NTP
National Toxicology Program

NTP Approach to Testing of PFAS Chemicals

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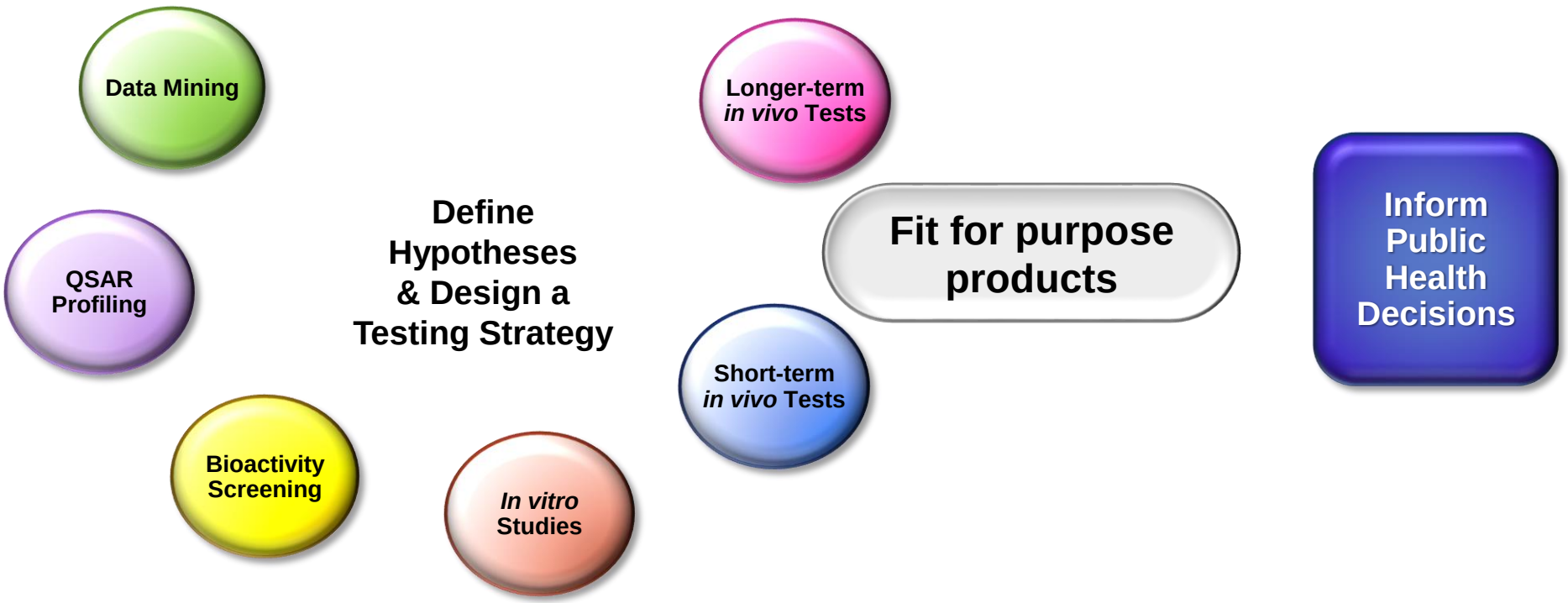
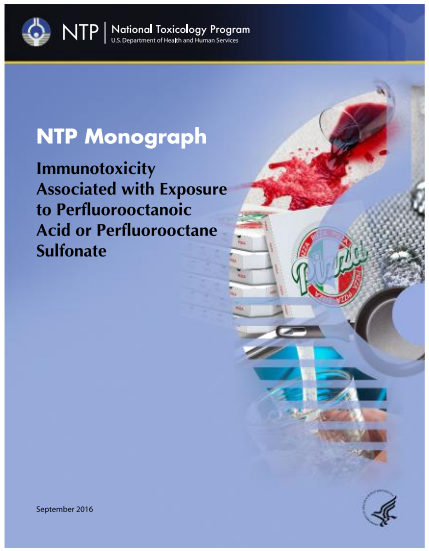
REACT PFAS

- Responsive Evaluation and Assessment of Chemical Toxicity (REACT)
  - REACT is a general approach that the NTP is developing to address environmental and public health challenges.
  - It is focused on fit for purpose solutions and involves literature mining, computational, in vitro and in vivo toxicological methods
  - REACT PFAS is a REACT project focused on Per and Polyfluorinated Alkyl Substances (PFAS)

DNTP Translational Toxicology Pipeline Plan



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
- Continuing effort working with NCEA to evaluate level of evidence for other PFAS

PFAS Guideline Toxicity Studies

- 28-Day Toxicity Studies: Comparing of Seven PFAS
  - Data available here: https://ntp.niehs.nih.gov/results/path/index.html
  - TOX Report 96: Sulfonates (2019) (PFBS, PFHxS, PFOS)
  - TOX Report 97: Carboxylates (2019) PFHxA, PFOA, PFNA, PFDA)
    - The shorter chained PFASs (perfluorohexanoic acid (PFHxA) and perfluorobutane sulfonic acid (PFBS)) induced similar toxicities as the longer chained analogues,
    - Shorter chained chemicals required higher doses.
    - There were similar effects within the liver and thyroid hormones that occurred for short-chain and long-chain PFAS.
- PFOA Two Year: Comparison of PFOA perinatal and non-perinatal effects
  - Data available https://ntp.niehs.nih.gov/results/path/index.html
  - Technical Report draft (TR-598) to be posted 2019 for peer review

REACT PFAS Challenges

- Too many chemicals for traditional approaches
  - EPA Library of 148 and NTP library with an additional 20 PFAS.
- How do we develop a screening approach?
  - Prototypes PFOA and PFOS.
  - Wide variety of structures, PFOA and PFOS may not be good prototypes.
    - 53 structural classes;
    - 10 classes in the EPA 75 chemical library
  - Use QSAR modeling to aid in the choice of in vitro models and assays.

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?

PFAS Assessment 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.
- This is a collaborative effort with the NCCT/USEPA
- Use the PFAS from the NTP 28 day studies as anchor chemicals for read across.
- Likely need to run other PFAS as anchors.

REACT PFAS Efforts

- EPA Library of ≈ 148 chemicals
  - Collaborative effort between NTP and EPA
  - Chemicals selected based on read across approaches
  - Forward looking library
- NTP PFAS Library
  - 10 Mil Spec AFFFs
  - ≈ 20 unique PFAS not in EPA library
  - Based on other agency interest and availability
- Chemical Specific Studies
  - Autoimmunity and PFAS in mice (Germolec)
  - GenX
    - Toxicokinetics (Birnbaum)
    - Transporters (Birnbaum)
      - Blood Brain Barrier
      - Renal
    - Placental Toxicity (Fenton)

EPA PFAS Screening Library Assays

Endpoint of Interest	NTP	EPA
High throughput transcriptomics		MCF-7 cells (maybe another cell type)
Hepatotoxicity	3D HepaRG cell viability and transcriptomics	
Developmental toxicity		Zebrafish embryo assay
Developmental neurotoxicity		Multi-Electrode Array assay in neonatal cortical cells and neurite outgrowth
Immunotoxicity	Cytokine alterations in human vascular endothelial cells (BioSeek)	
Hepatic Clearance	Metabolic clearance in 50 donor-pooled hepatocyte suspensions	
Plasma protein binding		Serum protein binding assay using human serum
Enterohepatic recirculation		Qualyst B-CLEAR hepatocyte transporter assay
In vitro disposition	In vitro disposition in cell lines under study	

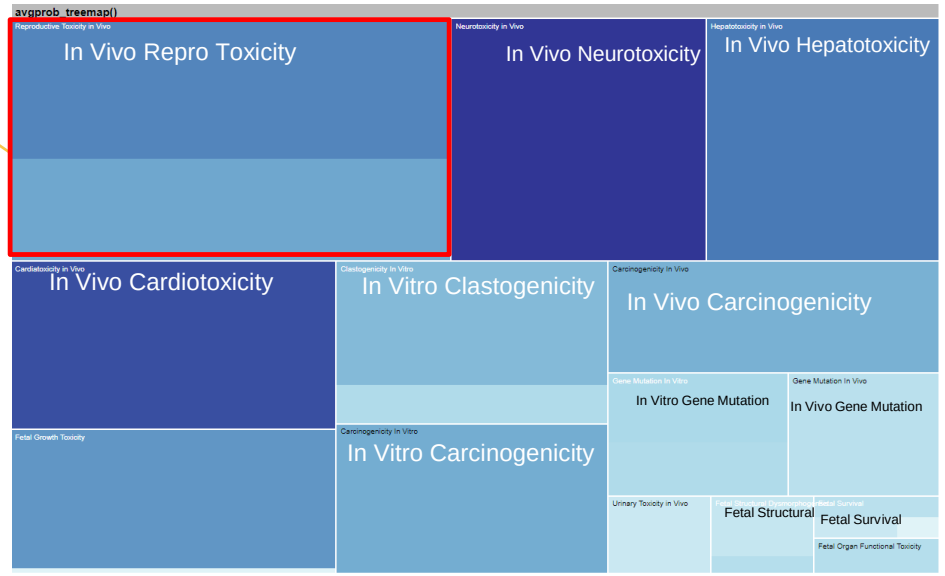
Leadscope Model Predictions

Almost all PFAS are predicted to have high probability of neurotoxicity and cardiotoxicity.

	Reproductive Toxicity in Vivo (parent)	
	Rodent Reproductive Adverse Effect (child)	Rodent Sperm Abnormality (child)
Cmpd#1	0.9	0.5
Cmpd#2	out of domain (set as 0.001)	0.4
Cmpd#3	0.8	0.8
Cmpd#4	0.8	0.8
…	…	…
Cmpd#100	0.6	out of domain (set as 0.001)
	avg = 0.6	avg = 0.5
	% of active = 95%	% of active = 75%

size

color



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Exploratory Assays

Endpoint of Interest	Assay
Immunotoxicity	Human and rat cytokine alterations in peripheral blood lymphocytes (contract)
Placental model	JEG cell viability and transcriptional changes(NTP Laboratory).
Milk protein production	Inhibition of milk protein production in MCF-7 cells (NTP Laboratory).
Renal Transport	Renal proximal tubule permeability assay in humans (NTP Laboratory and contract)
Embryoid Bodies (Human Embyronic Stem Cells)	Transcriptional and immunohistochemical markers of differentiation (NTP Laboratory).
Cardiotoxicity	On contract or collaboration

Products

- In vitro assessment of ≈ 150 PFAS.
  - Multiple biological endpoints to be used in read across
- IVIVE data and models for estimating oral equivalent exposures for PFAS.
- Grouping PFAS based on biological activity.
- Potential for follow up in vivo studies as needed.

REACT PFAS Efforts to Date

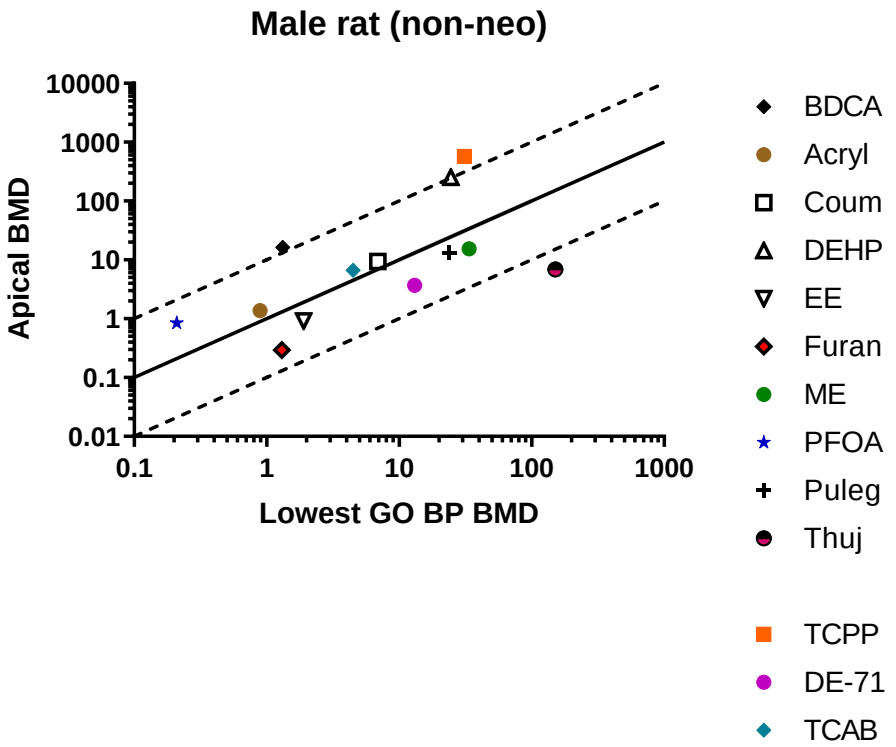
- Developed a data analysis pipeline in CEBS
- Developed a transcriptomic analysis and reporting pipeline
- Have evaluated part of the NTP Library for
  - Milk production
  - JEG cell viability
  - HepaRG cell viability and mitochondrial toxicity
- Developing rat renal transport model.
- GenX studies have begun.
- Obtained EPA Library.
- Developing analytical methods (have methods for 56 PFAS)
- PFOA 5-day in vivo transcriptomic study

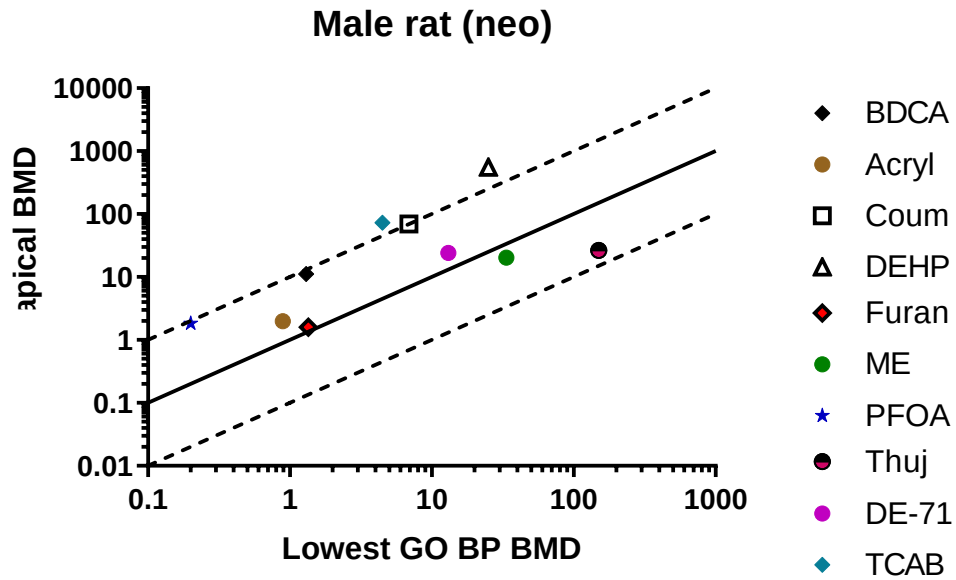
Short-term In Vivo Studies

What would be useful in vivo studies to evaluate prototype PFAS?

- 5 day Transcriptomic studies
  - Used to estimate points of departure from chronic toxicity studies
- 28 toxicity study
  - Provides a guideline study that adds to the PFAS we have already evaluated
- Dose-range study for Modified One Generation
  - Typically 5 dose levels (n=10)
  - Mostly looking at reproductive capability.
  - Animals do not go past PND 21
- Any others???

5-day Transcriptomic changes vs Apical changes from chronic study





Staff team leads at NIEHS

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

REACT PFAS Disciplinary Leads

- **Hepatotoxicity – Steve Ferguson**

- **Immunotoxicity – Dori Germolec**

- **Endocrine Toxicity – Sue Fenton**

- **In vitro toxicokinetics –**
- **Mike DeVito/Suramya Waidayanatha**

- **IVIVE – Nisha Sipes**

- **Embyroid Bodies – Erik Tokar**
- **GenX studies – Linda Birnbaum/Sue Fenton**

- Remember the overall project is a collaborative effort with the NCCT/USEPA
  - Data will come out on individual screens as completed
  - Data will eventually be combined for read across approaches