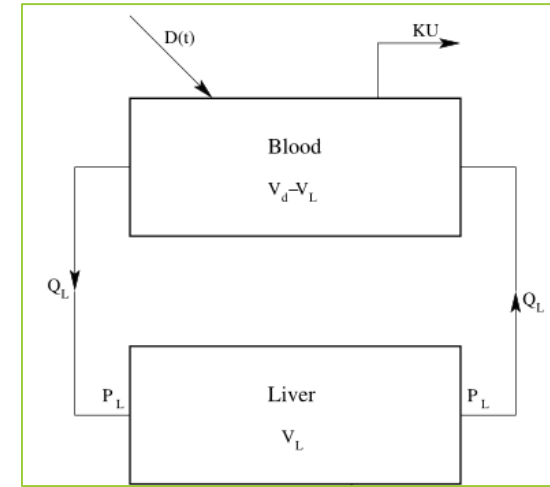


Team #5: Other Applied R&D

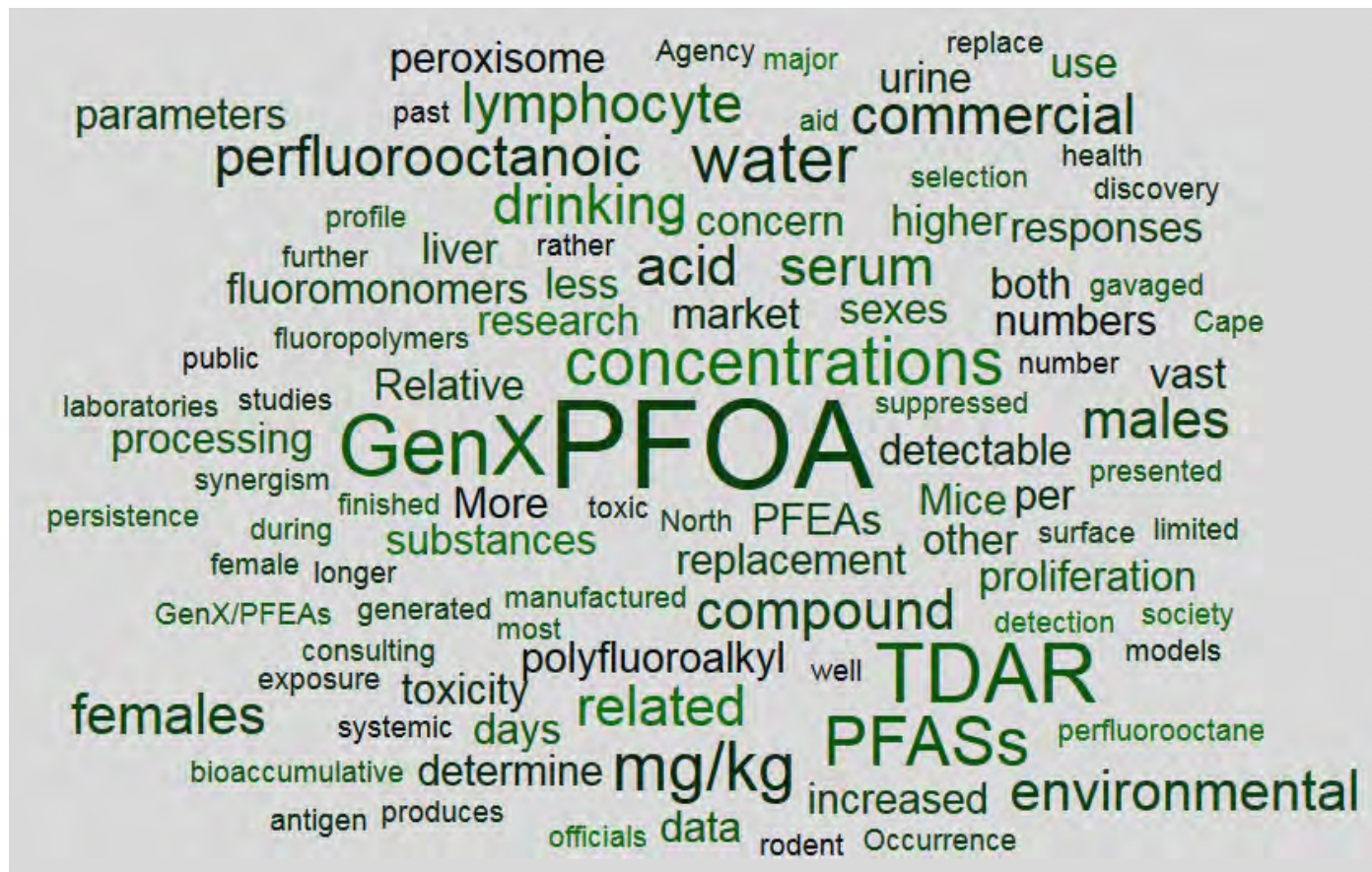


PRESENTED BY: JAMIE DEWITT

TEAM CO-LEADS: JAMIE DEWITT (ECU)
AND REBECCA FRY (UNC-CH)



Why do we need this type of PFAS research?



Context

Team 5 is providing diverse contextual information associated with identified PFAS in NC.

Team 5a: The presence of PFAS in landfill leachate



Lead: Dr. Morton Barlaz, NCSU

Collaborator: Dr. Jean-Rene Thelusmond, NCSU

Overall objective:

- Assess the relative importance of MSW landfills and domestic wastewater as sources of PFAS to POTWs and potentially to surface water



Team 5a: The presence of PFAS in landfill leachate



Sub-objectives/research questions:

- Estimate the mass of PFAS that is discharged to Publicly Owned Treatment Works (POTWs) from municipal solid waste landfill leachate
- Estimate the mass of PFAS entering POTWs in NC via municipal wastewater
- Estimate the mass of PFAS leaving POTWs in NC after treatment
- Estimate the release of PFAS from landfills that receive construction and demolition waste

Team 5a: The presence of PFAS in landfill leachate



Research approach:

- *Landfills*

Sample leachate at point where it is discharged to a wastewater treatment plant; may be a tank, gravity sewer line, or a pumping station

- *Wastewater treatment plants*

Sample from flow proportioned composite samplers after verification that sampling system does not contain Teflon

- *Construction and demolition (C&D) waste landfills*

Creative sampling as there are only a few lined C&D

Team 5a: The presence of PFAS in landfill leachate



Research approach:

- *Focus is on mass release*

$$\text{mass} = \text{concentration} * \text{volume}$$

- *Concentrations are measured*
- *Volumes are known for landfills and WWTPs but must be estimated using models for C&D landfills that are unlined*

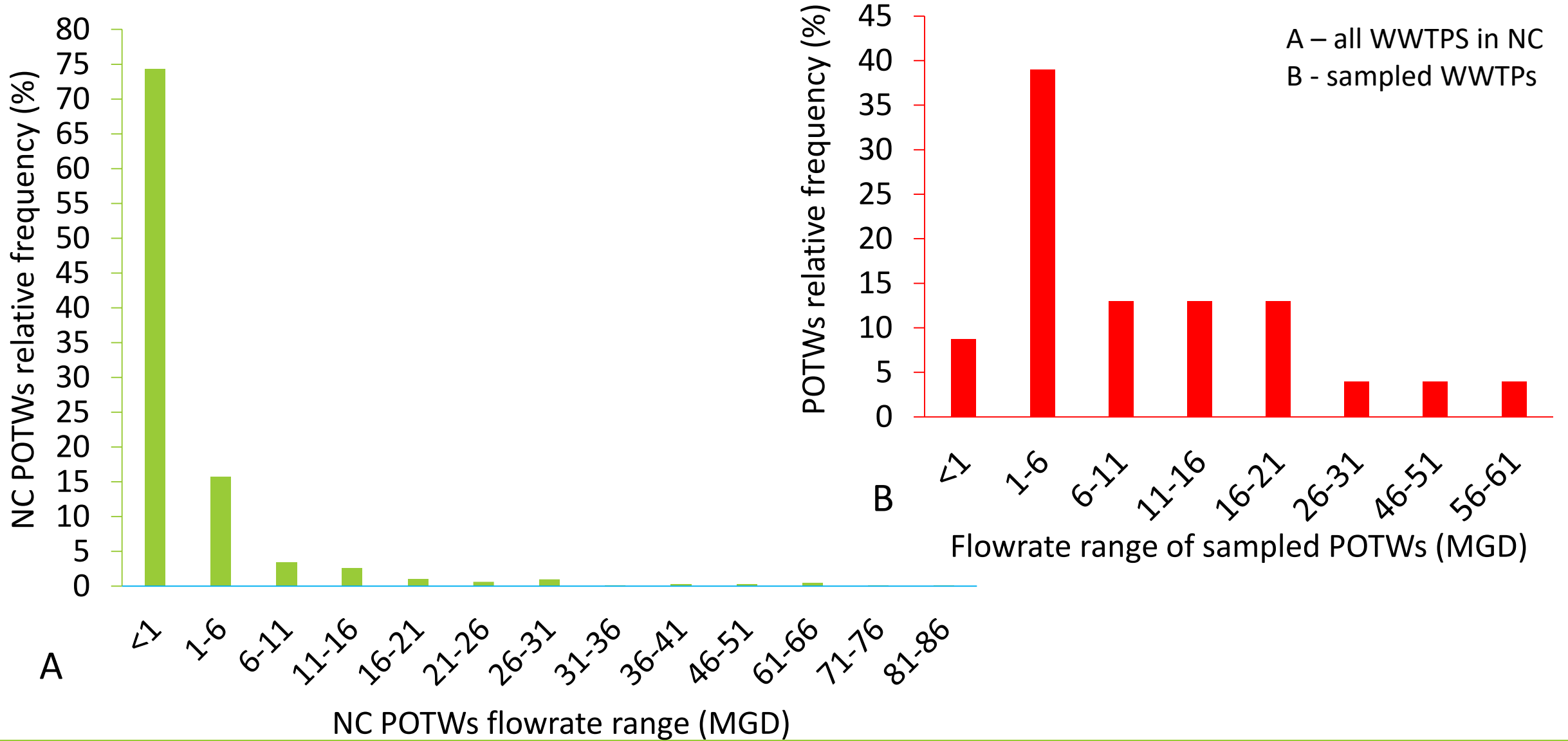
Team 5a: The presence of PFAS in landfill leachate



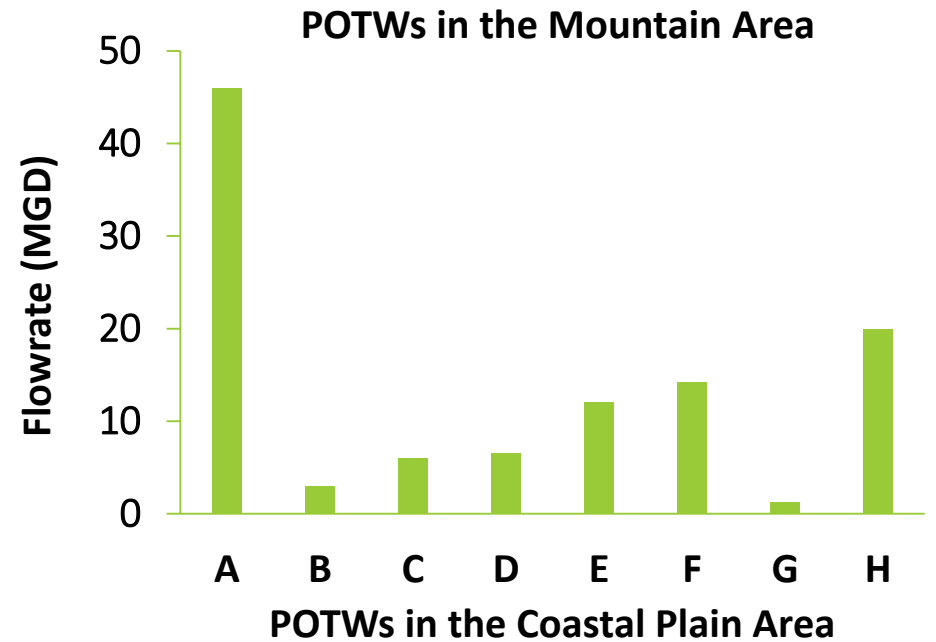
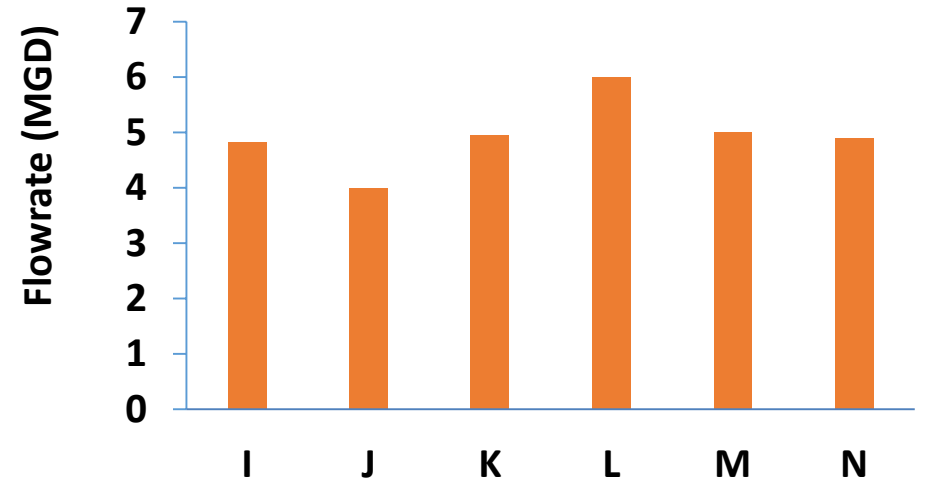
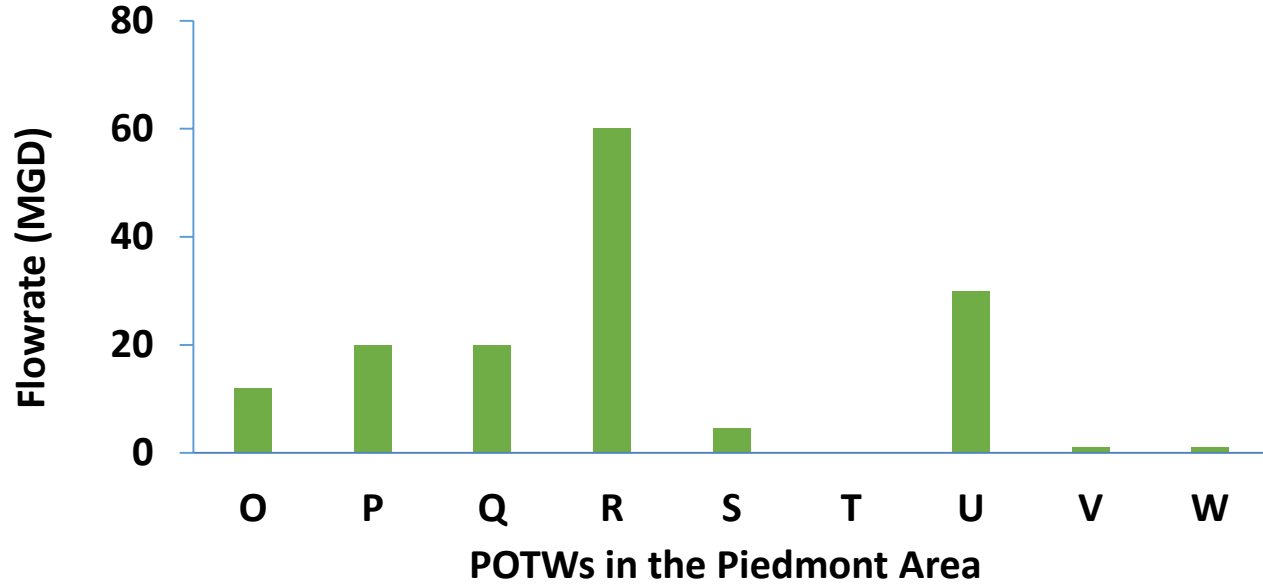
Current status:

- *Team 5a has sampled **23** WWTPs, **11** landfills, and **4** C&D landfills*
- *Team 5a has sampled **2** WWTPs weekly for four weeks*

Profile of wastewater treatment plant size in NC



Flowrate of the sampled POTWs in NC by region



Team 5b: The presence and accumulation of PFAS in NC wildlife species



Lead: Dr. Scott Belcher, NCSU

Collaborator: Dr. Theresa Guillette, NCSU

Overall objective:

- Are PFAS present and accumulating in NC wildlife?



Team 5b: The presence and accumulation of PFAS in NC wildlife species



Sub-objectives/research questions:

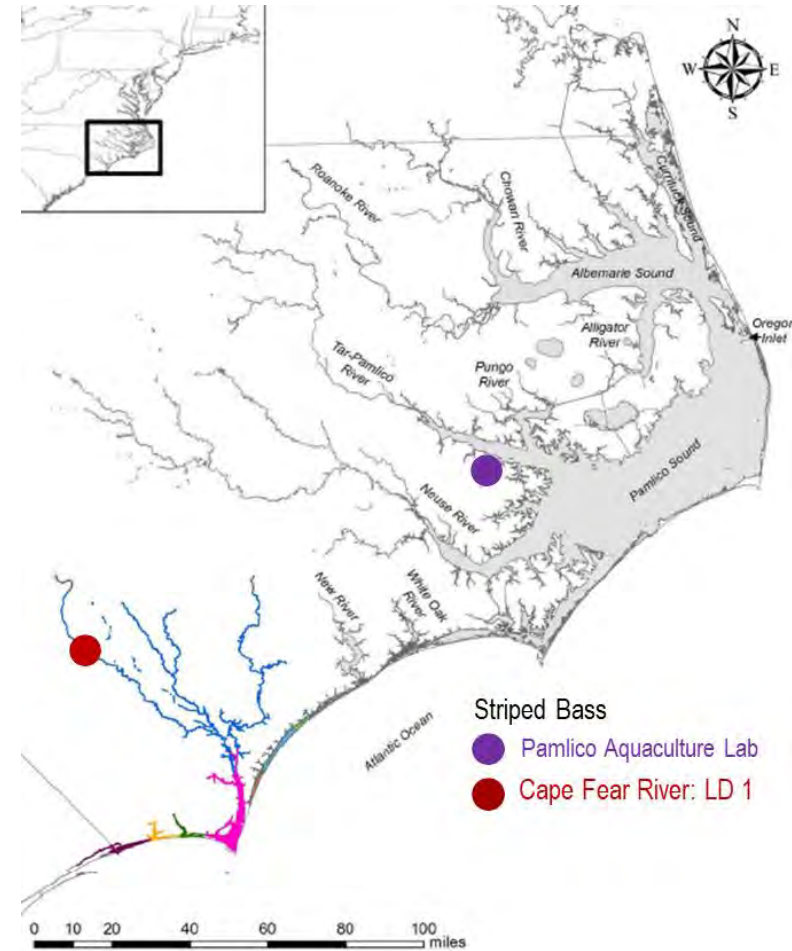
- Which PFAS are present in NC wildlife and how long do they remain?
- Do replacement PFAS bioaccumulate?
- Are levels of PFAS in NC wildlife associated with indicators of adverse wildlife/ecosystem health? What can these data tell us about human effects?
- Can consuming fish caught from the Cape Fear River increase PFAS in humans?

Team 5b: The presence and accumulation of PFAS in NC wildlife species



Research approach:

- *Striped bass and catfish*
Collecting from Cape Fear River as well as from populations without known PFAS contamination (“healthy” controls)



Team 5b: The presence and accumulation of PFAS in NC wildlife species

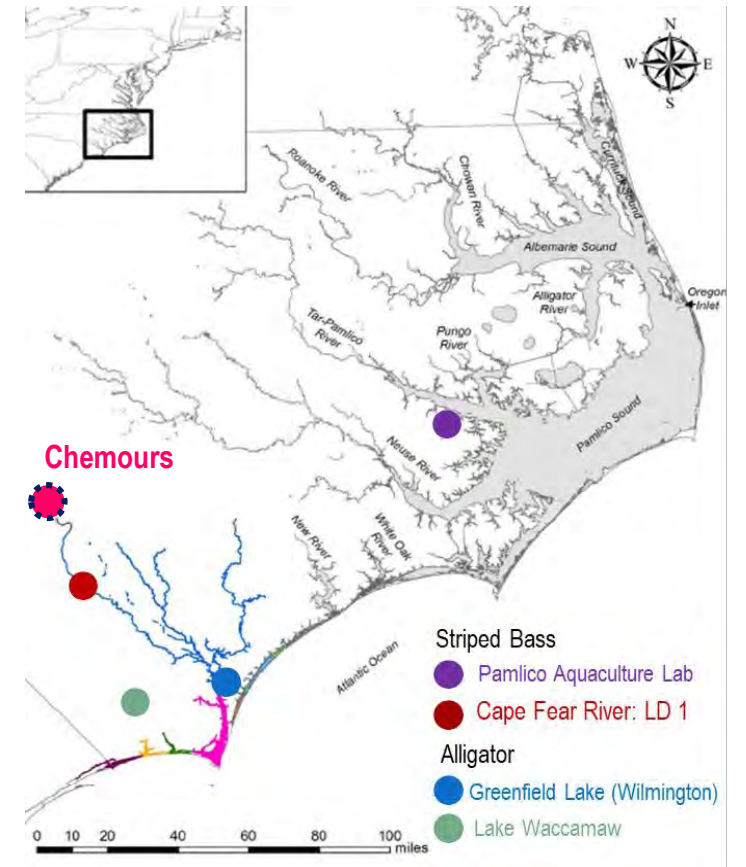


- *Alligators*

Active capture of adult (6'+) alligators

Collection of blood/serum and body measures

Sex determination



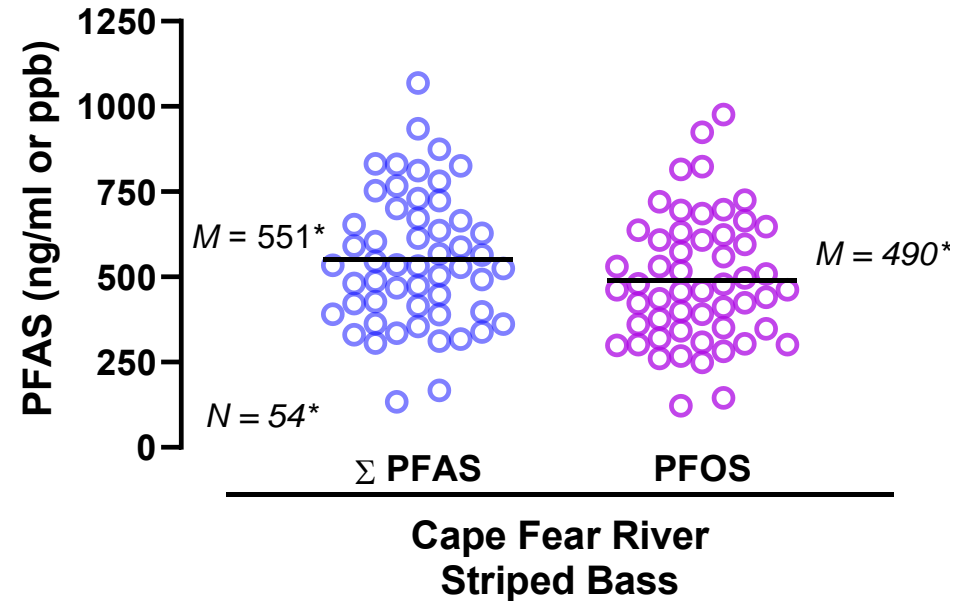
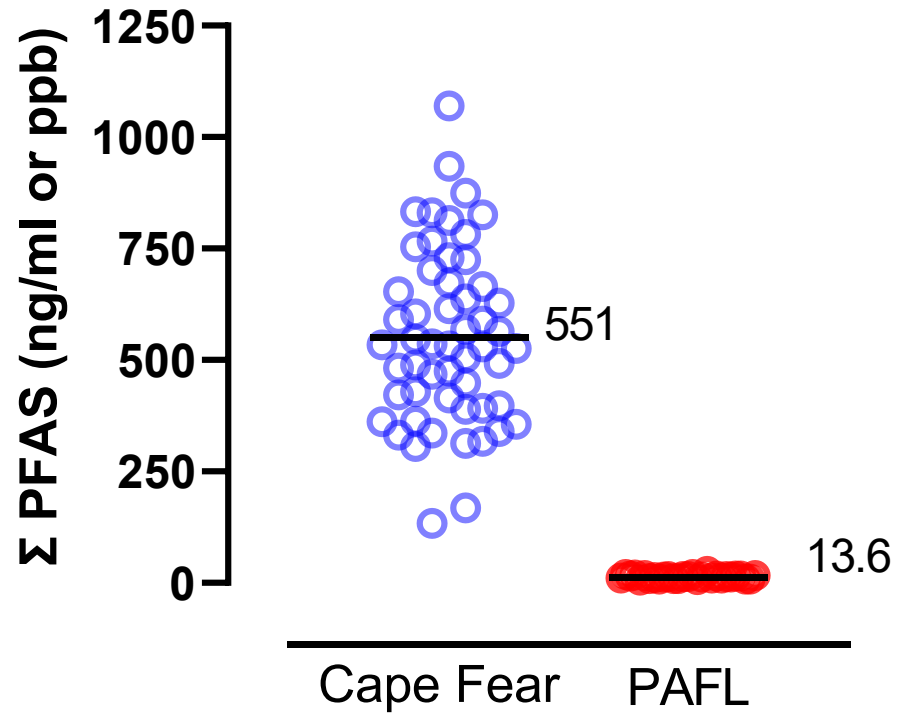
Team 5b: The presence and accumulation of PFAS in NC wildlife species



Current status:

- *Team 5b has collected **63** fish from Cape Fear River Lock and Dam 1*
- *Team 5b has collected **80+** fish from reference sites*

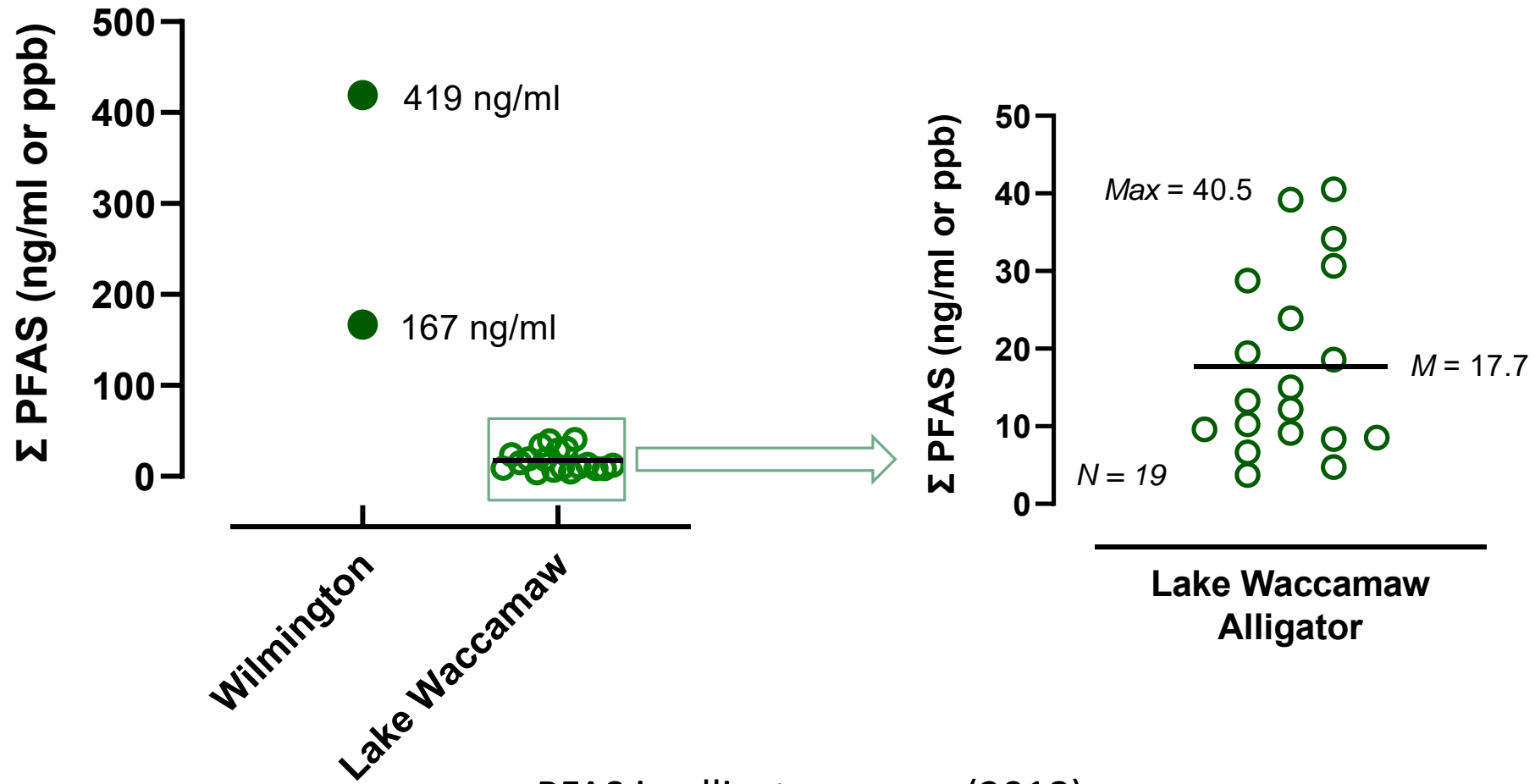
- *Team 5b has sampled **16** alligators from Lake Waccamaw (a reference population)*
- *Team 5b has sampled **~16** alligators from the Cape Fear River and Wilmington areas*



PFOS = 89% of Total PFAS

* concentration of 4 samples >LOQ

- High levels of PFAS were detected in every serum sample
- Reference hatchery fish were contaminated ($M = 13.6$ ng/ml)
- Total PFAS is >40 higher in striped bass from the Cape Fear River ($M = 551$ ng/ml)
- PFOS accounted for 89% of PFAS
- PFOS was “phased out” beginning in 2002



PFAS in alligator serum (2018)

Alligator serum LC-MS/MS – total PFAS was >10X higher in alligators from Wilmington

Team 5c: Immunotoxicological effects of PFAS in a rodent model

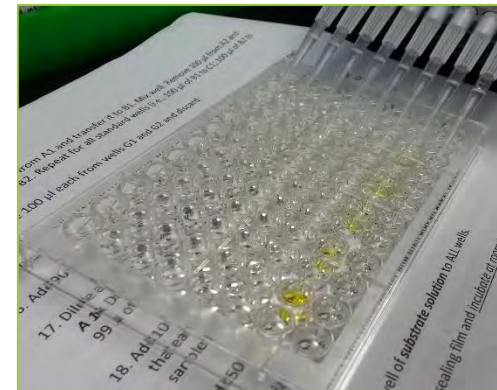


Lead: Dr. Jamie DeWitt, ECU

Collaborator: Dr. Tracey Woodlief and Samuel Vance, ECU

Overall objective:

- Do emerging PFASs impact the immune system to the same degree as legacy PFASs?



Team 5c: Immunotoxicological effects of PFAS in a rodent model





Sub-objectives/research questions:

- Determine effects of PFAS on major immune cell subpopulations in primary (thymus) and secondary (spleen) lymphoid organs
- Assess functional responsiveness of the adaptive immune system following exposure to PFAS
- Assess functional responsiveness of the innate immune system following exposure to PFAS

Team 5c: Immunotoxicological effects of PFAS in a rodent model



Research approach:

 Orally exposed male and female C57BL/6 mice. PFAS delivered in water. 



Daily body weights and in-life observations.

Urine and feces 24-hr prior to dosing and after 1, 5, and 15 days of dosing.

Sheep red blood cell injections at 25th day of dosing.

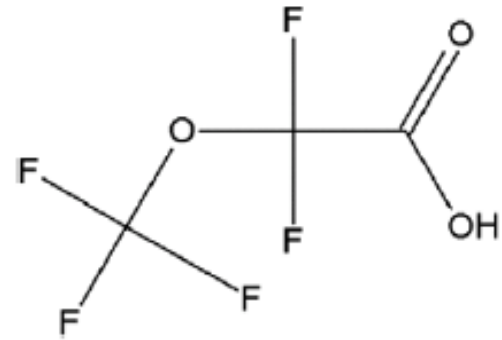
Evaluation of immunophenotype, TDAR, NK cell activity, and peroxisome proliferation, after exposure period. Collection of additional organs for other analyses by collaborators (i.e., brain and lung).

Team 5c: Immunotoxicological effects of PFAS in a rodent model



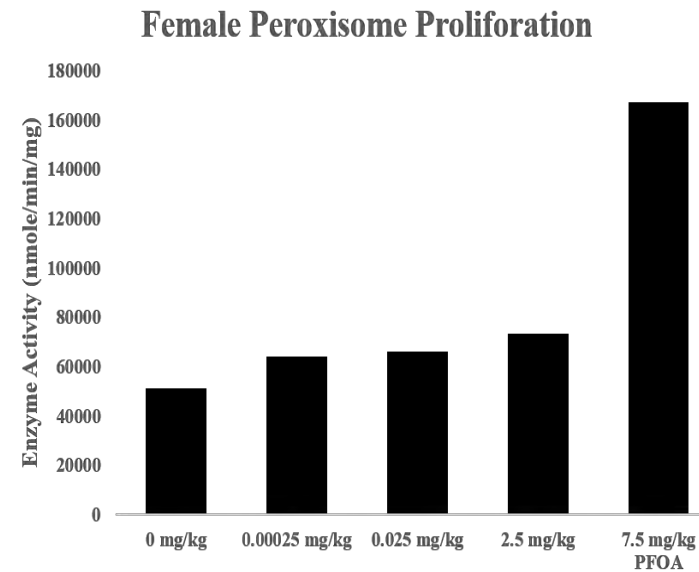
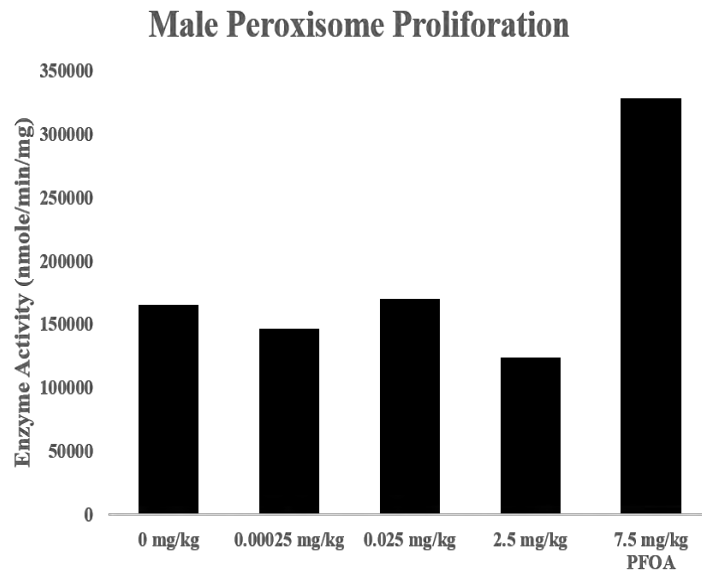
Current status:

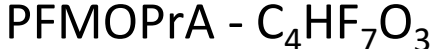
- *Team 5c has conducted studies with **PFMOAA** (3-carbons), **PFMOPrA** (4-carbons), and **PFMOBA** (5-carbons)*
- *Studies with Nafion BP2, PFHxA, and a mixture are planned*



PFMOAA - C₃HF₅O₃
perfluoro-2-methoxyacetic acid

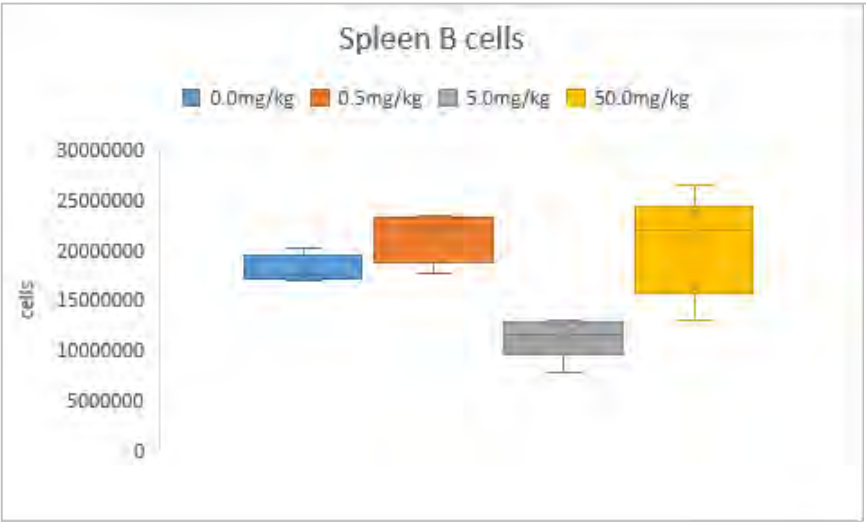
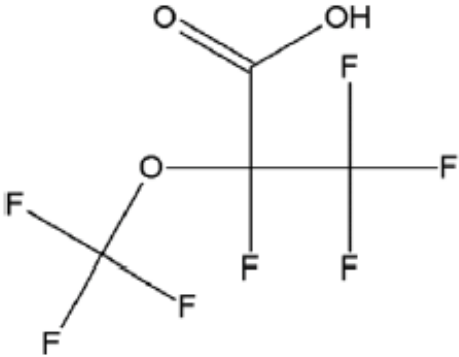
Dominant short-chain PFAS detected in Cape Fear River of North Carolina
in 2018 at
35,000 ng/L (Hopkins et al., 2018).
Part of Consent Order with NC DEQ/Sound Rivers.



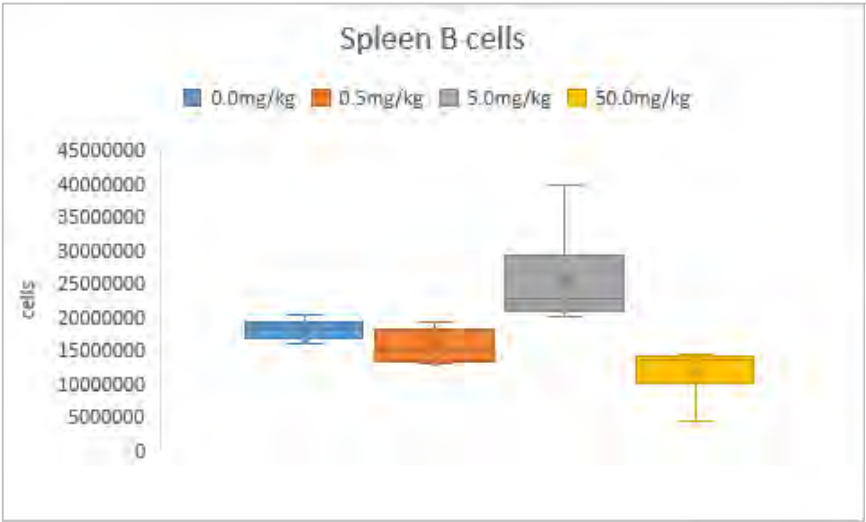


Perfluoro-2-methoxypropanoic acid

Short-chain PFAS detected in Cape Fear River of North Carolina in 2018
(Hopkins et al., 2018).



Males



Females

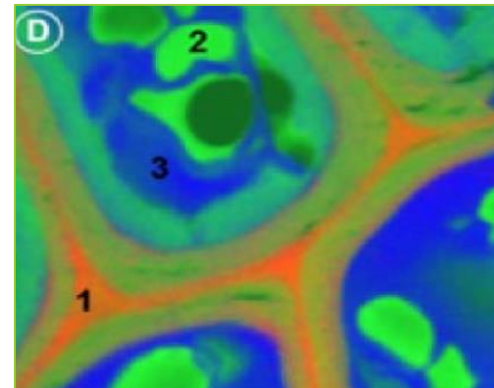
Team 5d: PFAS uptake and accumulation by plants



Lead: Dr. Owen Duckworth, NCSU

Overall objective:

- Assess uptake and accumulation of PFAS by relevant crops and how soil properties and management practices may impact PFAS uptake and distribution within plants



Team 5d: PFAS uptake and accumulation by plants



Sub-objectives/research questions:

- Quantitative linkage between organic matter content of soil and uptake of PFAS into crops prevalent in eastern NC
- Improved recommendations about soil management practices that can reduce uptake of PFAS from soil by plants
- Molecular scale information about where PFAS go in plant tissues

Team 5d: PFAS uptake and accumulation by plants



Research approach:

- *Soil amendments*

Compost addition to soil to determine if this reduces plant uptake of PFAS from soil

- *Evaluation of PFAS in plants*

Measurement of PFAS in whole plant and in specific parts of plants to see where PFAS go

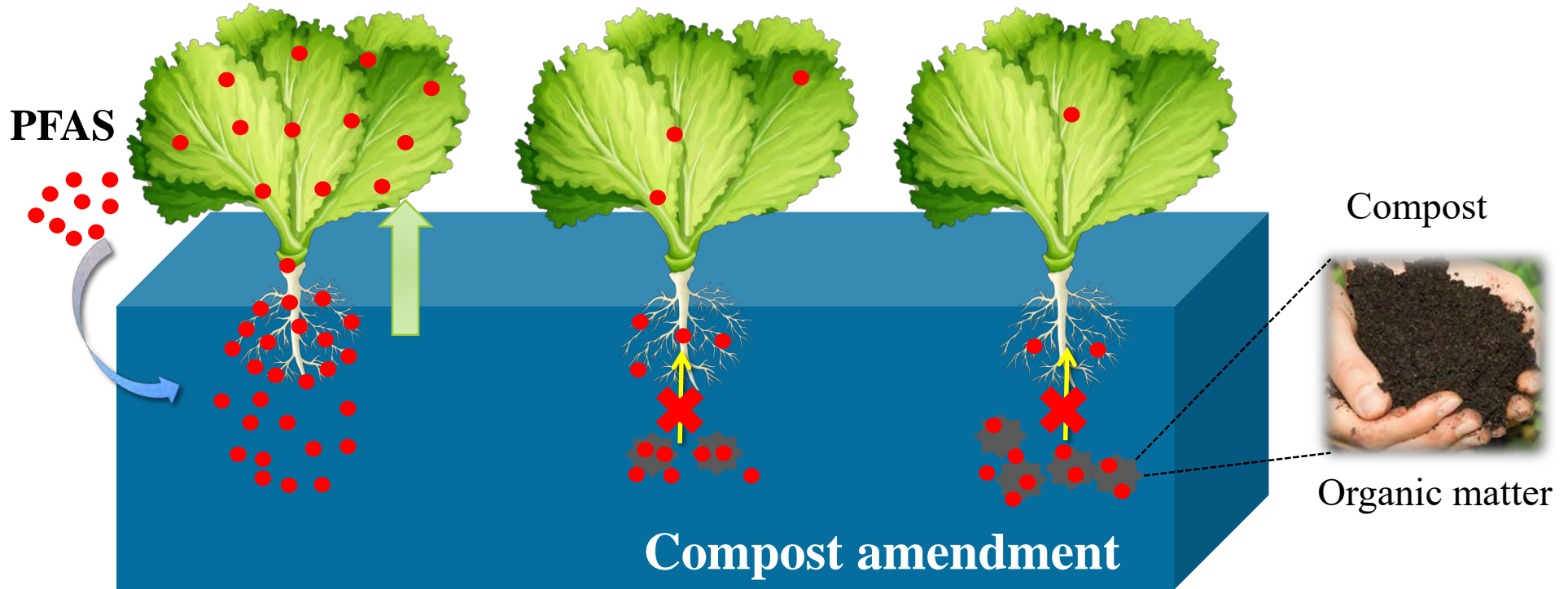
Team 5d: PFAS uptake and accumulation by plants



Current status:

- *Team 5d has conducted experiments with compost-amended soil spiked with PFAS and is waiting for plants to reach harvestable size*

Effects of compost addition on reducing the plant uptake of PFAS from soil



Hypothesis: Increasing the compost content could increase the sorption of PFAS chemicals thus reduce plant uptake.

Lettuce uptake experiment (to be harvested)

Two spiking concentration: 10 and 100 $\mu\text{g}/\text{kg}$



0% compost

5% compost

10% compost

20% compost

Expecting results:

- ✓ Application of compost could **increase** the lettuce biomass.
- ✓ Application of compost could **reduce** the lettuce **accumulation** of PFAS chemicals.

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?



Lead: Dr. Rebecca Fry, UNC Chapel Hill

Overall objective:

- Determine effects of PFAS on placental health and function



Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?



Sub-objectives/research questions:

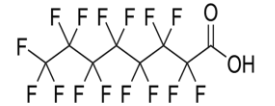
- What are levels of PFAS in:
Drinking water
Placenta
Cord blood
Maternal serum in pregnant women in NC
- What is effect of PFAS on placental health and function?

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?

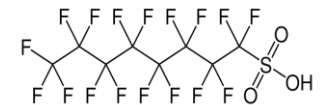


Research approach:

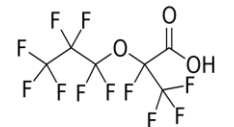
- *Well water sampling*
Collection of drinking water from pregnant women who obtain water from wells
- *Cord blood and maternal serum sampling*
Collection of blood for analysis of PFAS
- *Placenta sampling and work with placental cell lines*
Collection of placenta for evaluation cell-level changes and responses to PFAS in collected placenta cells and placenta cell lines



Perfluorooctanoic acid
(PFOA)



Perfluorooctanoic sulfate
(PFOS)



2,3,3,3-tetrafluoro-2-
(hepta fluoropropoxy)
propanoate
(GenX)

Team 5e: Do PFASs in drinking water pose a risk to pregnant women and could they affect the health and function of her placenta?



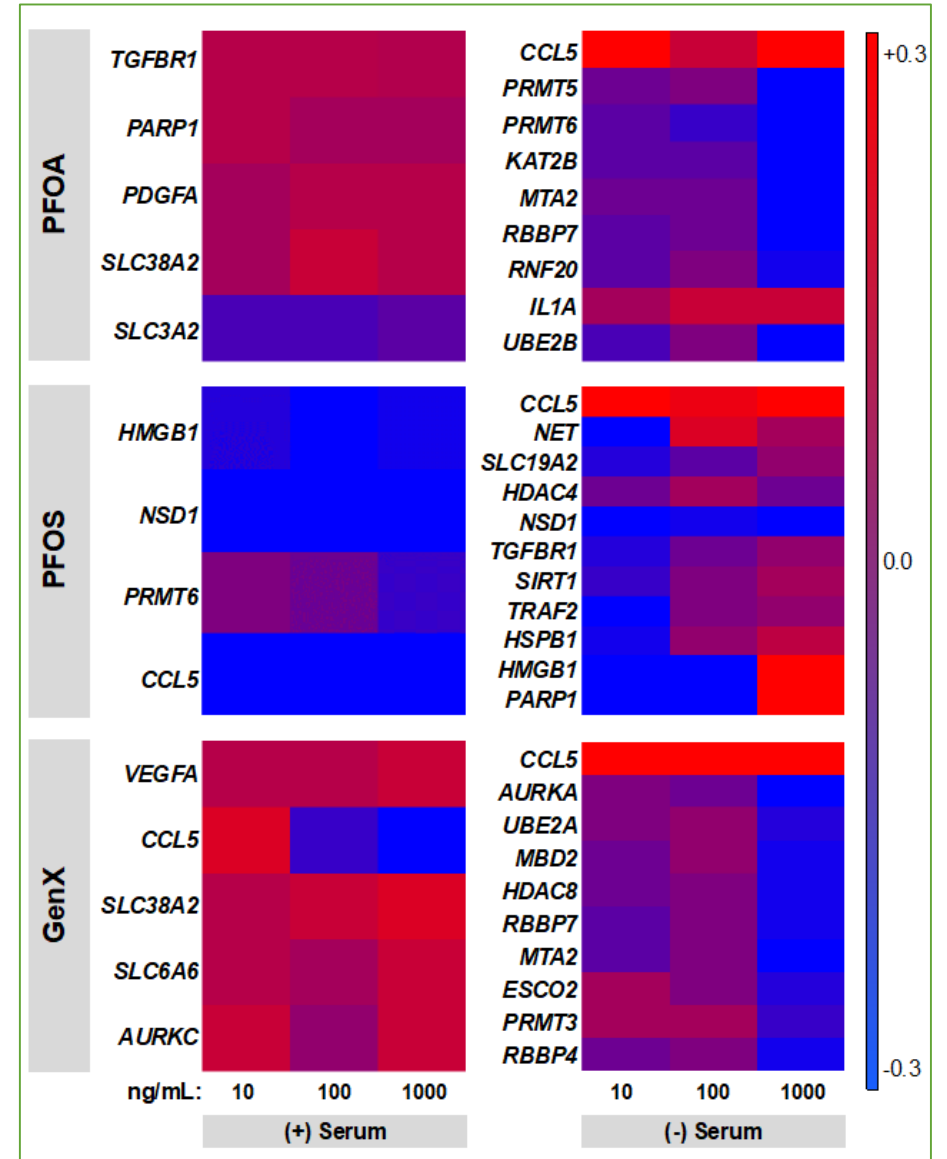
Current status:

- *Team 5e has recruited human subjects and collected urine and serum*
- *Team 5e has collected and continues to collect drinking water, cord blood, and placenta samples*
- *Team 5e has started measuring PFAS in water, serum, placenta, and cord blood*
- *Team 5e has run and continues to run cell-level experiments*

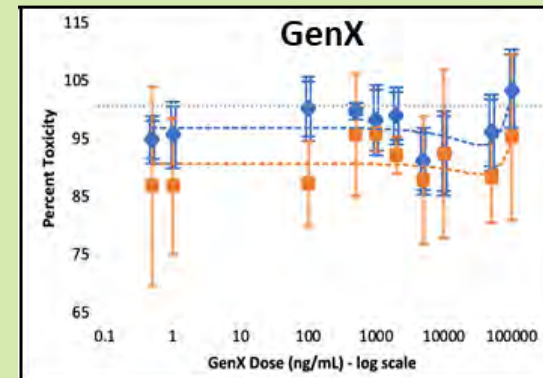
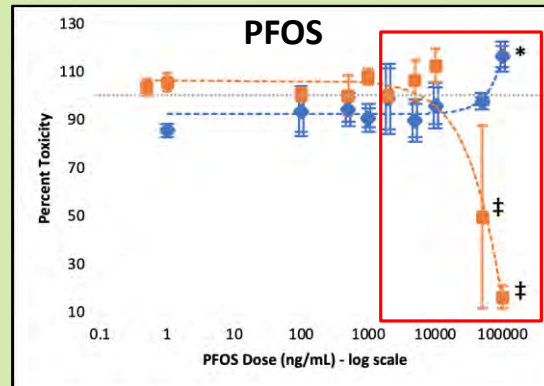
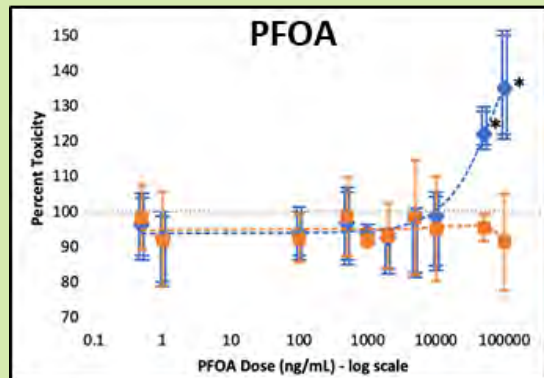
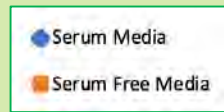
- Monitored for 14 PFAS in 12 well water samples
- Found 5 PFAS detected in several well water samples

	PFPeA	PFHxA	PFHpA	PFHxS	PFOA
Limit of detection (LOD) (ng/ml)	0.02	0.02	0.02	0.1	0.02
% of samples with detectable levels	16.7	25.0	33.3	8.3	25.0
Median	0.015	0.015	0.012	0.055	0.015
Range	< LOD -0.015	< LOD -0.016	< LOD -0.013	< LOD -0.055	< LOD -0.027

- Changes in gene expression associated with inflammation (CCL5) when placental cells were treated with PFAS
- Additional changes in gene expression in placenta cell line treated with PFAS



Changes in PFAS toxicity on a placental cell-line in two types of cell culture media



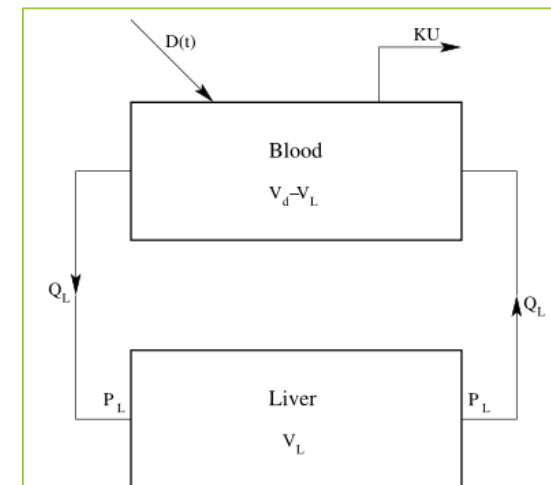
Team 5f: Predictive models for PFAS movement in the environment and in living organisms



Lead: Dr. Nick Luke, NC A&T

Overall objective:

- Can we develop models to predict where PFAS go in living organisms and in the environment?



Team 5f: Predictive models for PFAS movement in the environment and in living organisms



Sub-objectives/research questions:

- Quantitate analysis of immunotoxicity and systemic toxicity data generated by PFAST Network collaborators, including half-life estimates to generate a physiologically based pharmacokinetic (PBPK) model
- Quantitate analysis of environmental concentrations of PFAS generated by PFAST Network collaborators to develop models to predict fate and transport of PFAS in the environment

Team 5f: Predictive models for PFAS movement in the environment and in living organisms

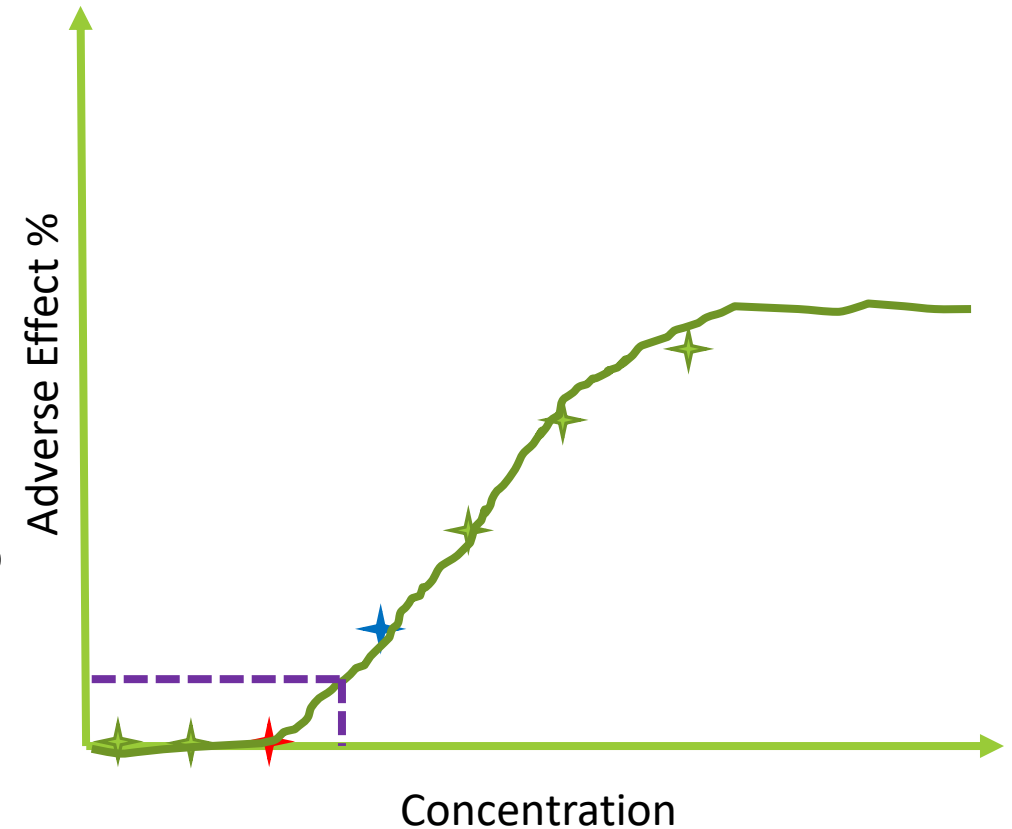


Sub-objectives/research questions:

- *Benchmark dose modeling*

Experiments conducted to find at what level of exposure adverse effects begin to occur

- **NOAEL (No Adverse Effects Level)** the highest concentration at which no adverse effects are observed
- **LOAEL (Lowest Adverse Effects Level)** the lowest concentration at which no adverse effects are observed
- **BMD (Benchmark Dose)** a quantitative model is formed to fit the adverse effects data, and a reference dose is identified using the model. The reference dose may be between the NOAEL and LOAEL.



Team 5f: Predictive models for PFAS movement in the environment and in living organisms

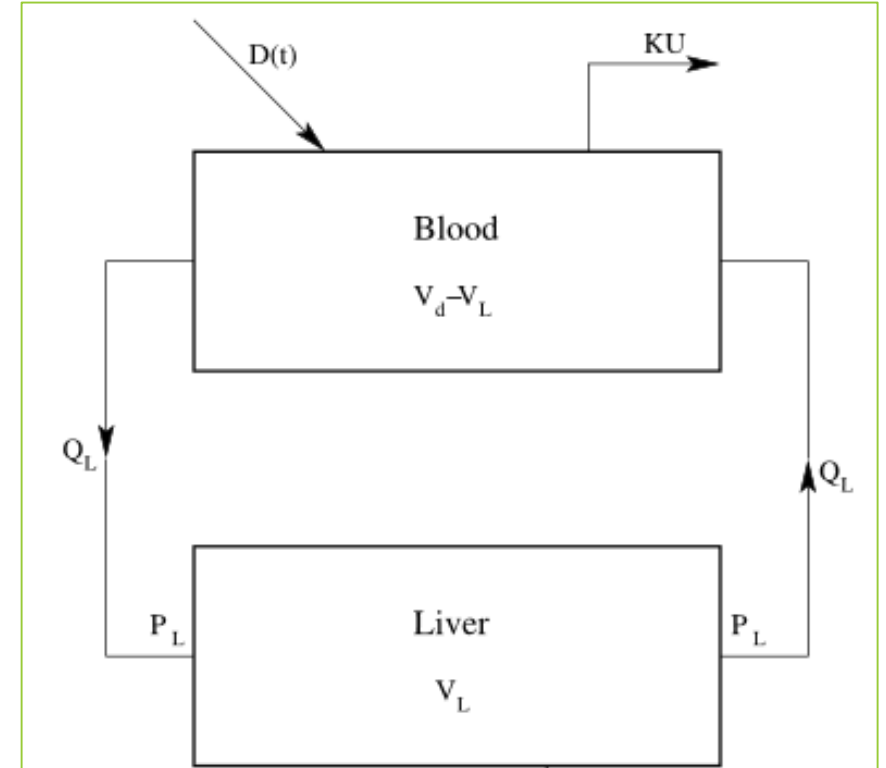


Research approach:

- *Physiologically based pharmacokinetic modeling (PBPK)*

The body is separated into a series of compartments

- A mathematical model is created to represent how a chemical is distributed throughout the body, excreted from the body, and metabolized in the body.
- The model is calibrated using experimental data.
- The calibrated model can be used to examine how long a chemical will remain within the body, how much of the chemical builds up within the body.



Schematic for a 2 compartment PBPK model



Team #5: Other Applied R&D

THANK YOU! WE WELCOME YOUR QUESTIONS



Photo references

From left to right on title slide:

- <http://efc.web.unc.edu/2013/11/05/solid-waste-finance/>.
- <http://www.starnewsonline.com/sports/20180816/scientists-look-for-genx-in-lake-waccamaw-alligators>.
- <https://www.slideshare.net/syednayyerarvi/immunotoxicity>.
- Karunakaran C. et al., 2015, Introduction of soft X-Ray spectromicroscopy as an advanced technique for plant biopolymers research, PLoS ONE 10, e0122959.
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- Luke N. et al., 2010, Development of a quantitative model of pregnane X receptor (PXR) mediated xenobiotic metabolizing enzyme induction, Bulletin of Mathematical Biology, 72:1799-1819.