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

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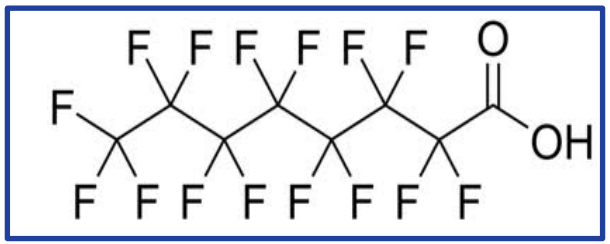
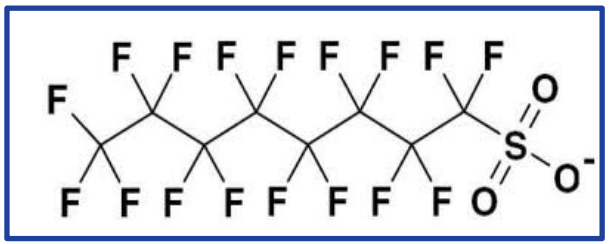
EDC Strategies Partnership

Per- and Polyfluoroalkyl Substances (PFAS)

- **Non-stick, water/grease/friction repellant, stain resistance**
  - **Over 5,000 compounds; many unknown formulations**
  - ***PFOA* (C8) was used in Teflon (GenX replacement)**
  - ***PFOS* (C8) was in Scotchgard and Gore-Tex (Adona replacement)**
- **Hundreds of other applications , e.g. cosmetics, dental floss, wiring, food contact surfaces, etc.**
- **Aqueous film forming foam (AFFF) containing mixture of PFAS; wide distribution across the U.S.**
  - **Over 600 military installations, airports, firefighter training sites**
- **Of high interest to US EPA, FDA, CDC and all states with industries or military installations**



PFOA



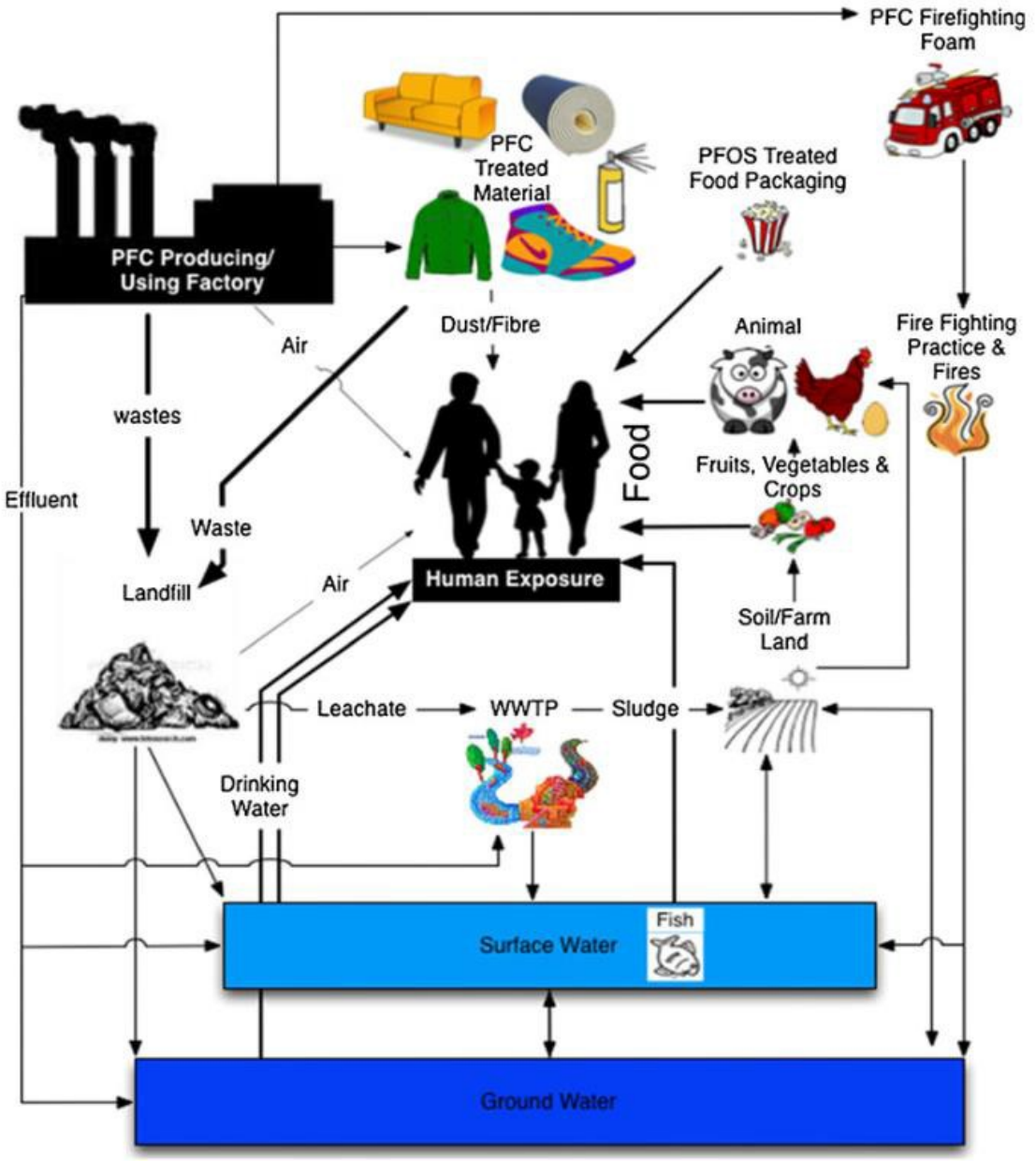
PFOS

Multi-media/Multi-route Exposure Pathways

Ingestion, inhalation, dermal via:

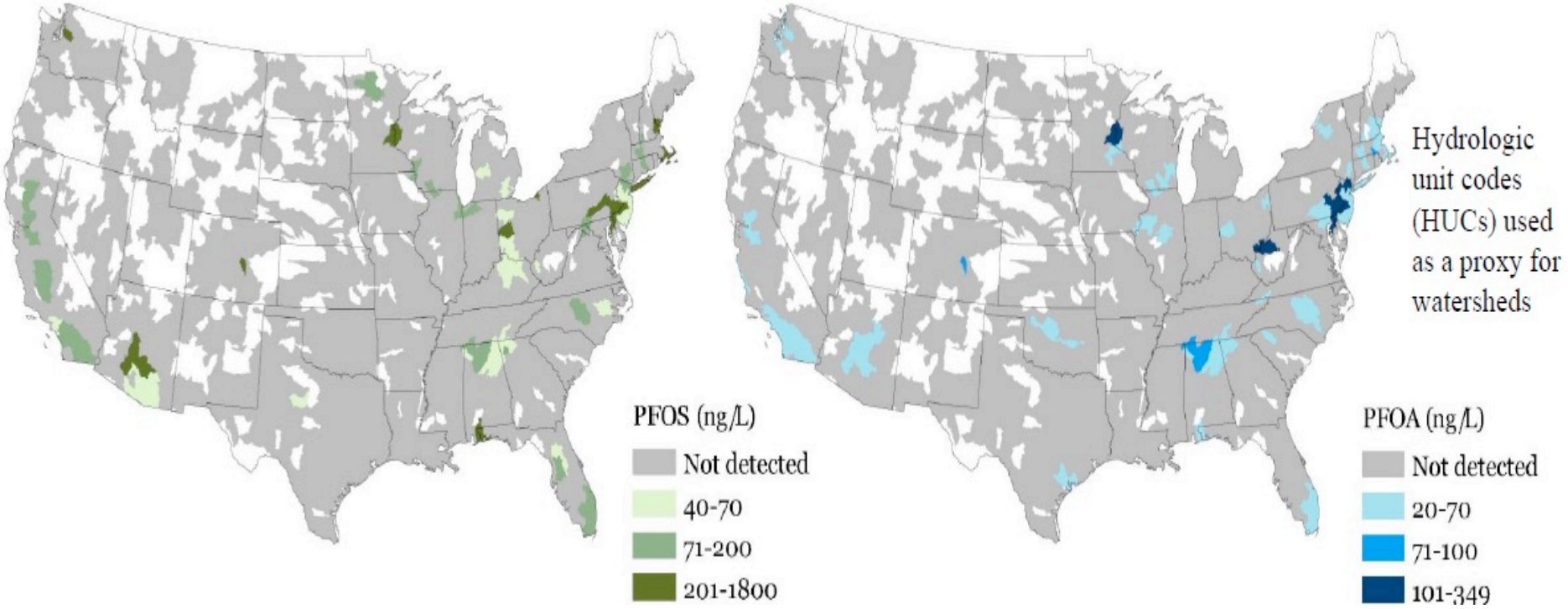
- industrial sites
- fire training/fighting facilities
- landfills
- wastewater treatment plants/biosolids
- consumer products/dust
- food items (e.g., fish/shellfish)
- food packaging

From Oliaei 2013,
Environmental Science Pollution Research



Who wants this kind of legacy?

PFOA & PFOS are not produced in the U.S. anymore!



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

Exposure to PFOA and PFOS

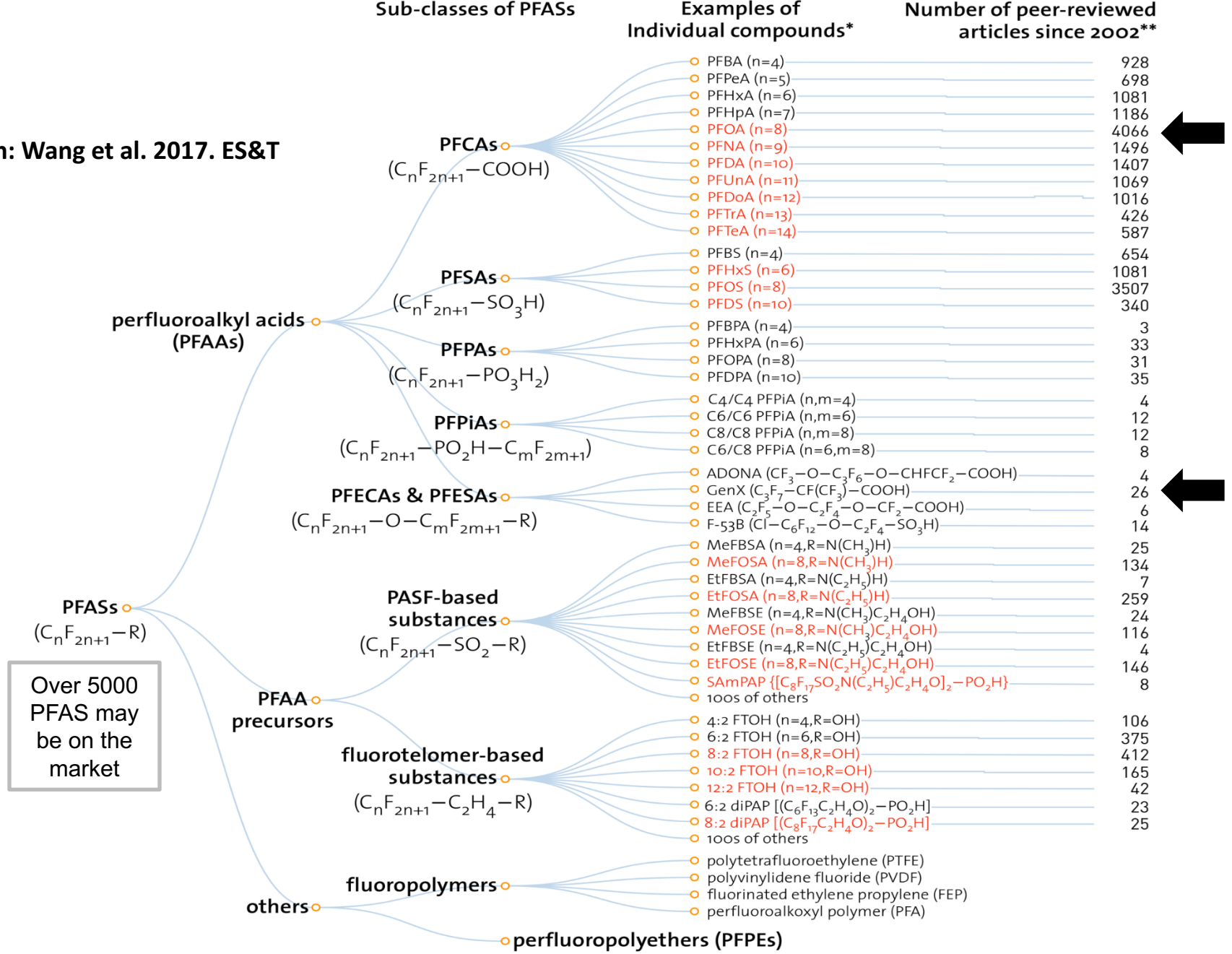
- **PFOA and PFOS are the most commonly detected perfluoroalkyl acids in environment and human serum**
- **PFOA and PFOS most studied for health effects**
- **PFOA and PFOS**
  - U.S. production eliminated; use and emissions reduced in U.S. and much of Europe through voluntary agreements
  - Not expected to degrade under typical environmental conditions
  - Not metabolized
  - Slower human elimination rates
    - Half-lives (2-8 years) humans vs. days or weeks in other animals

Geometric mean serum concentrations (µg/L) for US population

Survey years	PFOA	PFOS
1999-2000	**5.21** (4.72-5.74)	**30.4** (27.1-33.9)
2005-2006	**3.92** (3.48-4.42)	**17.1** (16.0-18.2)
2011-2012	**2.08** (1.95-2.22)	**6.31** (5.84-6.82)

Biomonitoring data from NHANES

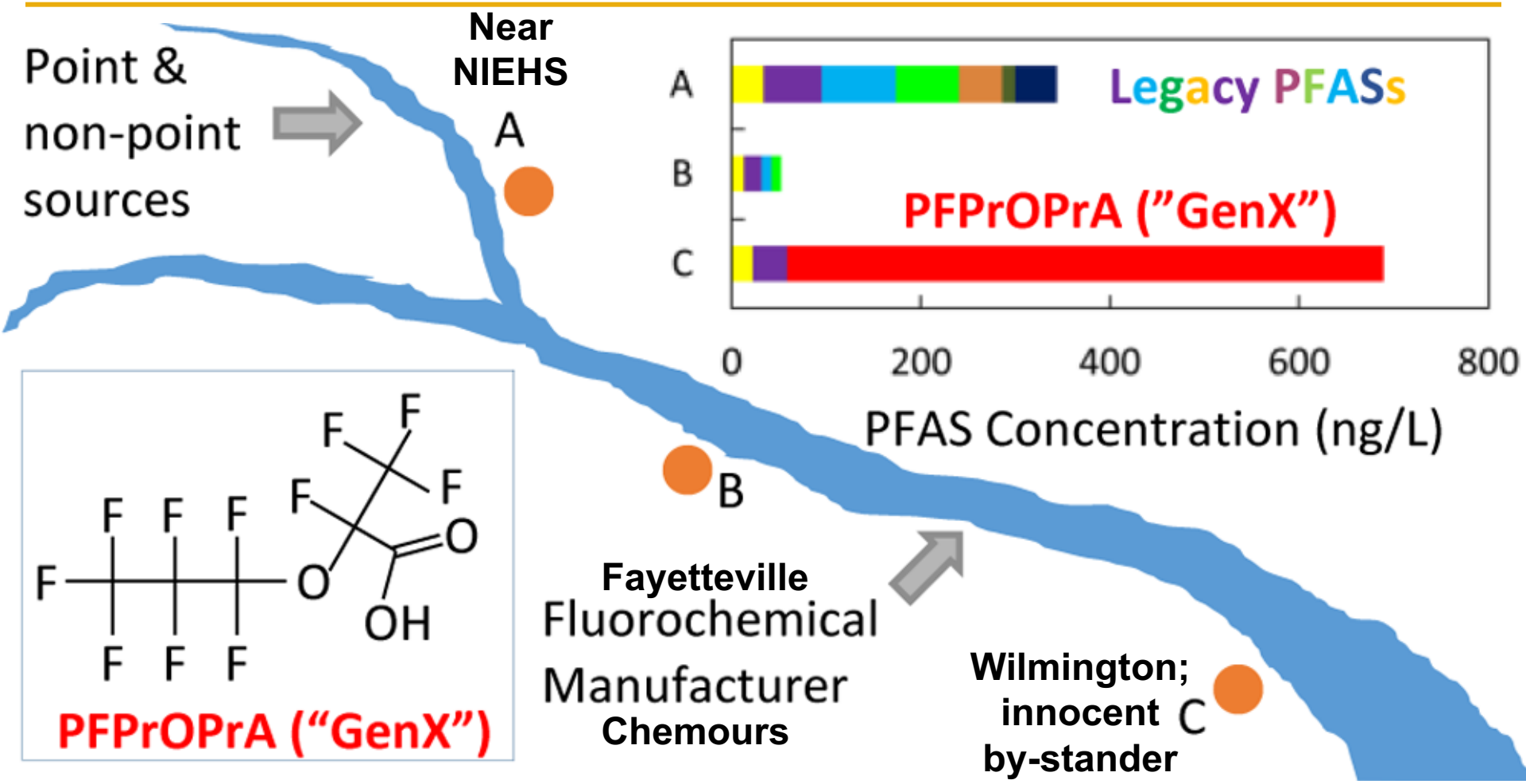
Figure from: Wang et al. 2017. ES&T



* PFASs in **RED** are those that have been restricted under national/regional/global regulatory or voluntary frameworks, with or without specific exemptions (for details, see OECD (2015), Risk reduction approaches for PFASs. http://oe.cd/1AN).

** The numbers of articles (related to all aspects of research) were retrieved from SciFinder® on Nov. 1, 2016.

Point source NC water pollution

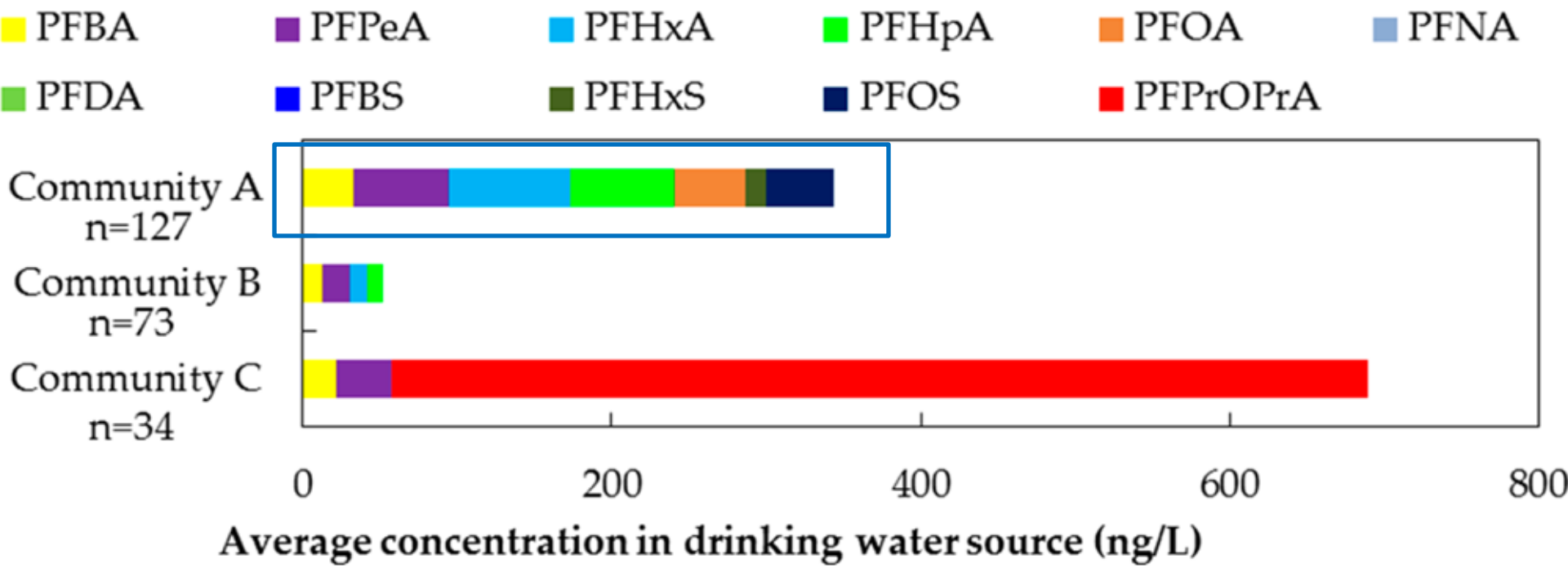


Environ Sci & Technol Letters – online only 2017

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

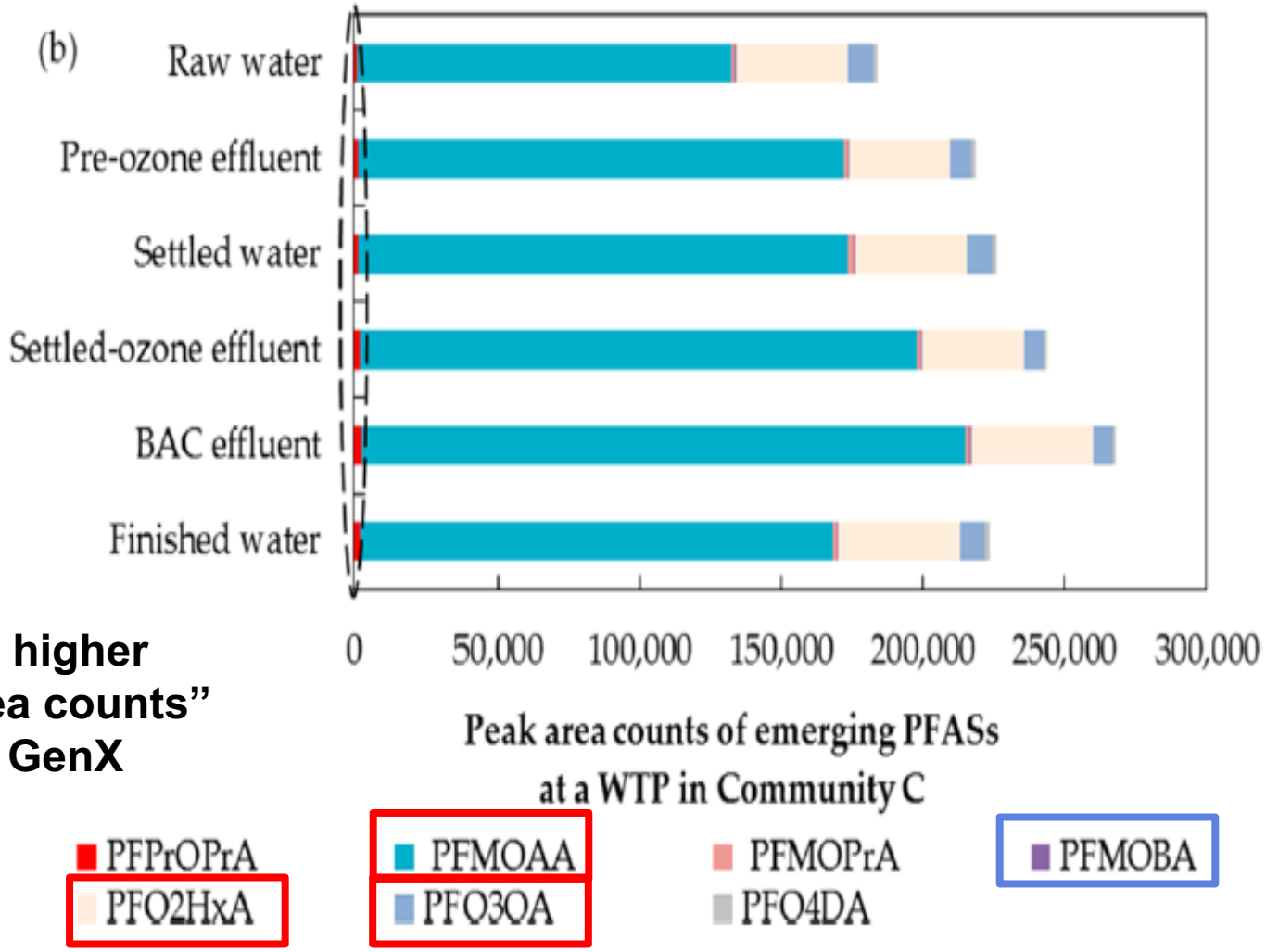
PFOS and PFOA over lifetime HAL



From Sun et al., 2016 ES&T Letters bio-solids recycling and industry sources

This is a mixtures problem

GenX, PFESA, and PFECAs



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

From Sun et al., 2016 ES&T Letters these are from industry sources

Evidence in Epidemiology (& rodent) Studies

PFOA exposure associated with:

- **Lower birth weights in infants (meta-analysis) [humans/mice]**
- **Enhanced weight gain in prenatally exposed young adults [h/m]**
- **Altered cholesterol levels [human/rat/mice]**
- **Kidney and testis cancer (C8 Science Panel) [rat]**
- **Immune system suppression (OHAT systematic review);[human/mice] immunization less effective, ulcerative colitis (C8 Science Panel)**
- **Gestational hypertension (pre-eclampsia; C8) [human]**
- **Thyroid dysfunction (C8 Science Panel) [human/rat/mice]**
- **Mammary gland (breast) changes [human/mice]**
  - **Delayed breast development in puberty/delayed menarche**
  - **Decreased ability to nurse offspring**

NTP Efforts

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

Primary goal:
To provide enough targeted information for Centers/Agencies/Departments/Institutes or states to make timely 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 toxicity based on PFOA/PFOS tissue targets
  - *In vivo* assessments of specific PFAS on an as needed basis
  - Enhanced communication with our research colleagues

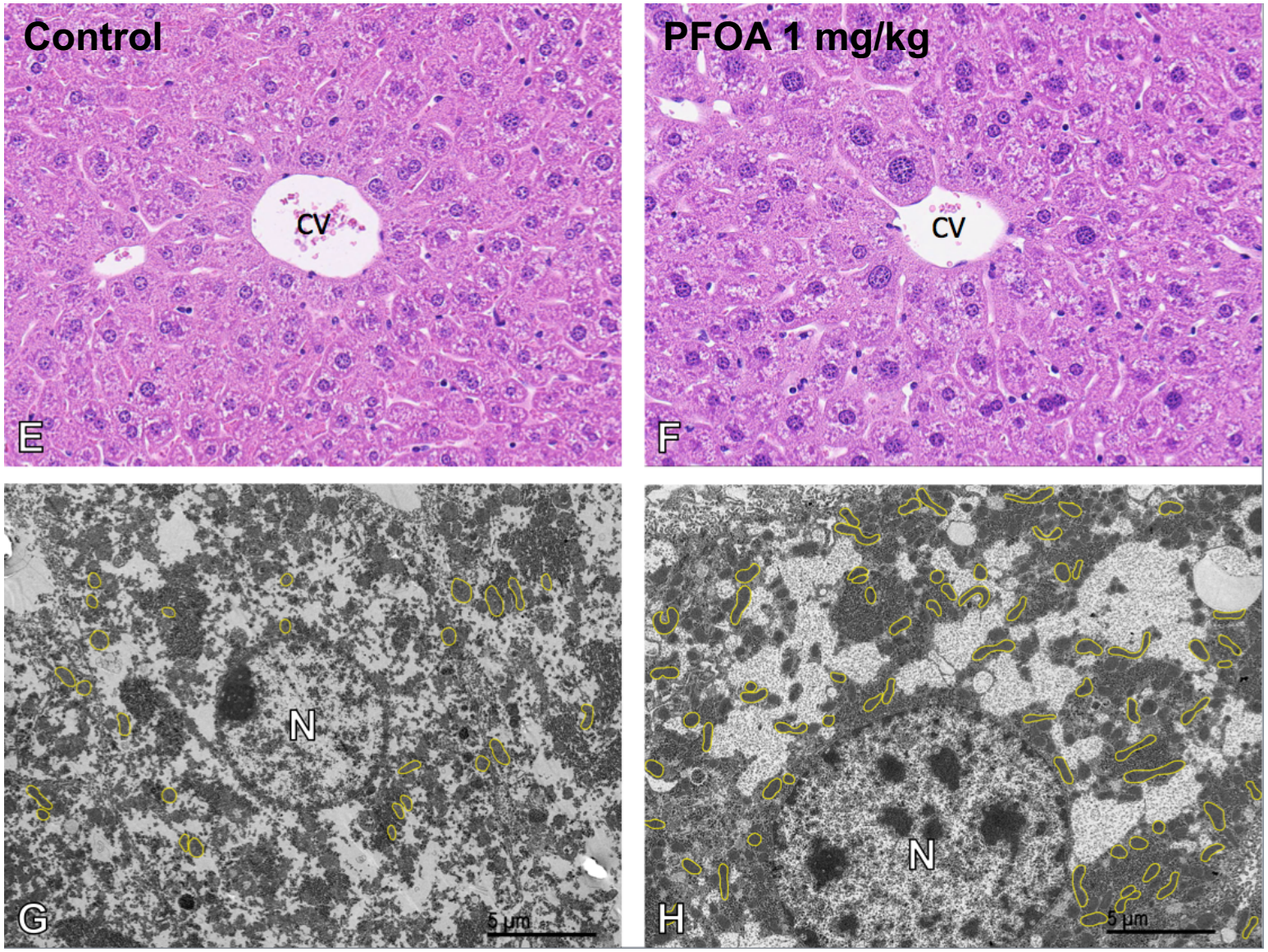
Blinded Evaluation of PFAS at NTP

Specific *In Vitro* Assays

- Most using 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

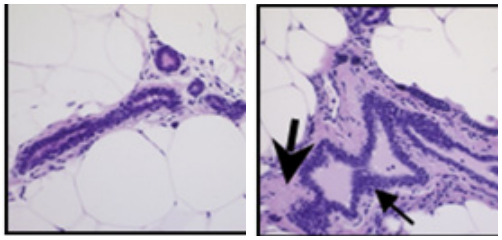
Hepatocellular Hypertrophy in CD-1 Mice



Late life effects on the mammary gland

CD-1 mice, GD 1-17 exposure, @ 18 mon

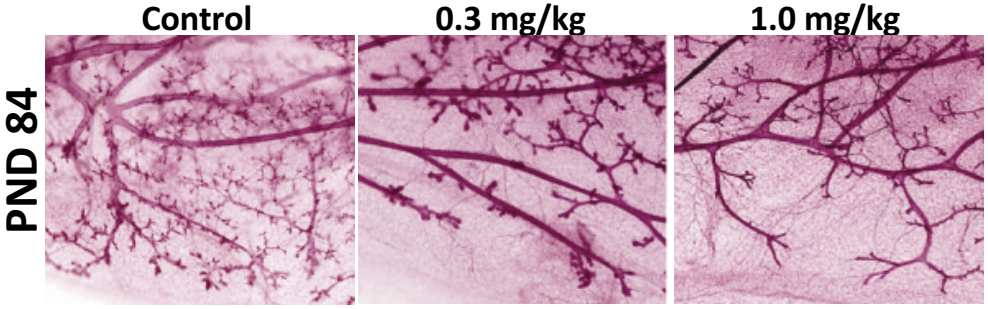
Control



5 mg/kg

White *et al*., 2009

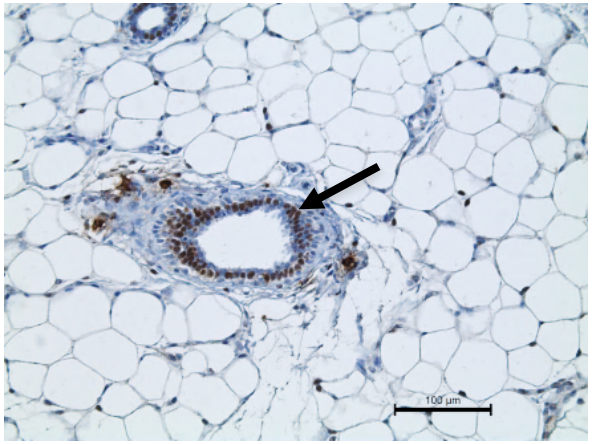
CD-1 mice, GD 1-17 exposure



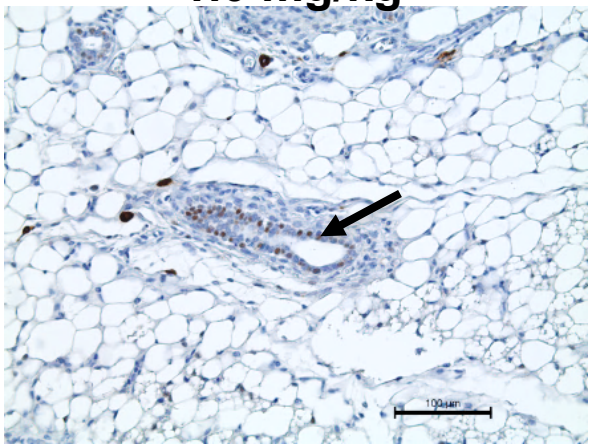
Macon *et al*., 2011

PFOA Mechanisms in the Mammary Gland

Control



1.0 mg/kg



*Note ER-α staining reduced in ductal epithelium (arrow) of adult animals prenatally PFOA exposed and dramatic remodeling of the fat pad

Cells other than epithelium are responding to PFOA!!

Prenatal PFOA & Early Adult Obesity

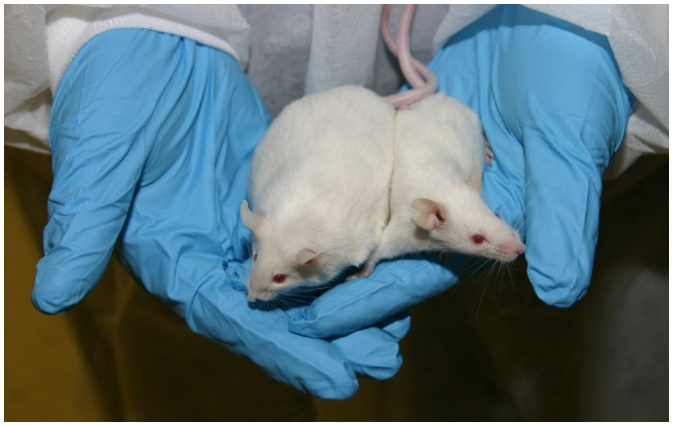
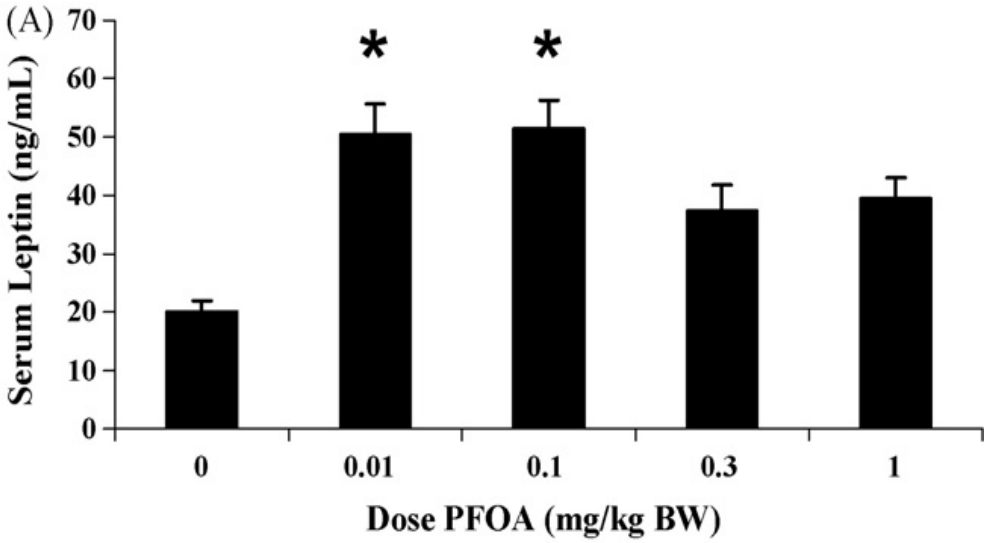
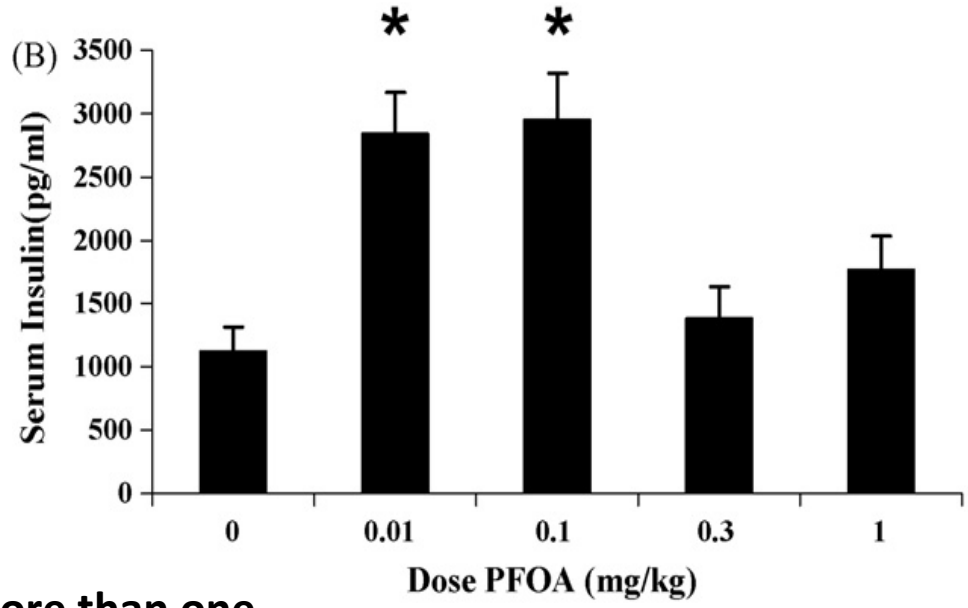


Photo from *Environ Health Perspect* Focus

Data in Hines et al, 2009, *Mol. Cell Endocrinol. 304: 97-105*





Supported in epidemiological studies:

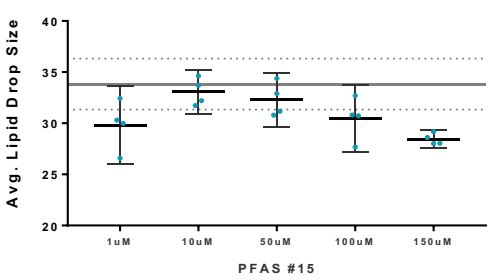
1. Increased gestational weight gain
Int J Environ Res Public Health. 2016
2. Overweight in 20 yr old Danish daughters exposed in utero.
Environ Health Perspect. 2012

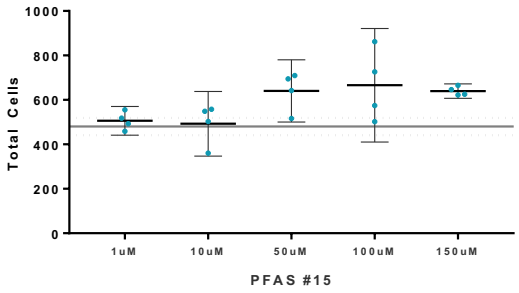
Mechanisms are not understood – Likely more than one.

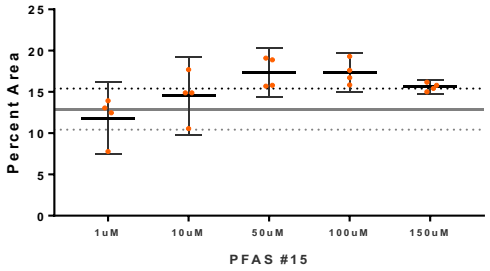
Adipogenesis and Lipid Production

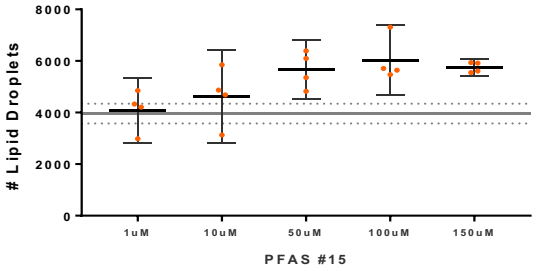
Blinded Treatment of Murine 3T3-L1 Preadipocytes

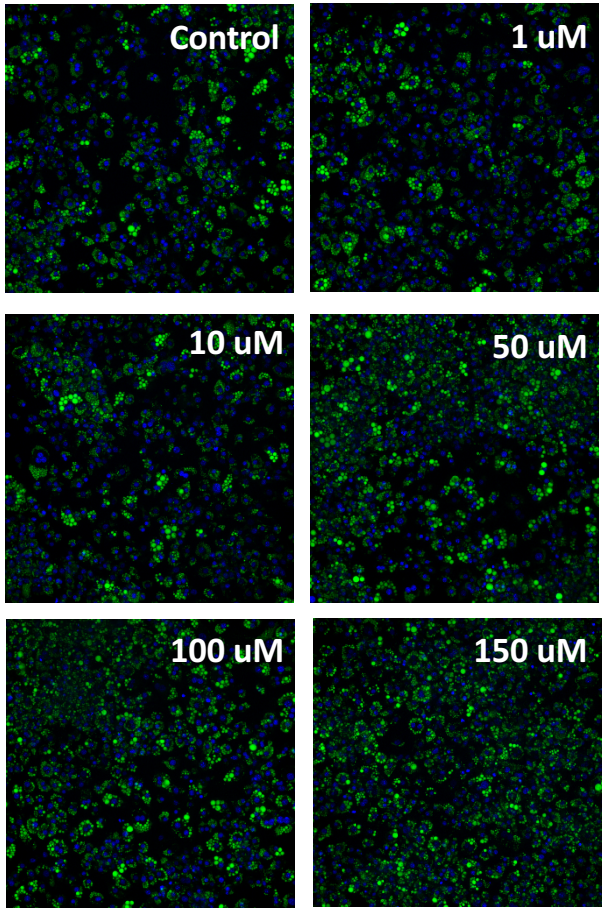
- Preadipocytes were grown to confluence and differentiation was induced with an MDI differentiation cocktail
- At Day 8, cell count and number of lipid droplets were increased, while the average lipid droplet size decreased, resulting in the overall lipid area remaining unchanged











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

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

Preliminary data: Do not cite

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?**

Kevin Mauge-Lewis
UNC CiTEM

2. NC water problems

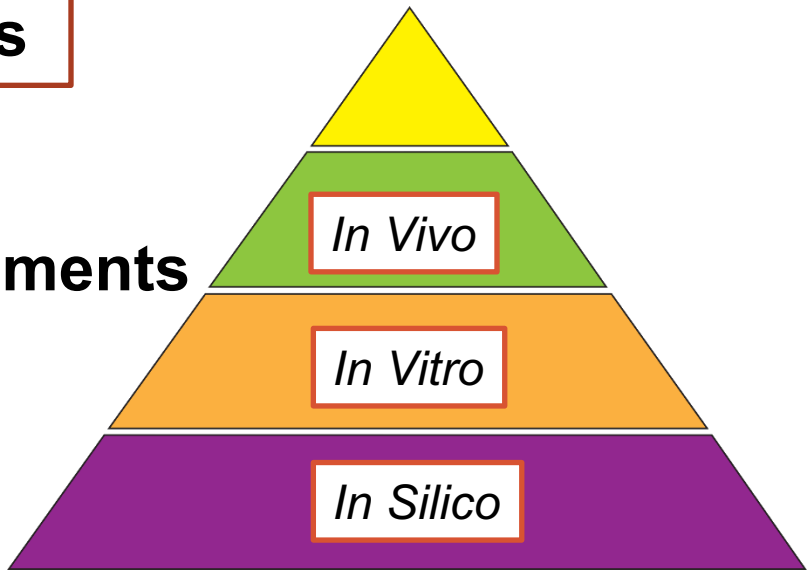
- **Test water concentrate from Cape Fear River basin**
- **Test as many single chemicals in that extract as we can purchase or isolate**

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

Future *In Vivo* Assessment Options

- 5-day toxicogenomics studies
- 28-day toxicity studies
- Development toxicity assessments

  (GD 6 – PND 21)



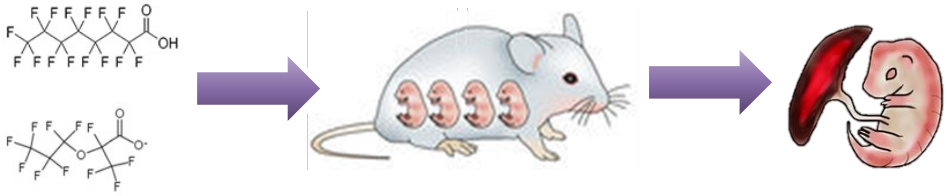
- Perinatal 90-day studies (GD 6 – PND 90)
- Studies in alternative models
- Targeted, hypothesis-based rodent studies
- Reporting all audited data in CEBS (in vitro and in vivo)
- Published as technical reports and manuscripts

In vivo gestational exposure to PFOA or GenX

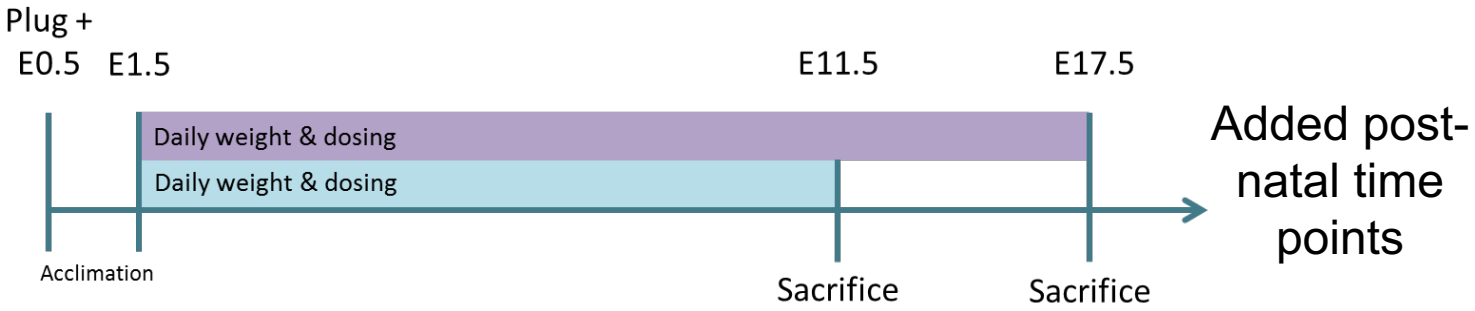
Study Design

Group	E11.5	E17.5
Control (water only)	N = 13	N = 13
1 mg/kg/day PFOA	N = 11	N = 12
5 mg/kg/day PFOA	N = 11	N = 12
2 mg/kg/day GenX	N = 12	N = 12
10 mg/kg/day GenX	N = 11	N = 12

Mouse strain: CD-1



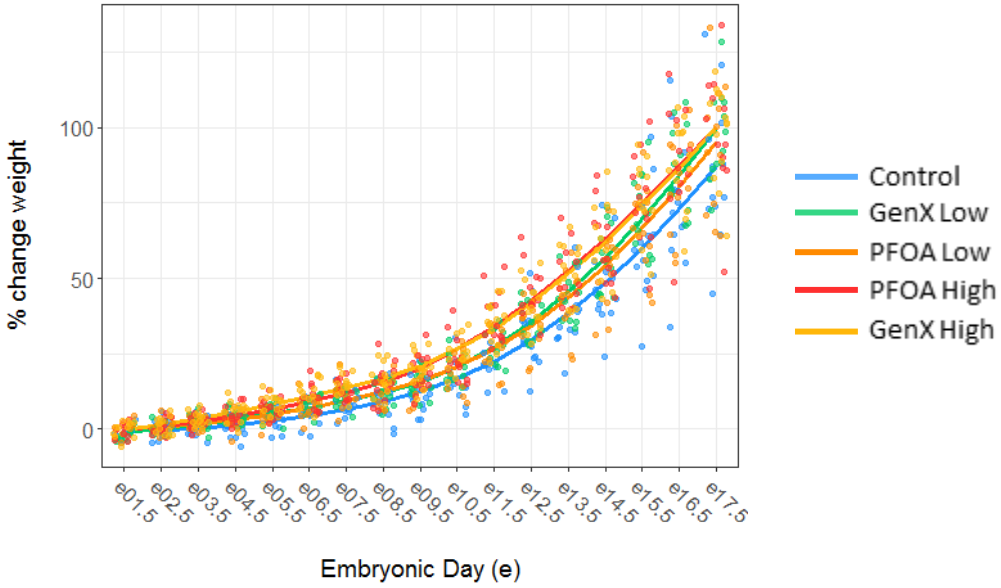
Bevin Blake
UNC CiTEM



*Treatment groups were blinded to researchers with a color-coding system and experimental groups were kept blinded until follow-up studies were completed. (Control = water)

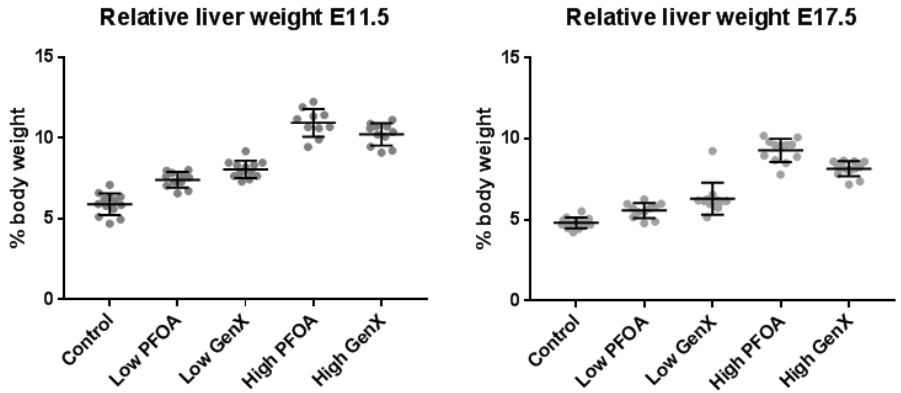
Preliminary data: Do not cite

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 $p \leq 0.05$



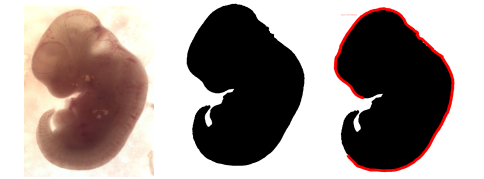
Pregnant mice gestationally exposed to high and low levels of PFOA or GenX exhibited increased relative liver weights at embryonic day 11.5 and 17.5, shown as percent of total body weight. N = 11-13, mean ± SE.

Preliminary data: Do not cite

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

How was data collected and analyzed?

- **Randomly chose 3 fetuses per dam**
- **Sex was determined (genotypic)**
- **Placenta was flash frozen**



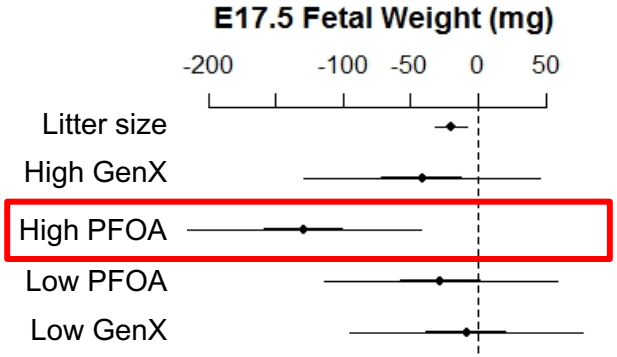
E11.5
17.00 mm

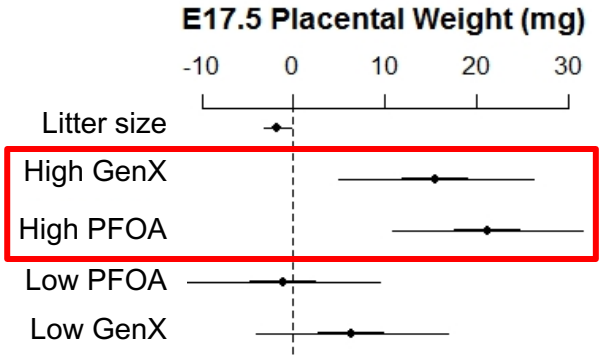
E17.5
36.54 mm

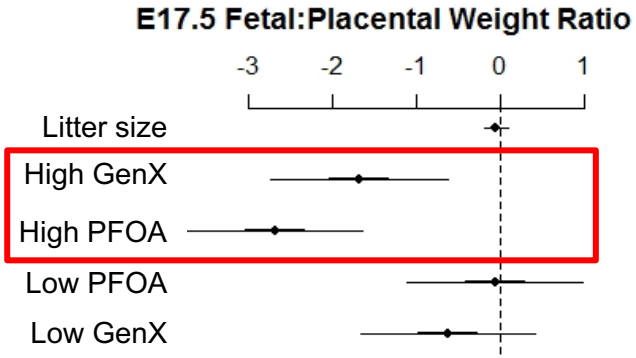
Mixed effect model estimates controlling for random effects of the litter and fixed effects of treatment group relative to controls (centered at 0). High PFOA and High GenX perturbed placental size and fetal placental ratios. N = 11-13 litters, 3 observations per litter.

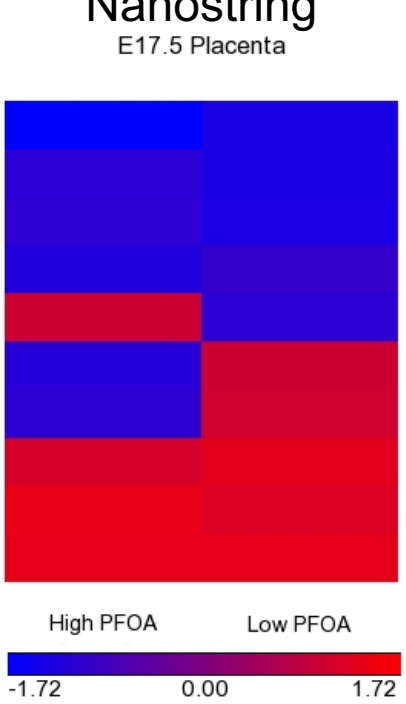
Preliminary data: Do not cite

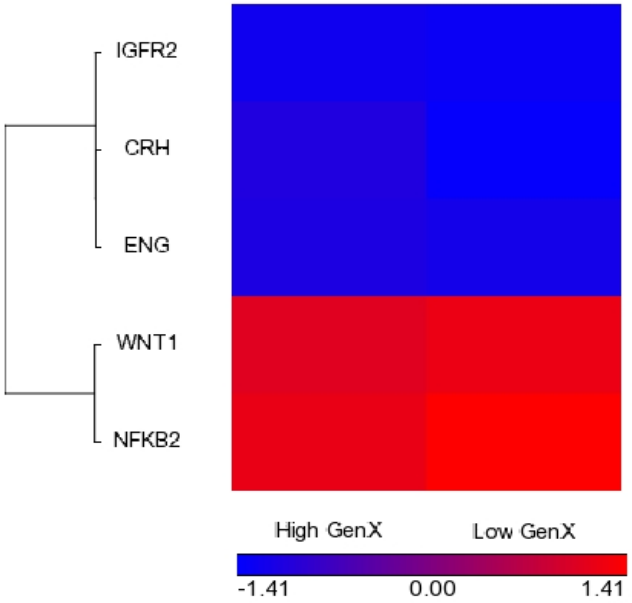
E17.5 Data











Preliminary data: Do not cite

Summary

We all need to work together……

- Challenges in testing so many compounds with numerous tissue targets. May be replaced without knowledge to the consumer.
- Half-lives and metabolism of most are not known – may be differences within strain, and between sexes
- Need modern tools for testing – transcriptomics, metabolomics, new HTS, 3-D models, thyroid, immune, and kidney models needed
- Inclusion of developmental stages in HTS – how to incorporate for the screening process
- Mode or mechanism of action studies needed - should include human relevant exposures (which we also don't know for more than about 15 – internal dose)

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)
Andy Rooney (OHAT lead)
Suramya Waidyanatha (Chemistry lead)

John Bucher
Linda Birnbaum
Brian Berridge

Chris Weis
Jed Bullock

Collaborators

US EPA
Mark Strynar
James McCord
Ann Richard

NTP Labs-based studies:

Bevin Blake
Kevin Mauge-Lewis
Harlie Cope
Julie Rice
Paul Dunlap
Susan Elmore, DVM
Tanner Russ (NIEHS Scholars Connect Program)

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

2019 EHP: https://ehp.niehs.nih.gov/doi/full/10.1289/EHP4555?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

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**

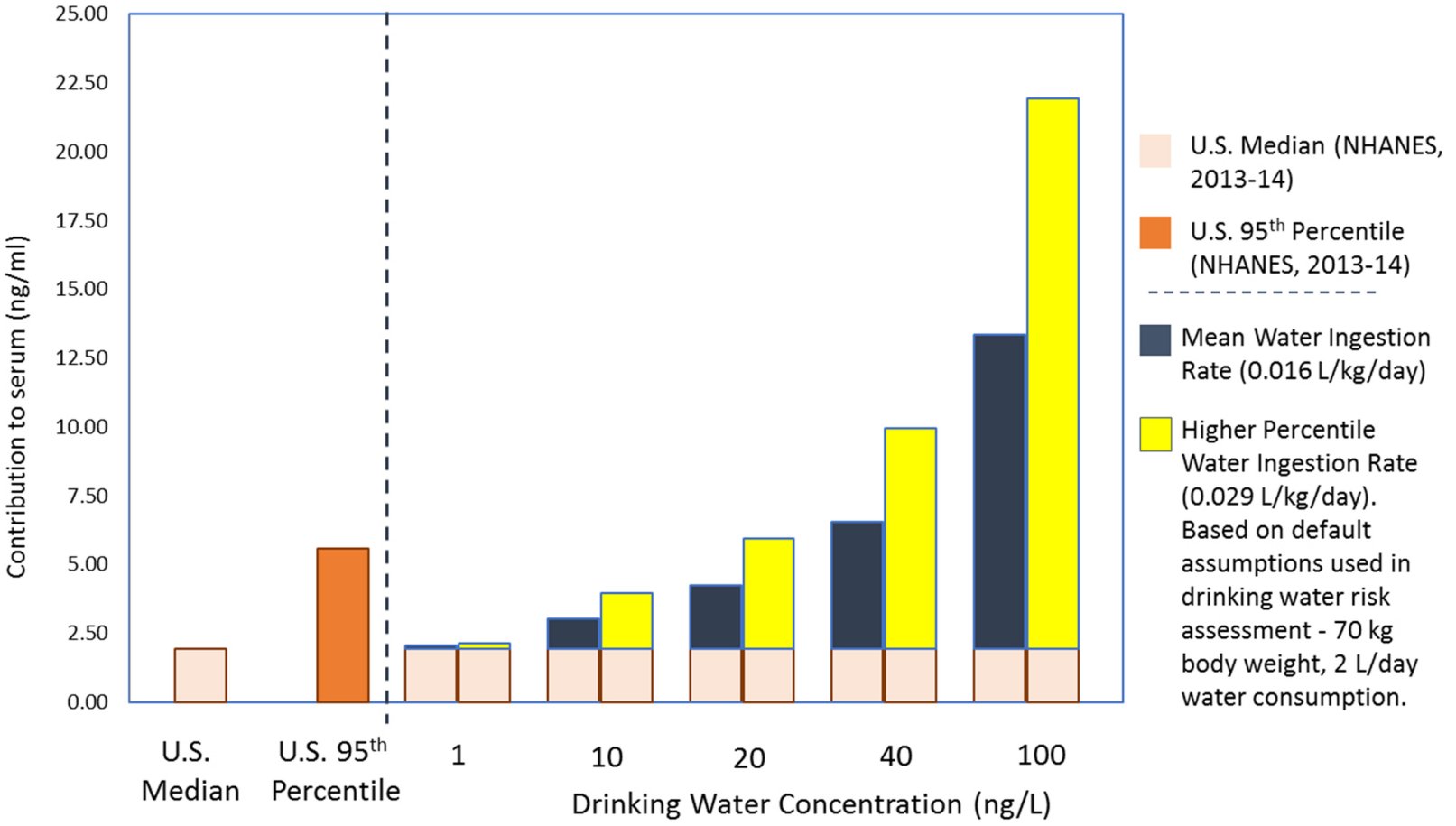
Reporting of GLP Toxicity Data

- **28-Day Toxicity Studies**
  - Data tables available now: https://ntp.niehs.nih.gov/results/path/index.html
  - TOX Report 96: Sulfonates (reports are in review for 2019)
  - TOX Report 97: Carboxylates

- **PFOA Two Year Carcinogenesis**
  - Data tables available soon.
  - Technical Report draft (TR-598) to be posted in 2019 for peer review

Predicted PFOA blood levels

Increased with contamination of drinking water or greater ingestion rate



-- Post et al., PLoS Biol 15(12): e2002855.