# Impact of perfluorooctanoic acid exposure on the urogenital physiology in adult mice

Charles Park
Department of Physiology
East Carolina University
Greenville, North Carolina 27858
parkc20@students.ecu.edu

## INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds found throughout NC. Due to their ability to make a fire-resistant coating, PFAS is ubiquitous on many consumer products like furniture, carpets, and the lining of microwavable popcorn bags. They are also very resistant to breakdown and, when leaked into the environment, can enter our water supply and bioaccumulate in animals and humans. A CDC monitoring program collecting serum and urine found that PFA contamination is widespread in humans. Humans exposed to a subset of PFAS called "long-chained" perfluoroalkyl acids (PFAA) exhibited adverse effects on cardiovascular, endocrine, immune, reproductive systems, and development (links to kidney and testicular cancer have also been found). Due to their persistence, ability to bioaccumulate, and toxicity, long-chain PFAAs like perfluorooctanoic acid (PFOA) have been phased out of manufacturing. Since long-chained PFOA exposure negatively affects urogenital morphology and function, as well as localized immune system activation in adult male and female mice.

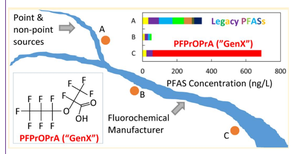



Fig. 1 PFAS in the Cape Fear River (2)

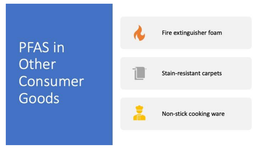



Fig. 2 Other consumer goods

| BRAND | TYPE | LIKELY PFAS-TREATED* |
|---|---|---|
| MOD | Medium | |
| MOD | Small | |
| Zeek's | Large | |
| Zeek's | Small | |
| Little Caesar's | Large | |
| Little Caesar's | Small | X |
| Ally's | Small | |
| Papa Murphy's | Standard Size | |
| Domino's | Medium | |
| Pizza Hut | Large | |
| Pagliacci | Large | |
| Papa John's | Large | |
| Papa John's | Small | |
| California Pizza Kitchen (Frozen) | Standard Size | |
| DiGiorno (Frozen) | Standard Size | |
| Tombstone (Frozen) | Standard Size | |
| Stark Raving (Frozen) | Standard Size | |
| Newman's Own (Frozen) | Standard Size | |
| Freschetta (Frozen) | Standard Size | |
| Act II | Butter Lovers | X |
| Act II | Kettle Corn | X |
| Act II | Buttery Kettle Corn | X |
| Act II | Movie Theater Butter | X |
| Act II | Light Butter | X |
| Act II | Mini Bags Butter | X |
| Pop Weaver | Kettle Corn | X |
| Pop Weaver | Extra Butter | X |
| Pop Weaver | Butter | X |
| Pop Weaver | Light Butter | X |
| Orville Redenbacher's | Movie Theater Butter | X |
| Orville Redenbacher's | Ultimate Butter Pop Up Bowl | X |
| Clover Valley | Extra Butter Flavor | X |
| Clover Valley | Kettle Corn | X |
| Lowrey's | Bacon Curls Microwave Pork Rinds | X |
| Lowrey's | Bacon Curls Microwave Pork Rinds - Hot & Spicy | X |
| Pop Secret | Homestyle | X |
| Pop Secret | Movie Theater Butter | X |
| Regal Cinemas | Movie Theater Butter Flavor | X |

Fig. 3 PFAS in food (1)

## MATERIALS & METHODS

**Animals and treatment:**
Male and female C57BL/6 mice (6-8 weeks old) were exposed to PFOA for 15 days via oral gavage. Control mice received sterile water with 0.5% Tween-20 and PFAS treatment mice received PFOA (7.5 mg/kg) dissolved in sterile water with 0.5% Tween-20. The selected dose is based on previous studies from the DeWitt lab and causes immunotoxicity but no systemic toxicity.

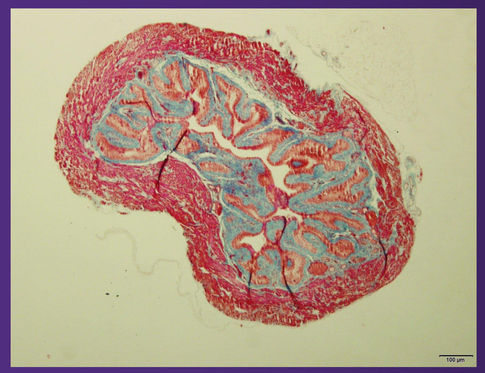
**Histological analysis:**
Penile, vaginal, and bladder tissue were collected, fixed in 10% formalin overnight and embedded in paraffin. We used a microtome to obtain tissue sections (0.6 mm thickness) and stained them with the following: picro sirius red stain (PSR), hemotoxylin and eosin stain (H&E), and Masson's trichrome stain (MT). The H&E slides will be analyzed for signs of immune cell infiltration and inflammatory response (edema). PSR and MT will be used to analyze collagen composition and smooth muscle composition respectively in the urogenital tissues. PSR stains will be analyzed using polarized scope microscopy. MT stains will be analyzed using the Zeiss Axio Imager. Bladder smooth muscle wall thickness was measured every octant via ImageJ.

**Quantitative PCR:**
We will extract and purify mRNA from a separate set of bladder tissues and run RT-PCR to examine gene expression of inflammatory markers (TLR4, TNF alpha, TGF-b, IL-10, and IL-1b), markers of urothelial barrier function (occludin, uroplakin II) and a house keeping gene (18S). The level of expression of inflammatory markers in the bladders of mice exposed to PFAS will be compared to bladders from control. We plan to examine the expression of these inflammatory markers in mRNA extracted from penile and vaginal tissue as well.

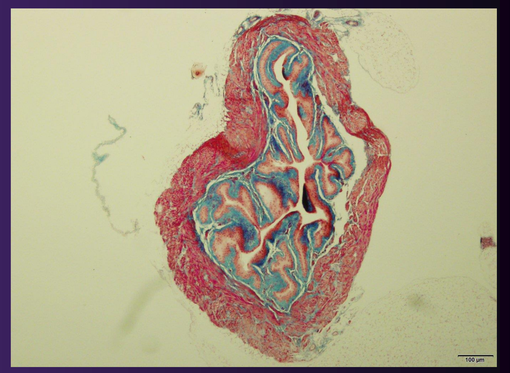
**Smooth muscle physiology:**
Bladder contractility was tested using another set of bladder tissue. Whole bladders were isolated, cut into strips, and mounted in the myograph tissue bath. Bladder strips were exposed to potassium chloride (KCl), adenosine triphosphate (ATP) and carbachol, a muscarinic receptor agonist. Penile contractility was also assessed using KCl, phenylephrine (PE), and acetylcholine (ACh). Vaginal contractility was assessed using KCl, norepinephrine and sodium nitroprusside.
We also plan to generate another cohort of mice to perform void spot assays to assess in vivo bladder function and apomorphine testing to assess sexual function.

Unpaired Student T test was used to compare bladder wall thickness between control and treated bladders. The level of evidence obtained from this study is categorized as animal and laboratory studies.

**Acute PFOA exposure** does **not** affect adult male **bladder contractility, smooth muscle composition,** and gene markers of **inflammation**




Control female bladder




PFOA-treated female bladder

**Significant differences** in **bladder wall thickness** found with **no** accompanying **functional differences**

## RESULTS

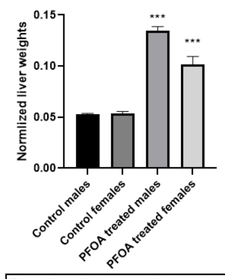



Fig.1 Liver weights normalized to total body weight of male and female C57BL/6 mice treated with PFOA vs control. Data are represented as mean ± SEM. p value < 0.0001. n=15-16

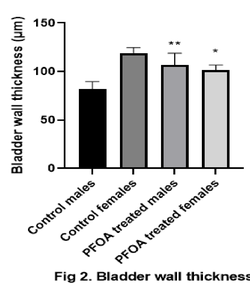
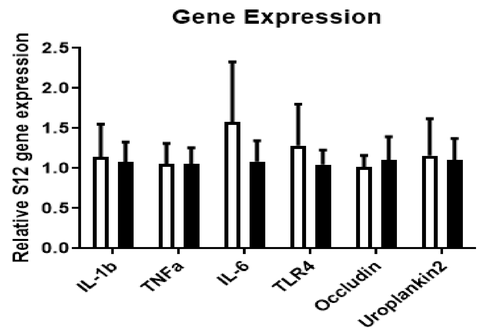



Fig 2. Bladder wall thickness




## DISCUSSION

- There were no changes in bladder contractility between PFOA treated vs control mice. Similarly, we did not find any differences in bladder smooth muscle area between the same groups of mice. We did find significant increase in bladder wall thickness in mice exposed to PFOA compared to control. No changes in the expression of inflammatory markers or urothelial barrier genes were found between bladders from treatment vs control mice.
- We found significant differences in bladder wall thickness following PFOA exposure. However, this morphological change did not translate into a functional change in contractility. Overall, there were no changes in the bladder's gene expression of markers of inflammation with PFOA exposure. This leads us to believe that more investigation is needed to understand if PFOA is influencing the urogenital tissues.

## REFERENCES

1. Stohler, S. (2021). *PFASs in Popcorn bags and PIZZA BOXES: Toxic-Free Future*. Toxic. https://toxicfreefuture.org/research/pfass-popcorn-bags-pizza-boxes/.

2. Sun, M., Arevalo, E., Strynar, M., Lindstrom, A., Richardson, M., Kearns, B., Pickett, A., Smith, C. and Knappe, D., 2016. Legacy and Emerging Perfluoroalkyl Substances Are Important Drinking Water Contaminants in the Cape Fear River Watershed of North Carolina. *Environmental Science & Technology Letters*, 3(12), pp.415-419.

## ACKNOWLEDGEMENTS

A special thank you to the Summer Scholars Research Program for funding this research opportunity this summer. Also, thank you to the DeWitt lab for sharing their mice and expertise. Lastly, thank you to Brody School of Medicine and the amazing Hannan Lab for their support.