Report to the Environmental Review Commission from The University of North Carolina at Wilmington Regarding the Implementation of Section 20.(a)(2) of House Bill 56 (S.L. 2017-209)

Executive Summary

The following report contains detailed scientific information regarding the work conducted, completed, still in progress, or planned by various researchers and organizational units at UNCW. It is organized based on the four primary objectives, similar to the organization of the scope of work document and the interim report previously provided. Highlights of findings to date and initial conclusions under each objective include the following:

--Objective 1: Based on weekly collection and non-targeted screening through mass spectrometry of raw and finished drinking water at the Sweeney Water Treatment Facility operated by the Cape Fear Public Utility Authority (CFPUA), several new PFAS compounds, not previously reported in the scientific literature, have been tentatively identified.
--Objective 2: Analyses of sediment samples collected from multiple locations indicate that: 1) GenX is present, with significant variability between sites; 2) both point and non-point sources are likely contributors; and 3) sediments appear to be acting as a repository of GenX that may be released into the overlying water column.
--Objective 3: Studies of biodegradation of GenX in sediments will require longer-term incubation experiments, including attention to seasonal and spatial effects.
--Objective 4: An initial study of the effects of exposure to GenX on the growth, survival, and filtration rates of juvenile oysters suggests that very high concentrations may decrease filtration and increase mortality rates, yet there was little bioaccumulation of GenX in oyster tissues.

Going beyond the objectives specifically outlined in HB56, UNCW researchers also:

1) analyzed sediment samples for compounds other than GenX and detected an additional seven PFAS compounds;
2) collected and analyzed several rain samples in which the presence of GenX was confirmed, possibly as the result of emission of a precursor compound (hexafluoropropylene dimer acid fluoride) and subsequent hydration;
3) documented the presence of GenX in biosolids from the CFPUA water treatment process; and
4) organized and hosted a symposium that included the major groups from across North Carolina working on GenX studies, leading to continuing collaborations.

UNCW remains appreciative of the opportunity to provide its expertise in service to the state in regard to these important issues.

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Objective 1: Quantification and identification of perfluorinated alkyl substances (PFAS) in raw and finished drinking water collected at the Sweeney Water Treatment Plant

Personnel Involved: Four senior faculty, one post-doctoral associate, one graduate student, and two undergraduate students.

Status: Scientists in the Department of Chemistry and Biochemistry at University of North Carolina Wilmington have been collecting weekly raw and finished drinking water since November 2017 from the Sweeney Water Treatment Facility operated by the Cape Fear Public Utility Authority (CFPUA). Water samples are processed and analyzed using non-targeted screening methods which rely on data generated by liquid chromatography/high resolution mass spectrometry. To date, we have identified several new PFAS in both raw and finished drinking waters that have not been reported in the scientific literature. The exact structure of these compounds is still being determined and requires obtaining standards of the pure materials, when available, to aid in identification. One compound (PFMOAA, structure I) has been positively identified through the custom synthesis of a standard. Structure elucidation of the remaining PFAS and any newly identified compounds will be carried out using multidimensional mass spectrometry to aid in identification.

Objective 2: Develop a method to quantify GenX in sediments collected along the Cape Fear River

Personnel involved: Four senior faculty (same personnel as Objective 1), one post-doctoral associate, two graduate students, and two undergraduate students.

Status: Four sets of sediment samples have been collected at approximately equal spatial intervals at four locations from north of Fayetteville just above the Chemours facility, just south of the Chemours facility with additional sites near Lock and Dam #1 (Table 1 and Figure 1). Sediment samples have also been collected on two estuarine transects using a grab sampler on the RV *Cape Fear* at seven locations within the estuary beginning at the seawater end member and moving upstream to the freshwater end north of the Port of Wilmington (Fig. 2). Additional transects will be collected during different seasons to evaluate how concentrations of GenX may change over time in river sediments. This portion of the research will continue through June 30, 2019.

The first phase of Objective 2 relates to the development of a method for the extraction of GenX from sediments with a timeline of 4 months beginning in December. This was required because no standard technique exists for determination of GenX in sediments. This phase of the project is now complete. The procedure we have developed is outlined below. The quality assurance protocol as indicated by percent recovery of the surrogate (a compound referred to as M8PFOA) can be found in Table 2. The average percent recovery among these 17 sediment samples was 110% indicating that our procedure is effectively recovering GenX from sediments of the Cape Fear River system. Procedural blanks as well as field blanks are routinely analyzed along with sediment samples.

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Sediment samples were oven dried (40°C), gently disaggregated, and passed through a polyethylene/nylon sieve to obtain the <500 μm size fraction. The weight percent coarse (>500 μm) and fine (<500 μm) material were then recorded. Approximately 5 grams of dried sediment (exact weight recorded) was placed in high-density polyethylene tube for extraction.
The surrogate (M8PFOA) was then added and allowed to incubate overnight. The following day 10 mL of methanol were added to the sample and shaken for 10 min followed by 30 minutes of sonication with periodic cooling of the sample. This process was repeated 3 times with fresh solvent. Extracts were filtered through 0.2 μm syringe filters, combined in a high-density polyethylene tube, and reduced in volume under a gentle stream of nitrogen to approximately 1 mL. GenX in the sediment extracts was quantified using an Agilent 1290 UHPLC/Bruker AmaZon SL ion trap LC mass spectrometer housed in the UNCW Department of Chemistry and Biochemistry.

Sediment concentrations of GenX can be found in Table 3. These are the first GenX concentrations ever reported for sediments. ***Note that the concentrations for sediment presented in this report are in units of ng/g (nanograms per gram, or parts per billion by weight) and cannot be directly compared to the health advisory goal set for GenX in drinking water of ng/L (nanograms per liter, or parts per trillion by volume).***

Data presented in Table 3 are important because they indicate that GenX is a ubiquitous component of the sediments sampled to date and that there is significant variability between sites. Sources likely include both point and non-point sources (possibly atmospheric deposition). The highest GenX concentration (21.6 ng/g dry sediments) was observed at site 2 near Lock and Dam #2 on the Cape Fear River (Fig. 1). The lowest concentration was observed at site 3 near the outfall of the Chemours facility. Significant concentrations (4.0 ng/g) were also observed above the Chemours outfall (site 4) and in the lower Cape Fear River (14.1 ng/g) near the port of Wilmington. It should be noted that sediments collected at a location on the river often reflect sediments deposited upstream that are then transported downstream. Also, the makeup of the sediments (e.g. sandy versus organic-rich) often control the level to which PFAS are attracted to and ultimately become associated with the sediments. As the research progresses, more information will become available to shed light on the transport and fate of this compound in the Cape Fear River and estuary.

An initial conclusion from this study is that sediments are acting as a repository of GenX that may be released into the overlying water column, potentially impacting sensitive estuarine ecosystems as well as drinking water utilities, even if it is no longer being released into the environment.

<u>Objective 3:</u> Conduct GenX biodegradation studies in sediments

Personnel Involved: Four senior faculty (same personnel as Objective 1), one post-doctoral associate (same personnel as Objective 1), one graduate student, and one undergraduate student.

Status: The third goal of HB 56 relates to the biodegradation of GenX in sediments. Often microbial communities present in sediments and exposed to specific substances are able to alter these substances. We will begin long-term (1 year) incubation experiments beginning May 2018 with sediments exposed to GenX to monitor potential loss of the compound over time. Selected

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sediments will be incubated in the dark at ambient temperature for a period of several months. Aliquots will be removed at predetermined intervals and the concentration of GenX determined as a function of incubation time. An analogous series of biodegradation experiments will be carried out with spiked GenX samples in order to evaluate concentration effects. Depending on results, additional studies may be carried out to determine seasonal and spatial effects on the loss of ambient levels of GenX or loss of GenX spiked into the sediments.

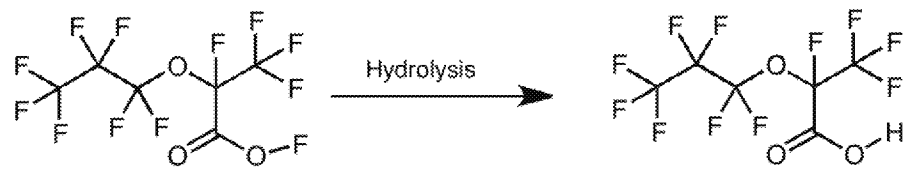
Additional Products:

Although our group's scope of work only focuses on GenX in sediments of the Cape Fear River, we have also performed other tasks of importance to the citizens of North Carolina regarding this unregulated compound.

Sediment: The focus of HB 56 is on GenX, however the sedimentary extraction and analysis by LC/QTOF provides high resolution mass spectrometric data that can be used for non-targeted exploration. Other than GenX (compound IV) in the sediments, there are an additional seven PFAS compounds that have been detected (Fig. 3 and 4). To the best of our knowledge this is the first reported occurrence of these compounds in sediments. Compounds I-III and V-VI have been previously reported in the Cape Fear River water column and drinking water. The structures of these compounds are shown in Fig. 4. Peaks VII and VIII have not been reported in the literature. The measured accurate masses of peak VII and peak VIII are 532.92270 u and 698.91672 u, respectively. Based upon the mass deficiency and isotopic pattern, both compounds appear to be perfluorinated and contain one atom of chlorine. Structure elucidation is ongoing and involves multidimensional mass spectrometry experiments.

Rainwater: Our group has been conducting National Science Foundation-funded rainwater research for over two decades resulting in one of the most comprehensive long-term data sets in the world. Using our rain collection facilities we collected and analyzed several rain samples for GenX. Based on these results we informed NCDAQ that GenX was indeed present in some rain samples leading to their current rainwater study of atmospheric transport of this compound.

The chemical characteristics of GenX do not indicate that it should be transported from water to the atmosphere. Barring direct physical injection of GenX in an aqueous aerosol, it was possible the GenX we detected in rainwater was from a precursor compound being emitted into the atmosphere. Based upon the scientific literature, the precursor identified is the hexafluoropropylene dimer acid acid fluoride (CAS 2062-98-8). Experiments performed in our laboratory showed the hydration (equation 1) to the corresponding carboxylic acid known as GenX (CAS 13252-13-6) occurs in less than 10 minutes. The latter compound is what is detected in drinking water and other matrices.



hexaflouropropylene dimer acid acid fluoride (CAS 2062-98-8)

perfluoro-2-propoxypropanoic acid (CAS 13252-13-6)

Equation 1

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Biosolids: After discussions with CFPUA about the biosolid collection from the water treatment process we offered to analyze this material for GenX. Although our sediment methodology was still in the development phase, we were able to conclude that GenX was present in biosolids from CFPUA. This resulted in the current measurements and study of GenX in biosolids being conducted by private laboratories by CFPUA.

Statewide GenX symposium: Our group organized and hosted a symposium on December 10, 2017 that included the major groups working on GenX studies within the state. This included university researchers (e.g., UNCW, NCSU, Duke, ECU), state agencies (e.g., DEQ and DAQ), water treatment facility representatives from CFPUA and Brunswick County, and non-profits (e.g., NC River Watch, Southern Environmental Law Center, NC Coastal Federation). Presentations updated all parties involved on the status of the GenX issue as well as the research challenges relevant to addressing this statewide problem. Collaborations were established between the variety of groups and organizations to leverage current resources to answer important questions related to GenX.

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Table 1. Date and location of sample collections

Date of Collection	Middle Cape Fear River (sites 1–4) Sediment and water column	Lower Cape Fear River Sediment and water column
9/11/17		X
9/19/17	X	
10/23/17		X
11/2/17	X	
1/30/18	X	
2/6/18		X
3/26/18	X	

Table 2. Recovery of surrogate M8PFOA.

Sample ID	Recovery
site 1_1	117%
site 1_2	84%
site 1_3	118%
site 2_1	92%
site 2_2	107%
site 2_3	100%
site 3_1	99%
site 3_2	95%
site 3_3	82%
site 4_1	129%
site 4_2	122%
site 4_3	125%
HB1	104%
HB2	114%
HB3	93%
M61_1	133%
M61_2	135%
M61_3	124%
Average	110%

Table 3. GenX concentration in Cape Fear River sediments collected on 9/19/17

Sample Site	Gen X concentration (ng/g dry sediments)
site 1	12.8
site 2	21.6
site 3	3.1
site 4	4.0
M61	14.1

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Figure 1: Map of sampling locations along the middle Cape Fear River including a site above the Chemours facility (site 4) and a site just below the Chemours facility (site 3)

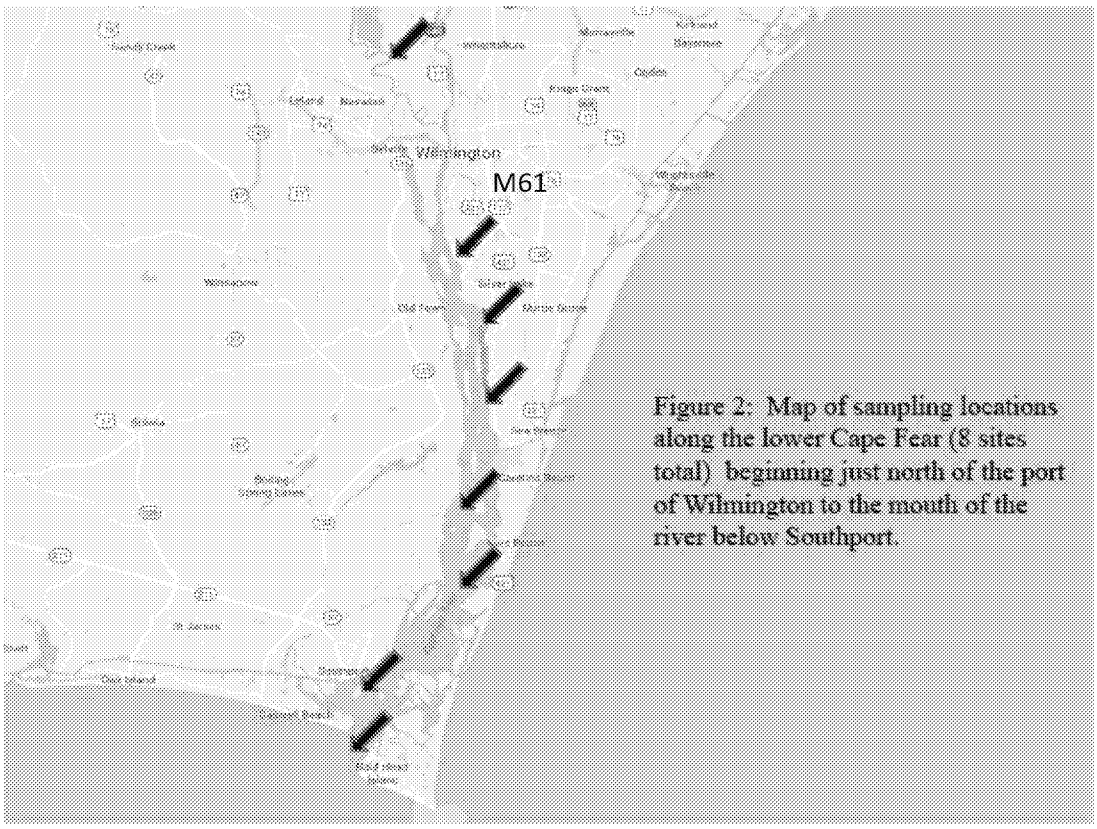


Figure 2: Map of sampling locations along the lower Cape Fear (8 sites total) beginning just north of the port of Wilmington to the mouth of the river below Southport.

Figure 3: Extracted ion chromatogram generated from LC/QTOF data of extracted sediment from M61 located in the Cape Fear River. The numbers above the peaks indicate several PFAS compounds detected through non-targeted analysis. As a reference point peak IV is GenX. Tentative reported structures of peaks I-VI can be found in Fig. 4.

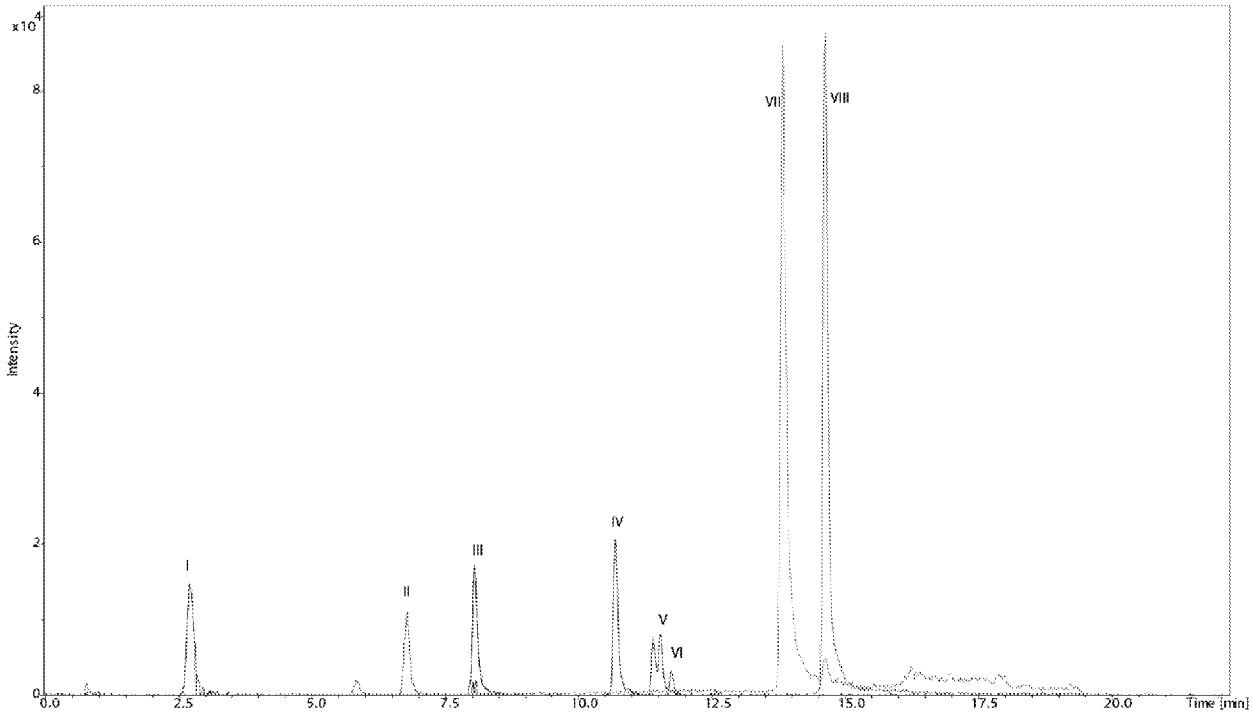
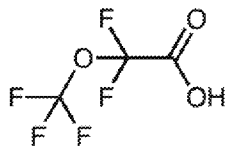
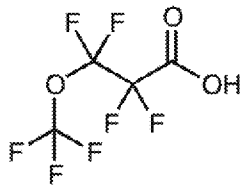


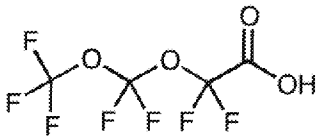
Figure 4: Structures and chemical names of PFAS compounds detected in sediment collected from Cape Fear River. The peak numbers corresponds to those shown in figure 3.



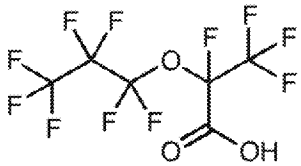
Peak I
perfluoro-2-methoxyacetic acid



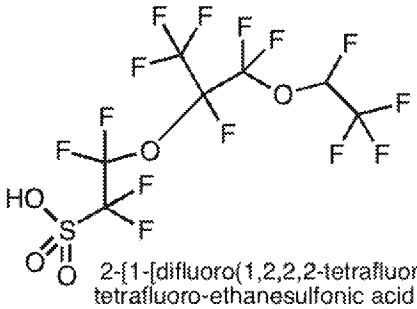
Peak II
perfluoro-3-methoxypropoanoic acid



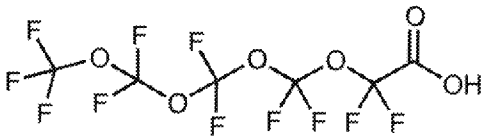
Peak III
perfluoro(3,5-dioxahexanoic)acid



Peak IV
perfluoro-2-propoxypropanoic acid



Peak V
2-{1-[difluoro(1,2,2,2-tetrafluoroethoxy)methyl]-1,2,2,2-tetrafluoroethoxy}-1,1,2,2-tetrafluoro-ethanesulfonic acid



Peak VI
perfluoro(3,5,7,9-tetradecanoic)acid

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Objective 4: Bioaccumulation in the ecosystem, initially through examination of levels of GenX that may result in ecological impairment in oysters and of the potential for oysters to uptake and accumulate GenX.

Personnel Involved: Three senior faculty, two graduate students, one undergraduate Honors student, and a middle school student.

Status: We conducted preliminary experiments to examine the levels of GenX that may result in ecological impairment in juvenile oysters. Juvenile oysters were obtained from UNCW's Shellfish Hatchery. Four test treatments (0, 1, 10, and 100 parts per billion; ppb nominal concentrations obtained by diluting 100% stock) of GenX with three replicates for each treatment (12 juvenile oysters/replicate) were used to examine the effects of GenX on the growth, survival and filtration (clearance) rates of juvenile oysters (10-20 mm). Oysters were exposed to GenX in two-liter beakers in a static system three times per week for four weeks. Oysters were fed a mixture of algal diet daily. Mortality was noted during water change (2X/week). Size and clearance rates using flow cytometry were measured on days 14 and 28 post-exposure. Clearance rates of oysters were measured by decrease in concentration of *Isochrysis galbana* (3 million cells/ml) over two hrs. Algal samples were fixed with glutaraldehyde and concentrations of cells measured using a Guava flow cytometer.

At the end of the 28-day exposure period, surviving oysters were frozen at -80C for determining the bioaccumulation of GenX in oyster tissues (Delinsky et. al 2009). Each sample set contained matrix calibration curve samples (spiked adult oyster from UNCW Shellfish Hatchery), a method blank (ultrapure deionized water), and a matrix blank (adult oysters from UNCW hatchery). Oysters were shucked and tissues were stored in -80°C until analysis. Oyster tissue samples were thawed and homogenized for 10 seconds using a Retsch MM 400 Mixer Mill (frequency = 30). Oyster tissue used for the matrix calibration curve was pooled to reduce individual variability. A 1g aliquot of homogenized tissue was added to 3mL of ultrapure deionized water and vortexed. Matrix calibration curve samples were spiked with appropriate levels of GenX. All samples were diluted in 0.01 NaOH in MeOH and vortexed. Samples were vortexed and placed on an orbital shaker with gentle shaking action for 16 hours, followed by 30 minutes of vigorous shaking. Samples were centrifuged for 5 minutes at 2000 rpm. A 3 mL aliquot of the supernatant was added to 27mL of ultrapure deionized water and vortexed.

Solid Phase Extraction (SPE) was performed on the diluted supernatant using a Visiprep vacuum manifold. Waters oasis 3 cc WAX cartridges (60 mg sorbent, 60μm particle size) were conditioned with 4 mL of 0.03% NH4OH in MeOH, 4 mL of MeOH, and 4 mL of ultrapure deionized water. The entire 30mL samples were loaded into the cartridges. To wash, the cartridges were loaded with 4 mL of 25mM sodium acetate buffer (pH = 4). Our analyte was eluted into 15 mL centrifuge tubes using 4 mL of 0.03% NH4OH in MeOH. The elute was evaporated with nitrogen gas to 1mL and vortexed. A 280 μL aliquot of the concentrated elute was added to a 2 micron filter spin column with 120 μL of 2mM ammonium acetate buffer (pH = 6.5). Spin columns were centrifuged at 8000 rpm for 1 minute. Contents of each spin column were placed into respectively labeled MICROSOLV autosampler vials for LC/MS mass spectrometry analysis.

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Oyster size did not vary between treatments. Given that these experiments were carried out under static conditions and fed algal paste diet, lack of growth in oysters was not surprising. While one to two oysters encountered mortality in control, 1 and 10 ppb treatments, nine out of 36 oysters encountered mortality in the 100 ppb treatment suggesting that 100 ppb was stressful to juvenile oysters under the experimental conditions. Similarly, while filtration rate was not affected in oysters from treatments 0, 1 and 10 ppb, oysters at 100 ppb showed a reduced clearance rates compared to other treatments (Fig 5).

Tissue concentrations of GenX in juvenile oysters at control (0 ppb), 1 and 10 ppb were < 0.5 ppb, while oysters exposed to 100 ppb had 1.7 ppb (0.0017 ppm; Fig. 6). Trace levels of GenX in control oysters may be due to the proximity of experimental chambers to the 1, 10 and 100 ppb treatments. Since water in the beakers has to be bubbled to maintain favorable oxygen levels for oysters to survive, aerosolization of GenX in bubbles may have contributed to trace levels of GenX in control treatments. Although oysters were exposed to high levels of GenX, it did not appear that bioaccumulation of GenX was high in oyster tissue. For example, while oysters were exposed to 100 ppb of GenX three times per week for four weeks, tissue concentration in oysters from this treatment was 1.7 ppb. This may be due to either adsorption of GenX to the test chamber (glass) or lack of bioaccumulation of GenX due to its polar nature.

Results from the preliminary experiment suggests that very high concentrations (between 10 and 100 ppb nominal concentrations) may decrease filtration in oysters. Also, while oysters were exposed to 1-100 ppb of GenX, there was little bioaccumulation of GenX in oyster tissues. GenX concentration in 0, 1 and 10 ppb treatments ranged between 0.1 and 0.2 ppb, while oysters even in 100 ppb treatment had 1.7 ppb of GenX in their tissues. GenX concentrations reported in Cape Fear River ranged between 800 – 2400 parts per trillion (0.8 – 2.4 ppb). These concentrations are far lower (100-fold) than the concentrations that resulted in decrease in filtration and higher mortality under experimental conditions. It should be cautioned that these are preliminary experiments to examine the levels of GenX that may result in impairment of ecological responses in oysters. We are currently in the process of conducting further experiments involving adult oysters using environmentally realistic concentrations of GenX using non-glass exposure chambers.

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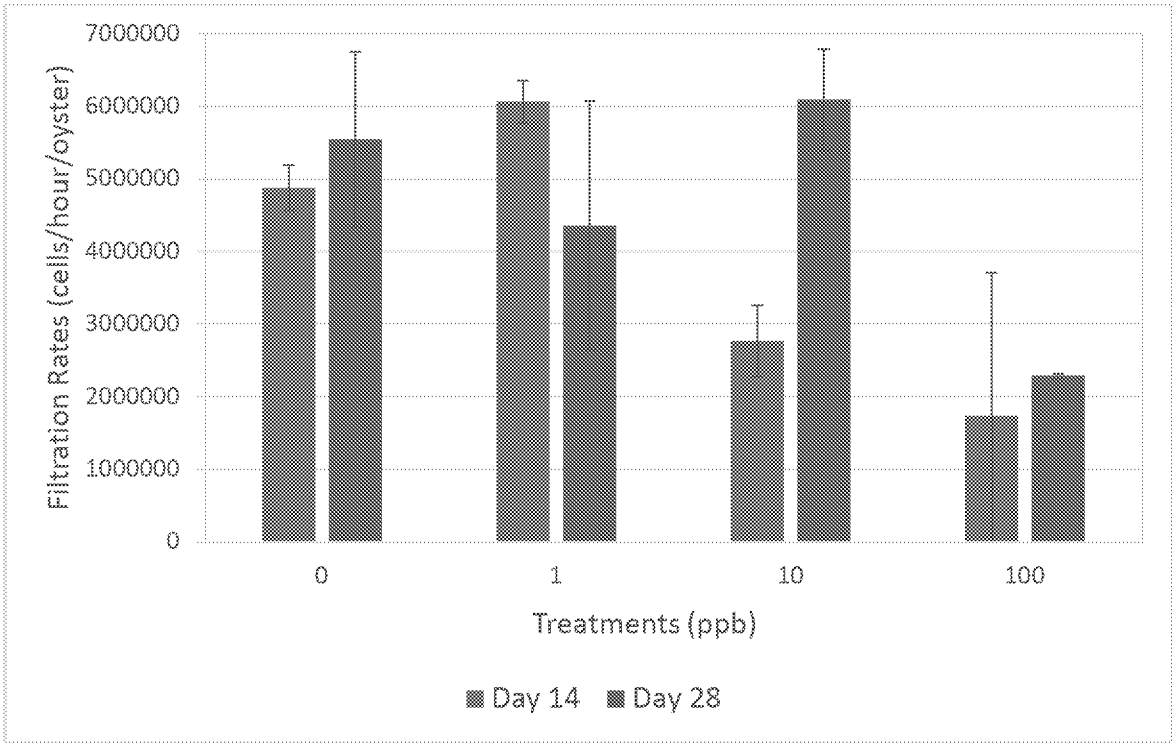

Fig. 5: Filtration (clearance rate) of juvenile oysters exposed to 0, 1, 10, and 100 ppb of GenX. Results suggest that oysters exposed to 100 ppb may result in decrease in filtration capacity of oysters. This level is far higher than levels of GenX found in Cape Fear River.

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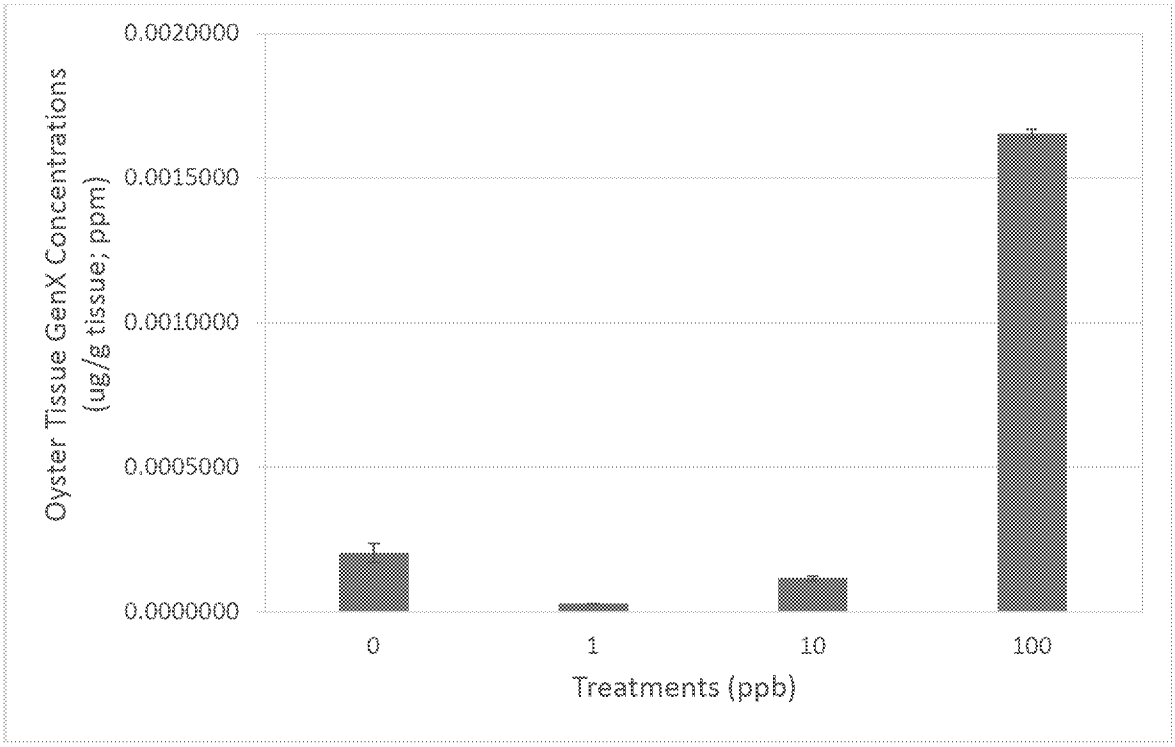

Fig. 6: Tissue concentrations of GenX in juvenile oysters exposed to 0, 1, 10 and 100 ppb of GenX three times/week for four weeks. Results suggest that even oysters exposed to 100 ppb accumulated very little GenX (1.7 ppb).

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Future oyster experiments:

Two sets of experiments are planned for the coming months. First set of experiments will involve collecting oysters from Cape Fear River from upstream to downstream (along a salinity gradient) to determine bioaccumulation of GenX (if any) in oyster populations in the wild. Since GenX source is at the upstream location, one would anticipate higher concentrations in water at upstream locations and lower concentrations in the water in downstream locations. These may be reflected in tissue accumulation of GenX in oysters. Since GenX is a polar compound (soluble in water as opposed to oil and pesticides), it is possible that oysters may not accumulate and they merely reflect concentrations found in the water surrounding them. Alternatively, given their high filtration rate, if there is any tissue bioaccumulation, it has potential for physiological impacts on oysters and/or bio-concentration in higher trophic levels as other organisms consume oysters. We anticipate accomplishing this task by early May. To date, we collected oysters from two downstream locations (Fig. 7). Oyster tissues from these two locations were extracted according to the methods described above and awaiting tissue analyses of GenX. We plan to collect oysters from three upstream locations in the Cape Fear River in the next two to four weeks and analyze tissue concentrations of GenX.

Fig. 7: Locations of oyster collection from downstream locations in Cape Fear River. Tissue concentrations of GenX (if any) will be determined from these oysters as well as oysters from upstream locations in the river.

A second set of experiments will be comprised of exposing clean oysters to various concentrations of GenX (0 to 5000 parts per trillion) under controlled laboratory conditions. Oysters will be exposed to GenX for three weeks. Three replicate tanks will house 25 oysters each for each treatment level. Two oysters from each tank will be sub-sampled on Day 1, 3,

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5, 7, 14 and 21 days to examine the bioaccumulation of GenX under various concentrations. This will inform us as to how quickly oysters may take up and accumulate (or not) GenX and will enable us to model potential bioaccumulation in the field given particular GenX concentration. We anticipate starting this experiment in the last week of April and completing it by June.

Future experiments will examine depuration kinetics (i.e., how quickly do oysters rid themselves of GenX should cleaner conditions prevail, with no GenX present). Additional experiments will examine potential biological impacts on oysters and will be the subject of studies to examine sub-lethal responses of oysters upon exposure.

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