NTP National Toxicology Program

Evaluation of poly/perfluoroakyl substances (PFAS) for potential health effects

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Fall FRTR meeting

Comparative Study of Straight Chain PFAS

NTP rat studies started in 2006 (2004 nomination)

Evaluated seven PFAS plus used a PPARα positive (Wyeth-14,643) for comparison

- **PFOS, PFHxS, PFBS**
- **PFDA, PFNA, PFOA, PFHxA**

From Charles River Labs photo stock

Endpoints (n=10/dose/sex):

- **Organ Weights**
- **Histopathology**
- **Clinical Pathology (Clinical Chemistry; Hematology)**
- **Andrology and Estrous Cycling**
- **Hormones (Thyroid = T3, T4, fT4, TSH; Testosterone)**
- **Liver activity (PPARα/CAR genes; Acyl-CoA enzyme activity)**
- **Plasma and liver (male) PFAS levels**

Reporting of GLP Toxicity Data

- **28-Day Toxicity Studies**
  - Data available now: https://ntp.niehs.nih.gov/results/path/index.html
  - TOX Report 96: Sulfonates
  - TOX Report 97: Carboxylates
- **PFOA Two Year Carcinogenesis**
  - Data available very soon: https://ntp.niehs.nih.gov/testing/types/cartox/index.html
  - Technical Report draft to be posted late 2018/early 2019 for peer review

Toxicity of class largely defined by PFOA & PFOS

- **Major Health Outcomes**
  - **Endocrine Disruption**
  - **Development**
  - **Hepatotoxicity**
  - **Immune**
  - **Behavior**
  - **Cancer**

Looking for order in the PFAS universe

Chemical "Universe" problem

EPAHFR - EPA Chemicals associated with hydraulic fracturing

1640

Endocrine Disruption Screening Program (EDSP) Universe of Chemicals

9411

PFAS Master List of PFAS Substances

5061

PFASOECD (4730)

PFASKEMI (2396)

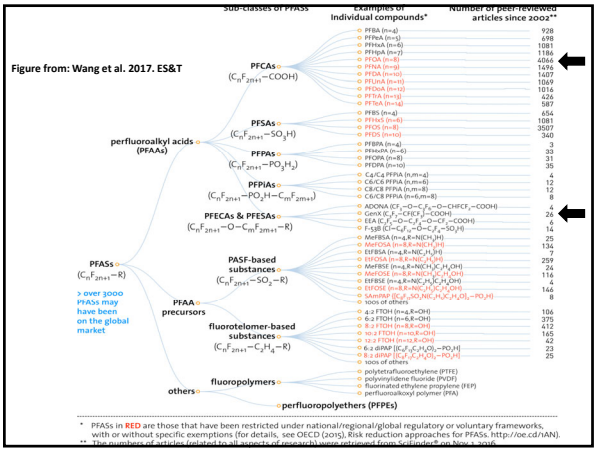
PFASTRIER (592)

EPAPFASINV (430)

EPAPFASINSOL(43)

EPAPFAS75S(74)

Slide courtesy of Ann Richard, US EPA



Challenges in Studying PFAS Health Effects

1. 5000+ on market – one by one will be replaced
2. Multiple routes of exposure that we don't fully understand (lacking data)
3. Half-lives and persistence are not predictable based on structure
   - Sex-based differences within a species
   - Species differences in clearance
4. Development as a sensitive period for this class
5. Mode of action not understood for any of the PFAS
6. Issues to address by in vitro testing: where is the chemical, solubility of compounds, IVIVE
7. Mixtures exposure problem

How can NTP generate faster responses?

Developed focused work-groups for REACT:
Responsive Evaluation and Assessment of Chemical Toxicity

Primary goal:
To provide enough targeted information in relatively short time frames for Centers/Agencies/Departments/Institutes or states to make decisions

- Currently, evaluating newer PFAS in an integrated fashion by using in silico, in vitro, and in vivo approaches
  - In silico assessment of the class using Leadscope QSAR
  - In vitro assessments of potential liver and other target tissue toxicity, chemical clearance, and developmental toxicity
  - In vivo assessments of PBPK, potential general, developmental, and immune toxicity
  - Communicate with our research colleagues to save time/money

Targets of interest

- Fetal development
  - Birth weight decrements (transient at low doses; permanent at high doses)
- Adipose
  - Overweight if developmentally exposed (transient?), underweight at high doses
- Breast/Mammary gland
  - Decreased breastfeeding duration/efficiency/ability
  - Mammary developmental delays with no change in other pubertal timepoints (in studies that have evaluated this tissue) – permanent change in those studies that have evaluated latent effects
- Liver
  - Hepatocellular hypertrophy, lipid deposition, enlarged relative liver weight
  - Liver disease (altered enzyme levels, cancer, etc)
- Endocrine disruption
  - down regulates ER pathways in MG and liver
  - Thyroid target: altered TT4 and fT4, but little effect on TSH
- Kidney
  - altered glomerular filtration rate; cancer

Ongoing Work on Uncharacterized PFAS

EPA library of 75 chemicals (underway…..)

- NTP/EPA collaborative effort plan

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

Blinded Evaluation of 45 PFAS at NTP

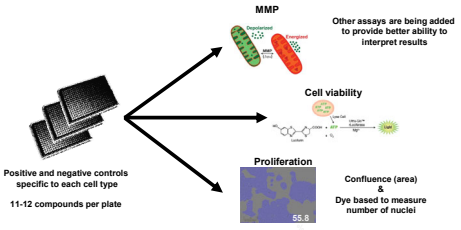
Specific In Vitro Assays

- Most grown in 384-well models

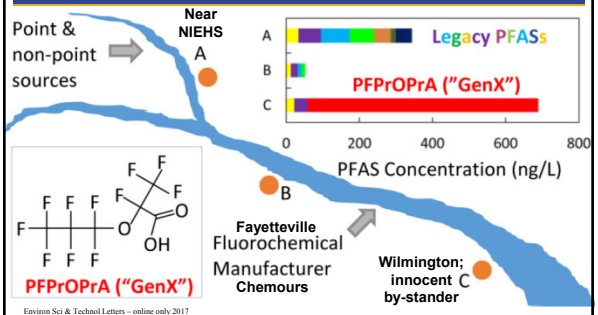
Endpoint of Interest	Assay
Adiposity	3T3-L1 high throughput assays for adipogenic and lipogenic effect (mouse)
Hepatotox	Metabolomics in HepaRG; cytotoxicity assays; mitochondrial function (human and rat)
Immunotox	NTP Immunotoxicity Contract
Placental Model	Using human JEG-3 cells for screening; Mouse model for evaluating fetal growth potential
Mammary gland model	Human MCF-7 cell proliferation assays and mouse HC-11 cytotoxicity & milk protein production assays
Renal Transport	Renal proximal tubule permeability assay in rats and humans (contracted)
Embryoid Bodies	Looking at transcriptional markers of differentiation and cell viability

Cell-based Screening Approach

Screening a panel of 45 PFAS (blinded to treatment) for effects on cell viability, mitochondrial membrane potential (MMP) and number, and cell proliferation rates in human and rodent cell lines



Point source NC water pollution



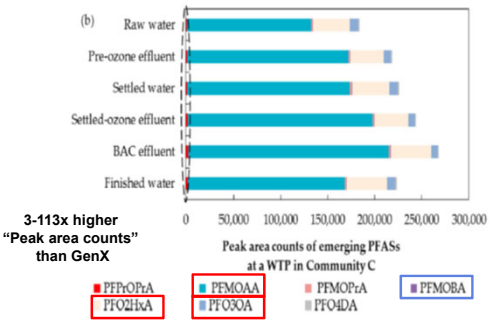
Environ Sci & Technol Letters – online only 2017

Legacy and Emerging Perfluoroalkyl Substances Are Important Drinking Water Contaminants in the Cape Fear River Watershed of North Carolina

Mei Sun, Elisa Arevalo, Mark Strynar, Andrew Lindstrom, Michael Richardson, Ben Kearns, Adam Pickett, Chris Smith, and Detlef R. U. Knappe

This is a mixtures problem

GenX, PFESA, and PFECAs

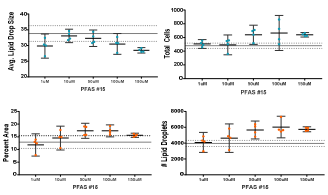


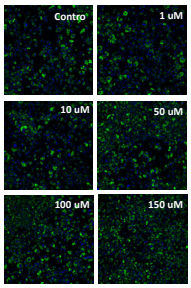
3-113x higher "Peak area counts" than GenX

Adipogenesis and Lipid Production

Blinded Treatment of Murine 3T3-L1 Preadipocytes

- Preadipocytes were grown to confluence and differentiation was induced with an MDI differentiation cocktail
- Cell count and number of lipid droplets were increased, while the average lipid droplet size decreased, resulting in the overall lipid area remaining unchanged





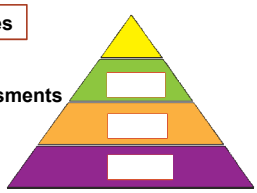
Gray line: control mean
Dashed gray lines: 95% confidence interval of controls

This is the work of Harlie Cope, post-bac IRTA

Preliminary data: Confidential

Some In Vivo Assessment Options

- 5-day toxicogenomics studies
- 28-day toxicity studies
- Development toxicity assessments (GD 6 – PND 21)



- Perinatal 90-day studies (GD 6 – PND 90)
- Targeted, hypothesis-based animal studies
- Reporting all audited data in CEBS (in vitro and in vivo)
- Published as technical reports and manuscripts

In vivo gestational exposure to PFOA or GenX

Study Design

Increased to n=11-13

Treatment Groups N = 6 - 8
Control (water)
1 mg/kg/day PFOA
5 mg/kg/day PFOA
7 mg/kg/day GenX
10 mg/kg/day GenX

Mouse strain: CD-1

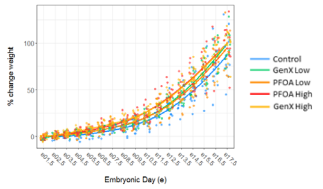
Bevin Blake
UNC CiTEM

Added post-natal time points

*Treatment groups were blinded to researchers with a color-coding system and experimental groups will be kept blinded until follow-up studies are completed. For data interpretation purposes, the control group has been identified (Control = water)

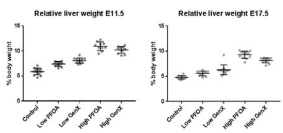
Preliminary data: Confidential

Maternal weight gain and liver weight in treated dams



Treatment	Increase in gestational weight gain relative to controls	
High GenX	19.1%	*
High PFOA	14.5%	*
Low GenX	12.5%	*
Low PFOA	8.7%	

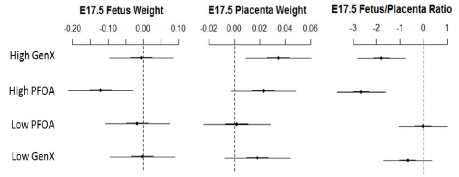
* = significant at $p \leq 0.05$



Pregnant mice gestationally exposed to high and low levels of PFOA or GenX exhibited increased relative liver weights at embryonic day 11.5 and 17.5, shown as percent of total body weight. N = 11-13, mean ± SE.

Preliminary data: Confidential

Fetal weight and length at E17.5 and E11.5



Mixed effect model estimates controlling for random effects of the litter and fixed effects of treatment group relative to controls (centered at 0). High PFOA and High GenX perturbed placental size and fetal placental ratios. N = 11-13 litters, 3 observations per litter. Mean ± 95% CI. These results suggest that PFOA and GenX may affect growth potential via different mechanisms.

Preliminary data: Confidential

A Problem of Mixtures

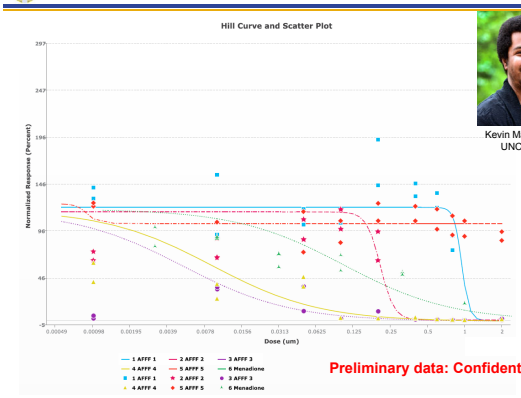
Two current collaborations to address these issues:

1. AFFF
- **Testing 10 AFFF for content, cyto-toxicity, etc**
- **Transcriptomics**
- **What fraction of the AFFF confers the activity?**

2. NC water problems
- **Test water concentrate from Cape Fear River basin**
- **Test as many single chemicals in that extract as we can purchase or isolate**

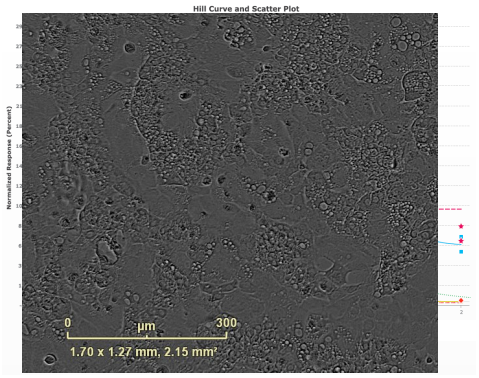
***Hope to develop collaborations on epidemiologic projects focused on legacy PFAS mixtures**

Evaluation of AFFF in Human Liver Cells



Kevin Mauge-Lewis
UNC CITEM

Preliminary data: Confidential



Steatosis Caused by AFFF #5 Exposure

Preliminary data: Confidential

2% concentration, 72 hours
- Cellular viability remained unaffected
- Lipid formation is visible

Current Challenges

We all need to work together……

- Communicate compounds that are being tested, together or separately – diluent is important for in vitro testing, don't want to duplicate efforts, difficult to acquire many of those we are interested in
- Half-lives and metabolism of most are not known and cannot be predicted by size or substitution group; the M≠F for several, adult and offspring are not equal
- Use additional high throughput methods to test large numbers of compounds at once - Txomics
- Inclusion of developmental stages in HTT
- Mode or mechanism of action studies should be done at human relevant exposures (which we also don't know for more than about 15)

REACT Team in NTP

Mike DeVito (REACT Lead)
Scott Auerbach (In silico lead)
Chad Blystone (In vivo lead)
Sue Fenton (In vitro lead)
Dori Germolec (Immunotoxicity lead)
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