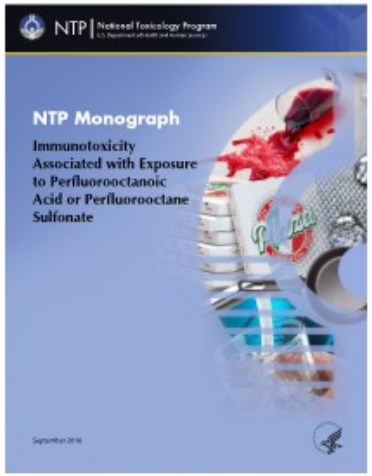
Staff team leads at NIEHS

- Literature Analyses – Andrew Rooney
- Chemistry – Suramya Waidyanatha
- In silico – Scott Auerbach
- In vitro – Sue Fenton
- In vivo – Chad Blystone
- Mixtures – Mike DeVito
- Reporting Plan – Mike DeVito





NTP Monograph

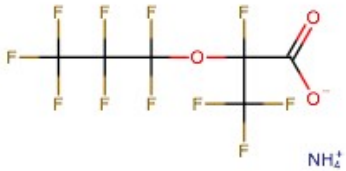


NTP Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate

- The NTP concludes that PFOA and PFOS are *presumed to be immune hazards to humans* based on a high level of evidence that PFOA and PFOS suppressed the antibody response in animal studies and a moderate level of evidence from studies in humans.
- https://ntp.niehs.nih.gov/go/749926

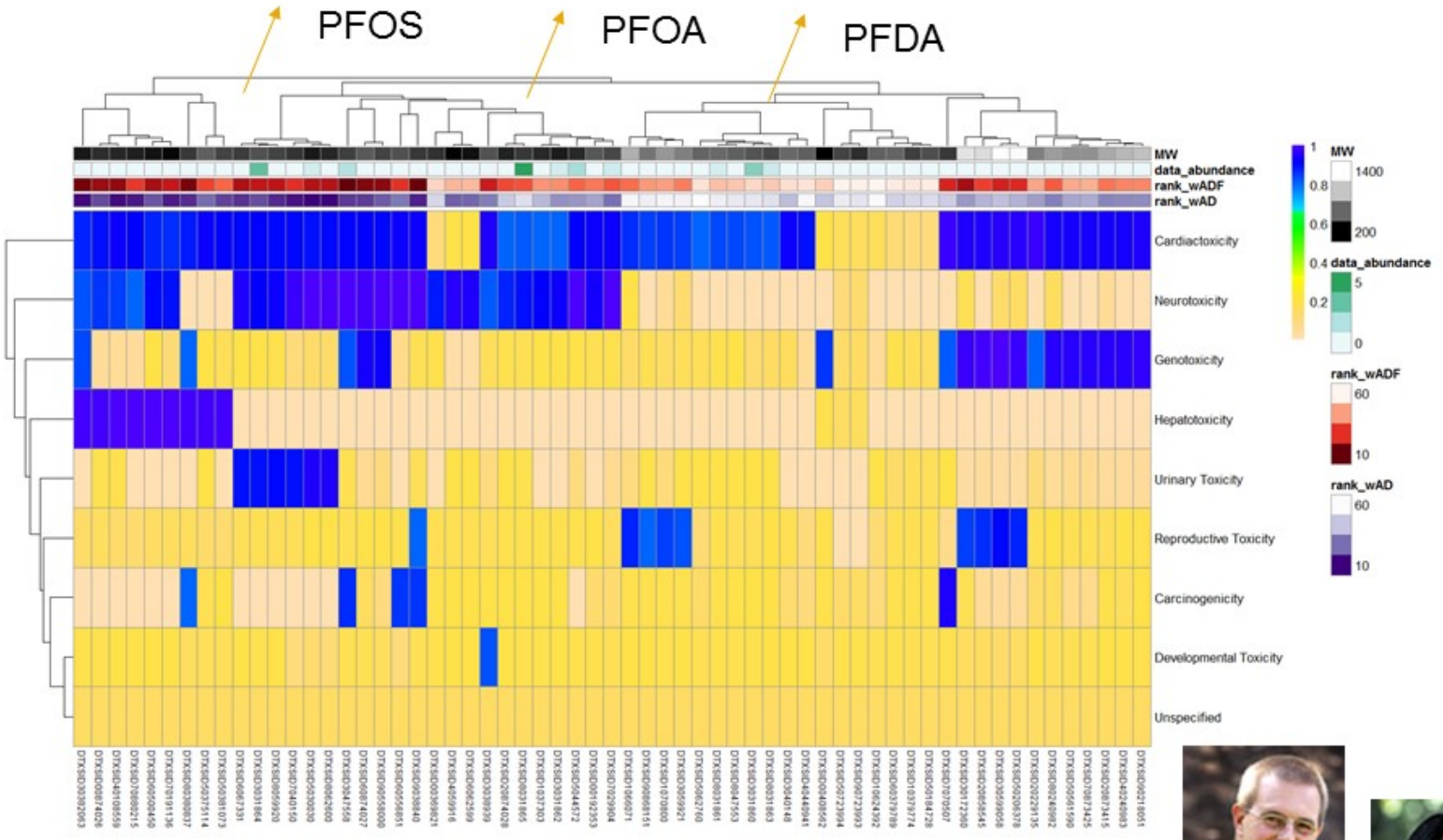
NIEHS/DNTP PFS *In Vivo* Studies

- Autoimmunity and PFAS in mice
- GenX developmental toxicity study in mice
- GenX in vivo pharmacokinetic studies
- GenX has been found in high concentrations in the Cape Fear River near Wilmington NC.



GenX

In Silico Predictions



NTP and EPA Collaborative Effort

Proposed *in vitro* assays for toxicological characterization of the EPA's 75 PFAS Chemical Library

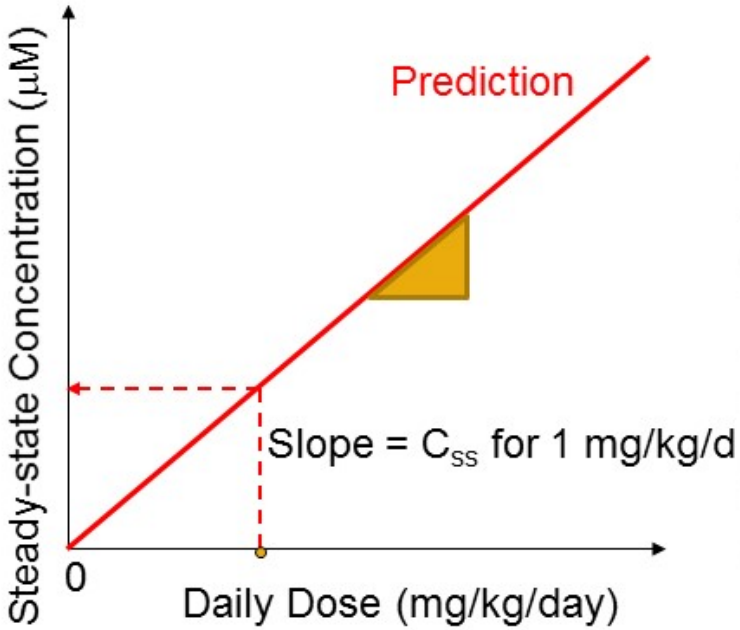
	NTP	EPA
Endpoint of Interest		
Hepatotoxicity	X	
Developmental Toxicity		X
Immunotoxicity	X	
Mitochondrial Toxicity	X	
Developmental Neurotoxicity		X
Hepatic Clearance	X	
Plasma Protein Binding		X
Enterohepatic Recirculation		X
In Vitro Disposition	X	X

Proposed Exploratory *in vitro* assays for toxicological characterization at NTP

Endpoint of Interest	Assay
Hepatotoxicity	Metabolomics in HepaRG
Immunotoxicity	NTP Imunotoxicity Contract
Placental Model	Using JEG cells
Mammary gland model	MCF-7 cell milk protein production
Renal Transport	Renal proximal tubule permeability assay in rats and humans

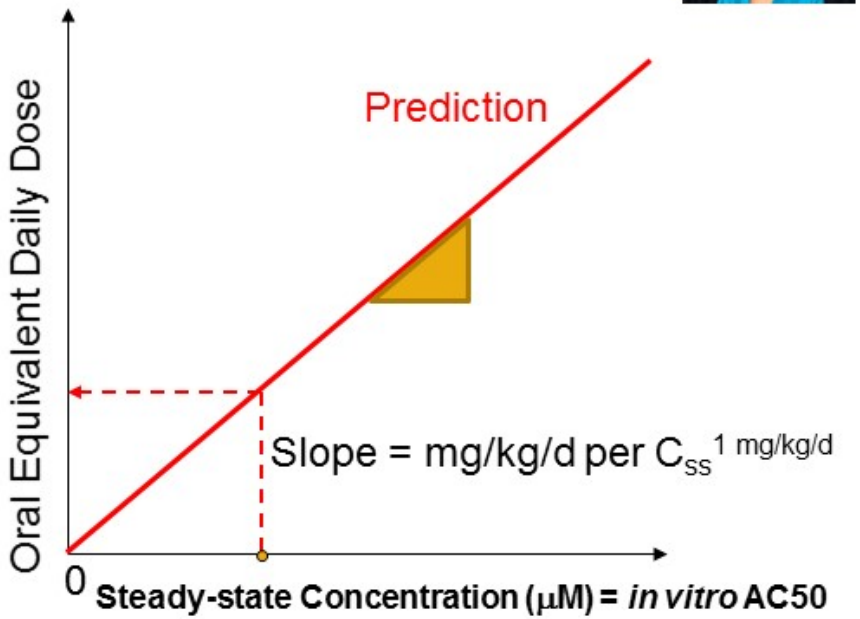
In Vitro To In Vivo Extrapolation: IVIVE

Steady state in vitro-in vivo extrapolation assumption:
blood::tissue partitioning ≈ cells::medium partitioning



$$C_{ss} = \frac{\text{oral dose rate}}{(GFR * F_{ub}) + \left(Q_l * F_{ub} * \frac{Cl_{int}}{Q_l + F_{ub} * Cl_{int}}\right)}$$

Wetmore *et al.* (2012)



- Swap the axes (this is the "reverse" part of reverse dosimetry)
- Can divide bioactive concentration by C_{ss} for a 1 mg/kg/day dose to get oral equivalent dose

Slide from John Wambaugh

In vivo studies

- Based on in vitro groupings, potency, IVIVE, environmental and human exposure.
  - 5-day rat hepatic transcriptomic assay
  - 28 day toxicity studies
  - Other in vivo studies possible for a limited number of PFAS

Products from REACT

- In vitro characterization and read-across grouping of PFAS chemicals
- Estimates of oral equivalent dose to attain Cmax or Css equivalent to *in vitro* Points of Departure.
- In vivo studies on limited numbers of chemicals that provide sufficient anchors for read-across.

REACT Approach: Note of Caution

- Not every tool will work for every class of chemicals!
  - 5 day adult transcriptomic study may not predict the point of departures for developmental effects
  - Need to understand when a tool is useful and when it is not
  - We need to adapt to the problem

PFAS Mixtures Assessment

- Are the effects of PFAS mixtures dose additive?
  - NTP will evaluate dose addition using laboratory-prepared mixtures. Initial mixtures will be based on water sample analyses from Mark Strynar (ORD/USEPA).
- Can the toxicity of commercial mixtures of Aqueous Fire-Fighting Foam for MIL Specs (AFFF), be estimated based on the PFAS content?
  - NTP will evaluate the AFFF mixtures and prepare PFAS mixtures at the same mixing ratios as in the formulation.
  - Compare and contrast the effects of the AFFF mixture to that of the PFAS mixtures

Summary

- Published a systematic review on PFOA immunotoxicity.
- A number of in vivo studies are at various stages of development.
  - Publications from NTP Laboratory on PFOA.
  - Carcinogenicity and toxicity studies of PFOA.
  - 28-day toxicity studies in rats on 8 PFAS.
- Developing an approach that provides a rapid response to a large class of chemicals and mixtures
- Integrated approach that will incorporate data and information from:
  - In silico models.
  - In vitro models.
  - In vivo models.

Acknowledgements

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Questions