# ABSTRACT

ZANE, CODY PATRICK. Hazard Assessment of Fluorochemicals Present on Firefighter Gear. (Under the direction of Dr. Bryan Ormond).

Per- and polyfluorinated chemicals (PFCs) are a class,4,000 strong, of emerging chemicals of concern. They have permeated much of the environment, from drinking water supplies to household dusts. Much of this has come from their widespread use as a water-, oil-, and dirt-repellent finishes, non-stick coatings for cookware, industrial effluent, and firefighting foams. Most countries have a significant portion of their population with PFCs measured in their blood serum, and studies indicate a plethora of health effects associated with exposure to these chemicals.

That being said, certain occupations may face an increased risk of exposure to PFCs, firefighting being one of them. Firefighters come into contact with a wide array of chemicals due to their occupation: phenols, phthalates, polycyclic aromatic hydrocarbons, heavy metals, carbon monoxide, etc. However, most of those chemicals are due to situations they encounter on the job. A few years ago, the firefighting community started to become weary of the personal protective equipment, known as turnout gear, they wear while facing those situations. They were misinformed when they were told that their gear was made from PFCs. Yet, it was still unknown whether they had increased exposure to PFCs from latent chemicals on their gear.

The following dissertation aimed to develop a risk assessment for the firefighting community for their exposure to PFCs. This was completed via a three-branch approach: an in-depth literature review to determine health effects and current exposure levels for firefighters, quantification of two PFCs in unused gear from a 20-year range, and a durability assessment of the PFCs present on the gear.

Ultimately, it was determined that firefighters have minimal risk of exposure to PFCs from their gear. Quantification of the 20-year range of turnout gear determined the highest level of perfluorooctanoic acid (PFOA) to be at 14.83 ng/cm2. With dermal exposure being the most likely pathway to exposure from PFCs on gear, this concentration is 1,000 times lower than the lowest concentration tested for dermal studies of PFCs. The study found no detectable limits of perfluorooctane sulfonate (PFOS).

The durability studies determined that the PFCs, present in the form of durable water repellent (DWR) finishes, were durable up to 120-hours of UV exposure and 50 standard NFPA 1851 wash cycles, separately. Outer shell material was exposed to UV up to 120 hours. No drop in ISO 6530 water repellency or goniometer contact angle was indicated. Outer shell material was also exposed up to 100 standard NFPA 1851 wash cycles. Results from ISO 6530 testing indicated a decrease in water repellency after 50 washes, but no more than a 20% loss of water repellency.

Additionally, this dissertation developed and validated an extraction procedure for PFCs from textile materials. The study indicates that a wide array of extraction techniques are viable but based on sample size, time, and solvent volume the sonication and pressurized solvent extractor methods, without evaporation, were determined to be the most beneficial.

While the following dissertation was thorough and answered many questions the firefighting community had about their gear there are still things to consider. Much of the published research indicates elevated levels of sulfonated PFCs in firefighters blood. This may be due to use of aqueous film forming foams but further investigation is needed. Although, that does not mean that they do not have higher exposure rates

# Hazard Assessment of Fluorochemicals Present on Firefighter Gear

by
Cody Patrick Zane

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Fiber and Polymer Science

Raleigh, North Carolina
2020

APPROVED BY:

| | |
|---|---|
| Dr. Bryan Ormond<br>Committee Chair | Dr. Melissa Pasquinelli |
| Dr. Emily Griffith | Dr. Ronald Baynes |

# DEDICATION

I dedicate the following dissertation to my grandmother, Grace Cecelia Zane, better known to me as Grammie.  Gram, I may not have had long with you in this life but you taught me so many important life lessons that I will never forget.  Unfortunately, it took me a few years after you passed to finally realize what you were teaching me.  Regardless, I know that every ounce of what you taught me went into getting this degree.  I could not have done this degree without those lessons.  They kept reminding me to find and surround myself with those I love and work with them, to help us all achieve our own goals.  Most importantly, you taught me pure, unadulterated compassion.  Love and care for everyone, regardless of what they have done to you because hatred will only eat you up.  I love you so much, miss you everyday, and thank you for everything you have done, and will do, for me.

# BIOGRAPHY

Cody Patrick Zane is an internationally unrecognized scientist, baker, chef, student leader and scholar, who specializes in bread, smoked meats, mental health activism, analytical chemistry and personal protective equipment (PPE). Born in the wee hours of a Tuesday morning, after his mother decided to do The Twist while being nine-months pregnant, Cody turned out to be a tiny terror to his parents and the reason for many of their grey hairs.

Despite all the mayhem he caused in his young life, Cody found his way as he stumbled from one passion to the next. In 2011, Cody started his baccalaureate degree in Polymer and Color Chemistry in the College of Textiles at North Carolina State University where he graduated Magna Cum Laude in 2015. Unsure of what to do next, Cody entered graduate school, at his alma mater, for a Masters of Science in Textile Chemistry, under the advisement of Dr. Melissa Pasquinelli and Dr. Nelson Vinueza, which he graduated Suma Cum Laude in 2017.

It was at this juncture where Cody started understanding how all of his passions aligned. He enjoyed teaching, mentoring, and research/projects that had direct impacts on communities. As someone who could not sit still, he decided he would go on to complete a Ph.D. in Fiber and Polymer Science (yet again at North Carolina State University) under the advisement of Dr. Bryan Ormond in the Textile Protection and Comfort Center, as well as being a Mental Health Ambassador with the Counseling Center on campus.

Simultaneously, Cody lead a charge for his University community to Stop the Stigma surrounding mental health for graduate students and to discover, and understand, the occupational exposure risk of firefighters to per- and polyfluoroalkyl substances. At the completion of his degree, Cody has a desire to go into industry to continue working on PPE for first responders and military personnel.

# ACKNOWLEDGMENTS

This page is not enough for me to acknowledge every person I think has helped me get to the competition of this degree.  If you know me and I have not mentioned you here, please understand that you have done something to help me on my path and I appreciate you wholeheartedly.

While there are so many people I could acknowledge there are a handful I would like to mention.  First and foremost, Bryan Ormond, my advisor and friend.  You have supported me through every single thing that I have put my mind to and I definitely would not be the research and activist I am today without having you as my academic father…maybe academic brother.

Next I would like to acknowledge my crazy, dopey, loving family.  Sometimes, as the youngest, I feel as if there is no where for me to go that hasn't already been achieved by everyone else in this family.  But every day you dispel that belief, remind me how strong I am, what good I am doing, and give me unconditional love.  I love you so much.

My Bella…we may not have been together for my entire time in grad school but you have supported me through the hardest times.  You are my pillar and I don't know where I would be today without you.  You remind me everyday to love the day and take charge of it.  I love you so much that a Unidentified Ferby Object could not break it.

Lastly, I have to acknowledge Miss Zeta Omega Zane (Lovebug) and Mr. Chi Rho Zane (BeauxBeaux).  They may never be able to read this, but that doesn't mean I am not going to thank them for the load of unconditional love they wash me with everyday.  Your Cody could not have asked for two better puppies to help him through this experience.  You always knew when I needed love and support and would cuddle me to help me feel better.  You will always have a place in my heart, I love you.

## TABLE OF CONTENTS

## LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1: Purpose of Research and Research Objectives

## 1.1. Purpose of Research

In the last few decades, there has been a growing concern about the chemicals that firefighters come into contact with when completing tasks for their job. Most of the research completed thus far has been focused on typical chemical families (polyaromatic hydrocarbons, phthalates, phenols) in which firefighters encounter due to the fire scenarios they fight. However, in the last few years, there has been increased concern with the chemicals on their gear and those they use to smother certain classes of fires. These chemicals fall into the family of per- and polyfluorinated compounds (PFCs), also known as per- and polyfluorinated alkyl substances (PFASs). Of foremost concern are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). Studies of these chemicals present in drinking water have indicated a plethora of adverse health effects[1-5]. Those studies conducted on firefighter blood serum have indicated elevated levels of PFCs, not just PFOA and PFOS[6-10] Most of these studies concluded that the elevated levels of PFCs are due to the use of aqueous film-forming foams (AFFFs), a fire suppressant used for Class B (i.e. fires involving flammable liquids, such as gas, diesel, or oils) fires containing an array of PFCs[11]. Unfortunately, there are no current research studies that have evaluated other possible pathways for firefighter exposure, such as the presence of these PFCs on the gear due to manufacturing of from usage of AFFFs. With this in mind, the scope of this research is focused on a hazard assessment of the fluorochemicals present on firefighter turnout gear.

## 1.2. Research Objectives

### 1.2.1. Understanding of PFC Concentration on Turnout Gear

In a review of the literature, there is nothing published on the amount of PFCs present on firefighter turnout gear. Studies on consumer goods have indicated low levels, <4E$^{-7}$ µg/cm$^{2}$, of PFCs present[12]. As most fire scenarios do not require the use of AFFFs, exposure from their gear, or the fire environment, is the most likely pathway for firefighters to come into contact with PFCs on their job. Therefore, it is crucial to understand firefighter-specific job exposure to these chemicals, to gain a more comprehensive understanding of the hazards that may be present. We hypothesize similar concentrations of PFCs present on the turnout gear compared to known levels on consumer products. To prove or disprove this hypothesis, this research plan involves a seven-step process: 1) development of analytical methods, 2) development of extraction procedures, 3) measurement of PFC levels on turnout gear from a range of manufacturing years, 4) *in vitro* assessment of PFC dermal absorption, 5) laundering durability of PFCs on turnout gear, 6) assessment of off-gassing of PFCs from turnout gear, and 7) comprehensive review of contamination and hazard assessment.

#### *1.2.1.1. Development of Analytical Methods for PFOA and PFOS*

There are several analysis methods already in the literature, mostly using liquid chromatography coupled with triple quadrupole mass spectrometry (LC/QQQ). However, this technique is a much more expensive, and less accessible, method compared to more traditional gas chromatography with single quadrupole mass spectrometry (GC/MS), of which there are also analysis methods for PFCs[13]. Having a GC/MS method would give small labs, running samples for standards committees, the opportunity to run these samples, since they typically cannot afford LC/MS systems.

#### *1.2.1.2. Develop Procedures for Extraction of PFCs from Textile Substrate*

As mentioned earlier, there have been a few studies to determine PFC contamination on consumer textile products, but they use different extraction methods. Owing to the use of high-performance fibers in firefighter turnout gear, some of these methods may or may not work. A few different methods are available for use of extraction of the PFCS, but, as eluded to in Section 1.2.1.1, they would all be full extraction procedures. Some procedures are simple one step sonication procedures with the fabric in a solvent, others are more involved Soxhlet extractions taking hours, or sonication procedures with multiple evaporation steps. This step in experimentation will utilize all these different methods to determine the best extraction procedure for PFCs from turnout gear fabrics. There is one deviation to the previous methods mentioned, our lab will utilized a pressurized solvent extractor (PSE) in place of a Soxhlet extraction. The PSE is an automated extractor which is faster than a Soxhlet extraction, while typically being more efficient.

#### *1.2.1.3. Measurement of PFC Levels in Turnout Gear from a Range of Manufacturing Years*

The Stockholm convention banned the use of PFOS in 2009, while the U.S. manufacturing industry phased out the use of PFOA in 2015. This may mean that levels of these chemicals on firefighter turnout gear may decrease over a selected year range of gear. Ultimately, this should mean that firefighter job-exposure to PFOA and PFOS from their gear should decrease as older gear begins to be phased out. According to the National Fire Protection Association 1971 standard, there is a 10-year retirement age of turnout gear. Unfortunately, this is not always followed as the standard is not a regulation only a guideline to follow. However, this does not mean they are not exposed to PFCs from AFFF, contaminated drinking water, or other PFCs now used on gear.

### 1.2.2. Assessment of UV Durability of PFOA and PFOS Water Repellent Finishes on Turnout Gear

In addition to the concerns over dermal exposure from contact with PFC-laden fabric, there has been recent concern about inhalation hazards associated either with degradation of PFCs due to UV exposure or from PFCs off-gassing from turnout gear due to high heat exposures. Studies indicate that the polytetrafluoroethylene (PTFE), a polymeric PFC, backing of moisture barriers from firefighter turnout gear is effected by UV exposure[14,15]. However, other studies conducted on small molecule PFCs, such as PFOA or PFOS, do not indicate any degradation due to UV exposure[16]. Since the PFCs present on firefighter gear are in the form of durable water repellent (DWR) finishes, another type of polymeric PFC, there is a likelihood of degradation due to UV exposure. The following study will complete a UV durability assessment of the DWRs present on the turnout gear. The study will utilize a weatherometer to mimic the effects of UV exposure in a controlled setting.

### 1.2.3. Assessment of Laundering Durability of PFOA and PFOS Water Repellent Finishes on Turnout Gear

There have been a few studies on the durability of water repellants, such as PFOA, or durable water repellent finishes on consumer textile products, and even less specifically focused on firefighter gear. The following literature review found no standards, by any standards committee (e.g., ASTM, ISO, AATCC, NFPA, etc.), on laundering durability of water repellents.

The Textile Protection and Comfort Center at NC State has a concurrent project looking at "how clean is clean" of exposed firefighter turnout gear. During experimentation for this project, control fabrics will be run through certain washing procedures, of note is NFPA 1851 which outlines guidelines fire departments should use to decontaminate gear after exposure, to help set a baseline for their washing efficiencies. This project will take their control fabrics and run them through the extraction procedure determined from section 1.2.1.2 to determine the durability of the water repellent finishes when subjected to standard washing procedures.

#### 1.2.4. Comprehensive Review of Contamination and Risk Assessment

Ultimately, the steps taken above will allow a risk assessment to be made on the risk of firefighter exposure to PFCs from their gear. We will be utilizing the United States Environmental Protection Agency (EPA) risk assessment guidelines as a framework in order to conclude any risk to the firefighters. Step one of the EPA guidelines is hazard identification, which is used to determine if a stressor/chemical poses a threat to human or ecological health, the following literature review covers this step. Step two is a dose-response assessment, which is used to determine a numerical relationship between the effects seen and the exposure, the *in vitro* dermal studies and literature review will cover this step. Step three is an exposure assessment, which examines all the information you know about an exposure (e.g. frequency, timing, levels of contact), all of the other steps proposed cover this step. Lastly, in step four, risk characterization, all the information and data is brought together to determine if they support the conclusions about the nature and extent of the risk.

# CHAPTER 2: Introduction

## 2.1. Firefighting

### 2.1.1. History of Firefighting and Their Gear

The first recorded battalion of firefighters were the Cohortes Vigilum, or Cohorts of Watchmen, created by Augustus in the early first century. Augustus found the need for the Cohortes Vigilum after a major fire broke out in Ancient Rome. Unlike modern firefighters, the Cohortes Vigilum didn't have any protective gear and fought fires using ladders, buckets of water, grappling hooks, and water/vinegar-soaked blankets. If a fire became too involved, i.e., it was getting too big, the Vigilium would try to tear down roofs, walls and adjacent buildings to keep the fire from spreading. [17]

Once the Roman Empire fell, it was another 1100 years until another form of "firefighting" came about in the Middle Ages, which could be considered fire prevention. Watchmen were put in place at night to watch for fires, while full buckets and ladders had to be within easy reach. These tactics were carried over to early American settlements, where the first written fire regulation was put into place in 1630 banning wooden chimneys and thatched roofs. [17,18]

In 1653 and 1666 the great Boston and London fires broke out, respectively, fought with bucket brigades. It was at this time consideration was put into fire fighting, instead of fire prevention, alone. Most of the innovation was put into the equipment used by early firefighters. For example, water pumps were created and placed on the back of wagons to increase the speed at which the fires could be fought. Of note was the creation of the National Fire Protection Association (NFPA) in the 1800s to govern and standardize fire protection efforts across the country. However, it wasn't until the early 1900s when firefighting tactics and gear changed considerably. [17-19]

Loyd Layman, a fire chief from 1930-1960, helped create a set of tactics which are the basis to modern firefighting tactics, see Table 2.1. Of the tactics Layman helped create "the indirect method of attack" was the most influential. This tactic calls for firefighters to spray the water around the substance that is burning, reducing the heat in the area. Layman help develop this technique during his time with the U.S. Coast Guard when learning how to extinguish oil fires in confined spaces. This new style of fighting allowed firefighters to move interior, i.e., to go within the burning structure, on a structure fire, which called for innovation in their gear. [19-21]

*Table 2.1: Division of modern firefighting tactics, as described by NFPA. Adapted from*[21]

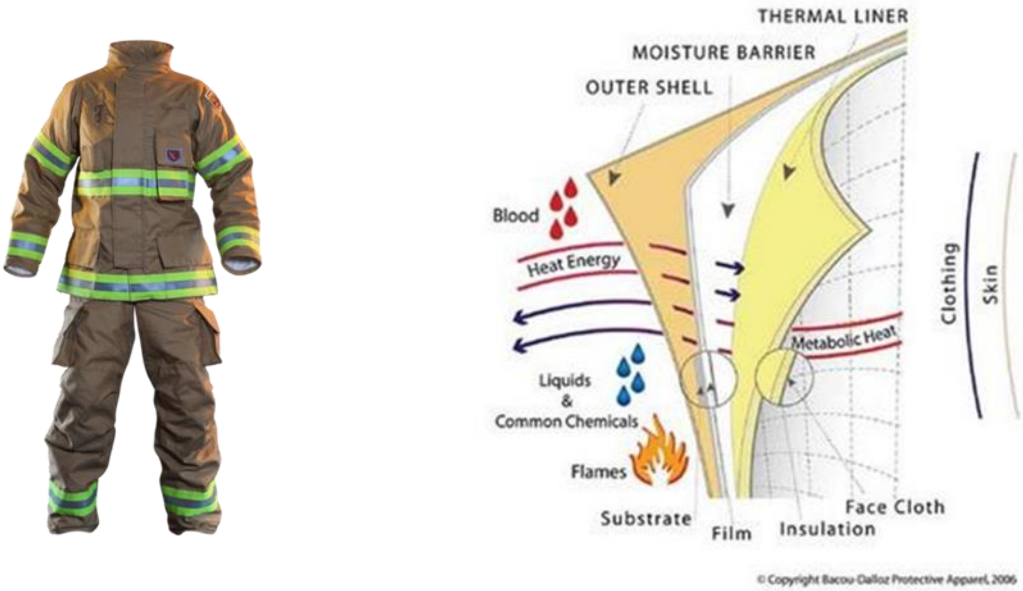
| **Division** | **Description** |
|---|---|
| Size Up | Assess the fire situation by getting a 360° view |
| Identify Flow Path | Read the fire and understand: smoke flow path, ventilation, and entry and exit points |
| Soften Target | Apply a stream of water onto fire to cool area before entering |
| Read Smoke | Ventilation-limited fire rapidly intake air, which could mean fire needs more room to grow |
| Vent close to fire origin | Venting too far from source could increase fire |
| Coordinate ventilation with hose attack | Use water in tandem with ventilation to reduce risk of fire growing |
| Vent, Enter, Isolate, Search (VEIS) | Allows the ability to search the area for survivors |
| Close Door | Keeps room detached from main fire, reducing growth |
| Control the Access Door | Limits airflow to fire, allowing attack crew to get a leverage on the fire |

The textile material firefighter wear, affectionately called "bunker gear", due to being laid out next to their bunk, or "turn out gear", is vital to modern firefighting tactics. Early versions of the gear consisted of a three-quarter length jacket and boots, see Figure 2.1. The outer shells of both the jackets and boots were made of rubber which would deflect any embers coming from the fire. Meanwhile, they were lined with cotton or wool on the inside which insulated them from the heat of the fire or kept them warm while fighting fires during the winter. [17,22]

*Figure 2.1: Early version, prior to 1970, of firefighting gear with ¾ length jacket and boots.* [22]

Due to the risk associated with the type of ensemble, in 1987 the NFPA took charge and created the NFPA 1500: Standard on Fire Department Occupational Safety, Health, and Wellness Program which banned the three-quarter length jacket and boots ensemble[23,24]. NFPA 1500 states that turnout gear must pass NFPA 1971: Standard on Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting[24,25]. Due to section 6.5.1, in NFPA 1971, the turnout ensembles must provide continuous protection from thermal and moisture hazards[25]. The legacy three-quarter length jacket and boots could not pass this standard owing to the hole at the interface of the jacket and boots[22].

Thus modern firefighter gear was created, as seen in Figure 2.2. NFPA 1971 standardizes the three different layers of the turnout ensemble: the outershell, moisture barrier, and thermal liner[25]. The purpose of the outershell is to protect from direct flames, abrasions, and cuts. This layer is typically made of fibers with high flame resistance, such as polybenzimidazole (PBI), meta-aramid (Kevlar®), para-aramid (Nomex®), or polybenzoxazole (PBO)[26]. The moisture barrier is utilized to protect against the transfer of liquid material onto the skin, while also moving perspiration away from the body. This is vital to the protection of the suit as liquid aids in the heat transfer to the body and causing severe burns. The moisture barrier is typically a laminated material with one side being PBI, meta-aramid, para-aramid, or flame-retardant (FR) rayon and the other side being polytetrafluoroethylene or polyurethane[27]. Lastly, the thermal barrier gives the most protection from ambient heat and is quilted with a facecloth and batting. The facecloth can be a polyimide, PBI, FR rayon, meta-aramid, or para-aramid[28].



© Copyright Bacou-Dalloz Protective Apparel, 2006

(a) (b)

*Figure 2.2: (a) Modern firefighter turnout gear passing NFPA 1971 requirements, (b) Depiction of the three layers, in turnout gear, and their uses.*[29, 30]

### 2.1.2. Cancer in the Fire Service

As of the 2010 International Agency for Research on Cancer (IARC), volume 98, report, the occupation of firefighting has been labeled as a possible carcinogen[31]. Unlike their conclusion that the profession of painting is carcinogenic and shift work is probably carcinogenic, firefighting had limited evidence for carcinogenicity in humans and inadequate (meaning no) evidence of carcinogenicity in animals[31]. However, extensive studies, such as the meta-analysis completed by LeMasters and co-workers, have concluded an increased risk of cancer to firefighters[32]. Table 2.2 summarizes the analysis LeMasters completed on 32 cancer studies done on firefighters. They found ten probable links to cancer, based on their analysis. They also conclude that testicular cancer should also be raised to probable based on anecdotal information they received from firefighter about the black soot they have on their groin region all the time[32]. A plethora of other studies, outside the 32 LeMasters used, confirm the correlation between career firefighting and cancer[33-37]. Unfortunately, there is no consensus as to any specific cancers being correlated with career firefighting, making it difficult for firefighters to get compensation from state presumptive legislation.

*Table 2.2: Summary of Likelihood of Cancer Risk and Summary Risk Estimate (95% CI)*[32]

| Cancer Site | Likelihood of Cancer Risk | Summary Risk Estimate (95% CI) |
|---|---|---|
| Multiple Myeloma | Probable | 1.53 (1.21-1.94) |
| Non-Hodgkin Lymphoma | Probable | 1.51 (1.31-1.73) |
| Prostate | Probable | 1.28 (1.15-1.43) |
| Testis | Possible | 2.02 (1.30-3.13) |
| Skin | Possible | 1.39 (1.10-1.73) |
| Malignant Melanoma | Possible | 1.32 (1.10-1.57) |
| Brain | Possible | 1.32 (1.12-1.54) |
| Rectum | Possible | 1.29 (1.10-1.51) |
| Buccal Cavity and Pharynx | Possible | 1.23 (0.96-1.55) |
| Stomach | Possible | 1.22 (1.04-1.44) |
| Colon | Possible | 1.21 (1.03-1.41) |
| Leukemia | Possible | 1.14 (0.98-1.31) |
| Larynx | Unlikely | 1.22 (0.87-1.70) |
| Bladder | Unlikely | 1.20 (0.97-1.48) |
| Esophagus | Unlikely | 1.16 (0.86-1.57) |
| Pancreas | Unlikely | 1.10 (0.91-1.34) |
| Kidney | Unlikely | 1.07 (0.78-1.46) |
| Hodgkin’s Disease | Unlikely | 1.07 (0.59-1.92) |
| Liver | Unlikely | 1.04 (0.72-1.49) |
| Lung | Unlikely | 1.03 (0.97-1.08) |
| All Cancers | Unlikely | 1.05 (1.00-1.09) |

This uncertainty comes from the exponential amount of chemicals that firefighters come into contact with when dealing with a fire scenario: halogenated flame retardants, polyaromatic hydrocarbons, phthalates, phenols, vaporized acids, and heavy metals, just to name a few classes of chemicals[32,38-41]. Many correlate this array of chemicals with modern amenities, furniture, and building materials[38,42]. Today, fires burn approximately eight times faster than those of 50 years ago, which Underwriters Laboratories demonstrated by burning a room with legacy furniture and one with modern furniture[43]. The room with modern furniture hit flashover conditions in 3.5 minutes, while it took the legacy room 29.5 minutes to reach flashover conditions, which also speaks to the ability for response time[43]. Flashover, fire point, and flameover are all firefighter terms used to describe a similar point in time during the fire. When a certain temperature (fire point) is reached at which materials give off flammable gasses to sustain the fire, which ignite at the ceiling level (flameover), and could be dangerous for firefighters when it occurs in a closed space (flashover), such as a bed room[44].

Subsequently, most firefighters will come on the scene by the time a fire becomes fully involved, meaning that the heat and smoke in the structure are at such a level that external application of water must be applied before entering the structure[44]. Moving within and around such hot structures, weighed down by their personal protective equipment (PPE) increases their body temperature significantly. Ultimately, a rise in skin temperature could also correlate to an increase in skin absorption[38].

Studies on inhalation exposure and weaknesses in the turnout gear ensemble[40,41,45], have lead to great changes in firefighting PPE, such as self-contained breathing apparatuses (SCBAs) and particulate-blocking hoods. However, changing the minds of firefighters is difficult. Despite knowing the proper cleaning of gear, on-site decontamination, and the wearing of hoods can help prevent cancer, many firefighters are still reticent to the change[46]. This all comes down to the idea of tradition and perceived norms of the career and community[46]. Thankfully, minds are changing, and the culture is shifting, but there are still great strides to be made.

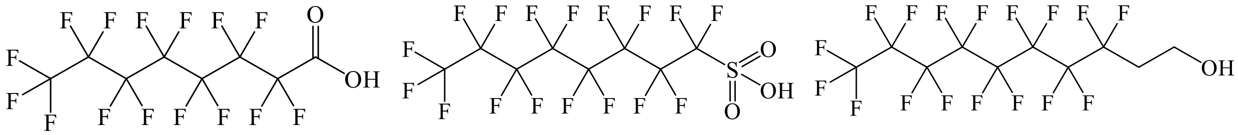
### 2.1.3. Firefighting and Perfluorinated Compounds/Chemicals (PFCs)

Firefighters come into contact with a plethora of chemicals, and no one exposure has been linked to the possible carcinogenicity of the occupation. However, there has been recent concern over the occupational exposure to perfluorinated compounds/chemicals (PFCs). As the following literature review will discuss, there are a plethora of health effects associated with PFC exposure. Firefighter exposure to PFCs can occur from non-occupational exposure (e.g., drinking water contamination) or from aqueous film-forming foams or contact with their gear. The following sections will discuss these exposure pathways in more detail.

#### *2.1.3.1. Aqueous Film Forming Foams*

First developed in the 1960s from a collaborative effort between the Navy and 3M, aqueous film-forming foams (AFFF) were developed to provide a more efficient method of dousing jet fuel fires[47]. Due to the difference in density and the heat generated by a jet fuel fire, water will either sit below this type of fire or boil immediately upon contact. Aqueous film-forming foams, however, utilize perfluorooctane sulfonate (PFOS), structure in Figure 2.3b, as an emulsifier and surfactant to create a film to sit on top of the fire, reduce the oxygen available for combustion and, eventually, smother the fire. The PFOS, and other possible fluorinated chemicals, also help the foam spread more easily across the Class B fire[48,49]. Some formulations contain fluorotelomers, examples of some fluorotelomers can be seen in Figure 2.3c. These fluorotelomers, easily degrade into other PFC's, mainly perfluorooctanoic acid (PFOA), essentially making AFFF's a mixture of

PFOS and PFOA[50-52]. However, when in use, AFFFs are not a majority PFOS and fluorotelomers; stations will typically create a 3-10% chemical solution with water[53].



(a) (b) (c)

*Figure 2.3: (a) PFOA, (b) PFOS, and (c) an example of a fluorotelomer*

Aqueous film-forming foams have been required on Navy ships since 1967 after 134 sailors died from a fire caused when a misfired rocket hit a jet-fuel tank causing it to explode and burn all night on deck[48]. Since then, the environmental contamination from AFFFs was fast and furious. The typical practice for the Navy, in the 1970s, with AFFFs was to wash the foam off the ship into the ocean, bays, and harbors. Thousands of gallons of AFFFs were used and washed away each year, mostly due to training exercises, causing environmental contamination in nearby groundwater[54,55]. Many commercial airports, airplane hangars, oil refineries, fire departments, heliports, and petrochemical transfer and production sites have been utilizing AFFFs for decades[47]. As of 2000, the makeup of AFFF use was 75% military, 13% municipalities (e.g., fire departments), and 5% by the hydrocarbon processing industry (e.g., oil refineries), respectively in that order, while 7% is for other uses (Figure 2.4)[56]. Concurrently, there were serious considerations to stray away from fluorinated-based AFFFs, due to the information coming to light from the DuPont litigation around its Washington Works plant and the toxicity research from 3M and DuPont.

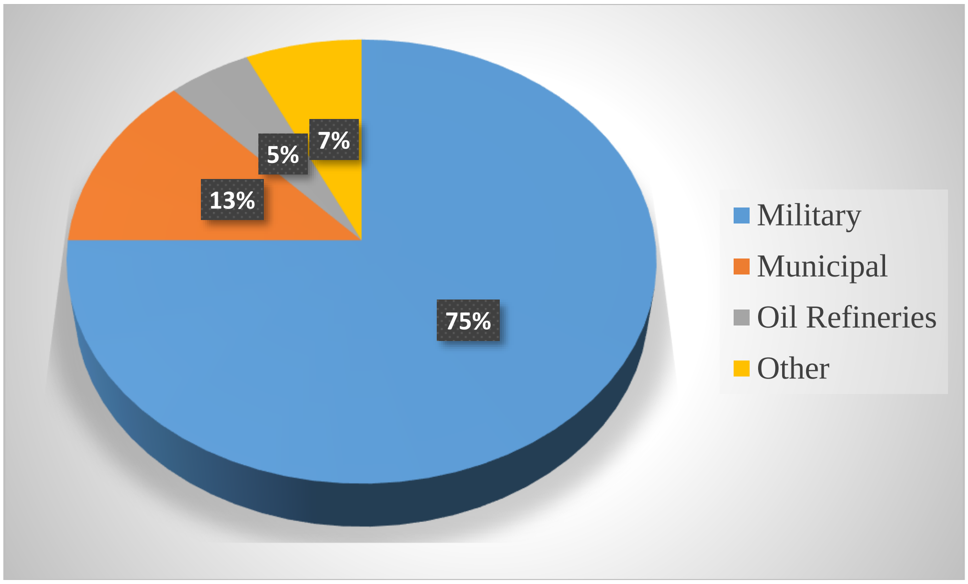


*Figure 2.4: Usage of AFFFs across industries, as of 2000. Adapted from*[57]

As the Environmental Protection Agency (EPA) started to scrutinize PFOS and PFOA the companies producing AFFFs (DuPont and Dynax were at the forefront of the companies) started changing their formulas so they could continue using fluorinated surfactants in their formulations[48]. As will be discussed in detail later on, shorter-chain PFCs were considered to be safer alternatives, and this is what the AFFF companies began to use in their formulations. Simultaneously, they formed the Firefighting Foam Coalition (FFFC) to present data for the use of fluorinated surfactants in AFFFs. One experiment done by the FFFC indicated there AFFF with PFCs could smother a fire within 30 seconds, while it took non-PFC AFFFs 60 seconds, or greater, to smother a fire completely[58]. The claim stated that non-PFC AFFFs barely meet the International Civil Aviation Organization standard for AFFFs to put out jet fuel fires within 60 seconds and, as such, are inferior substitutes for PFC-AFFFs[58].

Meanwhile, after 3M halted production of PFOS in 2000, 3M scientist Ted Schafer was given the task of creating non-PFC AFFFs. By 2002, Schafer created a formulation made from biodegradable organic surfactants and sugars which would smother a jet fuel fire with tiny bubble rather than a thin film[49,59]. Tests using this new formulation doused fires in 46 seconds, the same amount of time a 3M PFC-AFFF took[48,49,59]. However, due to the Navy's requirement for dousing fires within 30 seconds, this formulation was never adopted by the Navy[48].

To use AFFF's, either at an airport, military base, or fire station, firefighters must be trained and certified to use this fire suppression agent. For instance, Australian firefighters must be certified every 90 days to be able to use AFFFs[7,8]. Certification exercises for AFFFs differ slightly, but the constants are setting some level of jet fuel ablaze and dousing the fire with 75-100 liters of AFFFs, then washing away the excess foam down the nearest drains[7,8,47]. Ostensibly, this could make any current or former US military fire- or crash-training site, of which there are 664, an EPA Superfund site[47]. PFCs have been found at an extraordinary level around bases in Florida, Nevada, and Michigan, measuring in at 14,600 ppb, 6,270 ppb, and 100,000 ppb, respectively[47]. Figure 2.5 and 2.6 indicates areas across the country where PFCs have been found in drinking water due to military fire- or crash-training sites[47]. It has been estimated that between 500-700 different compounds have been detected in water surrounding these sites, while only 30-50 of those are PFC chemicals[48,60]. There has been an array of other environmental studies conducted, as well, documenting the issue with AFFF use in or around airports and training facilities[54-56,61-63].

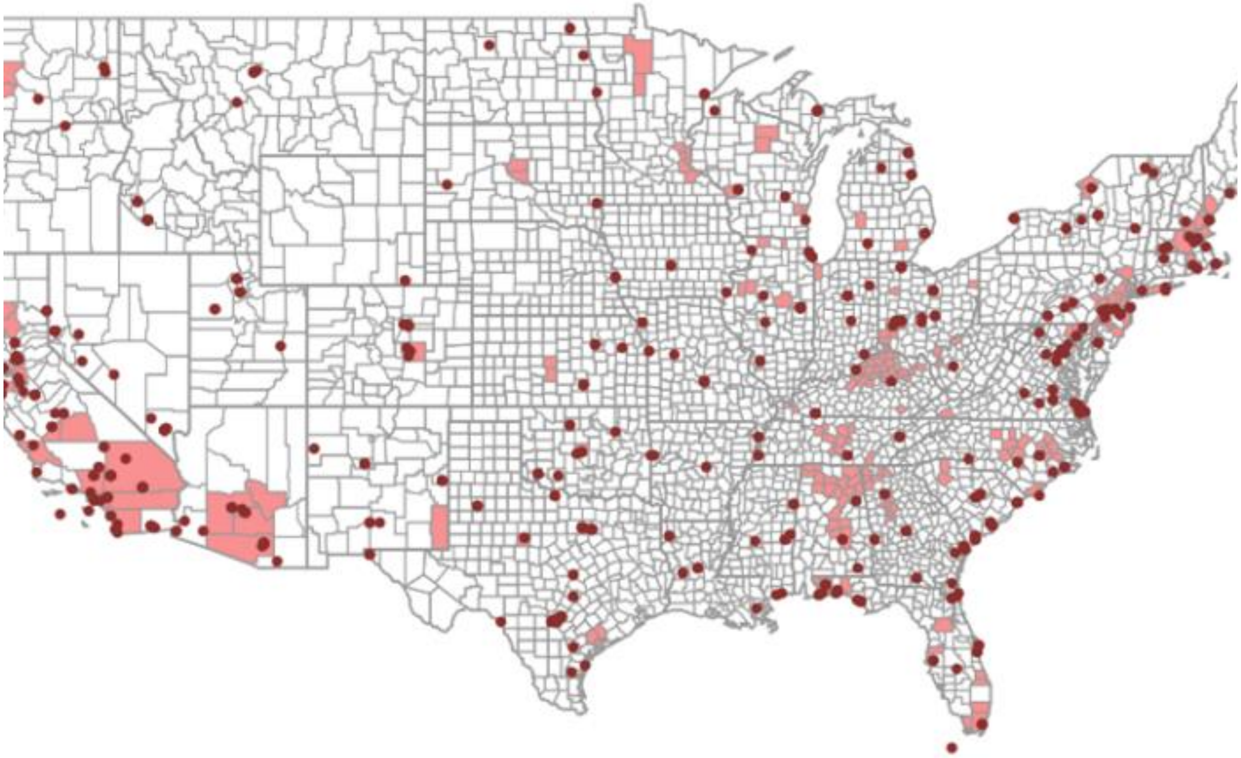


*Figure 2.5: Contamination of drinking water due to military- and crash-training site. As culminated by Lerner and co-workers. Taken without permission from*[48]

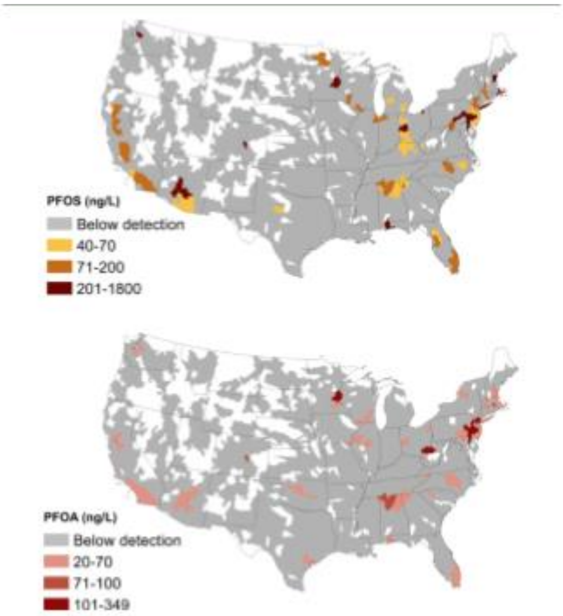


*Figure 2.6: Sites of contaminated ground water, across the United States, due to AFFF use at military training ground. Taken without permission from*[60]

In November of 2017, Congress passed the National Defense Authorization Act. This act funds the Air Force and Navy $72 million for clean-up efforts of PFOA and PFOS, as well as $7 million for a nationwide health study on PFC chemicals. More importantly, it requires both the Air Force and Navy to research AFFF alternatives[48]. Unfortunately, this may still be a moot point, as the Navy requires fluorinated chemicals to be in their AFFFs. They refer back to trials conducted with non-PFC AFFFs which doused fires between 35-39 seconds, which is over their 30-second requirement. Similarly, the National Defense Authorization Act was not clear in stating non-PFC AFFF alternatives, which means the alternatives could have short-chain PFCs and not contain PFOA or PFOS[48].

#### *2.1.3.2. Firefighters Exposure*

The evidence given above shows the environmental impact of AFFFs and the extent at which it has been studied. However, the effect and level of exposure to its users, firefighters, is not as well recorded. There have been a few studies that indicate firefighter PFC blood levels are elevated[6,9,10,64] compared to the United States national average[65].

The largest study conducted thus far was completed by Tao and co-workers on New York State World Trade Center (WTC) responders from the September 11th, 2001 attack on the WTCs. While there is no specific mention of AFFF used, there was extensive dust, and smoke inhalation by the 458 workers Tao and co-workers sampled. There have been other studies conducted looking at PFC contamination due to dust inhalation[66-72], making Tao and co-workers analysis valid. They found eight PFCs in the 458 samples, with PFOS, PFOS, perfluorohexanesulphonic acid (PFHxS), and perfluorononanoic acid (PFNA) being the most consistently measured PFCs[10]. Analysis suggests that PFNA contamination occurred due to more smoke inhalation rather than less smoke inhalation. Similarly, PFHxS contamination could have occurred due to more dust inhalation than less dust inhalation.  However, another of their analyses indicated that PFOA and PFHxS contamination came from smoke inhalation rather than dust inhalation.  Regardless, all the analyses point to PFC exposure to firefighters, most likely from industrial or consumer products used within the WTC, although the use of AFFFs cannot be discounted.

Moreover, Dobraca and co-workers[6] conducted a study on 101 southern California firefighters during their yearly physicals to determine PFC blood concentrations.  Contrary to the other studies in this review, Dobraca and co-workers only found two PFCs elevated, in comparison to national averages, in the firefighter's blood serum: perfluorodecanoic acid (PFDeA) and perfluoroheptanoic acid (PFHpA).  Dobraca and co-workers suggested the PFHpA elevation was due to AFFF use, which is contrary to all current knowledge of AFFF formulations.  They had no suggestion as to the elevation in PFDeA.

In addition, Shaw and co-workers[9,73] found elevated levels of PFHxS, PFOS, PFNA, and PFOA, in their study of 12 northern California firefighters, when compared to the national averages[65].  Jin and co-workers found similar results when studying 36 career firefighters in the Mid-Ohio Valley[74].  While their results match those of the other studies in this review, these studies suffer from low sample sizes and no evidence that the data they collected follows a normal distribution.

While most of the evidence given in this review has focused on American studies, this is a global issue, even for firefighters.  There were two concurrent studies ran by Rotander and co-workers[7,8] in Queensland, Australia which determined 17 different PFCs, four never been reported before, found in ~200 firefighters blood serum[75].  Their analyses discovered a strong correlation between PFOS and PFHxS ($R^2 = 0.93$), even when taking into account a control group of non-firefighter college students[7,8].  Across all 17 PFCs measured, their concentrations ranged from

<0.03 ng/mL – 391 ng/mL, with the largest concentration ranges occurring for PFOA, PFOS, and PFHxS[7,8]. However, when compared to the national averages for Australia and Canada ,PFOS and PFHxS were shown to be much higher than the national averages, see Figure 2.7[7]. Meanwhile, the PFOA blood concentrations of the firefighters were around the same level as the national average[7]. Rotander and co-workers[7,8] concluded that the increased blood concentrations of PFOS and PFHxS came from AFFF use, which is a reasonable conclusion considering the chemicals within the fire suppressant and the frequency at which they must use them in Australia. As an aside, it is unclear why Rotander and co-workers were comparing to Canadian national averages.

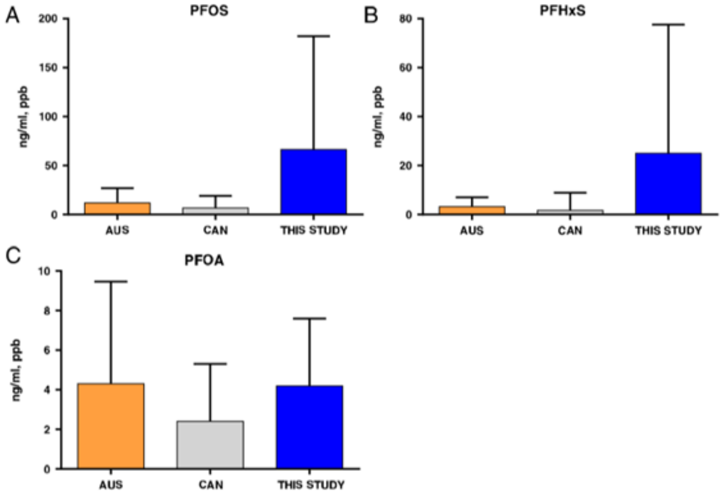


*Figure 2.7: Levels of (a) PFOS, (b) PFHxS, and (c) PFOA in 149 firefighters in Australia, as compared to the Australian and Canadian national averages. Taken without permission from*[7]

A similar, considerably smaller, study was conducted in Oulu, Finland, by Laitinen and co-workers, on eight male fire brigade workers at the Oulu Airport[53]. After three, one-hour training sessions, over three months, there was an increased amount of PFNA and PFHxS, when compared to their baseline readings taken two-weeks prior to the first training session. Meanwhile, their PFOA, PFOS, and PFDA blood concentrations fluctuated across the different training sessions. Which led to Laitinen and co-workers concluding the correlation of AFFF use with PFNA and PFHxS exposure, contrary to what they believed was in the AFFF formulations they were using in the training exercises. They put this down to fluorotelomers being the main chemical in the AFFF formulations, which subsequently broke down to PFNA and PFHxS. While Laitinen's results are similar to the previous studies, their sample size was incredibly small, and their analysis of the results was weak not having completed any statistical analysis.

## 2.2. Perfluorinated Compounds/Chemicals (PFCs)

### 2.2.1. History

Chemistry has long been intertwined with other aspects of society, chiefly of which are regulatory, legal, and social movements. However, the knowledge to understand how chemistry affects the public has long been out of the reach of the layman. This process was defined by Michaels and Monforton as *scientization*: 'the transformation of political conflict…into debate among scientific experts, ostensibly separate from the social context in which it unfolds'[76,77]. A major driving force for *scientization* is the interplay between scientific research and social forces. Of particular interest in social forces is the difference between production-oriented science and impact-oriented science. Production-oriented science 'aims to generate deliverables', which can be seen as the desire for newer technologies, while impact-oriented science 'enhances our understanding of environmental and human health impacts of production processes, products and externalities', which can be conducted by the companies creating a product or chemical (leading to conflicts of interest) or by external sources [78]. Unfortunately, the later is what drives government policy but is usually reactive instead of proactive. One only needs to look at the scenario that occurred in Love Canal, NY in the 1970s with the improper disposal of chemical byproducts or the many atrocities about early pesticides discussed in Rachel Carson's 1962 book, Silent Spring[77,79,80].

Founded in late 1970, much attributed to the events brought to light by Rachel Caron, the United States Environmental Protection Agency (US EPA) was given charge to control the industrial sector and chemical usage in the United States. Unfortunately, with close to 84,000 chemicals registered with the US EPA, there is a lack of data on the routes of exposure to consumers, the toxic levels, and the consequences which come from contact with the chemicals [77]. The Toxic Substances Control Act (TSCA) of 1976, under Section 8(e), states that chemical manufacturers are required to report all results on new chemicals that indicate actual or potential harmful health effects to humans [77,81]. However, even with the TSCA in place, the regulatory structure of the United States supports the rapid approval and production of new chemicals, due to the limited power granted to the EPA[77,82]. Essentially, placing the burden of proof for determination of toxicity and health effects on communities around the country, especially when the regulatory board presumes all pre-1976 chemicals to be safe [81,82].

To monitor the release of chemicals of concern, the US EPA has the Toxics Release Inventory. Unfortunately, the list only consists of 700 chemical compounds, leaving many possibly dangerous chemicals, from the 84,000 mentioned above, off the list. Of particular interest recently, is a chemical known as PFOA, which is known affectionately by the public as C8 (due to the eight carbons in its backbone, see Figure 2.3). This chemical falls into a family of chemicals, roughly 3,000 strong, called per- and polyfluorinated compounds (PFCs) or per- and polyfluorinated alkyl substances (PFASs). For this review, PFCs will be utilized from here on out. Per- and polyfluorinated compounds are extensively used industrial compounds which consist of carbon chains with fluorine substituted on its backbone. Due to the unique properties of fluorine, these chemicals have mainly been utilized for their water and oil repellency but have also been used as fire suppression agents or processing agents. As with most industrial chemicals, these compounds are toxic, bioaccumulative, and persistent in the environment.  The particular history of PFOA is inconsiderate, unregulated, and unseen by most of the public, see Figure 2.8.

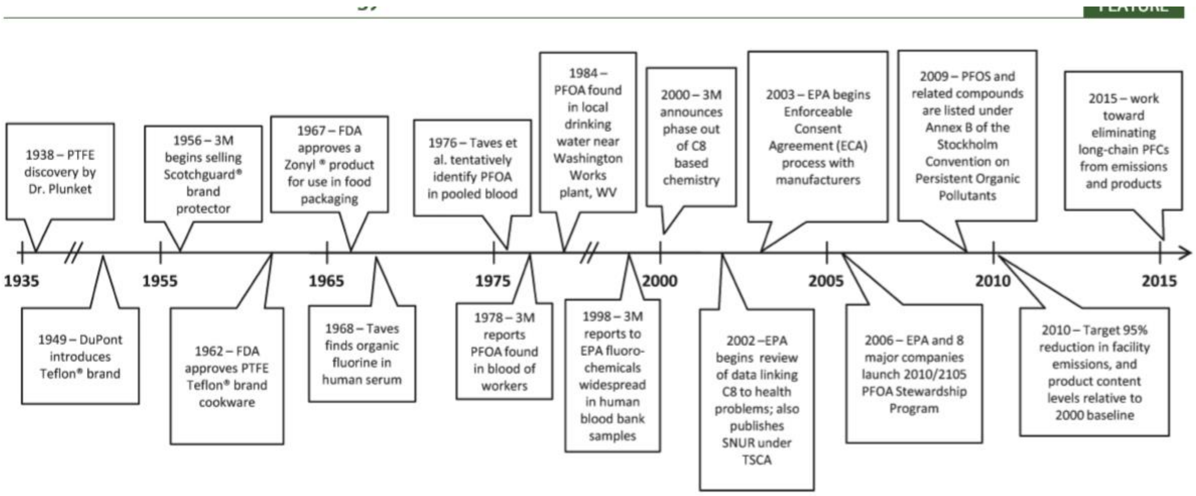


*Figure 2.8: Graphical history of PFOA.  Taken without permission from*[83]

In an accidental discovery in 1938 by Dr. Roy J. Plunkett, a DuPont Scientist, polytetrafluoroethylene (PTFE) was discovered [84].  Polytetrafluoroethylene was discovered while researching chlorofluorocarbon refrigerants, thought to be a better alternative to the common refrigerants of the day: sulfur dioxide and ammonia[84].  Dr. Plunkett had produced a large quantity of tetrafluoroethylene, a gas, and stored it in small cylinders at -78.5ºC (the temperature of dry ice)[84].  When he went to extract the gas from the cylinders he found no gas but a white powdery substance, which turned out to be PTFE[84].  He abandoned the research on the new refrigerant to determine the properties of this new substance.  The discovery of PFOA is, typically, attributed to this 1938 discovery of PTFE, as it was an unknown substance that Dr. Plunkett was finding on his PTFE as he was determining its properties[84].  However, it wasn't until 1949 when its use in processing Teflon® (commercialized PTFE) was discovered and 1951 when DuPont began full-scale manufacturing of Teflon® using PFOA as a processing agent (plasticizers, wetting agents and emulsifiers)[85]. By the 1960s there were two major chemical manufacturers responsible for PFOA production, and subsequent contamination: 3M and DuPont.

3M was the major chemical manufacturer for producing PFOA[86]. They would then sell it to DuPont to aid in the processing of PTFE and dispose of improperly. Otherwise, put in the words of a DuPont lawyer, from an internal email sent in 2000, “the material 3M sells us that we poop to the river and into the drinking water along the Ohio River”[86]. Within a few short years of full-scale processing, both companies began to have concerns of PFOA’s potential toxicity[77,86,87]. By 1968 Taves and co-workers found measurable amounts of organofluorine compounds in human blood, which was suspected to be from 3M products [88]. These findings were then supported by research done in 1976 by Guy and co-workers, who also found measurable amounts of organofluorine in human serum[89]. Around the time of the Taves and Guy studies, 3M ended up sponsoring three in-house research projects on fluorinated compounds: two with primates, one with pregnant rats. Overall, the findings indicated fluorinated compounds cause adverse health effects, congenital disabilities, and mortality[77].

All of the information DuPont and 3M were accumulating around PFOA exposure was, at first, kept quite. However, 3M was the first to break and reported their findings to the EPA in the 1980s while DuPont was insisting PFOA caused no harmful health effects [77,86]. The data DuPont was using to make those claims were produced at Haskell Laboratories, established in 1935 by DuPont to test the toxicity of DuPont chemicals[86]. Despite claiming PFOA and other fluorinated compounds did not cause the reported adverse health effects, DuPont still removed seven of its female workers from its Teflon® Division in 1981[77,86]. 3M had also removed 13 female employees from its Decatur, Alabama chemical plant, giving a press release explaining their action[77]. By 1982 DuPont and 3M met with EPA officials with new data suggesting that their first research findings were incorrect[77]. The new findings retracted the possibility that fluorinated compounds could cause congenital disabilities in pregnant female rats, but the EPA officials were skeptical of the results[77]. By 1984 DuPont was conducting confidential testing on the tap water near their Washington Works plant in West Virginia, they never told the public what they were doing or why they were conducting the tests. It would be another 15 years before any of this information came to light to the contaminated public around the DuPont Washington Work plant.

### 2.2.2. DuPont Liability

As stated above, manufacturing of PTFE (and use of PFOA) began in the early 1950s, and it was not long before DuPont and 3M began noticing detrimental health effects to their employees working around these chemicals[90]. Surprisingly, in-house health effect studies started in the 1960s at DuPont's Haskell Laboratories[86]. Unfortunately, they decided to brush aside these facts, even the facts from academia corroborating their results[86,88,89]. While 3M was informing the, then newly formed, EPA about the results of their studies, DuPont was keeping their results undisclosed. The executive board decided to disregard the results and "wait until they were sued" to do anything about the chemical[86].

Their time came about 40 years after initials studies began, when a cattle farmer, Wilbur Tennant, with land near DuPont's Washington Works Plant in Parkersburg, West Virginia, experienced the death of his whole heard of 280 cattle, over time[77,91]. He claimed that DuPont was poisoning the waterway that flowed onto his property, from which his cattle drank, although DuPont claimed the Tennants merely mismanaged their cattle[91]. To preface this story, DuPont purchased about 20 acres of land from Tennant in 1980 agreeing to use the land for only for non-hazardous waste[77,91]. As can already be assumed, this was not the case as DuPont was using the land as the central disposal site for PFOA containing waste. Over the next ten years, the Tennant family began noticing the loss of wildlife around the area with minnows in the creek disappearing to deer lying dead on their property, close to the creek[77,91]. This included the gradual death of Tennant's herd of cattle. Internal DuPont documents indicate that the stream's PFOA levels were at 100 parts per billion (ppb), 100 times greater than the company safety limit[91].

After nine years of unsuccessful outreach to DuPont, the Tennant's hired attorney Robert Billot to bring DuPont to court on their behalf. Although DuPont tried to limit Billot's access to their PFOA studies and information, they were ultimately unsuccessful. During his research for the Tennant case, Billot discovered a plethora of problems with PFOA, including the 3M studies from the '70s, and that they were violating Section 8(e) in the TSCA[91]. After being subpoenaed, DuPont share over 900 pages of PFOA related documentation, which Billot ended up handing over to the EPA[77]. Suffice it to say, Billot won the case for the Tennant's, in 1999, for an undisclosed amount of money. However, he opened a Pandora's box of startling information that would shape the next few years of DuPont litigation and his career.

The Tennant litigation led to two major lawsuits for DuPont: one against the EPA for violating the TSCA and a class-action lawsuit, headed by Robert Billot, on behalf of the residents surrounding the Washington Works Plant in West Virginia. The EPA lawsuit was finalized in 2005 and culminated in the largest civil, administrative penalty the EPA ever obtained, until that date. DuPont ended up being fined \$10.25 million, settling eight different environmental violations, seven of which involve violations of Section 8(e) in the TSCA[92,93]. DuPont also committed a further \$6.25 million toward two Supplemental Environmental Projects (SEPs), which are environmental projects used to mitigate the cost of the fine and enhance the knowledge around the reason for the fine[92]. The first SEP was a three-year project aimed at investigating the possible breakdown of fluorotelomer-based products into PFOA, valued at \$5 million[92]. Important research to conduct considering Zonyl, a fluorotelomer-based DuPont product, lines many food packages: pizza-slice paper, microwave popcorn bags, and ice cream cartons, to name a few[93]. In the end, it was determined that many fluorotelomers do breakdown into PFOA or some other type of PFC[52]. The second SEP was an educational project named the Microscale and Green Chemistry Project, valued at $1.25 million[92]. It was implemented in Wood County, West Virginia schools to reduce exposure to chemicals through microscale experiments as well as finding safer alternatives to chemicals.

Simultaneously, Robert Billot was unearthing increasingly more horrifying facts that DuPont was trying to hide. As mentioned above, DuPont created Haskell Laboratories in 1935 to conduct toxicity testing on potentially harmful chemicals DuPont was creating and using. The first project given to George H. Gehrmann, the unofficial director of the laboratories, was to determine if there was a link between bladder cancer and the use of beta-naphthylamine and benzidine, two chemicals used extensively in the '30s for dye production[94,95]. However, when Wilhelm Hueper, one of Haskell Lab's first pathologists discovered the link, he was forbidden to publish his finding and eventually fired[86]. Internal reports show that DuPont knew to handle PFOA "with extreme care" by 1961 after Haskell Laboratories had completed studying the chemical, seven years after the first misgiving was noted by a DuPont employee[86].

Unfortunately, they continued to use the chemical to produce PTFE and continued conducting, sometimes terribly unethical, toxicity studies. Reports Billot ascertained indicated that DuPont conducted human toxicity studies with PFOA. In 1962, volunteers smoked PFOA laced cigarettes, at differing doses. The high-dose subjects complained of fever, chills, coughing, and aching nine-to-ten hours after inhalation[86]. By 1965, a memo went out to most of the DuPont executives informing them that PFOA exposure was linked to the enlargement of several organs in rats, another study completed by Haskell Laboratories[86]. Over the next few years, evidence continued to mount about PFOA exposure. Taves and Guy published from academia in 1968 and 1974, respectively, about PFC exposure to the population[88,89]. 3M had started conducting their tests on monkeys and rats in 1979[77,86].

Meanwhile, DuPont had begun to notice effects to employees who worked near the chemical for several years and began testing their employee's blood after 3M had finished research indicating the PFOA bioaccumulates, which DuPont did share with their employee's[86]. DuPont's tests demonstrated that health effects due to PFOA exposure were similar to animal studies, with employees having increased rates of endocrine disorders and high liver function tests[86]. However, they still refused to report these findings to the EPA, claiming they did not see any substantial risk to the exposure[86].

At this point in the story, DuPont had finally sat down with EPA officials, as discussed earlier, to show studies on how PFOA does not cause congenital disabilities to rats, as the reported 3M study indicated. However, they still removed all women from their Teflon® division but not before a few women had children born with congenital disabilities. Unfortunately, due to the time in American history, it was easy to brush the women aside and lie, that their baby's congenital disabilities were not due to their position within the Teflon® division[86].

As mounting information was confirming PFOA's hazardous effects, DuPont began surreptitiously monitoring the water in the communities surrounding the Washington Works Plant, starting in 1984. Within the 900 pages of documentation Billot received from his subpoena, he found a record of how much PFOA contaminated waste DuPont had dumped into the adjacent Ohio River, see Figure 2.9 for locational awareness. Much to their horror, he learned that DuPont was dumping approximately 50,000 pounds of PFOA into the Ohio River each year, in addition to the 7,100 tons of PFOA contaminated sludge they dumped near Tennant's property in an unlined landfill (see Figure 2.10)[90,96]. Between the years of 1951-2003, this culminated in 2.5 million pounds of PFOA disposed improperly, at the Washington Works Plant in Parkersburg, West Virginia[86]. This was against instruction from the manufacturer's information sheet on PFOA, from 3M, which stated this chemical should be incinerated or placed in lined landfills[90]. However, DuPont did not listen to the information sheet and was also releasing PFOA into the air, which reached 87,000 pounds of PFOA air emissions at their Washington Works Plant, in 1999, and 25,000 pounds at their New Jersey Plant[86].

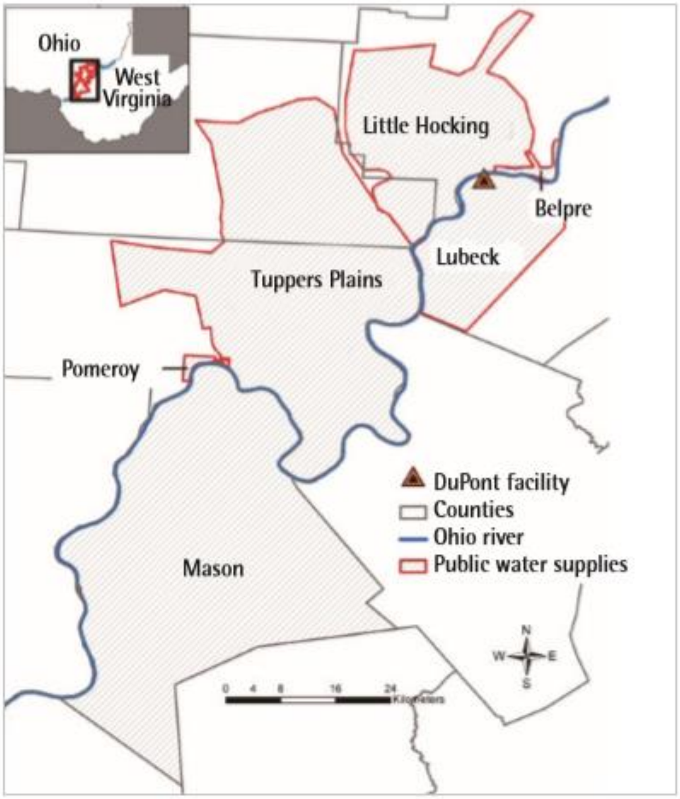


*Figure 2.9: Six water districts surrounding DuPont's Washington Work Facility. Taken without permission from*[97]

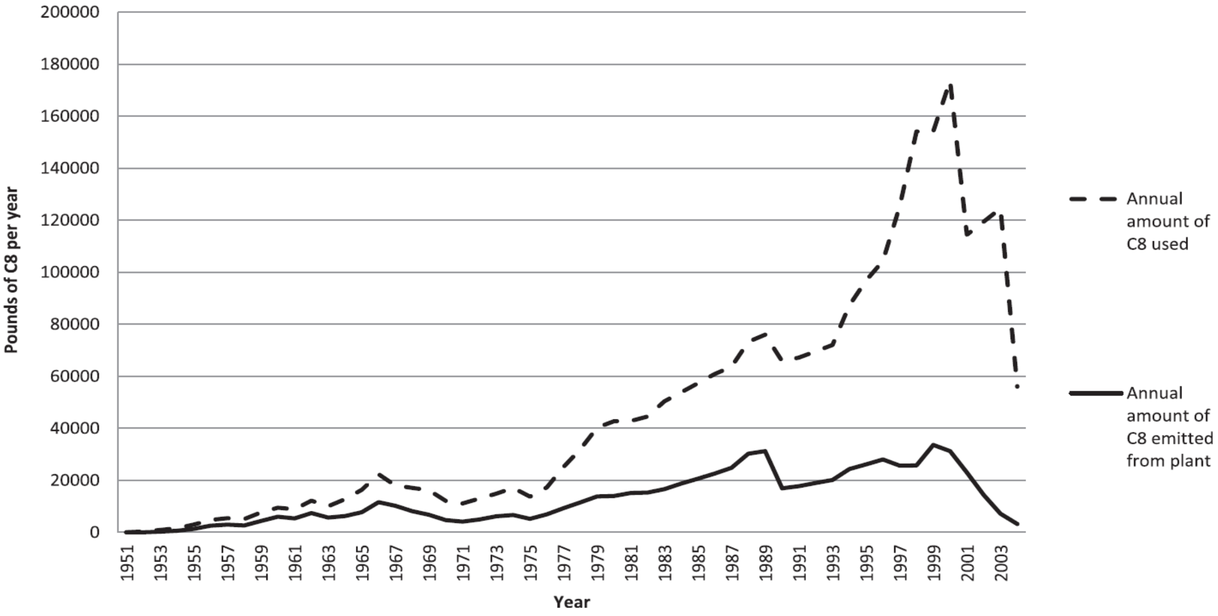


*Figure 2.10: PFOA usage and emission from DuPonts Washington Works facility in West Virginia, from 1951 to 2003. Taken without permission from*[83]

By May of 2000, 3M had decided to discontinue production of the precursor for PFOA and PFOS[91]. This put DuPont in a bind; they purchased PFOA from 3M to produce Teflon®, which would mean they would either have to discontinue Teflon® or find an alternative processing agent. However, DuPont took door number three: start producing PFOA in-house. Shortly after 3M discontinues PFOA production DuPont opened a production facility in Fayetteville, North Carolina (a future site for another environmental disaster of DuPont's)[91]. While this plant was being constructed the, then, CEO of DuPont, Charles Holiday, released a statement to the communities surrounding the new plant stating that this chemical was safe and after 50 years of use there have been no observed health effects[91].

The initial class-action lawsuit was completed in 2005 and DuPont had to pay for a $70 million health exposure study (discussed below), which led to DuPont finally settling for $671 million in 2017 to pay for those afflicted by the chemical's effects[77,91,98,99]. The $70 million for a health exposure study went into creating the C8 Science Panel, which was spearheaded by Kyle Steenland, Tony Fletcher, and David Savitz and the C8 Health Project, which was overseen by Arthur Maher and Paul Brooks[91]. Their job, along with their teams, were to use the population in the Mid-Ohio Valley region subjected to the PFOA contaminated water supply and determine which health problems could be associated with PFOA exposure. Over eight years they drew blood, took surveys, and completed statistical analyses of the almost 70,000 participants from the Mid-Ohio Valley region. The C8 Science Panel even went so far as to track people down who used to live in the area for a minimum of a year to discover whether they had any health problems that may be linked to PFOA exposure. Overall, the Panel focused on 19 broad health problem areas, with sub-areas within each category, See Table 2.3

*Table 2.3: 19 Disease Classes the C8 Science Panel Conducted Epidemiological Studies On*

| | |
|---|---|
| Liver Disease | Cancer* |
| Pregnancy Induced Hypertension/Preeclampsia* | Thyroid Disease* |
| Heart Disease* | Preterm birth |
| Low Birth Weight | Miscarriages |
| Stillbirths | Birth Defects |
| Diabetes | Stroke |
| Respiratory Disease | Parkinson's Disease |
| Neurodevelopmental Disorders in Children | Infectious Disease |
| Autoimmune Diseases* | Osteoarthritis |
| Kidney Disease | |

*Indicates categories in which probable links were discovered

Their goal was to determine probable links between these diseases and PFOA exposure. Unfortunately, due to variations in populations, the exposure to other sources of PFOA and other chemicals, definitive links to these diseases and PFOA exposure could not be made. After a multitude of experiments and data analysis the C8 Science Panel concluded that there are six probable links to health problems and PFOA exposure: testicular cancer, kidney cancer, ulcerative colitis, high cholesterol, pregnancy-induced hypertension/preeclampsia, and thyroid disease(hypothyroidism in men and hyperthyroidism in women)[1-5].

This list of probable links is by no means extensive. Due to the small sample size, competing conclusions from multiple studies or lack of data, many other probable links were discounted. David Savitz did state that time could reveal other probable links that they were unable to ascertain during their eight-year-long study, while more research could overturn the probable links the Panel discovered[91]. While this has no real impact on the majority of the population, currently, the current probable links, determined by the C8 Science Panel, are the only health effect people living around the Washington Works Plant could sue DuPont over. For litigation, it means that DuPont cannot argue that PFOA causes these diseases but must argue that the plaintiff's disease was caused by things other than PFOA[90]. However, there has been other research since the C8 Science Panel that has indicated ovarian cancer, prostate cancer, lymphoma, reduced fertility, arthritis, hyperactivity in children, altered immune responses in children and hypotonia are all associated with PFOA exposure, as well[100-105].

After both lawsuits, DuPont began to work with the EPA, although it may have been a desire to prevent another lawsuit. In 2005, DuPont worked with the EPA on the Global Stewardship Program which aimed to reduce PFOA production and use by 95% by 2010 and elimination by 2015[82,106]. DuPont signed on right away and brought in the other seven companies responsible for PFOA production and use (3M/Dyneon, Daikin, Solvay Solexis, Arkema, Asahi, Clariant, and BASF/Ciba) into the Program as well[82,106]. By 2008, DuPont was filing reports on PFOA contamination in air, water, soil, and biota around its Washington Works Plant[82]. Peer reviewers, however, thought the reports suffered from missing information and severe limitations and asked DuPont to repeat the experiments. The EPA, on the suggestions from the peer reviewers, asked DuPont to extend the breadth of the PFOA contamination research to fish, game, and meat, as well as the blood and breast milk of the residents surrounding the Washington Works Plant, along with expanding the 2-mile water sampling area of the Ohio River[82]. Despite revisions made by DuPont to the reports, the EPA continued to request the extension of the research in 2010 and 2011. DuPont submitted it's final report with the EPA in November of 2012, without covering much of the requested research and stating it would no longer continue the research. Since all of this was voluntary, the EPA had no legal recourse to take with DuPont[82].

### 2.2.3. C8 Science Panel Determined PFOA Health Effects

The following sections are in-depth analyses of the six probable links between PFOA exposure, in drinking water, and an array of health effects. The C8 Science Panel determined these links after studying the, nearly, 70,000 inhabitants of the six water districts surrounding DuPont's Washington Works Plant in Parkersburg, West Virginia. When determining if there was a probable link between PFOA exposure and a particular human disease, the Panel had to follow the definition set out by the Settlement Agreement. "A 'probable link' in this setting is defined…to mean that given the available scientific evidence, it is more likely than not that among Class Members a connection exists between PFOA exposure and a particular human disease"[1-5,107-118]. The Science Panel did not just focus on their finding of the 70,000 inhabitants but used findings in the literature to determine whether a probable link existed.

The probable links were determined by calculating the population's relative risk (RR). An RR value of 1.0 (also considered a null value) would indicate no association between exposure and outcome. However, RR values above 1.0 indicate an increased risk with increased exposure. Accordingly, RR values between 0.0 and 0.9 indicate a decreased risk with increased exposure. The RR's were calculated with measures of rate ratios, odds ratios, hazards ratios, and standardized mortality ratios. To account for chance within the calculation, 95% confidence intervals were utilized as a measure of statistical precision[1-5,107-118]. In instances where there were ranges of RRs for different exposure groups p-values were utilized, as well, to account for chance within the calculation.

#### *2.2.3.1. Testicular/Kidney Cancer Probable Link*

Of the six probable links discovered by the C8 Science Panel, the following section on testicular and kidney cancer has the greatest effect on the firefighter population, as will be discussed in another section. Cancer is a difficult disease to link to any one particular source. In fact the EPA has a whole set of guidelines they have to follow in order to name anyone chemical as one of the following five classifications: carcinogenic to humans, likely to be carcinogenic to humans, suggestive evidence of carcinogenic potential, not likely to be carcinogenic to humans, and inadequate information to assess carcinogenic potential[119]. To be classified as one of those five, extension research must be conducted to determine a weight-of-evidence with the likelihood of the compound causing tumor formation in both humans and laboratory animals, the compounds chemical and physical properties, a comparison of its structure-activity relationship with other carcinogenic compounds, and in vivo, or in vitro, studies focusing on mode(s) of action and possible carcinogenic processes[119]. Altogether, this can lead to years and years of research before a chemical can even be classified.

Compounding on this issue, cancer is not one disease but specific to the location in which it originates. However, there are some similarities across cancer at the most basic level. Normally, cells divide at a set rate, in a particular way, and die at certain rates. Cancer cells have a mutation in their DNA, causing them to replicate at a much faster rate inhibiting the functionality of healthy cells. These cells can either stay in the location in which they originate, e.g., the lung or they can travel, also known as metastasizing, to other parts of the body. A metastasized cancer cell does not change forms of cancer. For instance, when lung cancer metastasizes it favors the bones, but it is not called bone cancer now that it is in the bones, it is still called lung cancer. This is because the cells still keep the characteristics of the original cancer cells, they now inhibit the functionality of healthy cells in a different part of the body. [120]

For the most part, that is where the similarities across all cancers end. Some cancers typically have fast replication rates, while others are slower. Some cancers will form tumors, but not all, blood-born cancers are a good example. The type of cancer, or the stage in which a particular cancer is found, could also change the type of treatment chosen: surgery, chemotherapy, or radiation. Over the last few decades, treatment options have become more targeted, but physicians my still choose to target a type of cancer with, at least, two treatment options. In effect, over the last 25 years cancer mortality rates have dropped 27%, with survival rates for kidney and testicular cancer being 75% and 97%, respectively. Table 2.4 reports the survival rates for other types of cancers for three different periods. [120,121]

*Table 2.4: Survival Rates (%) for Cancers from 1975-2014*[122]

| | 1975-77 | 1987-1989 | 2008-14 |
|---|---|---|---|
| All Sites | 49 | 55 | 69 |
| Brain | 23 | 29 | 35 |
| Breast (female) | 75 | 84 | 91 |
| Colon | 51 | 60 | 65 |
| Rectum | 48 | 58 | 69 |
| Esophagus | 5 | 9 | 21 |
| Hodgkin Lymphoma | 72 | 49 | 88 |
| Kidney & Renal Pelvis | 50 | 57 | 75 |
| Larynx | 66 | 66 | 92 |
| Leukemia | 34 | 43 | 95 |
| Liver | 3 | 5 | 19 |
| Lung & Bronchus | 12 | 13 | 20 |
| Melanoma | 82 | 88 | 94 |
| Myeloma | 25 | 27 | 52 |
| Non-Hodgkin's Lymphoma | 47 | 51 | 74 |
| Oral Cavity & Pharynx | 53 | 54 | 68 |
| Ovary | 36 | 38 | 48 |
| Pancreas | 3 | 4 | 9 |
| Prostate | 68 | 83 | 99 |
| Stomach | 15 | 20 | 32 |
| Testis | 83 | 95 | 97 |
| Thyroid | 92 | 95 | 98 |
| Urinary Bladder | 72 | 79 | 78 |
| Uterine Cervix | 69 | 70 | 69 |
| Uterine Corpus | 87 | 82 | 83 |

The C8 Science Panel considered all the cancer sites reported in Table 2.4, however, toxicological and epidemiological evidence indicated the following cancers as the most important: pancreas, liver, testicular, prostate, breast, and kidney. Most of the toxicological evidence came from the EPA's 2005 risk assessment on PFOA, and its salts (EPA 2005 reference), which indicated that Sprague-Dawley (CD) rats have a "tumor triad" induced by PFOA contamination. This "tumor triad" included liver, testicular, and pancreatic tumors.

Further evidence suggested breast tumors formed in CD rats, but this was contradicted by a study completed by Hardisty and co-workers[123,124]. Altogether, the EPA Risk Assessment concluded that PFOA has "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"[123]. Furthermore, the risk assessment gave evidence for PFOA being a peroxisome proliferator-activated receptor alpha (PPARα) agonist in rats as a major mechanism for tumor induction, which is support by findings from Lau and co-workers[125]. However, DeWitt and co-workers demonstrate that peroxisome proliferation is less apparent in humans as a mechanism for tumor production[126].

The Panel relied on four epidemiological studies, outside their own, when determining these probable links: two on worker cohorts within the United States, a study on the general population of Denmark, and a study on breast cancer within Greenlandish Inuit women[127-130]. The two worker cohort studies indicated no statistically relevant link to any cancers reported, despite having PFOA blood level concentrations between 100-5000 ng/mL[128]. However, this may have been due to the small sample sizes for the cohort. Leonard and co-workers had a total sample size of n = 37: 8 liver, 11 pancreas, 12 kidney, 3 thyroid, 1 testis, and 2 breast[127]. Lundin and co-workers had a larger total sample size of n = 138. Unfortunately, specific cancers did not have statistically significant sample sizes, topping out at n = 9. When they did have a statistically large enough sample size, as they did for lung cancer (n = 37), correction for smoking made any correlation statistically insignificant[128].

Eriksen and co-worker studied 55,000 Danish adults, ages 50-65, from 1993-2006, with average PFOA blood concentrations of averaging around 6.5 ng/mL, which is lower than worker studies but on par for general populations. This study did not suffer from the same issues as the worker cohort studies above as they had n = 713 for prostate cancer, n = 332 for bladder cancer, n = 128 for pancreatic, and n = 67 for liver cancer. That being said, this study did not find any significant trends ($p < 0.05$) between PFOA concentration and the cancers mentioned. However, there were small positive trends for prostate and pancreatic cancer. [129]

Furthermore, the study on Greenlandish Inuit woman, conducted by Bonefeld-Jorgensen and co-workers, also indicated no association between PFOA exposure and breast cancer ($p = 0.5$). The study focused on 31 Inuit women who had full-term pregnancies and PFOA blood concentrations averaging 2.5 ng/mL. However, there was a significant trend between PFOS and breast cancer ($p = 0.02$), with an average PFOS blood concentration of 45.6 ng/mL. [130]

The Panel conducted four of their own studies to aid in the determination of probable links between PFOA exposure and cancer. As stated above, the literature review on toxicological and epidemiological put a particular focus on pancreatic, liver, testicular, prostate, breast, and kidney cancers, although all cancers types were considered.

In their first study[131], The Panel evaluated the same worker cohort as Leonard and co-worker. However, they considered the time range from 1955-2009, instead of 1948-2002, which gave them more concrete data on PFOA blood serum levels. Table 2.5 shows the standardized mortality ratios (SMRs) for pancreatic, liver, testicular, prostate, breast, and kidney cancers for workers who were constantly employed by DuPont during 1952-2008 time range. As Table 2.5 indicates, there are small sample sizes for the six types of cancer; breast and prostate cancer have decreased combined SMRs due to a decreasing trend across quartiles, liver and pancreatic cancer have neutral combined SMRs due to unclear trends across quartiles, and testicular had too few cases to make any inference. Meanwhile, kidney cancer has a significant SMR for combined quartiles, which correlated to a p-value of $p = 0.02$ for the trend.

*Table 2.5: Standardized Mortality Ratios for Selected Cancers from Steenland and Co-Workers*[131]

| Cancer | No. of Deaths | SMR | 95% CI |
|---|---|---|---|
| Liver | 10 | 1.07 | 0.51-1.96 |
| Pancreatic | 18 | 1.04 | 0.62-1.64 |
| Breast | 4 | 0.65 | 0.13-1.90 |
| Prostate | 21 | 0.76 | 0.47-1.16 |
| Testicular | 1 | 1.80 | 0.05-10.03 |
| Kidney | 12 | 1.28 | 0.66-2.24 |

However, these were not the only data that made The Panel conclude kidney cancer as a probable link. The same study went on to examine workers with a 10-year and 20-year lag between working at DuPont and contracting the disease. Out of the six cancers of particular focus, kidney cancer was the only one to show up both in the 10-year and 20-year lag experiments with p-values of $p = 0.02$ and $p = 0.003$ for the trends, respectively. [131]

In the second study[132], a comparison of cancer rates between exposed and unexposed areas in Ohio/West Virginia region surrounding DuPont's Washington Works Plant was conducted. The analysis included all incident cancers diagnosed between 1996-2005 for five counties in Ohio and eight counties in West Virginia, covering six water districts (see Figure 2.9). The water districts are as follows: Little Hocking, Lubeck, Tupper Plains, Belpre, Pomeroy, and Mason. Analyses indicated a significant association between testicular cancer and high serum concentration and testicular cancer and living in the most contaminated water district. Furthermore, a positive association was found between kidney cancer and the top two quartiles of blood serum concentration. Meanwhile, ovarian and prostate cancer only had an association with the topmost quartile of blood serum concentration.

In the third study[4], The Panel used participants from the C8 Health Project who were recently diagnosed with one of the following cancers: bladder, breast, cervical, colon, liver, kidney, melanoma, ovarian, pancreatic, prostate, testicular, thyroid, and uterine. They chose to look at recent cancers due to the accuracy of PFOA blood serum measurements. However, there was no suggestion of a positive association for any of the indicated cancers. After down-selecting for medically verifiable cancers from a self-reporting system, the sample size for pancreatic, liver, and testicular cancers were too small to analyze.

In the final study[133], The Panel used data collected from the C8 Health Project and worker cohort studies to determine associations between PFOA exposure and cancers. Both sets of data gave an initial data set of n = 32,254, which was dwindled to n = 2,507 after validated self-reported cancer incidents. The authors concluded a positive association between PFOA exposure and kidney, testicular and thyroid cancers, abbreviated data from paper shown in Table 2.6. 10-year lag has to do with one of their analyses where they completed their original association while considering those with a 10-year lag in PFOA exposure. As Table 2.6 indicates, kidney, testicular, and thyroid cancers had positive RRs of 1.10, 1.34, and 1.10, respectively. However, looking at p-value[a] shows only testicular cancer as having a significant association based on the hypothesis statement of $p \leq 0.05$. Even changing the analysis of the data did not change the significance of the associations for kidney or thyroid cancer in p-value[b]'s column.

*Table 2.6: Relative Risk (95% CI) for kidney, testes, and thyroid cancers on cancer risk in Barry and co-workers cohort*[133]

| **Cancer** | **No. of cases** | **RR (95% CI)** | **p-Value[a]** | **p-Value[b]** |
|---|---|---|---|---|
| Kidney | | | | |
| No Lag | 105 | 1.10 (0.98, 1.24) | 0.18 | 0.10 |
| 10-year Lag | 105 | 1.09 (0.97, 1.21) | 0.34 | 0.15 |
| Testes | | | | |
| No Lag | 17 | 1.34 (1.00, 1.79) | 0.04 | 0.05 |
| 10-year Lag | 17 | 1.28 (0.95, 1.73) | 0.02 | 0.10 |
| Thyroid | | | | |
| No Lag | 86 | 1.10 (0.95, 1.26) | 0.25 | 0.20 |
| 10-year Lag | 86 | 1.04 (0.89, 1.20) | 0.57 | 0.65 |

[a]p-Value is for linear trend test in the log rate ratios across quartiles. [b]p-Value is form the continuous log estimated cumulative PFOA serum concentration models

Of chief concern is that The Panel considered testicular and kidney cancer as a probable link when there was a lack of evidence from other studies outside of their own. As will be seen in the following sections, this is a stray from their usual conclusions on probable links.

#### *2.2.3.2. Ulcerative Colitis Probable Link*

As mentioned above, the C8 Science Panel analyzed 19 different health diseases. Within the autoimmune disease category, they focused on the six most prevalent diseases found in the Mid-Ohio Valley region: inflammatory bowel disease (combining ulcerative colitis and Crohn's Disease), rheumatoid arthritis, type 1 diabetes, systemic lupus erythematosus, and multiple sclerosis. Of these five diseases, a probable link between PFOA and ulcerative colitis was the only one to be determined. This determination came strictly from Panel studies as they could not find toxicological or other epidemiologic studies.

An autoimmune disease is a disease in which one's body begins to attack itself because the immune system believes the body's healthy cells to be intruders. They are typically rare and non-fatal, but incurable[3,134]. Autoimmune disease can be managed, but they may be disabling and cause serious discomfort at times. While autoimmune diseases are rare, a review by Cooper and co-workers found that they could occur at a prevalence of about 10% and that co-occurrence of autoimmune disease is more prevalent[135].

Inflammatory bowel disease (IBD) can be characterized by the inflammation of the mucosal lining in the gastrointestinal (GI) tract (the esophagus to the rectum). While inflammation is not abnormal in a healthy individual, IBD is determined when a gut cannot down-regulate these responses[3,134]. All-together, IBD comprises of two main disorders: ulcerative colitis (UC) and Crohn's disease (CD). Both UC and CD have clinically distinct diagnostic conditions but, in reality, are on opposite ends of a broad spectrum[134,136]. 10% of people with IBD fall in the middle of UC and CD, and are typically diagnosed with “indeterminate colitis” until other symptoms, and diagnostic features present themselves[3,134]. The differences between UC and CD are small: UC is limited to the colon while CD can occur anywhere on the GI tract, UC only affects the top lining of the intestines while CD can affect deeper layers, and UC is a continuous disease while CD can start and stop along the GI tract[137]. Miller and co-workers[138] also completed a literature review study to determine possible correlations between a slew of autoimmune disease and environmental factors, such as chemical factors, physical factors, and biological factors. They determined many possible factors, for inflammatory bowel disease but they found that smoking could contribute to the development of CD while protecting against UC.

From the C8 Health Project, there were 747 self-reported cases of IBD, in which 245 were validated through medical histories. Of the 245 validated cases, there were 161 cases of UC and 96 cases of CD. The overall analysis of IBD indicated a positive trend of increasing risk with increased exposure. When the two sub-categories of IBD were analyzed, the previous positive trend was determined to come from the positive trend in UC and not CD. There was a strong dose-response curve between PFOA exposure and UC with risk ratios for quartiles of increasing exposure of 1.0, 1.89 (1.08-3.31), 2.58 (1.52-4.38), and 3.18 (1.845.51), with a p-value $\leq 0.0001$ for the trend[3].

Compared to the other probable links discovered by the Panel, the link with ulcerative colitis is, by far, the weakest. While the one study they conducted did point to UC, there was no other evidence to back up this claim. Even the cancer probable link had some misleading toxicological and epidemiological data to back up their claim.

#### *2.2.3.3. High Cholesterol Probable Link*

Unlike the other diseases, the C8 Science Panel studied, high cholesterol does not present with any symptoms and will only be discovered with a blood test. The diagnosis of high cholesterol has many associated factors: age, family history, other diseases, smoking, weight, activity, and diet are just a few. However, on average, high cholesterol is diagnosed when a total cholesterol level is above 240 mg/dL. Although, many doctors also take into account a person low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides before deciding whether to start their patient on medication. Cholesterol is important for the body to function; it is responsible for the generation of new cells and sex hormones. However, too much LDL (back cholesterol) can lead to heart attacks or strokes because it builds on the walls of arteries; hence, constricting blood flow[139].

Meanwhile, a large amount of HDL (good cholesterol) is good because they trap the LDLs and bring them back to the liver to be excreted[139]. Triglycerides help store excess fat from a diet, but a high concentration can be detrimental[139]. In general having an LDL concentration below 130 mg/dL, an HDL concentration above 60 mg/dL, and a triglyceride concentration less than 150 mg/dL is acceptable [140-142]. Although, for high-risk patients, doctors will aim to lower the LDL concentration to below 70 mg/dL[143,144].

Many studies completed on PFOA exposure in animals have been two-faced. In certain studies, PFOA or PFOS, exposure have shown to reduce serum cholesterol[145,146]. Martin and co-workers indicated that a three-day dosing of PFOA has an overall effect of downregulation on the genes and enzymes important to the synthesis of cholesterol[146]. However, in primate studies, serum cholesterol increase after dosing regiments with PFOA[145].

There were multiple studies conducted by the Panel[2,147,148]. In the study headed by Fitz-Simon, they found similar findings to the rest of the studies following, an increased level of PFOA increased LDL levels. However, they also discovered that the inverse of that finding is true, a 50% drop in PFOA blood concentrations corresponded to a 3.6% decrease in LDL levels[147].

For many of the studies conducted by the Panel, they typically use ZIP Code data to determine PFOA concentration or average the PFOA contamination over all six water districts that were contaminated by DuPont: Belpre, Little Hocking, Lubeck, Tupper Plains, Pomeroy, and Mason. However, there are large differences between the PFOA concentrations in these six water districts[149-152]. In one study[2], the Panel explored whether LDL levels could be associated with each separate water districts average PFOA levels. They concluded that an association could be made based on the water district, but it was weaker than ZIP Code referencing.

Subsequently, the Panel also conducted a three-year study, between 2008-2011, on 29,000 residents and 4,000 DuPont workers to determine whether PFOA exposure could predict coronary heart disease, high blood pressure, or high cholesterol[2]. They determined that PFOA exposure is associated with an increase in "high cholesterol with prescription medication." Although, there was no increasing trend across the quintiles but plateaued after the second quintile. When male and female subsets were separated, the male subset saw a more pronounced association.

Fletcher and co-workers took the data from the Panel and Health Project and attempted to determine an association between PFOA and PFOS with gene expression involved in cholesterol metabolism in humans[148]. The authors concluded that changes in gene expression depend on gender, see Figure 2.11. The changes they found support the findings from the other Panel studies suggesting that PFOA or PFOS increase cholesterol, and this could be a reason as to how these compounds increase cholesterol in humans. This data contradicts findings from Martin and co-workers who found that PFOA and PFOS decreased cholesterol in rats[146].

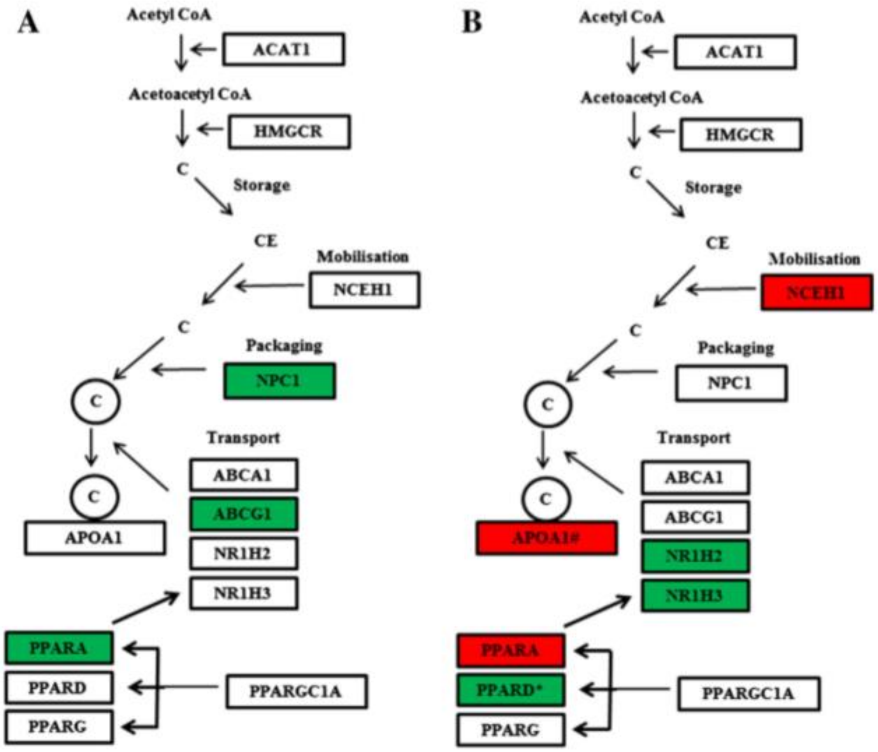


*Figure 2.11: Gender specific (A men, B women) expression changes in cholesterol transport genes in response to PFOA and PFOS. Down regulated genes are illustrated in green boxes, up-regulated genes by red boxes, and genes with no significant association are given by white boxes. Genes significant only in pre-menopausal women are indicated with a *, those significant only in postmenopausal women are marked by #. C- cholesterol, CE – cholesterol ester, Encircled C – cholesterol in the endosomal compartment. Taken without permission from*[148]

Outside of studies completed by C8 Science Panel, six studies of exposed workers, three studies on highly exposed communities, and one study on the general population on the association of PFOA, and PFOS, exposure with high cholesterol[101,104,153-158] were used to determine the probable link. Of the six papers concerned with exposed workers, two found no association between high cholesterol and PFOA/PFOS exposure[104,154], while three found an association[101,153,159].

Costa and co-workers followed the careers of 50 workers for 30 years, taking blood samples annually. They found a correlation between PFOA exposure and total cholesterol but found no significant correlation with HDL, LDL, or triglycerides[153]. Meanwhile, Sakr and co-workers found a positive relationship between PFOA and total cholesterol and LDL levels[159]. Conversely, Olsen, and Zobel found no relationship between PFOA exposure and total cholesterol or LDL levels. However, they did find a negative relationship with PFOA exposure and HDL levels, i.e., PFOA was found to lower HDL levels[101]. A positive relationship between PFOA and triglycerides was also found[101].

In the papers concerning an exposed population, each paper focused on some subset of the community affected by DuPont's contamination around their Washington Works Plant, in Parkersburg, West Virginia and were not a part of the C8 Health Project or C8 Science Panel. Of these three papers, one paper found no association between PFOA/PFOS exposure and increase cholesterol count[157], while two found an association[156,158]. Both papers concluding positive relationships had similar findings: PFOA increase total cholesterol and LDL levels but has no effect on HDL levels. Steenland and co-workers we able to calculate an 11-12 mg/dL increase in total cholesterol for PFOA and PFOS. Figure 2.12 represents the data obtained by Frisbee and co-workers, indicating the increase in total cholesterol and LDL levels for PFOA and total cholesterol, LDL and HDL levels for PFOS.

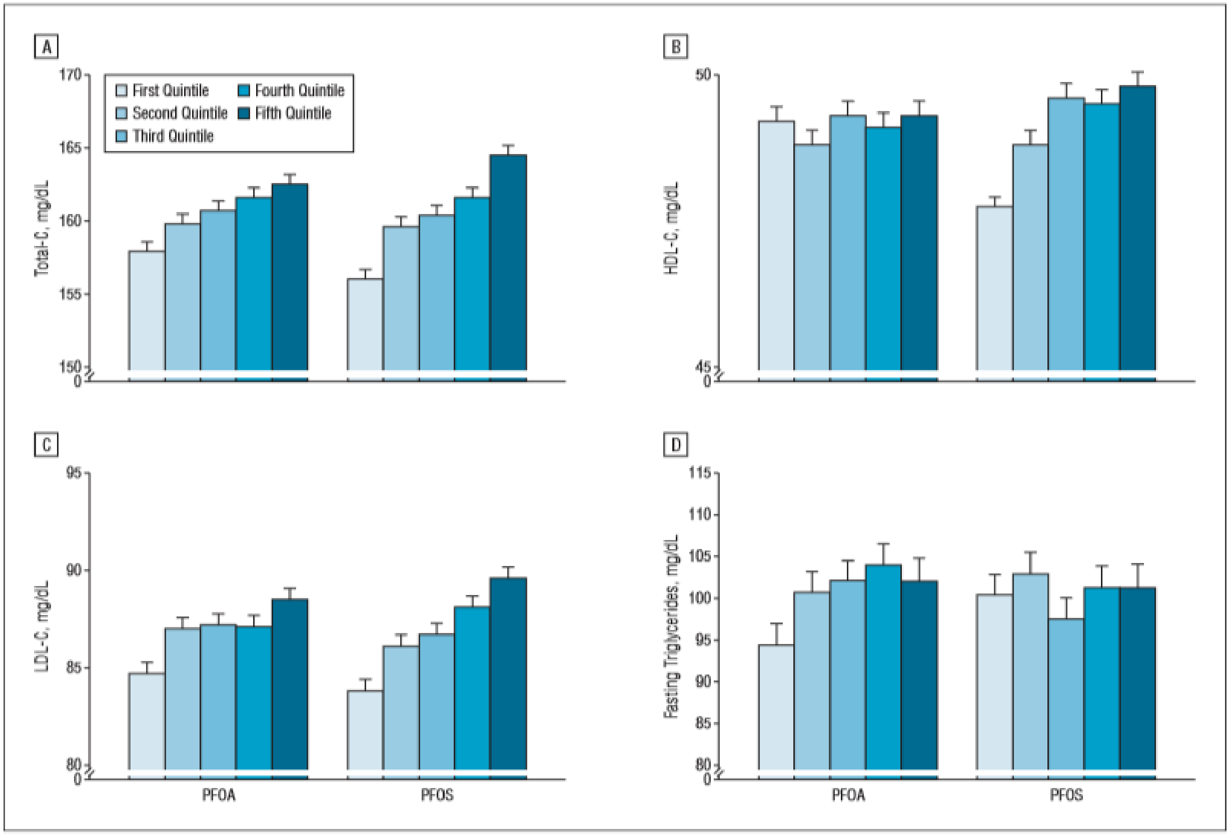


*Figure 2.12: Changes in covariable-adjusted estimated marginal means(general linear model analysis) across PFOA and PFOS quintiles. A, Total cholesterol (total-C) . B, High-density lipoprotein cholesterol (HDL-C). C, Low-density lipoprotein cholesterol (LDL-C). D, Fasting triglycerides. Lipid values are presented as mean (SE). To convert total-C, HDL-C, and LDL-C to millimoles per liter, multiply by 0.0259; fasting triglycerides to millimoles per liter, multiply by 0.0113. Taken without permission from*[156]

Nelson and co-workers analyzed data from the 2003-2004 National Health and Nutrition Examination (NHANES) Survey[65] for participants ranging from 12 to 80 years of age[155]. However, their analyses were mostly completed in the 20-80-year-old age range, as there was not enough data for the 12-19-year-old range. Figure 2.13 displays the increasing quartile results from Nelson and co-workers for the four chemicals they analyzed: PFOA, PFOS, PFNA, and PFHxS. They state that there is an association between these chemicals and total cholesterol (TC) and non-HDL levels, while there are fewer consistent trends across HDL and LDL levels. Which indicates that the total cholesterol association was due to the non-HDL levels, which may also indicate a high triglyceride level.

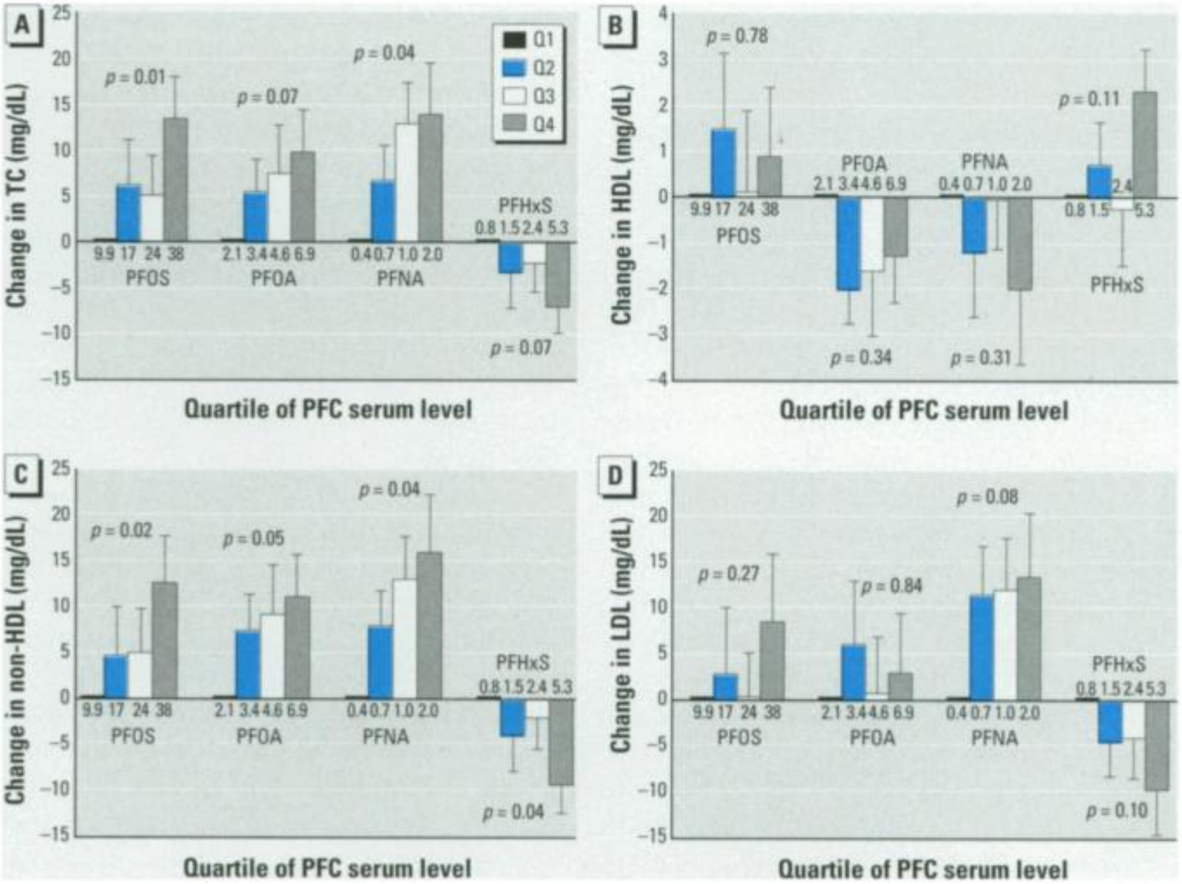


*Figure 2.13: Association between PFOA, PFOS, PFNA, and PFHxS exposure with (A) change in total cholesterol (TC), (B) changes in HDL, (C), changes in non-HDL, and (D), changes in LDL. Taken without permission from*[155]

### 2.2.3.4. *Pregnancy Induced Hypertension/Preeclampsia Probable Link*

Pregnancy-induced hypertension (PIH) is not a rare disease, but it is also not common, occurring in around 6% of pregnancies[160]. Diagnosis of PIH will occur around the 20th week of pregnancy if the mother's blood pressure reads with a systolic > 140 mmHg and a diastolic > 90 mmHg (140/90)[1,160]. Mothers with a history of high blood pressure may be difficult to diagnose with PIH. This disease progresses to preeclampsia when proteins are present in the urine[1]. Further progression of the disease results in eclampsia, which presents with the mother having seizures and can only be alleviated by delivering the fetus[160].

Due to inaccuracies in medical documentation and self-reporting, it was difficult for the C8 Science Panel to analyze PIH and preeclampsia as separate diseases. However, since both diseases could cause issues in pregnancy the C8 Science Panel considered documentation or self-reporting of PIH or preeclampsia as the same disease, PIH. Regardless, the Science Panel ruled PIH/preeclampsia as a probable link with PFOA exposure from six studies.

In the first study (n=1,548), conducted by Nolan and co-workers, there was no association between PFOA exposure and PIH when they examined water service area (based on a person's Zip Code) and PIH[161].

Savitz and co-workers found competing results based on differing analyses in the second study (n=224)[162]. In this study, they were examining PIH identified from birth records between 1990-2005 for five counties across Ohio and West Virginia. They found that PFOA exposure tended to be higher in infants who: were younger, had older mothers, had more highly educated mothers, had mothers who were nonsmokers or were apart of West Virginia families. Savitz and co-worker's analysis of exposure to PFOA and birth problems indicated no association between exposure and PIH, see Table 2.7[162]. However, when focused on 66% of the data in which they could accurately associate precise addresses to the subjects, they received a weak association. This was because exact street addresses is a more accurate way of analysis then ZIP Code averaging. This weak association was only for the top three quintiles for the highest PFOA exposure with odds ratios for the 3rd, 4th and 5th quintiles at 1.1 (95% CI = 0.7-1.6), 1.0 (95% CI = 0.6-1.6), and 1.3 (95% CI = 0.8-2.1)[1].

*Table 2.7: Association of PFOA with pregnancy-induced hypertension*[162]

| **Estimated PFOA** | **Live Births (n)** | **Cases (n)** | **Crude OR** | **Adjusted OR (95% CI)** |
|---|---|---|---|---|
| IQR Increase | 3,828 | 224 | 1.06 | 1.02 (0.86, 1.21) |
| 100-ng/mL Increase | 3,828 | 224 | 1.06 | 1.02 (0.86, 1.31) |
| < 40th Percentile | 1,527 | 82 | 1.0 | 1.0 |
| 40th to < 60th Percentile | 764 | 48 | 1.2 | 1.0 (0.7, 1.6) |
| 60th to < 80th Percentile | 759 | 51 | 1.1 | 1.0 (0.6, 1.5) |
| ≥ 80th Percentile | 778 | 43 | 1.1 | 1.0 (0.7, 1.5) |

< 40th Percentile = 1.0 to < 6.1 ng/mL. 40th to < 60th Percentile = 6.1 to < 10.2 ng/mL. 60th to < 80th Percentile = 10.2 to < 21.0 ng/mL. ≥ 80th Percentile = 21.0 to 717.6 ng/mL

The third study used to determine a probable link between PFOA exposure and PIH/preeclampsia is a companion study to the second study where they took the birth records from 1990-2005 and linked them to pregnancies reported in the C8 Health Project across 13 counties in Ohio and West Virginia[1]. Savitz and co-workers simultaneously analyzed PIH as reported on the birth certificates[1]. Unlike the second study, this study utilized the demographic information of geographic location from the C8 Health Project but did not receive any different results. Overall, PFOA exposure still had no association with PIH. However, upon different analysis a positive association was suggested, with an increased risk in the 4th quintile, odds ratio 1.5 (95% CI = 1.1-2.1)[1,162]. Despite the overlap in the studies and the similar analysis results of no association, these studies analyzed a majority of different populations with less than 8% of all study two births being analyzed in study three[162].

The fourth study, by Darrow and co-workers published in 2013, investigated pregnancies between 2005-2010 after enrollment into the C8 Health project began[163]. There total study sample size was n = 1,630, but their sample size studying PIH was considerably lower, but still sufficient, at n = 106. After estimating the serum levels of PFOA and PFOS in the blood, taking into account the half-life of the compounds, Darrow and co-workers found an association between PFOA and PFOS exposure with PIH[1,163]. Table 2.8 summarizes their odds ratio and quintiles data. For PFOA, when compared to the 1st quintile (for all births), the 2nd, 3rd, 4th, and 5th quintiles odds ratios were considerably higher: 2.39 (95% CI = 1.05-5.46), 3.43 (95% CI = 1.50-7.82), 3.12 (95% CI = 1.35-7.18), and 3.16 (95% CI = 1.35-7.38), respectively, indicating a higher risk[163]. Although, it is relatively the same risk from the 2nd-5th quintile, suggesting there is no increase in risk with increasing exposure. The data for PFOS follows the same general trend as PFOA but not as high of a risk. When compared to the 1st quintile (for all births), the 2nd, 3rd, 4th, and 5th quintiles odds ratios were: 1.46 (95% CI = 0.69-3.11), 2.71 (95% CI = 1.33-5.52), 2.21 (95% CI = 1.07-4.54), and 1.56 (95% CI = 0.72-3.38), respectively[163]. However, unlike the trend for PFOA, the trend for PFOS indicates that exposures from 12.1 ng/mL – 21.3 ng/mL are associated with a slightly higher risk.

*Table 2.8: Association between serum PFOA and PFOS with PIH for all births from*[163]

| PFC Metric | Crude OR (n = 106) | Adjusted OR (95% CI) (n = 106) |
|---|---|---|
| PFOA | | |
| Per IQR Increase | 1.04 | 1.06 (0.99, 1.14) |
| Quintiles (ng/mL) | | *p-trend = 0.005* |
| 0 to < 6.9 | 1.0 (reference) | 1.0 (reference) |
| 6.9 to < 11.1 | 2.37 | 2.39 (1.05, 5.46) |
| 11.1 to < 18.9 | 2.72 | 3.43 (1.50, 7.82) |
| 18.9 to < 37.2 | 2.71 | 3.12 (1.35, 7.18) |
| ≥ 37.2 | 2.59 | 3.16 (1.35, 7.38) |
| PFOS | | |
| Per IQR Increase | 1.13 | 1.16 (0.94, 1.43) |
| Quintiles (ng/mL) | | *p-trend = 0.107* |
| 0 to < 8.6 | 1.0 (reference) | 1.0 (reference) |
| 8.6 to < 12.1 | 1.48 | 1.46 (0.69, 3.11) |
| 12.1 to < 15.9 | 2.43 | 2.71 (1.33, 5.52) |
| 15.9 to < 21.4 | 2.03 | 2.21 (1.07, 4.54) |
| ≥ 21.4 | 1.45 | 1.56 (0.72, 3.38) |

In the fifth study, blood serum PFOA and PFOS and self-reported preeclampsia were analyzed from 2000-2006, five years before the C8 Health Project began[164]. This constraint on time was done to ensure that the blood serum measurements from the C8 Health Project were relevant to the time of pregnancy. Table 2.9 displays their findings. Woman in above the 50th percentile were at a higher risk of preeclampsia (odds ratio of 1.5), with PFOA exposure, which was driven by the high odds ratio for the 50th-75th percentile, 1.8 (95% CI 1.0 – 2.3), and the 75th-90th percentile, 1.2 (95% CI = 0.7 – 2.1)[1,164]. Trends for PFOS exposure were similar except for the >90th percentile having the highest odds ratio of 2.0 (95% CI = 1.2 – 2.3)[164]. Which led to the conclusion that there is a weak association between PFOA and PFOS exposure with preeclampsia.

*Table 2.9: Association between PFOA and PFOS with Preeclampsia, from*[164]

| | **PFOA Exposure (n = 1,589)** | | | | **PFOS Exposure (n = 4,566)** | | | |
|---|---|---|---|---|---|---|---|---|
| Serum PFC | No. of Cases | Crude OR | Adjusted | | No. of Cases | Crude OR | Adjusted | |
| | | | OR | 95% CI | | | OR | 95% CI |
| < 50th percentile | 64 | 1.0 | 1.0 | | 280 | 1.0 | 1.0 | |
| ≥ 50th percentile | 92 | 1.5 | 1.3 | 0.9, 1.9 | 127 | 1.5 | 1.3 | 1.1, 1.7 |
| > 50th percentile | 95 | 1.0 | 1.0 | | 163 | 1.0 | 1.0 | |
| 50th - <75th percentile | 52 | 1.8 | 1.5 | 1.0, 2.3 | 117 | 1.4 | 1.3 | 1.0, 1.7 |
| 75th - 90th percentile | 27 | 1.3 | 1.2 | 0.7, 2.1 | 65 | 1.3 | 1.1 | 0.8, 1.6 |
| > 90th percentile | 13 | 0.9 | 0.9 | 0.5, 1.8 | 62 | 2.0 | 1.6 | 1.2, 2.3 |

In the sixth study, PFOA exposure and preeclampsia were analyzed between 1990 and 2005[162]. Due to the large sample size for 11,737 pregnancies (730 of which reported preeclampsia) and the large time span (much of the time having no PFOA blood serum data) PFOA blood levels were calculated using wind patterns, river flow, groundwater flow, and residential history[150,162,165]. Table 2.10 displays the quintiles data from Savitz and co-workers, indicating a modest association between PFOA exposure and preeclampsia. While the table indicates percentile data, the first and second quintiles were combined into the first, < 40th percentile. This percentile was used as the reference point for the following adjusted odds ratios for the third (40-<60th), fourth (60-<80th) and fifth (≥ 80th) quintiles: 1.2 (95% CI = 1.0-1.5), 1.1 (95% CI = 0.9-1.4), and 1.2 (95% CI = 1.0-1.6), respectively[1,162]. As stated in earlier data analyses, due to a lack of gradient across the quintiles, there is no increase in risk due to increased exposure.

*Table 2.10: Association between PFOA blood serum concentration and preeclampsia, from*[166]

| Estimated PFOA | Cases (n) | Crude OR | Adjusted OR (95% CI) |
|---|---|---|---|
| IQR Increase | 730 | 1.18 | 1.13 (1.00-1.28) |
| 100-ng/mL Increase | 730 | 1.07 | 1.08 (1.01-1.15) |
| < 40th Percentile | 237 | 1.0 | 1.0 |
| 40th to < 60th Percentile | 172 | 1.4 | 1.2 (1.0-1.5) |
| 60th to < 80th Percentile | 154 | 1.3 | 1.1 (0.9-1.4) |
| ≥ 80th Percentile | 167 | 1.4 | 1.2 (1.0-1.6) |

< 40th Percentile = 3.9 to < 6.8 ng/mL. 40th to < 60th Percentile = 6.8 to < 16.6 ng/mL. 60th to < 80th Percentile = 16.6 to < 63.1 ng/mL. ≥ 80th Percentile = 63.1 to 934.3 ng/mL

In modified analyses, Savitz and co-workers found similar trends between PFOA exposure and preeclampsia. When this data was calibrated to the PFOA levels measured in the C8 Health Project between 2005-2006, the trend was strengthened slightly: 1.2 (95% CI = 1.0-1.5), 1.3 (95% CI = 1.1-1.7), and 1.4 (95% CI = 1.1-1.7), for the third, fourth and fifth quintiles when compared to the combined first and second quintiles[166]. When the data set was constricted to pregnancies into three five-year periods, 1990-1994, 1995-1999, and 2000-2005, the same trend, as above, continued, see Table 2.11. A stronger association occurring for the 2000-2005 time range was noticed with odds ratios for the third, fourth and fifth quintiles as 1.5 (95% CI = 1.1-2.2), 1.3 (95% CI = 0.9-1.8), and 1.5 (95% CI = 1.0-2.0), respectively[166]. It is unclear from their methods section whether Savitz and co-workers used the estimated blood serum levels or the calibrated blood serum levels in calculating the associations for the three five-year periods.

*Table 2.11: Association of Estimated Maternal PFOA Serum Concentration with Preeclampsia, from*[166]

| | 1990-1994 | | | 1995-1999 | | | 2000-2005 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Estimated PFOA** | **N** | **Crude OR** | **Adjusted OR (95% CI)** | **N** | **Crude OR** | **Adjusted OR (95% CI)** | **N** | **Crude OR** | **Adjusted OR (95% CI)** |
| IQR Increase | 179 | 1.05 | 1.07 (0.85, 1.35) | 235 | 1.18 | 1.16 (0.94, 1.42) | 316 | 1.20 | 1.17 (0.97, 1.42) |
| 100-ng/mL Increase | 179 | 1.01 | 1.04 (0.92, 1.17) | 235 | 1.09 | 1.09 (0.96, 1.24) | 316 | 1.10 | 1.09 (1.00, 1.19) |
| < 40th Percentile | 94 | 1.0 | 1.0 | 75 | 1.0 | 1.0 | 68 | 1.0 | 1.0 |
| 40th to < 60th Percentile | 31 | 1.0 | 1.0 (0.7, 1.6) | 54 | 1.4 | 1.3 (0.9, 1.9) | 87 | 1.5 | 1.5 (1.1, 1.2) |
| 60th to < 80th Percentile | 18 | 0.8 | 0.8 (0.5, 1.3) | 52 | 1.4 | 1.4 (0.9, 2.0) | 84 | 1.3 | 1.3 (0.9, 1.8) |
| ≥ 80th Percentile | 36 | 1.2 | 1.2 (0.8, 1.9) | 54 | 1.3 | 1.3 (0.9, 1.8) | 77 | 1.5 | 1.4 (1.0, 2.0) |

< 40th Percentile = 3.9 to < 6.8 ng/mL. 40th to < 60th Percentile = 6.8 to < 16.6 ng/mL. 60th to < 80th Percentile = 16.6 to < 63.1 ng/mL. ≥ 80th Percentile = 63.1 to 934.3 ng/mL

#### 2.2.3.5. *Thyroid Disease Probable Link*

To understand thyroid diseases, a cursory knowledge of the endocrine system will be beneficial. The endocrine system is a network of glands throughout the body, see Figure 2.14, which regulate the function of organs by producing hormones. The two master glands in this system are the hypothalamus and pituitary. The hypothalamus gland is directly linked to the nervous system and directs the pituitary to start and stop making hormones. Thus, making the pituitary the main gland responsible for instructing all the other glands in the peripheral endocrine system to start and stop making hormones. For example, when a person is placed into their "fight or flight" response, the pituitary dumps adrenocorticotropic hormone (ACTH) into the blood, which directs the adrenals (which sit on top of the kidneys) to start producing cortisol (the stress hormone) and release epinephrine from the adrenal cortex. [167,168]

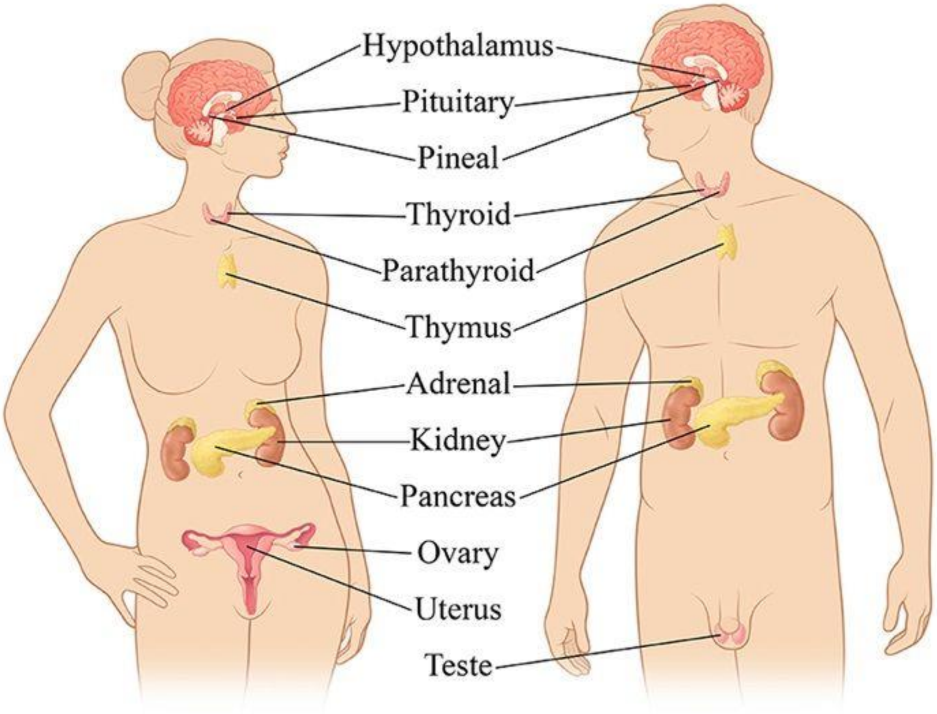


*Figure 2.14: Graphic of the endocrine system.*[169]

However, the endocrine system is not a one-way street, but a large negative/positive feedback loop, in which organs such as the liver, kidneys, and lungs play an important role. Figure 2.15, shows this loop type system. When put into a stressful situation, such a Ph.D. Defense, the brain realizes something is wrong, and the hypothalamus will start creating corticotropin-releasing hormone, which tells the pituitary to release ACTH into the blood. When the adrenals receive the ACTH, they will start producing glucocorticoids (cortisol), which is also known as the stress hormone. This will interact with multiple receptors in the body to create the responses seen when stress is induced. Thus, the cycle continues until the hypothalamus releases another hormone that directs the pituitary to stop making ACTH, this is sometimes called the hypothalamic-pituitary-adrenal (HPA) axis.[170,171]

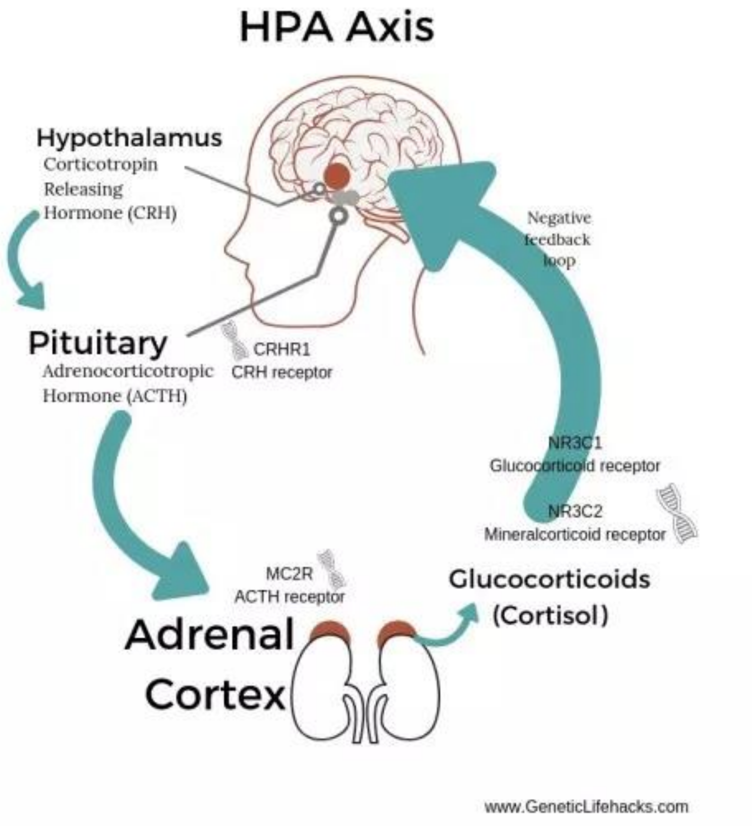


*Figure 2.15: Schematic of a positive/negative feedback loop for the hypothalamic-pituitary-adrenal (HPA) axis.*[172]

For the function of the thyroid gland, this same loop structure will hold true but with different hormones, with the hypothalamic–pituitary–thyroid (HPT) axis. Located within the neck, just below the Adam's apple, the thyroid gland is shaped like a butterfly. The thyroid gland receives iodine from food consumption to produce triiodothyronine (T3) and thyroxine (T4), which helps regulate metabolism. These hormones are balanced as the hypothalamus communicates to the pituitary to release thyroid-stimulating hormone (TSH). When the body produces too much T3 and T4, it is a condition called hyperthyroidism. Conversely, when the body produces too little T3 and T4, it is a condition called hypothyroidism. Diseases, such as Grave's Disease, Hashimoto's Disease, and goiter can cause these conditions to occur. Hypothyroidism is much more common, occurring in ~4.6 % of the American population over 12 years of age, while hyperthyroidism is less common, occurring in ~1% of women and < 1% in men. [167,173,174]

To determine whether someone has hypo- or hyperthyroidism, a blood test is done to measure T3, T4, and TSH levels, and a Free Thyroxine Index (FTI) can be derived from these markers. Typically, only T4 and TSH are used in determining whether a person has hypo- or hyperthyroidism. Low T4 and high TSH levels typically point to hypothyroidism because the pituitary is trying to stimulate the thyroid into producing more hormone by putting more TSH into the circulatory system. Conversely, high T4 and low TSH levels indicate hyperthyroidism as the pituitary is trying not to stimulate the thyroid as much since it is overactive. Other hormones that can be measured to determine if there is a thyroid problem are free T3 (FT3), total T3 (TT3), free T4 (FT4), and total T4 (TT4). These are what The Panel used to determine whether was a connection between PFOA exposure and thyroid disease.[5,175]

The C8 Science Panel conducted four studies[5,176,177]. For all of these studies, the sample size was down-selected using the information from the C8 Health Project, which surveyed the exposed population and collected blood samples[5]. They concluded that PFOA exposure causes hyperthyroidism in women and hypothyroidism in men.

For the first study[5], which examined the plant-workers and adults in the community for an association between PFOA exposure and diagnosed thyroid disease, 3,633 participants reported some form of functional thyroid disease. For this study, functional thyroid disease included diseases such as hypothyroidism, hyperthyroidism, goiter, Grave's disease, Hashimoto's disease, and excluded neoplasms, congenital disease, nodules without functional changes, cysts, and unspecified types. Of the 3,633 participants who reported a functional thyroid disease, 70% were able to be validated with medical records. Of the 70%, 400 were classified as hyperthyroidism and 1,442 were classified as hypothyroidism. The overall analysis, both sexes together, indicated a small increasing trend between functional thyroid disease and increasing PFOA blood serum levels (p-value = 0.09). However, when analyzing both sexes separately, women indicated a much stronger relationship (p-value = 0.03) than men (p-value = 0.85). When analyzing each sex and thyroid disease separately a relationship between women and hyperthyroidism (p-value = 0.26) was seen, the sample size for men (n = 16) was too small to determine a relationship with hyperthyroidism. In the case of hypothyroidism, women had no trend as their RRs for increasing PFOA blood serum levels were non-correlating, and men indicated a strong relationship (p-value = 0.02).

In a second study[177], Lopez-Espinosa and co-workers studying a population of 50 adults with average PFOS concentrations and above-average PFOA concentrations. These average concentrations were taken from the concentrations reported by NHANES reports[65,178,179]. They were trying to determine whether PFOA or PFOS were having negative effects on FT4 counts, as previous animal studies concluded, and if there was a bias in the measurements of FT4. The study of these human populations concluded there were no associations correlated with PFOA or PFOS, and there is no bias in their measurement methods.

For the third study[5], which examined thyroid hormones in adults with elevated PFOA levels in their blood serum, about 3,300 participant's TSH levels indicated some form of subclinical hypo- or hyperthyroidism. Participants with subclinical hypo- or hyperthyroidism are at greater risk of developing hypo- and hyperthyroidism. However, the analysis indicated both men and women for hypo- and hyperthyroidism have reduced risk (RR < 1.0). This is contrary to the analysis completed from the first study.

For the fourth study[176], which examined thyroid hormones in children with elevated PFOA levels in their blood serum, a measured RR level of 1.44 with a 95% CI of 1.02 to 2.03 was calculated. A similar association was seen when examining reported hypothyroidism (n = 39), a measured RR level of 1.54 with a 95% CI of 1.00 to 2.37. The correlation between PFOA exposure and hyperthyroidism could not be examined due to the small sample size. Although, the Science Panel did consider the likelihood of children developing hypo- or hyperthyroidism due to mother's blood serum levels during pregnancy. This association indicated an increased risk with increased exposure, RR = 1.61 with a 95% CI of 0.96 to 2.63.

There have been a few other non-occupational studies of exposed community populations across North America. A study of pregnant Canadian women with high PFOA blood level concentrations indicated no relationship to their thyroid disease (hypothyroxinemia) or any difference between them and a control group[180]. Two studies from New York and the Mid-Ohio region indicated no association between PFOA exposure and TSH or FT4[181,182]. Melzer and co-workers analyzed NHANES data on self-reported thyroid disease and found a strong odds ratio of 2.2 (95% CI of 1.4 and 3.7) between thyroid disease and the first and second quintiles of PFOA serum concentrations for females[179]. Knox and co-workers expanded on the work done here by the C8 Science Panel by further separating the male and female group into above and below age 50 groups. Knox and co-workers found an association between FT4 and PFOA but not TSH[183].

Olsen and co-workers have examined occupational exposure to PFOA and thyroid disease for multiple years. In 1998, they found no association between PFOA exposure and TSH levels after investigating two occupational populations (n = 111 and n = 80)[102]. A study they conducted in 2000 of 518 workers found a positive association between PFOA exposure and elevated T3 levels, but not TSH, TT4, or FT4[103]. In a cross-sectional study of the worker population study from 2000, Olsen and co-workers found a negative association between PFOA and FT4 and a positive association between PFOA and T3 but no associations between PFOA and TSH or TT4[184]. The previous two studies focused on the same sample set of data from the 518 workers, a majority of which had low PFOA and PFOS, between 0 and 2.9 ppm[103].

Two animal studies have observed effects due to PFOA exposure. Butenhoff and co-workers conducted a six-month study with male cynomolgus monkeys exposed to three doses of PFOA (3 mg/kg/day, 10 mg/kg/day, and 30/20 mg/kg/day) plus a control group. The 30/20 means they began dosing at 30 mg/kg/day, yet this dose was deemed too toxic and was lowered to 20 mg/kg/day. The study focused on a plethora of health parameters, although those reported here are only those regarding thyroid function. They found no significant changes in TSH, FT4, or TT4[105]. Although, for the high dose (30/20 mg/kg/day) group FT3 and TT3 did decrease of the study period[105]. However, as four out of the six monkeys in the high dose group died, this association may be meaningless. They were not able to find a “no observable effects” limit, although they state that 3 mg/kg/day is the lowest observable effect limit, despite having a monkey die in that dosing group[105].

Meanwhile, Martin and co-workers conducted a short-term study on Sprague-Dawley rats. Sample groups of 5 rats were dosed with either 20 mg/kg/day of PFOA, 10 mg/kg/day of PFOS, or three different triazole fungicides for one, three, and five consecutive days. Results indicate that the dosing of PFOA and PFOS decrease FT4, TT4 and TT3, see Figure 2.16[185]. PFOA and PFOS induce PPARα by increasing the expression of Cyp17a1. Cyp17a1 has been shown to be a marker in testosterone and cholesterol production. As indicated in this paper, an increase in Cyp17a1 expression led to a decrease in testosterone and cholesterol production, contradictory to the evidence given above for PFOA exposure leading to high cholesterol. However, the data presented in this paper is insufficient to determine if thyroid problems occur because of gene down-regulation for testosterone and cholesterol production or vice versa.

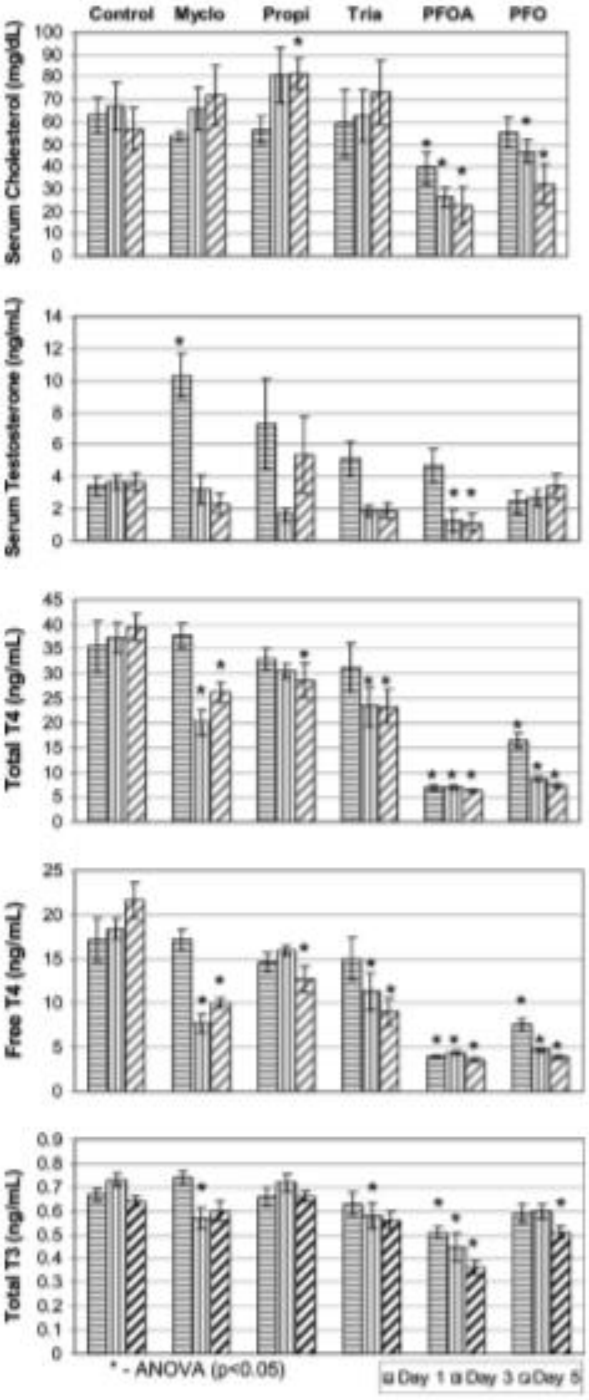
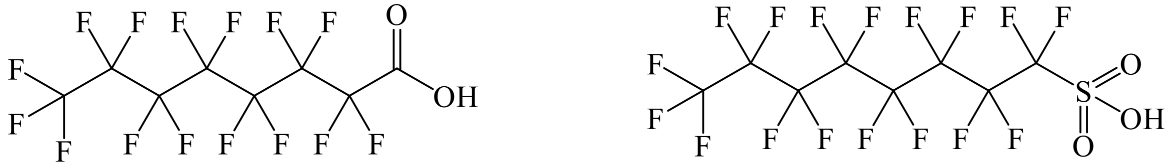


*Figure 2.16: Serum cholesterol, testosterone, total T4, free T4 and total T3 levels following 1-, 3-, or 5-day treatment of myclobutanil (Myclo), propiconazole (Propi), triadimefon (Triad), PFOA, and PFOS. Treatment groups significantly different from the controls (ANOVA) were designated using asterisks. Taken without permission from*[146]

### 2.2.4. Chemistry

While this class of chemicals is nearly 3,000 strong, it can be partitioned into multiple different sub-classes[125,186]. The broadest two sub-classes of PFCs are perfluorinated compounds and polyfluorinated compounds. Perfluorinated compounds have fully substituted all the hydrogens with fluorine (see Figure 2.17a-h)[83]. Polyfluorinated compounds, on the other hand, are compounds which are partially substituted with fluorine, leaving some hydrogens attached to the structure (Figure 2.17i-j)[83]. Furthermore, these sub-classes can be partitioned even further into two subcategories: per- and polyfluoroalkyl carboxylic acids (PFCAs) and per- and polyfluoroalkane sulfonates (PFSAs). To clarify, perfluorooctanoic acid (PFOA), chemical structure in Figure 2.17a, would fall under the PFCA category, while perfluorooctanoic sulfonate (PFOS), chemical structure in Fig 2.17b, would fall under the PFSA category. Other examples of PFCs would be fluorotelomer alcohols, perfluoroalkyl sulfonamides, perfluorophosphonic acids, Perfluorinated cyclosulfonates, perfluorosulfonamidoethanol, fluorotelomer phosphate esters, polyfluorinated ether carboxylates, polyfluorinated polymeric units, and polyfluorinated ether sulfonates[83]. For this literature review, focus will be given to PFCAs and PFSAs, with an even tighter focus on PFOA and PFOS.

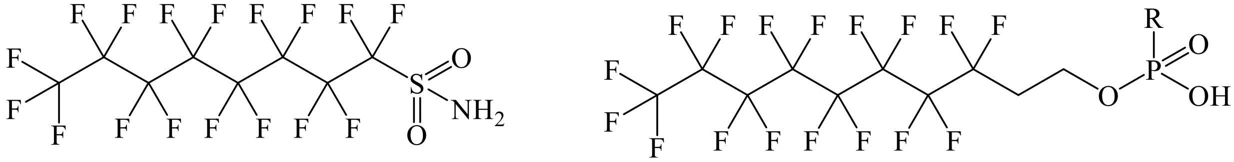


Perfluorocarboxylic Acids
(e.g., PFOA)
(a)

Perfluorosulfonic Acids
(e.g., PFOS)
(b)

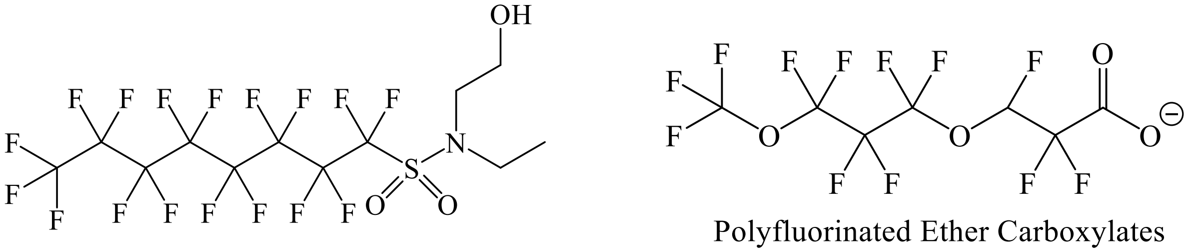
Fluorotelomer Alcohol
(e.g., 8:2 FTOH)
(c)

Perfluorophosphonic/phosphinic Acids
(e.g., if R = OH, PFOPA)
(d)



Perfluorosulfonamide
(e.g., FOSA)
(e)

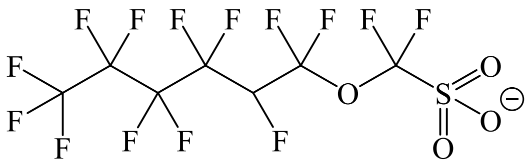
Fluorotelomoer Phosphate Esters
(e.g., if R = OH the 8:2 monoPAP)
(f)



Perfluorosulfonamidoethanol
(e.g., N-EtFOSE)
(g)

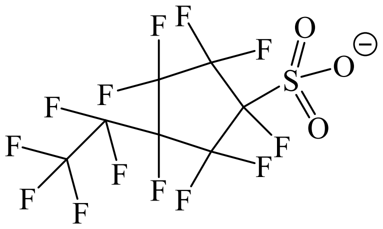
Polyfluorinated Ether Carboxylates
(e.g., 4,8-dioxa-3H-polyrfluorononanoate)
(h)

*Figure 2.17: Structures/classes of some of per- and polyfluorinated chemicals.*



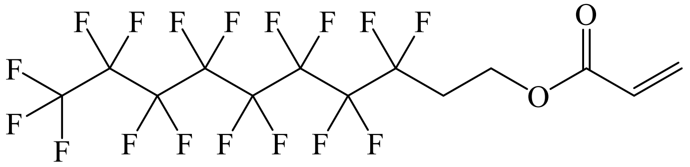
Polyfluorinated Ether Sulfonates
(e.g.,Polyfluoro [hexyl ethyl ether sulfonate])

(i)



Perfluorinated cyclo sulfonates
(e.g., PFECHS)

(j)



Polyfluorinated Polymeric Unit
(e.g.,1H, 1H, 2H, 2H-perfluorodecyl acrylate)

(k)

*Figure 2.17: Continued*

Both of these chemicals express desirable properties when it comes to water and oil repellency. For example, PFOA expresses amazing surfactant properties. When the structure comes into contact with water, the hydrophilic head (i.e., the carboxylic acid) will be in contact with the water while the hydrophobic tail (the C – F chain) will stick straight up out of the water. Perfluorinated compounds with shorter of longer chains do not stand straight up but bend somewhere along their C – F chain. When used as a water-repellant, the hydrophilic head is typically in contact with the textile material, while the hydrophobic tail sticks straight out making water glide right over the surface. Other basic chemical properties of PFOA and PFOS are indicated in Table 2.12. [187,188]

*Table 2.12: Properties of PFOA and PFOS*[188]

| Properties | PFOS (Free Acid) | PFOA (Free Acid) |
|---|---|---|
| CAS # | 1763-23-1 | 335-67-1 |
| Physical Description | White Powder | White Powder |
| Molecular Weight (g/mol) | 500 | 414 |
| Water Solubility @ 25°C (mg/L) | 680 | 9.5x103 |
| Melting Point (°C) | No Data | 54 |
| Boiling Point (°C) | 258-260 | 192 |
| Vapor Pressure @ 25°C (mmHg) | 0.002 | 0.525 |
| Partition Coefficient | 2.57 | 2.06 |

The use of elemental fluorine to produce PFCs is economically infeasible albeit dangerous. This is due to the fact that the heat of formation for C–F bonds (460 kJ/mol or 110 kcal/mol) and H–F bonds (566 kJ/mol or 135 kcal/mol), compared to C–C bonds (348 kJ/mol or 83 kcal/mol) is much greater, meaning more energy needed and given off when forming those bonds. If the heat in the reactor with elemental fluorine is not dissipated sufficiently, the reaction can become explosive. Thus, there are two main ways to commercially produce PFCs: electrochemical fluorination (ECF), and telomerization. [189,190]

In ECF, a carboxylic acid or sulfonic acid compound is dissolved in liquid hydrogen fluoride (HF). An electric current between 5-7V is passed through the substance. Water may also be added to the solution to increase the conductivity, but care must be taken to keep the amount of water below 1%. Anything above 1% w/w of water decreased the yield and anything larger than 10% w/w of water makes the reaction explosive. The yield of the reaction also depends on the length of the solute; short chains (2-4 carbons) can have yields around 80% while longer chain solutes (8-12 carbons) can have yields as low as 10%. The low yield can be attributed to the production of cyclic perfluoro ethers. In the case of the use of carboxylic acids as the solute, decarboxylation is one reasoning behind low yield. Using dicarboxylic acids has been a proven way of increasing the yield. Either way, ECF produces perfluorocarboxylic acid fluorides

($C_xF_yCOF$) and perfluorosulfonyl fluorides ($C_xF_ySO_3F$), the precursor compounds for PFCAs and PFSAs, respectively. [189,190]

There are multiple ways in which to use the telomerization process to produce PFCs, but they all derive from the method first developed by DuPont[189]. The general process follows a typical free-radical polymerization that is terminated when a specified chain length is produced. A free-radical reaction between a taxogen, such as perfluoroethylene ($CF_2=CF_2$), and a telogen, such as perfluoroethyl iodide ($CF_2–CF_2I$), produces straight-chain perfluorinated iodides ($(C_2F_4)_n–CF_2I$)[83]. These perfluorinated iodides are then the precursor to create the PFCAs, PFSA, fluorotelomer, and other PFCs.

### 2.2.5. PFCs Environmental Effects

As eluded to in previous sections, the environmental effect of PFCs from improper disposal of industrial or AFFFs has caused severe water contamination[150,152,165,191,192]. This has not only caused a measurable amount to be found in humans but animals as well: arctic ring seals[193], lake trout[194], whales[195], and other ocean fish[196-198]. Sadly, the contamination hasn't stopped. After the EPA Stewardship Program, companies sent their PFOA and PFOS production to other countries, which ended up spreading the contamination worldwide. Within the United States, alternate PFCs were discovered and have been discovered in local water systems. GenX being the most public contamination to the Cape Fear Water Shed[199]. Thankfully, a multitude of water treatment technologies have been shown to be effective in removing these chemicals from the water[200].

Consequently, the high production rate of PFCs means that they are found in a plethora of consumer products: nonstick surfaces (e.g. Teflon ™), water repellent finishes (e.g. Scotchgard®), surfactants and dispersants, paints, lubricants, mist suppression, carpet backings, textile products, and paper products[201-209]. It has been estimated that PFC usage in Europe breaks down to the following percentages: 50% for synthetic carpets and other textiles, 33% for paper and packaging, 15 % for industrial surfactant additives and 3% for AFFFs[210,211].

The food industry hasn't escaped the contamination of PFCs either. Along with the earlier mentioned contamination of fish, PFCs have been found in milk and dairy products[212]. They also line the inside of many fast-food and pre-packaged foods(e.g., microwavable popcorn bags and pizza boxes)[213,214]. The European Food Safety Authority conducted a six-year-long study to determine the extent of PFOA and PFOS contamination in the food chain in 13 European countries. Their analyses determined these PFCs to be found most frequently in fish and meat (mostly liver). They also calculated tolerable daily intake (TDI) values of 150 ng/kg/day (150 ppt/day) and 1500 ng/kg/day (1500 ppt/day) for PFOS and PFOA, respectively. Ultimately, they concluded that adverse effect are unlikely to be seen from consuming contaminated food[215]. However, this is in stark contrast to the EPA recommendation of 70 ppt allowable in the drinking water.

Unfortunately, the widespread use means that this problem with PFOA and PFOS is not just an American problem. It has become a global contamination problem, with PFOA and PFOS being detected in multiple countries: Germany, Canada, Greenland, Spain, Italy, Holland, Norway, Sweden, Denmark, France, China, Vietnam, South Africa, India, England and Australia[216]. In some places the contamination is known, well-publicized, and with clean-up efforts taking place. In Ronneby, Sweden, AFFFs contaminated the water supply with PFOS. When the contamination was discovered, clean water was dispersed immediately, an emergency grant was awarded to a local university to study the effects of PFOS on the population, and clean-up efforts began swiftly[47]. Meanwhile, in Dordrecht, Holland, an international location for a DuPont plant, it took an activist group from America to unveil the problem[216].

While the European Union (EU) ratified the Stockholm Convention, in 2014, to add PFOS to the list of persistent organic pollutants (PFOA has been in consideration to be added for the last few years), it has not stopped use or production of these chemicals. In some economically climbing countries, like China, environmental contamination is far worse. Unfortunately, the Stockholm Convention, which was adopted in 2001 and signed by 179 countries, did not completely stop the spread of PFOS. Loopholes were placed into the revision for the use of PFOS in certain products, primarily of which are AFFFs. China also still produces a precursor chemical to PFOS to be used to control fire ant populations[217].

In a study comparing the levels of PFC contamination in Chinese and European rivers, Heydebreck and co-workers found that the Xiaoqing River, in China, had a concentration 6,000 times greater of PFCs in it then the Scheur River, near DuPont's Dordrecht plant in Holland[216]. The contamination in the Xiaoqing River comes from the Dongyue Group, who also produces Teflon® and emits 350 pounds of PFOA waste a day, calculating to 63 tons a year[217]. However, PFOA is not the only chemical entering the Xiaoqing River, which houses multitudes of industrial/commercial plants along its 134 miles such as livestock pens, agricultural fields, and paper mills[217].

As production was grinding to a halt in North America and Europe, it was ramping up in China. Between 2004 and 2012, the same time frame of the EPA Stewardship program, China's PFOA and PFOS production tripled[217]. Studies estimate China's PFOA production between 64-292 tons per year and PFOS production between 110-220 tons per year[217]. This has corresponded to an increase in the blood serum levels of PFOA and PFOS in human which increased 54- and 747-fold between 1987 and 2002, again parallel with the decrease in the production of these chemicals in other parts of the world[217].

Many companies tout that shorter chain PFCs are the future of fluorinated chemistry, claiming lower toxicity, environmental persistence, and bioaccumulative effects. However, some studies indicate that short-chain replacements such as PFHxA of PFHxS can have longer half-life ranges of 8.0 and 9.0 years, respectively[218]. This is in stark contrast to the ranges of 2.3-3.4 and 3.1-5.4 years for PFOA and PFOS, respectively[218]. Likewise, there are a plethora of studies indicating that the toxicity of short-chain PFCs has similar or additional toxic effect from PFOA or PFOS. For instance, PFHxS has been shown to cause Gilberts Syndrome, a liver disorder, at levels as low at 0.5 ppb[219].

While the section began with contaminated drinking water due to industrial waste, it must also end with the contamination of drinking water due to the disposal of consumer goods. A newly emerging issue within the entire PFC problem is the end of life for many of the consumer goods mentioned above. Due to the environmental stability of these compounds, they could outlast the lining of many of the landfills they are currently found within[220]. They have already been measured in leachate in Germany, Sweden, and Denmark at levels between 10-30 ng/mL[221-224]. Contrary to Vecitis's review[200], the studies on leachate in Germany[221,222] found that wet air oxidation, biological treatment, and activated carbon filters are ineffective in removing PFCs from the leachate. Although, Taylor and co-workers found incineration to be effective in the removal of PFCs, with no detectable amount within the smoke from incineration[225].

# CHAPTER 3: Hazard Assessment of Fluorinated Compounds within the Fire Service

## 3.1. Methods of Analysis

### 3.1.1. LC/MS

By and large, liquid chromatographic-mass spectrometric (LC/MS) and liquid chromatographic tandem mass spectrometric (LC-MS/MS) methods are the most common way of analyzing PFCs. These analysis methods are used as the sole or reference method for analysis in water, soil, biological (such as the C8 Science Panel Studies), air, food, and consumer product sampling[226]. The main advantage of LC/MS and LC-MS/MS is the ease of separation of differing PFCs, with the LC, portion, conjugated with the ability to identify and quantitate simultaneously with the MS or MS/MS portion. Other methods, such as $^{19}$F nuclear magnetic resonance ($^{19}$F NMR) or capillary electrophoresis, don't have the same advantages and must be paired together for separation, identification, and quantitation[13]. In the past, PFC content has been measured at high quantities with $^{19}$F NMR then when LC-MS/MS has been utilized, which has been attributed to the lack of separation between the different PFC within the sampled measured[227]. Fluorescence detection coupled with just an LC has also been shown to be successful for perfluorinated carboxylic acids[228] but is hardly, if at all, utilized.

While LC/MS and LC-MS/MS methods are the most widely used, it is difficult to compare between methods across different studies due to the ability to analyze with differing configurations of the instrument. Onghena and co-workers[229] tried to complete a comparison by testing high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), and capillary liquid chromatography (CLC) with the same mass spectrometer configuration. Figure 3.1 indicates the scaling Onghena and co-workers used to grade the differing LC sources with a score of 5 indicated the least generated waste, the least cost, the most rapidity, the best selectivity, and the lowest LOD[229]. Ultimately, the study demonstrates how the best configuration depends on the type of analysis being completed.

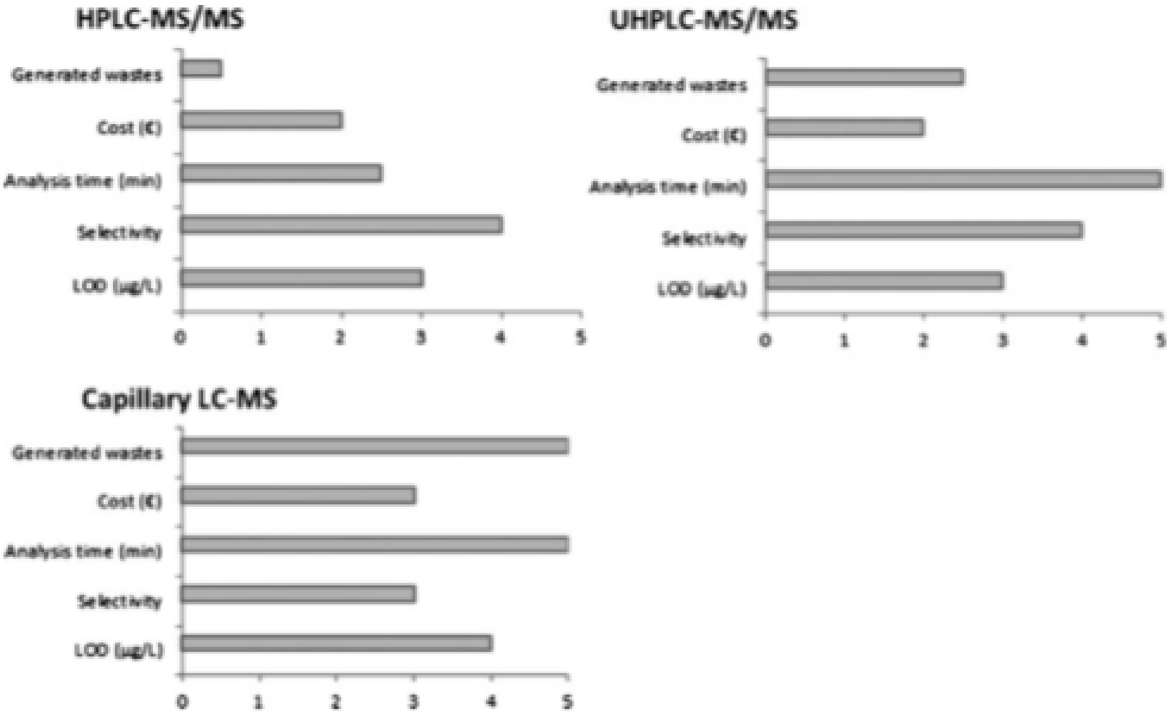


*Figure 3.1: Comparison of the functional characteristics of different HPLC systems with MS detection in determination of PFASs in river waters. Score 5 corresponds to the least generated wastes and costs, the most rapidity, the best selectivity and the lowest LOD values. Taken without permission from*[13]

The type of column used for the separation could change the analysis of the PFCs. Most studies, see Villagrasa and Lopez de Alda review[227], utilize either a short alkyl chain (C8) or long alkyl chain (C18) stationary phase for the separation of the PFCs. Schröder and co-workers demonstrated that the short alkyl chain stationary phases (C6, C8, phenyl, phenyl hexyl) could aid in the separation of branched isomers[230]. However, the same study also indicated that fluoroalkyl stationary phase columns would separate PFCs based on chain length and extent of branching for isomers, better than C18 and short alkyl chain stationary phases[230].

Likewise, there is an issue when trying to compare mass spectrometer configurations between studies. Some of the more popular analyzers for PFC analysis using LC/MS or LC-MS/MS are quadrupoles, triple quadrupoles (QQQ), and ion traps. Berger and co-workers[231] completed a study similar to Onghena and co-workers, looking at four different types of analyzers: ion trap, quadrupole, QQQ, and time-of-flight (ToF). While they do specify that ToF is the best overall due to high resolution, high selectivity, and low limit of detection, the data specifies that it depends on the analysis needed as to which mass spectrometric analyzer could be chosen.

Furthermore, ionizations sources may differ between studies. The overwhelming majority of studies, as amassed in the review by Villagrasa and Lopez de Alda[227], use electrospray ionization (ESI), in the negative mode, to analyze the PFCs. Figure 3.2a-b depict a set of total ion chromatograms for an LC-ESI-MS negative mode method for 15 different PFCs[232]. Atmospheric pressure photoionization (APPI) and atmospheric pressure chemical ionization (APCI) have also been utilized to analyze PFCs in differing matrices[227,233]. While APCI is a better ionization source compared to ESI it is utilized least between the three types of ionization sources. This is due to the range of PFCs ionizable by APCI being small compared to ESI and APPI[227].

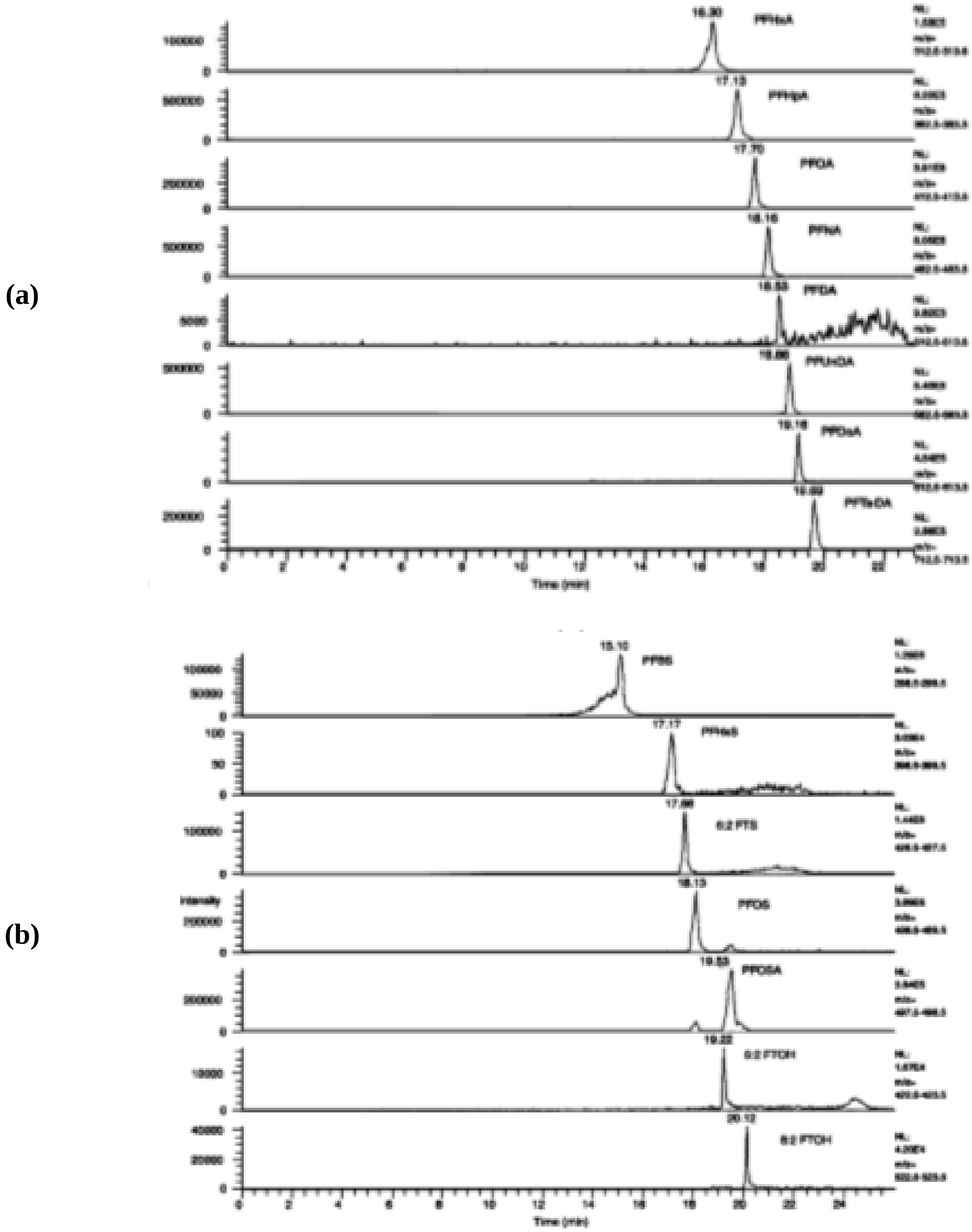


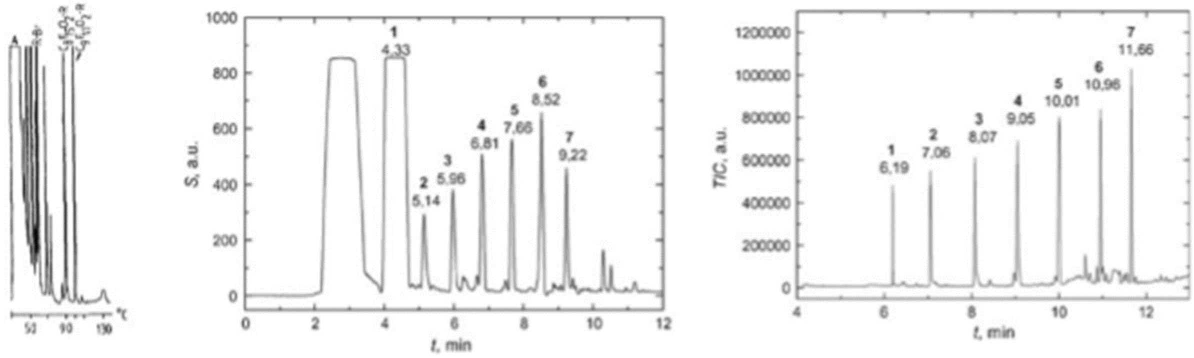
*Figure 3.2: LC–ESI(NI)–MS chromatograms obtained in the SIM mode for a standard solution containing (a) perfluorocarboxylic acids and (b) sulfonates and neutral FASs. Column: Phenomenex (Torrance, CA, USA) RP-C18 column (125×2 mm, 3 μm). Taken without permission from*[227]

The variability between instrument configuration from study to study might seem a large disadvantage to this type of analysis. When culminated together, LC/MS and LC-MS/MS is still the most powerful technique utilized when analyzing PFCs. That being said, a huge disadvantage to this analysis technique is the cost of analysis, making it uncommon for routine analysis and only for research purposes. Similarly, great care must be taken when using LC/MS and LC-MS/MS systems due to in-line contamination. The PFC contamination, as discussed above, is so widespread that special lines, solvents, and laboratory supplies must be used when preparing and running samples, especially when the highest sensitivity is desired[234,235].

### 3.1.2. GC/MS

In contrast to LC/MS and LC-MS/MS, gas chromatographic flame ionization detection (GC-FID), and gas chromatographic-mass spectrometric (GC/MS) analysis methods are cheaper, more efficient at separation, and have less in-line contamination. Overall, it is a less common analysis method for PFCs, but it can still be useful for the determination of some specific PFCs. The limiting factor for GC analysis comes down to the volatility of the PFCs analytes[13]. Those most easily analyzed are neutral PFCs, volatile PFCs (e.g., fluorotelomer alcohols (FTOH) and perfluoroalkyl sulfonamides), and perfluorinated acrylates[13,227,232,236-238]. A great example is from Shoeib and co-workers using GC/MS to analyze fluorotelomer alcohols (6:2 FTOH, 8:2 FTOH, and 10:2 FTOH) and perfluoroalkyl sulfonamides (N-methyl perfluorooctane sulfonamidoethanol (MeFOSE), N-ethylperfluorooctane sulfonamidoethanol (EtFOSE), and N-methyl perfluorooctane sulfonamide ethylacrylate (MeFOSEA)) in Arctic atmospheric samples, without any derivatization needed[239].

Similar to LC/MS and LC-MS/MS, GC analysis has some variables that can assist with the analysis of PFCs. Most of the literature sides with the use of GC/MS (single quadrupole mass spectrometers) with an electron impact (EI) or chemical ionization (CI) ion source. Contrary to LC analysis, EI and CI are, really, the only viable options for GC/MS analysis since the analytes are already in the gas phase, and EI and CI are gas-phase ionization sources. Regardless of the ionization source, GC/MS analysis of PFCs can obtain method detection limits in the range of 0.1-1.0 pg/m$^3$ (1E$^{-14}$-1E$^{-12}$ ug/mL)[236,239-242]. Conversely, GC-FID is multiple factors higher in method detection limits, only reaching 0.1-1.0 ug/mL (parts-per-million)[243,244]. Electron capture detection has also been utilized to analyze PFCs having a method detection limit between GC-FID and GC/MS[245-247]. Figure 3.3, gives a pictorial demonstration on the difference between the three detection methods for GC[13,246].



**(a)** **(b)** **(c)**

*Figure 3.3: Comparison of application of gas chromatography with different detection methods for determination of PFCAs.* ***(a)*** *Chromatogram recorded in the system with flame ionization detection for separation of benzyl esters of PFOA and perfluorononanoic acid in blood plasma.* ***(b)*** *Chromatogram recorded in the system with electron capture detection for mixture of C6 to C12 PFCAs 10 mg/L each derivatized to isobutyl esters.* ***(c)*** *GC-EI-MS chromatogram recorded for mixture of C6 to C12 PFCAs 10 mg/L each derivatized to isobutyl esters. Taken without permission from*[13]

While GC-FID, GC-ECD, and GC/MS have excellent method detection limits, the limited range of PFCs that can be analyzed is problematic. Fortunately, derivatization has been used to analyze those PFCs that are difficult to volatilize, such as polar PFCs like PFOS with a range of reagents: benzyl bromide, 2,4-difluoroaniline, diazomethane, methyliodide, butanol, methyl esters, or methanol[13,57,227,245,248-254]. However, care must be taken when analyzing perfluoroalkane sulfonates and sulfonamides. Due to the exceptional leaving group properties of sulfonate groups, the perfluoroalkane sulfonate esters formed during derivatization are unstable, making the method useless for analysis of certain samples, such as environmental samples of water for fluorinated surfactants[248,253,254]. Although, Martin and co-workers did show that CI could be used to analyze perfluoroalkyl sulfonamides without derivatization, see Figure 3.4[236].

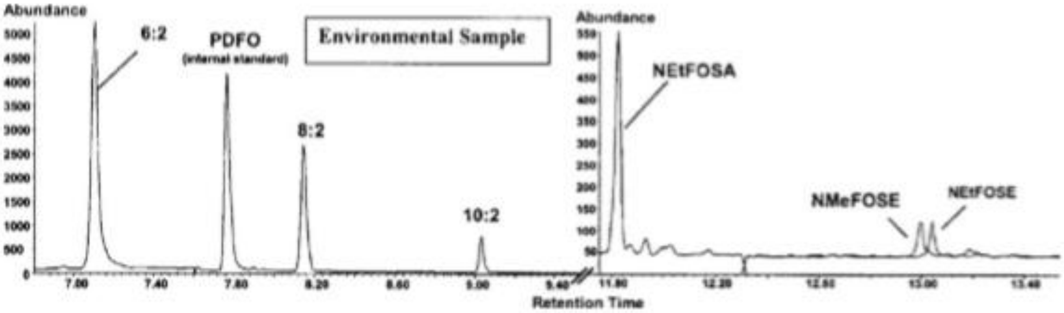


*Figure 3.4: Extracted and overlaid SIM chromatograms recorded in GC/MS system for determination of volatile fluorinated organics in air samples collected on XAD 2 resin and eluted with ethyl acetate. Taken without permission from*[13]

Altogether, GC-FID, GC-ECD, and GC/MS have superior separation power, detection limits, and cost compared to LC/MS and LC-MS/MS. However, when it comes to the range of PFCs that can be volatilized and analyzed, GC methods are by far inferior. Furthermore, not mentioned above is the time of analysis. Gas chromatographic methods are almost always longer (20-60 minutes per run) compared to LC runs (5-30 minutes per run) and even longer then UHPLC runs (2-5 minutes per run), making it difficult for high throughput laboratories. Despite the advantages and disadvantages for GC analysis compared to LC analysis, it is still a viable option for PFC analysis, especially for labs where cost is a major issue.

### 3.1.3. ToF-SIMS

Time-of-flight secondary ion mass spectrometry, Figure 3.5, is a useful analytical tool for surface analysis of samples. Unlike most mass spectrometric acronyms (e.g., ESI-QToF), the ToF-SIMS acronym is backward, where the analyzer (ToF) is put before the ionization source (SIMS). In SIMS a focused ion beam, sometimes described as the primary ions or primary ion beam, is sent to collide with the surface of a sample. These atomic collisions transfer the energy from the primary ion beam into the sample, allowing molecular fragments to overcome their binding energy and be emitted into an ion cloud, of secondary ions, above the sample surface. Most of these fragments will be neutral, making them useless for analysis. However, those that are positively or negatively charged can be extracted into the ToF analyzer. SIMS gives the ability to analyze one to two monolayers on the surface or a depth analysis down to a few nanometers, depending on the parameters of the experiment. [255]

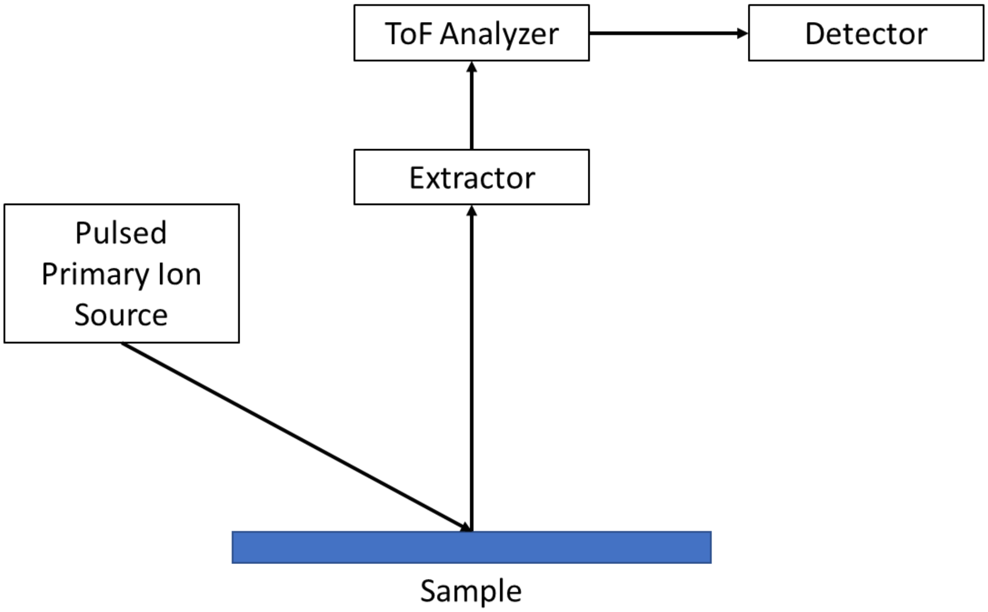
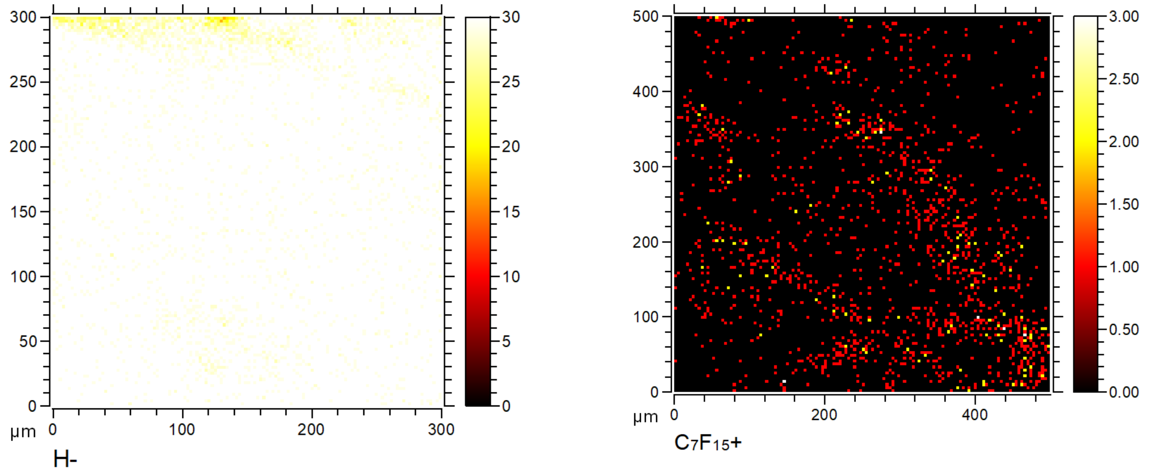


*Figure 3.5: Simplified schematic of a ToF-SIMS instrument. Adapted from*[255]

Once the surface has been ablated, and secondary ions are formed, the extractor is set to the opposite voltage of the ions desired (e.g., set to a negative voltage to analyze positive ions). Being attached to a ToF analyzer allows for high mass resolution of the fragments that are detected from the surface of the sample. Ultimately, giving high precision and accuracy to the determination of the fragments in the mass spectrum. [255]

Furthermore, this analysis allows for spectral interpretation of the data. Due it is fast scan rate, ToF-SIMS can take a multitude of mass spectral scans (upwards of 500) across the surface of the sample. Using software packages, a particular peak, say that for hydrogen (as seen in Figure 3.6), can be chosen and an image can be created. Each pixel on the image corresponds to one mass spectrum from the surface of the sample, and the heat map indicated the intensity of that ion across the surface of the sample. The more yellow in the image, such as Figure 3.6a, the more of that ion that is detected on the surface. The more black and red in the image, such as Figure 3.6b, the less likely that ion is on the sample surface. [255]



**(a)** **(b)**

*Figure 3.6: (a) ToF-SIMS spectra of H$^-$, indicated a large intensity of H$^-$ ions. (b) ToF-SIMS spectra of $C_7F_{15}^+$, indicating a low intensity of $C_7F_{15}^+$ ions.*

The use of ToF-SIMS for the analysis of PFCs has been well documents in the literature[256-259]. However, this literature review was unable to find any literature analyzing PFCs on fabric surfaces. That being said, the literature is united in their analysis of PFCs on their differing substrates. Each study used positive mode analysis to determine short-chain fragments of the PFCs present, see Table 3.1 for masses and fragment.

*Table 3.1: List of fragments and molecular weights possible from ToF-SIMS experiments of PFCs*[257-259]

| m/z | Fragment |
|---|---|
| 31 | $CF^+$ |
| 50 | $CF_2^+$ |
| 69 | $CF_3^+$ |
| 93 | $(CF_2)_2^+$ |
| 100 | $CF_3CF_2^+$ |
| 131 | $C_3F_5^+$ |
| 163 | $C_3O_2F_5^+$ |
| 169 | $C_3F_7^+$ |
| 181 | $C_4F_7^+$ |
| 185 | $C_3OF_7^+$ |
| 213 | $C_4O_2F_7^+$ |
| 231 | $C_5F_9^+$ |

Unfortunately, most of the studies use $C_xF_y$ fragments as their identifying fragments for the PFCs they were analyzing, due to the knowing the compounds they were analyzing[256,258,259]. In experiments, such as those described in Chapter 1, where the PFCs present are unknown using those short fluoro-chain fragments can be problematic. $C_xF_y$ fragments can indicate a PFC present, but they may also indicate the use of a fluoropolymer, especially when fluoropolymers are known to be present (i.e., moisture barriers). Hoshi and co-workers did use $C_xF_yO_z$ compounds during their analysis, although they were showing how differing primary beam conditions can affect the intensity of the fragments[257]. $C_xF_yO_z$ or $C_xF_ySO_z$ fragments would be more of an indication that

PFCAs or PFSAs are present on the gear, due to the functional group being attached, -OOH or -$SO_3H$ for PFCAs or PFSAs, respectively.

## 3.2. Skin Permeation

### 3.2.1. Past Studies

So far, this literature review has demonstrated an extensive amount of studies completed on the toxicity of PFOA and PFOS. However, all of these studies have been on the ingestion of these PFCs. Comparatively, there is a diminutive amount of studies completed on the absorption of these PFCs through the skin. A majority of those that have been completed have been performed on rats and mice, which is a poor substitute for human skin[260-264].

The skin is made up of three main layers: epidermis, dermis, and hypodermis, see Figure 3.7. The epidermis (Latin for “over skin”) is the outer most layer which protects your body and provides the color of your skin and has a thickness of 80 µm[265]. This layer of skin contains no blood vessels, only dead or dying tissue cells. The thickness of this layer varies throughout your body with differing amounts of stratum (Latin for layers). The thinnest skin will have four stratum and be located around the neck, groin, armpits, and eyes. Meanwhile the thickest skin will have five stratum and be located on the soles of the feet and palms of the hands. The thinner the epidermis layer, the more likely contaminates can penetrate this layer. [266,267]

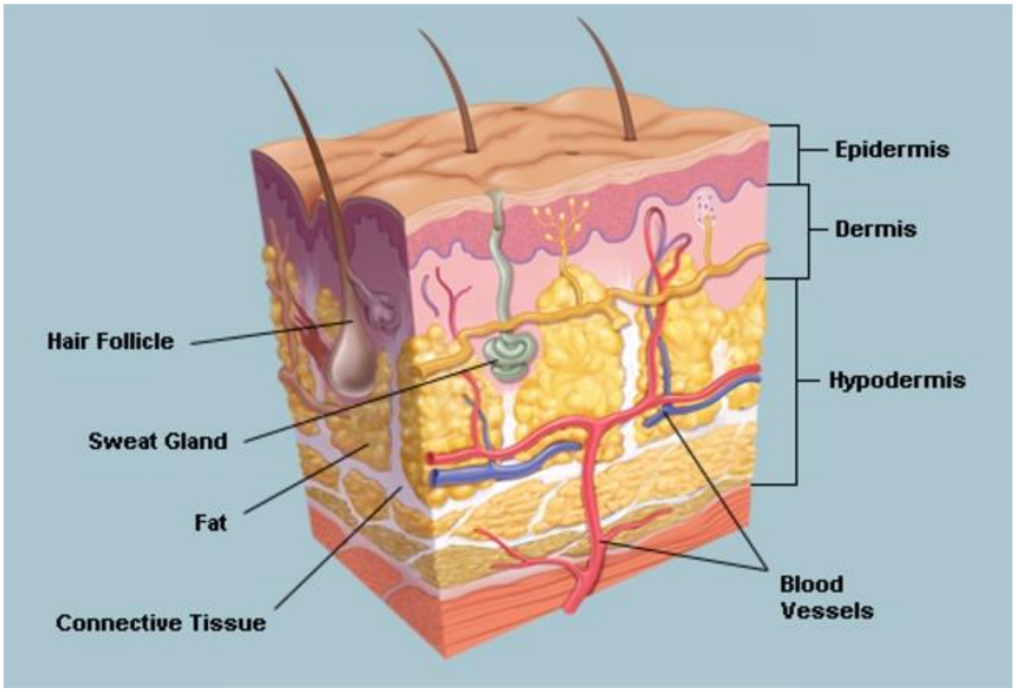


*Figure 3.7: Diagram of skin layers – epidermis, dermis, and hypodermis.*[268]

The dermis (Latin for skin) lies below the epidermis. Out of the three layers, the dermis has the most roles to play. It is responsible for sweat, hair, and oil production, it brings blood to the skin, and is responsible for the sensation of touch with the nerve endings that run through this layer. [266,267]

Finally, the hypodermis (Latin for below skin) lies below the dermis. This layer helps attach the previous two layers of skin to the muscle and bones. The blood vessel and nerve endings in the dermis are larger in the hypodermis, transporting blood and nerve signals to and from the dermis. This layer is also responsible for fat storage, which ultimately aids in the control of body temperature. [266,267]

Ultimately, the skin can be visualized as a brick and mortar structure, similar to the façade of many NC State buildings, see Figure 3.8. For contaminates, or drugs, to enter the barrier of the skin, one of four pathways must be taken: intercellular (between the cells), intracellular (through the cells), and transappendageal, which is an overarching term for the following two pathways: intrafollicular (along the follicle), or via sweat ducts[269].

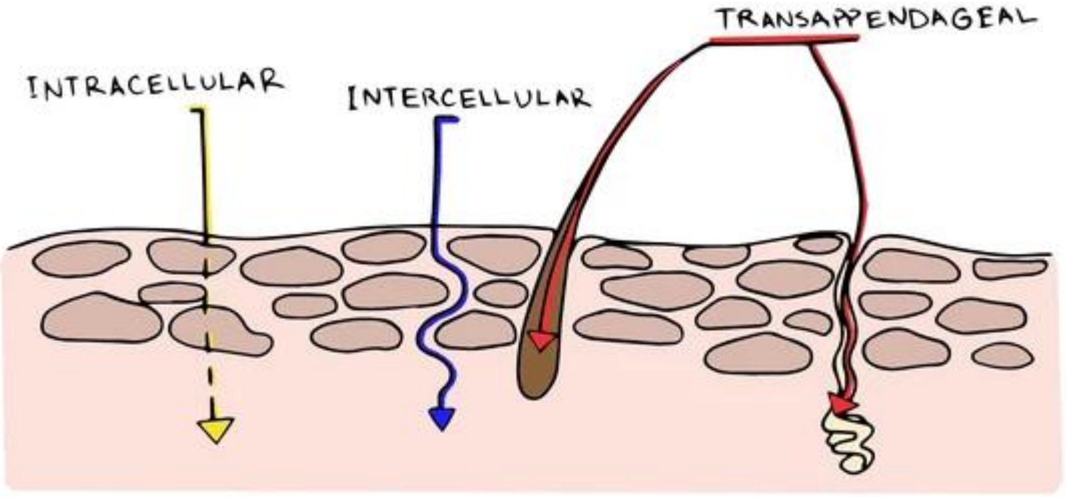


*Figure 3.8: Diagram of the ways in which chemicals enter the body: intracellular, intercellular, and transappendageal (which encompasses interfollicular and through the sweat glands).*[270]

These four pathways help explain why dermal studies on rats and mice are inconsistent with those on human skin.  Since most animals are covered in lots of hair, or fur, the main absorption pathway is intrafollicular, along with some differences in the structure in the skin compared to porcine and human skins[271].  Scott and co-workers demonstrated this with PFOS on rabbit, rat, and human skin, the chemical penetrated the rabbit and rat skin but did not permeate the human skin[272].  Due to its ionized form, PFOS should not be expected to permeate skin.  Which is an important factor in pharmacokinetics when determining drug pathways across membranes; ionized drugs will not cross a membrane unfacilitated, while un-ionized drugs pass freely across a membrane[273].

This dichotomy is also present with PFOA, as indicated by the differing results in Fasano and co-worker’s and Franko and co-worker’s studies[260,262].  Fasano and co-workers studied the ammonium salt of PFOA, ammonium perfluorooctanoate (refer back to Figure 2.19 for structures), determining a permeability coefficient of $9.49 \pm 2.86 \times 10^{-7}$ cm/h through human skin, using a concentration of 20,000 µg/mL[260]. However, Franko and co-workers determine a permeability coefficient of $5.5 \times 10^{-2}$ cm/h for neutral PFOA in water at 3,000 µg/mL through human skin, a 100,000 factor different in permeation[262]. Contrary to Fasano and co-workers, Franko and co-workers determined a permeability coefficient for ionized PFOA (in a buffered solution at pH 5.5) to be $4.4 \times 10^{-5}$ cm/h, a 100 fold difference from Fasano and co-workers. Further work also indicated that low dose (14 µg/mL) had a similar permeability coefficient to the ionized PFOA at $5.8 \times 10^{-5}$ cm/h, a 1,000 fold difference from the high dose[262].

However, most environmental exposure will not be a 3,000 µg/mL, unless working at one of the manufacturing plants. Most environmental exposure will occur near the low dose of 14 µg/mL, where most of it will be ionized. In order to have mostly un-ionized PFOA, Franko and co-workers had to buffer the solution down to a pH of 2.25, meaning most environmental PFOA will be ionized making the permeability coefficient even lower, maybe even as low as Fasano and co-workers measured.

### 3.2.2. Flow-Through Diffusion Cells

Most studies, nowadays, use a system called flow-through diffusion cells (FTDCs) to determine the penetration of chemicals through the skin[260,262,274-282]. The FTDC system is a step up from the older static cell diffusion system because it mimics environmental conditions much better while not excessively hydrating the skin[265]. This updated system also allows for the continual removal of the chemical from the skin, similar to blood perfusion in the skin layers[265].

The FTDC, seen in Figure 3.9, has three main components: (1) a peristaltic pump and tubing, (2) the diffusion cell held within heating blocks, and (3) the sample collecting apparatus which sits below the diffusion cells. The peristaltic pump and tubing pump the dissolved chemical onto the surface of the skin. These receptor fluids can be a saline solution or other solvent systems, but care must be taken because some media may not truly represent in vivo situations. The skin samples will lay within the heated diffusion cells. Skin from primates, rats, rabbits, mice, pigs, and humans may be used. Porcine skin is the most anatomically similar to human skin and may be preferred if disease-free human skin cannot be found. Lastly, the artificial medium in the sampling apparatus may vary but should be physicochemically similar to blood serum to best mimic oncotic pressure in vivo. [265]

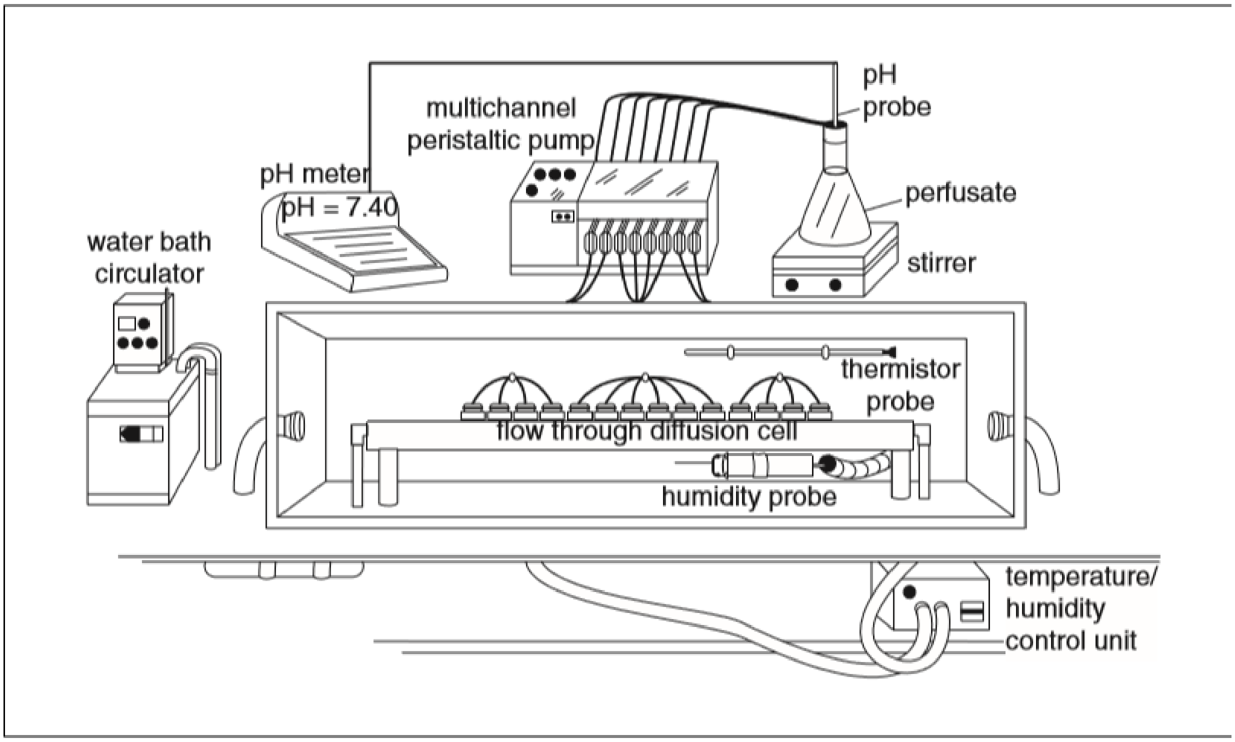


*Figure 3.9: Illustration of the FTDC system in an environmentally controlled chamber.*[265]

These experiments typically run between 8-12 hours, especially those that look to mimic worker exposure[265]. However, some studies[260,262] have done 24-hour diffusions. Bronaugh states that this is viable as long as perfusion is with a physiological buffer, like a balanced salt solution[275].

Overall the goals of FTDC testing is to dose the surface of the skin with a test chemical, collect samples of the artificial medium at specified time points throughout the experiment, and finally obtain samples of the different layers of skin at the end of the experiment to determine the depth of penetration[265].

## 3.3. Exposure Assessment

### 3.3.1. Washing Procedures

### *3.3.1.1. National Fire Protection Association 1851 Standard*

The National Fire Protection Association (NFPA) 1971, Standard on Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting is mainly a guideline for manufacturers to ensure they are creating uniform PPE for firefighters[25]. Fire agencies will also use NFPA 1971 as guidelines for purchasing protective ensembles for their departments. The NFPA 1851, Standard on the Selection, Care, and Maintenance of Structural Fire Fighting and Proximity Fire Fighting can be viewed an addendum to NFPA 1971 to aid in the care of the protective ensembles during their service life[283].

NFPA 1851 provides procedures for the selections, inspection, cleaning and decontamination, repair, storage, verification, testing procedures, and retirement/special incident procedure[283]. Of particular concern for this literature review as chapters 6 and 7, inspection, and cleaning and decontamination, respectively. The standard suggests that each member must complete an inspection of their gear after each use, i.e., any time they go out on a call. During inspections, the firefighter must look for[283]:

(1) Soiling

(2) Contamination

(3) Physical damage

(4) Damaged or missing reflective trim

(5) Loss of seam integrity and broken or missing stitches

(6) Overall integrity and compatibility of the three layers

If the inspection determines high levels of soiling and contamination, the gear must be cleaned, either by trained personnel within the department or sent to a verified independent service provider (ISP). Regardless, the gear will get cleaned with the same general parameters with the washer-extractor[283]:

- Water temperature not exceeding 40°C (105°F)
- Mild detergents (pH 6-10.5)
- The extractor must not exceed 100 G forces

Unfortunately, NFPA 1851 is not a federal mandate and is more of a guideline for taking care of turnout gear. Which means that the contamination from fire scenarios can build exponentially over time. The only state that has thus far mandated NFPA 1851 is Texas[284]. Some firefighters may wash their gear once a month or once every six months. This reviewer had even talked to a firefighter that has never washed their gear, which they inherited from their grandfather. This is due to the "badge of honor" culture surrounding firefighting, meaning the dirtier you look, the more brave and honorable you are in the eye of other firefighters[46]. Furthermore, the NFPA 1851 cleaning requirements have shown only to be 40% effective in cleaning turnout gear. The inefficiency of the standard is attributed to cross-contamination of the different garments in the washer-extractors[285].

#### *3.3.1.2. Durability of Water Repellent Finishes*

Ultimately, what NFPA 1851 fails to require is an inspection method for the water repellency of the outershell material after laundering. Chapter 12, Test Procedures, does include a section on water penetration barrier evaluation, but this is only to test the efficiency of the moisture barrier[283]. This failure is in disagreement with requirements from NFPA 1971, Section 7.1.2, and 8.48, which requires the garment and ensemble to pass a liquid penetration test, letting no liquid come through[25]. However, this is not to the fault of the NFPA committee, as there a currently no standards, from any standards committee, for determining the durability of water repellent finishes after laundering. There are ones to determine colorfastness[286], the durability of antibacterial agents[287], and for washing machine requirements[288].

Meanwhile, there is research indicating that water repellent finishes are not durable to laundering[289-294]. Some of those studies utilized physical properties of the fabric, e.g. contact angle measurements of water droplets on the surface[289,292] and fabric penetration tests[291,293], while others utilized analytical methods, such as ToF-SIMS[290], FTIR[289], X-Ray photoelectron spectroscopy (XPS)[290,294], atomic force microscopy (AFM)[294], and low-frequency mechanical spectroscopy (LFM)[294]. There was one study that went so far as to measure the effluent from their experimental home laundering set up to determine how much PFOA and PFOS were removed from the fabric[295]. They determined that 29.8% and 99% of the PFOS and PFOS, respectively, were removed from the textile swatches they were using for the study.

However, there still lies a gap in knowledge about the durability of these water repellents on the firefighting gear. While their gear employs high-performance materials, the durability of water repellents should not be all that different from those on consumer goods.

### 3.3.2. Thermal and UV Degradation of Turnout Gear

#### *3.3.2.1. Off-Gassing of PFOA*

As eluded to in earlier sections, firefighters' PPE is made of high performance, thermally stable, materials that help protect them from the heat environment in which they enter. As a part of their PPE, their self-contained breathing apparatus (SCBA) protects them from the toxic smoke and particulates generated by the conflagration. However, recent studies have indicated that the chemicals that come to rest on their gear during firefighting operations may off-gas from the material[296-302]. Off-gassing is the process of compounds volatilizing off the gear at elevated or ambient temperatures, particularly after a fire scenario. Some of these studies have indicated that the first 30 minutes, after a fire scenario, is the time in which most of the compounds off-gas from the turnout gear[299,301].

However, to the knowledge of this literature review, there is almost no literature indicating the off-gassing of PFCs from firefighter turnout gear. The only study this literature review found, at NC State's Textile Protection and Comfort Center, used head-space sampling, which indicated a possible fluorotelomer alcohol off-gassing from firefighter turnout gear[303]. Although there is a precedent for PFCs off-gassing from consumer goods, namely non-stick pans and microwave popcorn bags[304-307]. While these studies found that the chemicals do not penetrate the corn kernels or popped corn[304,305], they found that 80% of the compounds are released at the opening of the bag at concentrations of 0.5-6.0 ng/cm$^2$ [306,307]. Furthermore, non-stick pans, used at normal cooking temperature (179-233°C) were found to off-gas 11-503 pg/cm$^2$ of PFCs from their surface[307]. Although, in a study on the incineration of activated carbon filters used for water filtration, Watanabe and co-workers found that volatile organic fluorine compounds decreased from 13.2% to 0.1% at a temperature difference from 700°C to 1000°C, respectively[308]. Which indicates there is a limit to the thermal stability to these PFCs, although those temperatures won't be found in consumer appliances.

That being said, these temperatures may be reached in the fire scenarios that firefighters find themselves. Internal temperatures of fully involved fires have been measured above 600°C[309].

While the fluoropolymers (i.e., PTFE), which some moisture barriers are constructed from, has a safe operating temperature up to 260°C, the temperature it is exposed to in a fire scenario could exceed this by a few hundred degrees[310]. Although, it is worth mentioning that most of the heat burden will be taken by the outershell material. Upon exposure to this excessive heat, the PTFE could decompose into a plethora of volatile by-products: fluoroalkenes, hydrogen fluoride, carbon oxides, or lower molecular weight fluoropolymers (which may occur in particulate form)[310]. Altogether, this points to the need for understanding the off-gassing of PFCs from firefighter turnout gear.

#### *3.3.2.2. UV Degradation of Gear*

As mentioned in Section 2.1.1, the NFPA 1971 standard has certain requirements for firefighters' turnout gear. Broadly, the standard requires three layers to the turnout (outershell, moisture barrier, and thermal liner) to protect from thermal and moisture hazards[25]. Ultimately, the requirements for the standard narrow down the types of materials that can be utilized for each of the layers. For the outershell and thermal liners, the most common materials used are meta-aramids (i.e., Nomex ®), para-aramids (i.e., Kevlar ®), and polybenzamidizoles (PBIs). The moisture barriers are some combination of meta-aramids, and PTFE laminated to a polyurethane.

Outside of the requirement for the gear, NFPA 1971, and NFPA 1851, highly suggest keeping the turnout gear away from UV light. Research has shown that direct UV exposure greatly reduces the tear resistance, tensile strength, and extensibility of meta-aramids, para-aramids, and PBIs[311-313]. Depending on the humidity of the geographical region of where the gear is this degradation can either be due to oxidative reduction or hydrolytic reduction of the polymer chains[311-313]. The higher the humidity of the geographical region, the more likely hydrolytic reduction is occurring[311]. Either way, the exposure causes the polymer chain length to reduce, which reduces the high crystallinity of the fiber structure, hence causing a reduction in tear resistance, tensile strength, and extensibility[311-313]. A diagram of one possible degradation pathway for meta-aramid is depicted in Figure 3.10.

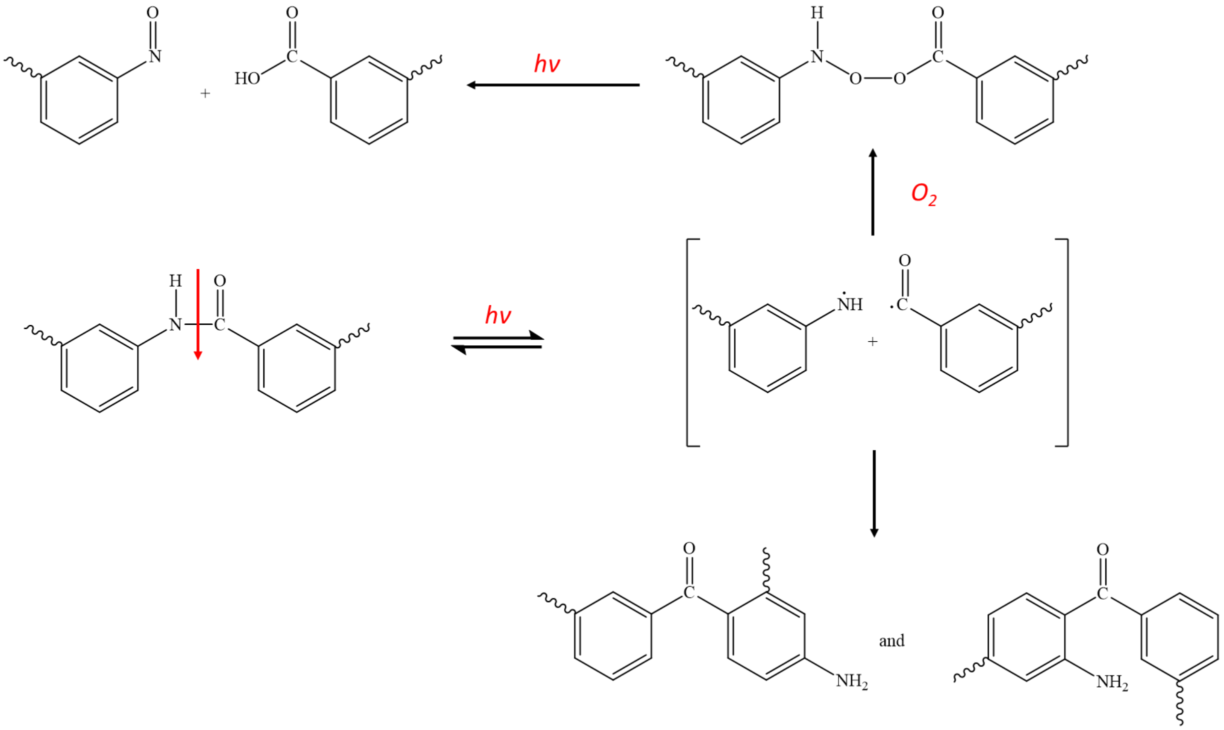


*Figure 3.10: Proposition of the schematic representation of the possible mechanics of photoaging of meta-aramid fibers. Taken without permission from*[255]

A study conducted by Aidani and co-workers[314] has shown that UV exposure can also cause a significant reduction in physical properties to a PTFE/meta-aramid moisture barrier. A majority of their results show the reduction in physical properties to the meta-aramid, but they do show an SEM image of the aging of PTFE after 48 and 96 hours of exposure, see Figure 3.11. Davis and co-workers[312] also indicated the loss of the water repellant finish on two different types of outershell material after 13 days of UV exposure. However, both of these studies did not, nor could not, identify the chemical changes due to UV exposure since their only analytical tool was Fourier Transform infrared (FTIR) spectroscopy. While FTIR is a good, non-destructive, analysis tool for structural elucidation, both Aidani and Davis did not mention how they were able to differentiate between peaks for the aramids and peaks for the PTFE/water repellant, respectively.

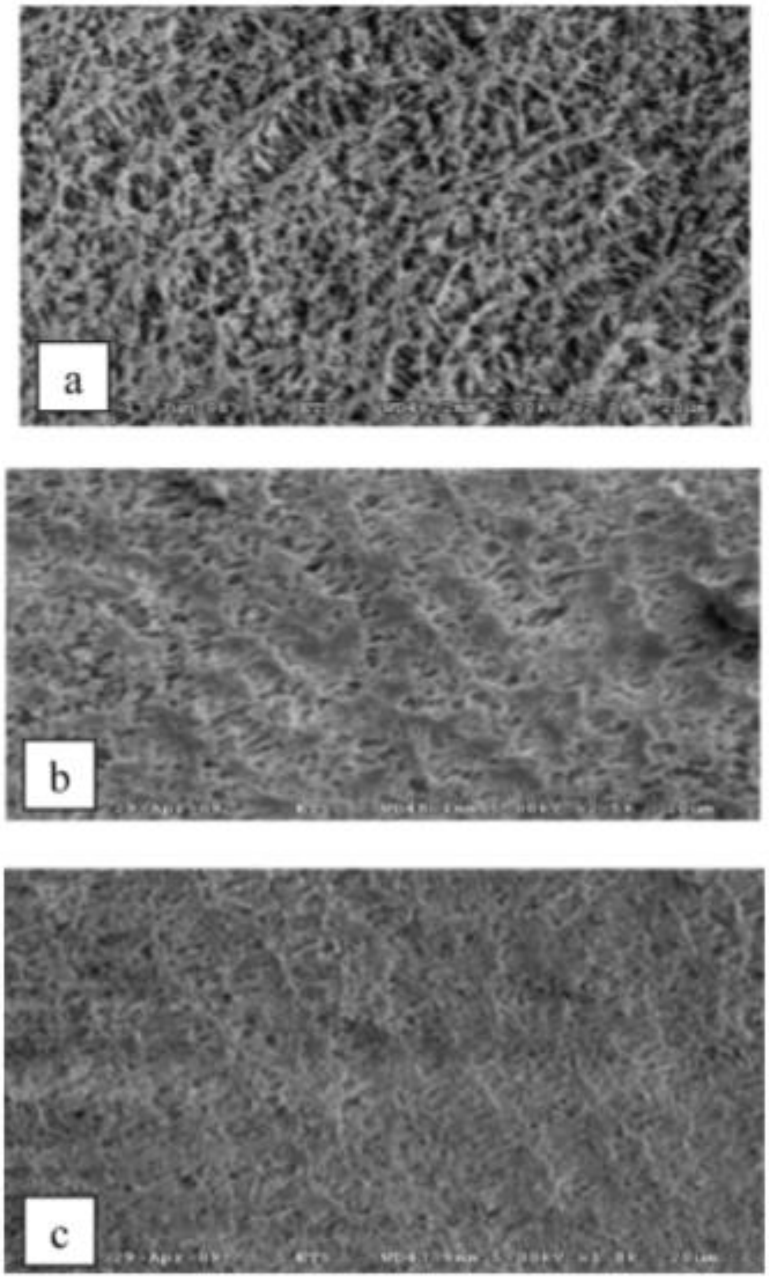


*Figure 3.11: SEM images of e-PTFE unaged (a) and aged at light intensity of 1.35 W/m2 and temperature of 70 C during 48 h (b) and 96 h (c). Taken without permission from*[255]

Studies conducted on the UV degradation on waste and municipal water containing PFCs have been moderately successful at best[315-318]. What they all have in common is the need for a catalyst, $Cl_2$ and $H_2O_2$ being the most common[316,318]. Although $Cl_2$ is inefficient (0-20% removal efficiency), while $H_2O_2$ is much more efficient (95-100% efficiency)[318]. There have been experiments using hydrated electrons[315] and $TiO_2$-rGO[317] as catalysts which prove promising in their efficiency at removing PFCs from waste and municipal water. Unlike the studies conducted, above, on firefighter gear, all of these studies on removal of PFCs from water used LC or LC-MS/MS to quantify the removal of the compound. Altogether, their analysis was much more accurate than with the use of FTIR. Which still leads to a gap in knowledge about the UV durability of PFCs on firefighter turnout gear. Degradation of PFCs due to UV exposure is a possibility, as the degradant of the gear's material may act as the catalyst in their degradation.

# CHAPTER 4: Comparison of Extraction Methods of Perfluorinated Chemicals from a Textile Substrate

## 4.1. Abstract

As more and more industrial chemicals are proven to be hazardous to the environment and humans, it is paramount to find ways to analyze these compounds. Per- and poly-fluorinated compounds are no different. While there is a plethora of analytical methods to measure PFC levels and studies looking at ingestion exposure, there are still areas that need to be considered, such as dermal and inhalation exposures. Exposure from industrial and consumer textile goods is a possible exposure route, but there have been very few studies conducted all using different methods to prepare samples. In this study, five different extraction methods were analyzed to determine their efficiency of extracting PFOA and PFOS off a griege textile fabric. Results indicate that there is no significant difference between any of the methods. However, two of the methods, just sonication and extractor dilution, could be considered better than the others based on their method parameters.

## 4.2. Introduction

Over the past several decades, issues surrounding per- and polyfluorinated chemicals (PFCs) have become more prevalent. At first, the issues surrounded exposure to PFCs through contaminated drinking water, areas surrounding chemical plants, airports, and firefighter/military training grounds[150,178,191,199,203]. However, the past several years, the issue has veered into exposure via consumer products: non-stick pans, prepared foods, and durable water repellent (DWR) finishes. Of note are the DWR finishes as they are placed on consumer textile goods to repel both hydrophilic and lipophilic substances. These types of finishes can also be used for industrial garments and personal protective equipment (PPE), such as firefighter turnout gear.

Durable water repellent finishes can be considered a polymeric PFC as they are polymeric coatings with fluorochemical side chains, as seen in Figure 4.1. Inherently, these compounds should not pose any exposure risk, as they have been cured onto the surface of the fabric. The point of the curing process is to cause the aqueous monomer units to polymerize, forming a coating on the outside of the fabric, essentially making the coating one large unit[319]. However, small molecule PFCs, such as perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS), are sometimes used as processing agents to attach the fluorochemical side chain to the backbone of the polymer[85]. Additionally, it is unlikely to get 100% polymerization of all the monomer units for DWR finishes, making these compounds available for exposure. Ultimately, the exposure concern lies over the residual small molecule PFC left after this process, not the polymeric PFC finish or possible degradation products due to certain exposures.

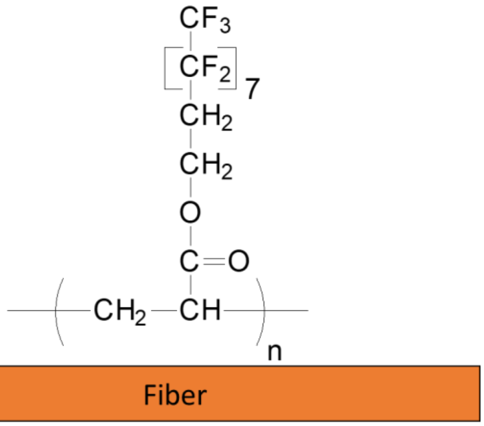


*Figure 4.1: Chemical structure of one type of durable water repellent finish, which also falls into the category of per- and poly-fluorinated compounds.*

Unfortunately, much of the literature surrounding exposure assessments of a wide range of PFCs have not found a lowest-dose response. Studies encompassed under the C8 Health Project have found a range of PFC blood level concentrations, 2.5 ng/mL – 5,000 ng/mL, which all had some associated level of disease response[85]. Furthermore, it is unclear which exposure pathway (dermal, inhalation or ingestion) are most likely for exposure. Ingestion has been the most extensively studied exposure pathway, so far, since this concern first began with exposure to the general population from drinking water[1-5]. While dermal has contradicting information whether PFCs absorb through the skin and there is none on inhalation.

To compound on that issue, there have been few studies that have investigated exposure concentration of PFCs from textile materials. Those that have, utilized unvalidated sonication methods on small amounts of fabric, without replicates[12]. Ultimately, this means there is no validated method for extraction of these compounds from textile materials. While the method was unvalidated, the study found low levels of PFCs (< 171-µg/m$^2$)[12]. When converted to µg/mL, using the size of their sample and amount of solvent for extraction, the highest dose of 171-µg/m$^2$ becomes 0.019-µg/mL. When compared to studies in the literature looking at dermal absorption of PFCs through skin, 0.019-µg/mL is 1,000 times smaller than the lowest dose used (14-µg/mL)[320]. At this dose, the PFC chemicals tested had a permeability coefficient of 5.8E-5 cm/h, meaning very little chemical permeated through the skin. Altogether, it could possibly be extrapolated that the dose found in the textile extraction study might not permeate through skin, making this an unlikely exposure pathway.

Since these chemicals are considered “forever chemicals”, it is paramount to find an extraction method which is validated, and takes into account as many parameters surrounding possible issues for extraction: time to extract, amount of solvent used, amount of fabric needed for extraction, and ability to remove DWR finishes. With a validated extraction method, results from different labs can start to be compared in order to understand the scope of this wide-ranging issue. The study below aims to answer those questions in order to be a starting point for understanding.

## 4.3. Materials and Methods

### 4.3.1. Extraction Methods

Five different extraction methods were tested in the following experiment: just sonication (JS), sonication-evaporation-reconstitution (SER), sonication-evaporation-sonication-evaporation-reconstitution (SESER), extractor-dilution (ED), and extractor-evaporation-reconstitution (EER). Each method utilizes greige, or unfinished, Pioneer™ (Kevlar®/Nomex® blend spun yarn, 6.6 oz/yd$^2$ twill weave) material[321] doped with a PFOA/PFOS[322] mixture, depending on the method a different doping concentration was utilized, which was calculated based on the desired end concentration of 350-400 ng/µL. Furthermore, each extraction method utilizes Fisher Chemical Optima® LC/MS-grade methanol[323] (MeOH) as the extraction solvent. Additionally, each extraction procedure uses a positive control sample (the method for the positive control differs between extraction procedure) and an internal standard of a 5 ng/µL $C^{13}$PFOA/$C^{13}$PFOS[322] mixture was utilized for quantitation, these chemicals were procured from Sigma Aldrich. Further details about specific extraction procedures can be seen below.

There are similarities for each of the methods described below. Ten Thermo Scientific EP clear vials with polypropylene (PP) lids[324] were labeled and initial weights were recorded, although the size of the vials differed between methods. Of the ten vials, one was used for a positive control, the other nine were used for fabric samples. Square samples (size differed between methods) were cut from the griege fabric and eight were doped with a PFOA/PFOS mixture (doping concentration differed between methods), one was left as a fabric control. When doping samples, care was taken not to over saturate the textile substrate at one time. After doping, fabric weight was recorded, and then the samples were placed in their respective vials, and a "vial + fabric" weight was recorded.

At the end of each extraction method, 2-mL of each sample was filtered using Thermo Scientific Luer-Lock plastic syringes (3-mL)[324] with a Thermo Scientific Choice 25-mm, 0.22-um nylon syringe tip[324] into a Waters 12 x 32-mm glass screw neck, and a preslit HPLC vial[325]. Samples were then sent to be analyzed. A summary of all the differences can be found in Table 4.1.

*Table 4.1: Summary of all parameters for extraction methods.*

| | JS | SER | SESER | ED | EER |
|---|---|---|---|---|---|
| Size (cm x cm) | 2 x 2 | 7.62 x 7.62 | 7.62 x 7.62 | 7.62 x 7.62 | 7.62 x 7.62 |
| Vial Size (mL) | 20 | 40 | 40 | 60 | 60 |
| Solvent Amount (mL) | 2 | 20 | 20 | ~30 | ~30 |
| Dilution Amount (mL) | 2 | 2 | 2 | 50 | 2 |
| Doping Amount (µL) | 50 | 500 | 500 | 1,000 | 500 |
| Doping Concentration (ng/µL) | 16 | 1.4 | 1.4 | 17.5 | 1.4 |
| Theoretical Amount to be Measured (ng/µL) | 0.4 | 0.35 | 0.35 | 0.35 | 0.35 |

#### 4.3.1.1. *Just Sonication (JS)*

Square samples (2 cm x 2 cm) were cut out of the greige material. The fabric were doped with 50 µL of 16-ng/µL PFOA/PFOS mixture for a total of 800 ng (16-ng/µL x 50 µL) of PFOA and PFOS on the material. Samples were placed in 20-mL vials after doping.

Two milliters of MeOH was then placed in the vials using an Eppendorf Repeater® M4 pipette with a 10-mL tip and care was taken to ensure fabric was fully submerged. The positive control vial had 2 mL of MeOH, plus 50 µL of 16-ng/µL PFOA/PFOS mixture. A “vial + fabric + solvent” weight was recorded, before sonication. Once all weights were recorded, the 20-mL vials were placed in a FisherBrand CPX 5800 Ultrasonic Bath 9.5L for 30 minutes on the sonication setting.

After the 30-minute sonication time, vials were removed, and water was thoroughly wiped off the vials. A “vial + fabric + solvent” weight was recorded after sonication to ensure no MeOH evaporated during the sonication time. Fabric was then carefully removed from each vial and a “vial + solvent” weight was recorded.

#### *4.3.1.2. Sonication-Evaporation-Reconstitution (SER)*

Square samples (7.62 cm x 7.62 cm) were cut out of the greige material. The fabrics were doped with 500 µL of 1.4-ng/µL PFOA/PFOS mixture for a total of 700 ng (1.4 ng/µL x 500 µL) of PFOA and PFOS on the material. Sample were placed in the proper 40-mL vial, by folding and rolling to ensure the ensuing 20-mL of MeOH would cover the entire sample, as seen in Figure 4.2.

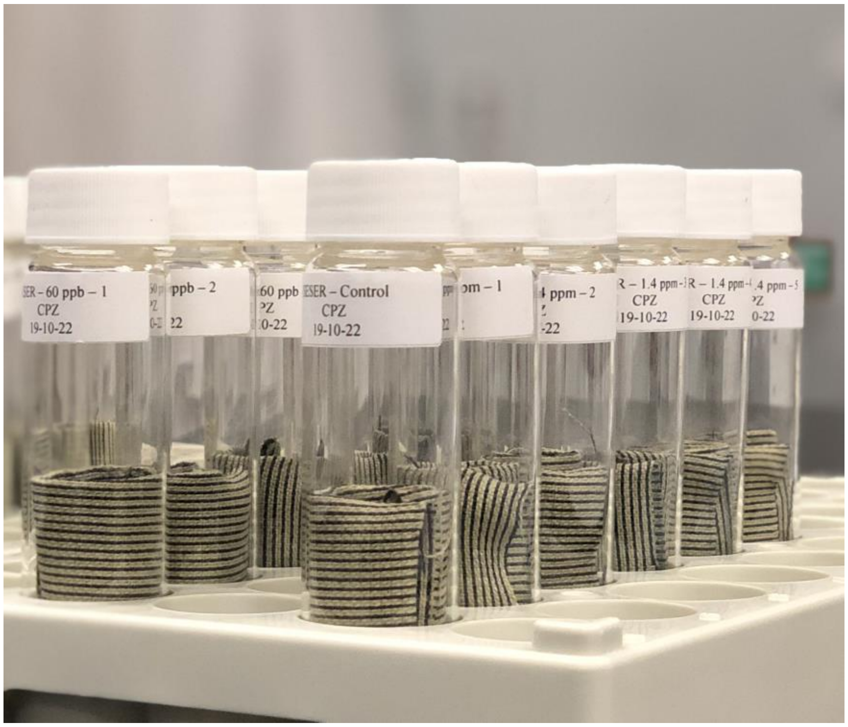


*Figure 4.2: Indicates how 7.62 cm x 7.62 cm fabrics were folded and rolled for SER and SESER extraction methods to ensure the fabrics would be submerged in the 20-mL of methanol.*

Twenty milliliters of MeOH was then placed in the vials using an Eppendorf Repeater® M4 pipette with a 10-mL tip and care was taken to ensure fabric was fully submerged. The positive control vial had 20 mL of MeOH, plus 500 µL of 1.4-ng/µL PFOA/PFOS mixture. A "vial + fabric + solvent" weight was recorded before sonication. Once all weights were recorded, the 40-mL vials were placed in a FisherBrand CPX 5800 Ultrasonic Bath 9.5L[323] for 30 minutes on the sonication setting.

After the 30-minute sonication time, vials were removed, and water was thoroughly wiped off the vials. A "vial + fabric + solvent" weight was recorded, after sonication, to ensure no MeOH evaporated during the sonication time. Fabric was then carefully removed from each vial and a "vial + solvent" weight was recorded. Each vial was placed in a Supelco® vacuum manifold and left overnight to evaporate the 20-mL of MeOH with a positive pressure flow of air flow. After all the solvent was evaporated, the empty vial was weighed.

#### *4.3.1.3. Sonication-Evaporation- Sonication-Evaporation-Reconstitution (SESER)*

Square samples (7.62 cm x 7.62 cm) were cut out of the greige material. Fabrics were doped with 500 µL of 1.4 ng/µL PFOA/PFOS mixture, for a total of 700 ng (1.4 ng/µL x 500 µL) of PFOA and PFOS on the material. Samples were placed in the proper 40-mL vials by folding and rolling to ensure the ensuing 20 mL of MeOH would cover the entire sample, refer to Figure 4.2.

Twenty milliliters of MeOH was then placed in the vials using an Eppendorf Repeater® M4 pipette with a 10-mL tip and care was taken to ensure fabric was fully submerged. The positive control vial had 20-mL of MeOH, plus 500-µL of 1.4-ng/µL PFOA/PFOS mixture. A vial + fabric + solvent weight was recorded, before sonication. Once all weights were recorded, the 40-mL vials were placed in a FisherBrand CPX 5800 Ultrasonic Bath 9.5L for 30 minutes on the sonication setting.

After the 30-minute sonication time, vials were removed and water was thoroughly wiped off the vials. A vial + fabric + solvent weight was recorded, after first sonication, to ensure no

MeOH evaporated during the sonication time. Each vial was placed in a Supelco[322] vacuum manifold and left overnight to evaporate the 20-mL of MeOH. After all the solvent was evaporated, the vial was weighed with fabric. After recording weight, the vial was reconstituted with 20-mL of MeOH for another round of sonication. Another weight was recorded after reconstitution with 20-mL then placed in a FisherBrand CPX 5800 Ultrasonic Bath 9.5L for 30 minutes on the sonication setting.

After the 30-minute sonication time, vials were removed and water was thoroughly wiped off the vials. A vial + fabric + solvent weight was recorded, after sonication, to ensure no MeOH evaporated during the sonication time. Fabric was then carefully removed from each vial and a vial + solvent weight was recorded. Each vial was placed in a Supelco vacuum manifold and left overnight to evaporate the 20-mL of MeOH with a positive pressure flow of air flow. After all the solvent was evaporated, the empty vial was weighed.

#### *4.3.1.4. Extractor Dilution (ED)*

Square samples (7.62 cm x 7.62 cm) were cut out of the greige material. Samples were doped with 1,000-µL of 17.5 ng/µL PFOA/PFOS mixture, for a total of 17,500 ng (17.5 ng/µL x 1,000 µL) of PFOA and PFOS on the material. This was done in two installments of 500 µL so as not to over saturate textile substrate at one time. After doping fabric, solvent was allowed to dry, fabric weight was recorded, then placed in plastic bags.

A BUCHI E-916 pressurized speed extractor was utilized to extract the chemicals off the textile substrate, this system uses 60-mL vials. The method was set to pressurize the 10-mL extractor cells to 100 bar at 100°C for three cycles using MeOH for the solvent and 5.0 grade nitrogen as the gas. Each cycle had a one-minute heat-up, five-minute hold, and two-minute discharge. After discharge, the sample was flushed with solvent for 1 minute and nitrogen gas for 2 minutes. Altogether, extraction time was approximately 45 minutes. When packing the cells, 8-9 grams of glass beads were used to fill the empty space of the 10-mL extractor cell to ensure less solvent was used during the extraction process.

For the positive control, a 10-mL extractor cell was completely filled with glass beads (~12 grams) then 1,000 µL of 17.5-ng/µL PFOA/PFOS mixture was spiked directly into the cell. Then the extraction process allowed to proceed as usual.

After extraction, vials were weighed to determine how much solvent was utilized during the extraction procedure since the BUCHI E-916 is not repeatable in solvent used per sample. That being said, all samples had less than 50 mL of solvent. This allowed for a dilution to 50 mL of total extract, using Fisher Chemical Optima® LC/MS-grade MeOH in a 50-mL volumetric flask, to ensure constant volume across all samples for quantification.

#### *4.3.1.5. Extractor-Evaporation-Reconstitution (EER)*

Square samples (7.62 cm x 7.62 cm) were cut out of the greige material. Samples were doped with 500 µL of 1.4-ng/µL PFOA/PFOS mixture, for a total of 700 ng (1.4 ng/µL x 500 µL) of PFOA and PFOS on the material. After doping fabric, solvent was allowed to dry, fabric weight was recorded, and samples were then placed in plastic bags.

The same BUCHI E-916 method for the ED extraction method was utilized here. The method for the positive control was also the same, with the one change of adding 500 µL of 1.4-ng/µL PFOA/PFOS mixture directly into the extraction cell.

After extraction, vials were weighed to determine how much solvent was utilized during the extraction procedure since the BUCHI E-916 is not repeatable in solvent used per sample. Each vial was then placed in a Supelco® vacuum manifold and left overnight to evaporate the

MeOH with a positive pressure flow of air flow. After evaporation, vial was reconstituted with 2 mL of MeOH.

### 4.3.2. Analysis Methods

Separation of mixture was completed on a Waters ultra performance liquid chromatography (UPLC)[325], equipped with an Acquity BEH C18 2.1 x 50 mm, 1.7 µm column with a vanguard precolumn attached. A gradient method was used (see Table 4.2) for a total run time of five minutes using 0.1% formic acid in water for mobile phase A and 0.1% formic acid in acetonitrile for mobile phase B. An injection volume of 7.0 µL was used and the column temperature was set to 35°C.

*Table 4.2: Gradient Method for PFOA/PFOS Analysis*

| Time | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0 | 0.4 | 90 | 10 |
| 1 | 0.4 | 90 | 10 |
| 2.5 | 0.4 | 5 | 95 |
| 3.5 | 0.4 | 5 | 95 |
| 3.51 | 0.4 | 90 | 10 |
| 5 | 0.4 | 90 | 10 |

Detection of compounds was completed on a Waters triple-quadrupole (QQQ) LC-MS system[325]. Mass spectrometer instrument parameters can be seen in Table 4.3, while MS/MS parameters, used for quantitation, can be seen in Table 4.4.

*Table 4.3: Triple Quadrupole Mass Spectrometry Parameters for PFOA/PFOS Analysis*

| | |
|---|---|
| Capillary Voltage (kV) | 3.25 |
| Cone Voltage (V) | 55 |
| Desolvation Temperature (°C) | 200 |
| Desolvation Gas Flow (L/h) | 550 |
| Cone Gas (L/h) | 50 |
| Collision Energy (V) | 30 |
| Source Temperature (°C) | 150 |

*Table 4.4: Tandem Mass Spectrometry Parameters for PFOA/PFOS Quantitation Analysis*

| Compound | Parent (m/z) | Daughter (m/z) | Dwell (s) | Cone Voltage (V) | Collision Energy (V) |
|---|---|---|---|---|---|
| PFOA | 413.2 | 169 | 0.08 | 18 | 20 |
| $C^{13}$ PFOA | 421.2 | 172 | 0.08 | 16 | 16 |
| PFOS | 499.2 | 130 | 0.08 | 76 | 42 |
| $C^{13}$ PFOS | 507.2 | 80 | 0.08 | 78 | 44 |

Using this method, baseline separation was achieved between the four compounds, as seen in Figure 4.3, although, the $C^{13}$ labeled compounds eluted at the same time as the non-labeled compounds, by design.

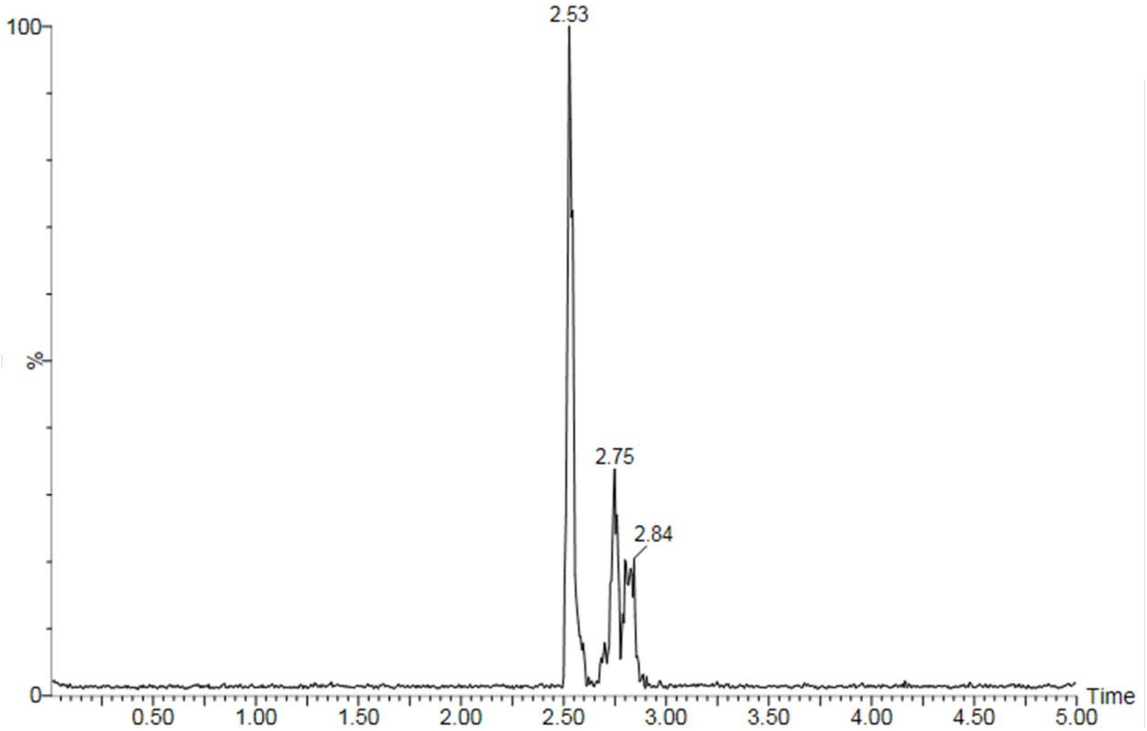


*Figure 4.3: Baseline separation of PFOA and PFOS using UPLC method. PFOA eluted at 2.53 minutes, PFOS eluted around 2.8 minutes.*

The same analytical standards used to make the PFOA/PFOS mixture that doped the griege fabric earlier were utilized to make the calibration curve standards. The calibration curve ranged from 0.025 ng/mL to 1,000 ng/mL, over 20 concentrations: 0.025, 0.05, 0.1, 0.2, 0.3, 0.5, 2, 5, 10, 15, 20, 40, 75, 100, 250, 350, 500, 600, 750, 1,000. For quantitation purposes, $C^{13}$PFOA and $C^{13}$PFOS were used as an internal standard at concentrations of 5 ng/mL. All calibration curves were linear with an $R^2$ value of 0.99 or higher. The limit of detection (LOD) and limit of quantitation (LOQ) for PFOA and PFOS can be found in Table 4.5.

*Table 4.5: Limit of Detection and Limit of Quantitation for PFOA and PFOS Analysis Method*

| Compound | LOD (ng/mL) | LOQ (ng/mL) |
|---|---|---|
| PFOA | 1.07 | 2.81 |
| PFOS | 0.91 | 2.24 |

## 4.4. Results and Discussion

### 4.4.1. Comparison of Parameters

#### *4.4.1.1. Sonication Methods: JS, SER, and SESER*

As analysis of PFAS chemicals from textile garments is a relatively new area of study. There is a need for a validated extraction method. A comparison of the five different extraction methods can be seen in Table 4.6.

The just sonication (JS), sonication-evaporation-reconstitution (SER), and sonication-evaporation-sonication-evaporation-reconstitution (SESER) methods used in this study were determined from the few previous studies completed on textile garments. Each of these methods has the benefit of using a small amount of solvent for extraction (a maximum of 42 mL for the SESER method) and as little as 2 mL for the JS method. In addition, for the JS method there is the benefit of using a small piece of the textile garment and only being a 30-minute extraction time.

Unfortunately, due to the evaporation time (especially the 2 evaporation cycles for the SESER method) the SER and SESER methods do not have a time efficiency aspect. The evaporation of 20 mL of MeOH took anywhere from 12-14 hours to ensure the complete evaporation of the solvent from the vials. Although, if a faster evaporation method was utilized, this technique may be a viable option.

Furthermore, due to the short time scale for extraction, it is unlikely that these methods would have sufficient time to equilibrate in between the fibers of the yarns in the woven structure. Thus, these methods are possibly only extracting any residual PFAS chemical on the surface or within the woven structure of the textile garment. This may be the intended purpose for some experiments as a determination of only dermally available chemical is the goal of the experiment. However, if the goal is to consider all PFAS chemicals present on or within a textile fabric, the JS, SER, and SESER methods do not operate at a high enough temperature to remove the durable water repellent (DWR) finish on the surface of a textile fabric.

*Table 4.6: Comparison of Five Extraction Methods*

| Method | Fabric Size (inch) | Approx. Solvent Used (mL) | Time (hours) | Fit for DWR |
|---|---|---|---|---|
| JS | 1x1 | 2 | 0.5 | No |
| SER | 3x3 | 22 | 12-14 | No |
| SESER | 3x3 | 42 | 24-30 | No |
| ED | 3x3 | 80-100 | 1.5 | Yes |
| EER | 3x3 | 50-60 | 30-35 | Yes |

#### *4.4.1.2. Pressurized Solvent Extractor Methods: ED and EER*

Consequently, the disadvantage of the sonication methods is a benefit of using the extractor dilution (ED) or extractor evaporation (EER) methods. The BUCHI E-916 pressurized speed extractor (PSE) has the ability to both heat up and pressurize a stainless steel cell to assist with extraction. The PSE also have the benefit of being an automated system which is a benefit over Soxhlet (even if these were not used in this study) and other types of extractions.

Due to the heat and pressure of the system, DWR finishes can be removed from the textile substrate with ease, which is what the precipitate that gathers at the bottom of the collection vials is assumed to be, see Figure 4.4. Although warning must be given, shorter condensing coils are needed since the DWR is a polymeric system. With the conventional condensing coils that come with the E-916, the polymeric DWR has been known to condense in the longer lines and clog the system. There is also a benefit to having excess eluent to run instrumental measurements across multiple days without having to rerun extractions.

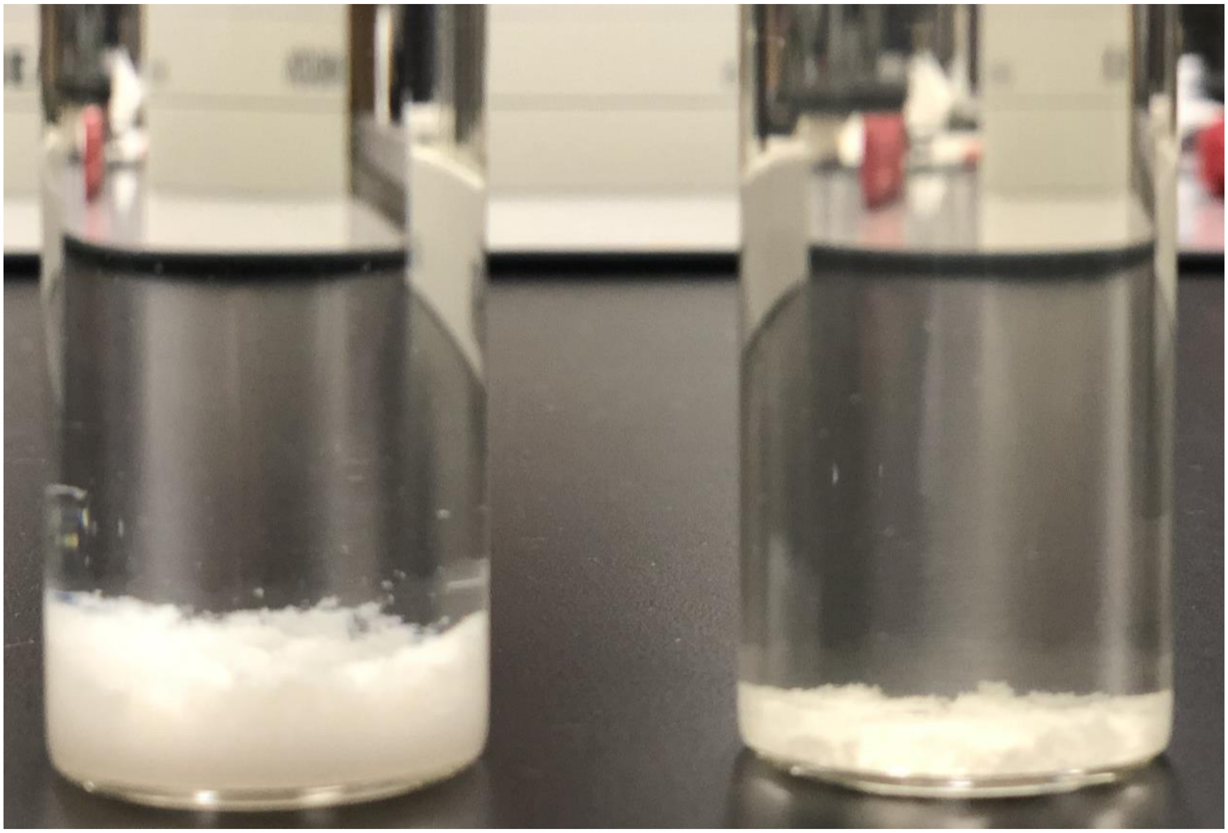


*Figure 4.4: Precipitate gathered at bottom of collection vials after PSE extraction from outershell materials.*

That all being said, there are some large disadvantages to the PSE. The extraction times can be quite time intensive. A 45-minute extraction cycle was used in this study, with a 15-minute cleaning cycle in between each extraction cycle (a cleaning cycle is needed to clean the cells to ensure no cross-contamination). Considering there is also time spent in removing or adding samples, to and from the extraction cells for the extraction or clean cycles, the time per extraction is about 75-90 minutes (this varies depending on how fast the users is at emptying or filling cells). Additionally, the BUCHI E-916 only has six extraction cell slots. When many extractions are necessary for a study, the time to extract all samples is magnified immensely.

In terms of solvent use, the ED and EER methods have the most amount of solvent used per extraction: 80-100 mL for the ED method and 50-60 mL for the EER method. This includes the amount of solvent used for extraction, cleaning, dilution/reconstitution.

### 4.4.2. Evaluation of Methods of PFC Extraction

#### *4.4.2.1. PFOA*

Figure 4.5 presents the average percent extraction rates of PFOA, ranging from 87-97%, from the griege outershell material: JS = 91.2%, SER = 88.7%, SESER = 87.3%, ED = 97.0%, and EER = 90.8%. Based on the only the averages, ED is the best method for removing PFOA from the outershell material. However, a single-factor ANOVA of the data indicates that none of the extraction methods are significantly different from each other, with a p-value of 0.122, which is higher than then significance level set at $p < 0.05$.

Additionally, each of the methods pass the requirements from the Association of Official Analytical Chemists (AOAC) for method validation. In the AOAC guidelines[326] for method validations for a single lab, an acceptable recovery range for part-per-billion concentrations is 70%-125%. The values for percent recovery for PFOA from griege outershell material falls within this range.

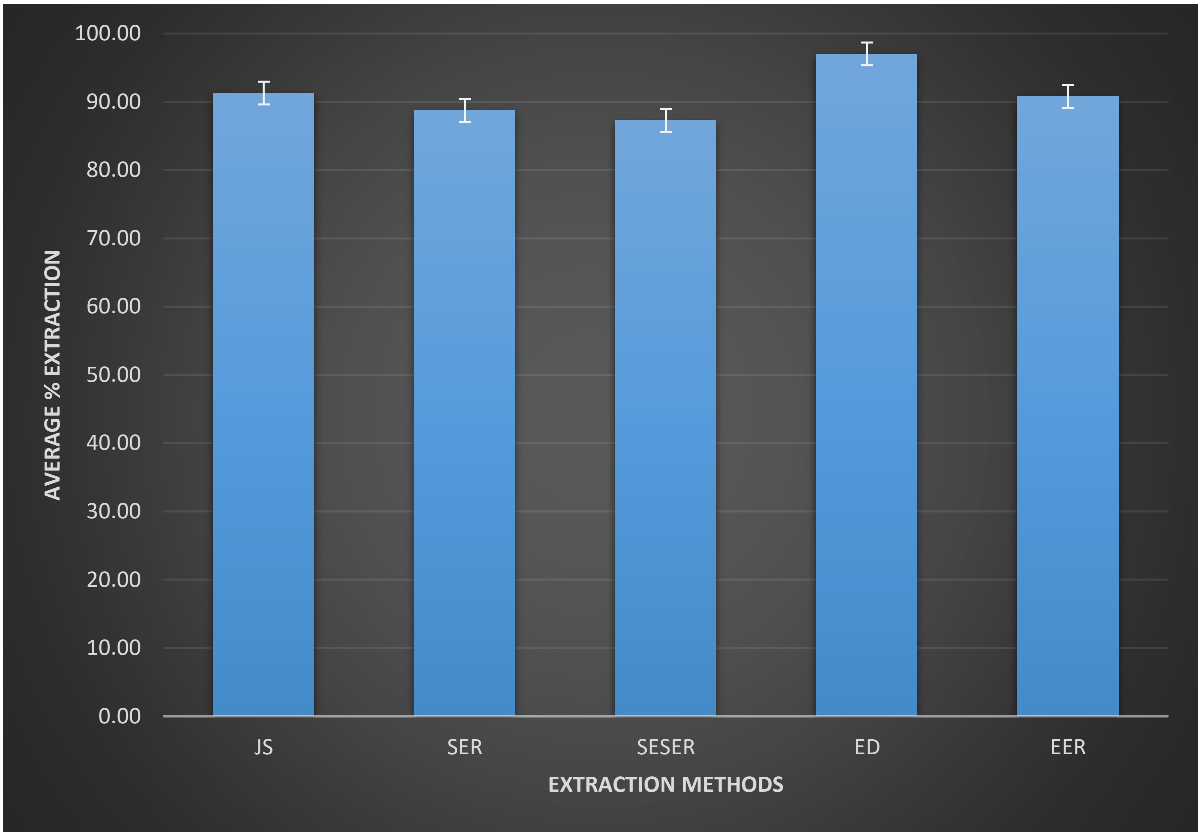


*Figure 4.5: Average extraction efficiencies of PFOA from griege outershell material for the five methods tested in this study: just sonication (JS), sonication-evaporation-reconstitution (SER), sonication-evaporation-sonication-evaporation-reconstitution (SESER), extractor dilution (ED), and extractor evaporation (EER).*

Furthermore, as eight replicates were completed for each method studied, a coefficient of variation (CV) or relative standard deviation (RSD) can be calculated to determine an acceptability range for repeatability. The AOAC requires a 15% RSD for repeatability for part-per-billion concentrations, which all of the methods studied fall into[326]. The RSDs range from 3 – 13%: JS = 9.28%, SER = 5.07%, SESER = 2.77%, ED = 13.25%, and EER = 6.85%.

Ultimately, based on the ANOVA analysis, any of these methods can be used to extract free PFOA from a textile material, it is still qualitatively unknown whether DWR finishes could be removed. However, based on the other parameters reported here, percent extraction, RSD, time for extraction, solvent used, sample size, and usability for DWR finishes, the extractions methods could be dwindled down to JS and ED as the most viable options for extraction of PFOA from textile material.

This analysis took into consideration, time, percent extraction, and RSD. Both JS and ED had the highest reported percent extractions. Unfortunately, they did have the highest reported RSD. However, when coupled with the amount of time each of these methods take, compared to the other three, JS and ED stand out as the paramount methods of extraction for PFOA from textile materials.

#### *4.4.2.2. PFOS*

Figure 4.6 presents the average percent extraction rates of PFOS, ranging from 96-106%, from the griege outershell material: JS = 96.3%, SER = 103.7%, SESER = 99.0%, ED = 106.9%, and EER = 104.3%. Based on the averages, ED is the best method for removing PFOS from the outershell material, albeit with an extraction efficiency of over 100%. However, a single-factor ANOVA of the data indicates that none of the extractions methods are significantly different from each other, with a p-value of 0.104, which is higher than then significance level set at $p < 0.05$.

As with the results from PFOA, all the results from the PFOS trials report values that fall within the AOAC guidelines for acceptable recovery ranges for part-per-billion concentration ranges[326]. Despite having some extraction efficiencies over 100%, which could have come from human error in doping the fabrics or making the calibration solutions, or from cross-contamination, as many analytical consumables have polytetrafluoroethylene (Teflon®).

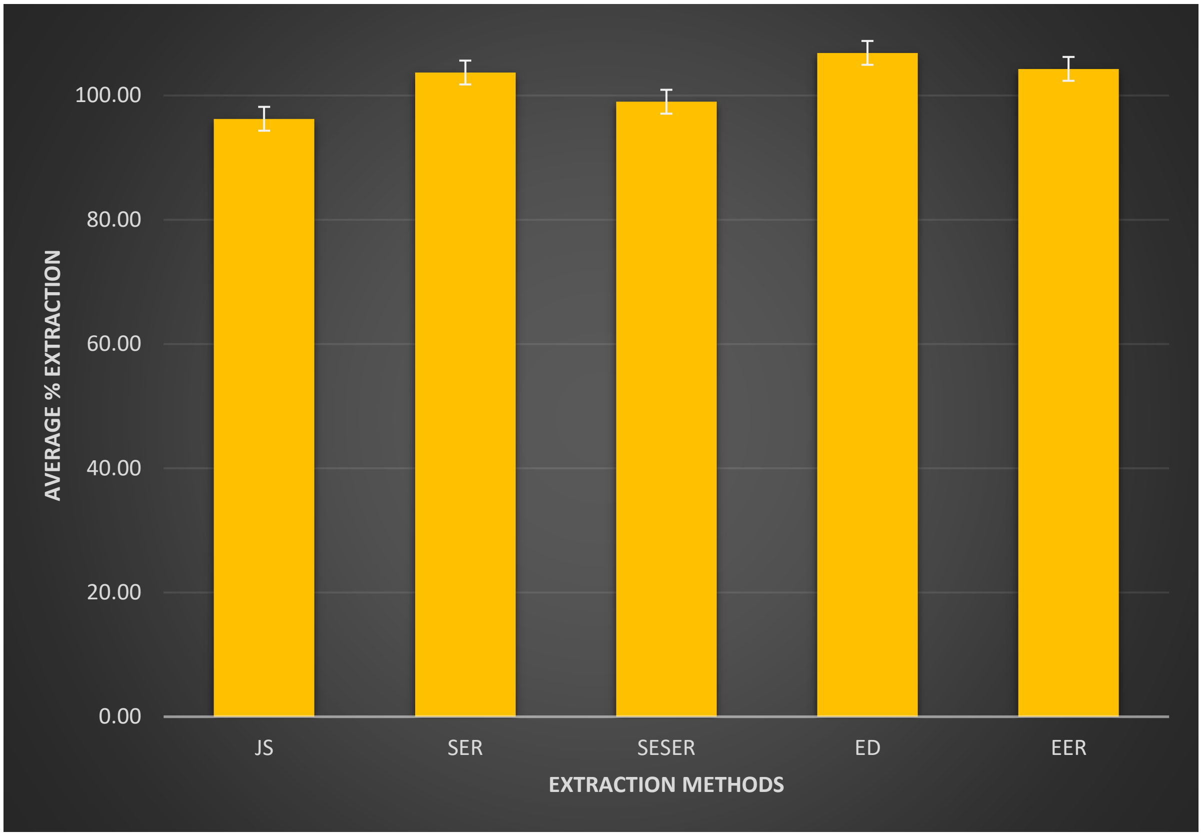


*Figure 4.6: Average extraction efficiencies of PFOS from griege outershell material for the five methods tested in this study: just sonication (JS), sonication-evaporation-reconstitution (SER), sonication-evaporation-sonication-evaporation-reconstitution (SESER), extractor dilution (ED), and extractor evaporation (EER).*

Additionally, with the eight replicates run, the RSD values all fell within the AOAC acceptable RSD range for part-per-billion concentrations: JS = 7.99%, SER = 7.17%, SESER = 4.18%, ED = 13.62%, and EER = 7.39%.

As with the PFOA analysis, when all parameters are taken into considerations both JS and ED could be considered the better methods, for PFOS extraction from textile substrates, with JS being slightly better than ED.  Time ends up being the most important parameter that makes JS and ED stand out against the rest, despite having higher RSD values.  While JS might have an extraction efficiency below 100% and a better RSD, the ability to remove DWR finishes must still be taken into account when considering which method to use, since they are not statistically different.

## 4.5. Conclusions

Ultimately, a validated method for extraction of PFCs must be created in order to assess findings across labs. Based on the findings in this study, there is no significant difference between the methods tested for PFOA or PFOS. However, based on the parameters of the extraction methods, just sonication and extractor dilution could be considered more efficient than the other three methods. Both methods are considerably faster than the other three. The main difference between them is whether there is a desire to remove any DWR finishes present on the surface of the fabrics being tested.

Unfortunately, due to the small scale of this study, the methods would need to be re-validated if a wider range of PFCs is desired. However, there would be no need to consider the SER, SESER, or EER methods, based on the data given in this paper.

That being said, work can still be completed on quantifying the difference between JS and ED for removal of the DWR finishes. This could be done in a multitude of manners: a mass balance of before and after sonication to determine a mass loss, using a non-targeted LC/MS method to determine what other PFCs were removed from a textile substrate, or using FTIR on any precipitate from the extractions.

## 4.6. Acknowledgements

- The Federal Emergency Management Agency (FEMA), Assistance to Firefighter Grants, Grant Number: EMW-2017-FP-00601.
- Dr. Ronald Baynes’ Laboratory in the College of Veterinary Medicine at North Caroline State University

# CHAPTER 5: Analysis of 20-Year Rang of Unused Firefighter Turnout Gear for Perfluorinated Compound Concentration

## 5.1. Abstract

Per- and polyfluorinated chemicals (PFCs) have been a rising concern over the past several decades, eventually being termed the “Forever Chemicals”. While there are well documented cases of exposure from drinking water, firefighter occupational exposure to these chemicals is relatively unknown. Some studies do indicate elevated levels of PFCs in firefighters’ bloods serum, but their exposure source is unknown. To date, there have been no studies to examine PFC concentration on firefighter turnout gear. This study examined a 20-year range, 2000-2020, of turnout gear for PFOA and PFOS concentrations. The findings indicate some quantifiable concentrations of PFOA in some turnout gear years, while there were no detectable amounts of PFOS.

## 5.2. Introduction

Over the past several decades there have been extensive studies on water exposure from per- and poly- fluorinated chemicals (PFCs), indicating a wide array of health effects associated with those exposures[1-5]. However, concerns over occupational exposure to PFCs for the firefighting community are relatively new, which came about surrounding exposure to these chemicals from the gear firefighters wear, which is called turnout gear[327]. To the best of this study’s knowledge, there have been no other studies examining this type of exposure. There have been studies which quantified the concentration of PFCs on consumer textile goods (outdoor jackets, baby bibs, rain ponchos, winter gloves, etc.), finding relatively small concentrations, < 171 $\mu g/m^2$, see Table 5.1[12,328,329].

*Table 5.1: Per- and Polyfluorinated Chemical Concentrations (µg/m$^2$) in Consumer Textile Goods*[12,328,329]

| | Canadian Study (n = 137) | Hanssen & Herzke (n = 18) | Gremmel et al. (n = 16) |
|---|---|---|---|
| PFOA | 0.02 – 99 | 0.09 – 3.1 | 0.02 – 171 |
| PFOS | 0.006 – 3.6 | - | 0.01 – 0.54 |
| PFNA | 0.004 – 12 | 0.19 – 0.21 | 0.02 – 28 |
| PFDA | 0.1 – 14 | 0.15 – 1.47 | 0.07 – 85 |
| PFUnA | 0.02 – 2.8 | - | 0.36 – 20 |
| PFDoA | 0.01 – 3.4 | 0.16 – 0.76 | 0.13 – 81 |
| PFTrDA | 0.02 – 0.22 | - | 0.03 – 3.7 |
| PFTeDA | 0.01 – 1.1 | - | 0.01 – 21 |

Furthermore, exposure happens via three routes: inhalation, ingestion, and dermally. In the case of textile garments, the dermal pathway is the most likely pathway for exposure, especially for the firefighting community. When this is considered in conjunction with the concentrations reported in Table 5.1, exposure may be unlikely[260,320]. There have been few dermal penetration studies completed on PFCs, with perfluorooctanoic acid (PFOA) being the majority of those studies. Findings indicate that very little of the dose penetrates pig skin during flow through diffusion studies, which correlates well with permeation through human skin. As seen in Table 5.1, the largest concentration found in the textile goods was 171-µg/m$^2$. Using the parameters in the paper, this concentration can calculate into 0.019-µg/mL. Studies completed on dermal absorption of PFCs utilized a dose of 14-µg/mL, 1,000 times larger than the concentration found on textile garments[320]. At the dosing concentration for the dermal paper, the PFC chemicals tested had a permeability coefficient of 5.8E-5 cm/h, meaning very little chemical permeated through the skin. Culminating in the conclusion that at the concentration found on textile garments, it is unlikely to dermally permeate.

Ultimately, this means that firefighter exposure to PFCs should not be any higher than the general population, which was seen in a few studies investigating PFC concentrations in firefighters' blood serum. The conclusions of most of these studies were that firefighters' blood serum concentrations of PFCs were no higher than the general population. A study completed by Rotander and co-workers, on 149 firefighters after a training exercise indicates that their PFOA (Figure 1c) levels, 4.6 -ng/mL, do not significantly differ from the Australian (4.2-ng/mL) or Canadian (2.3-ng/mL) populations[8,330]. However, firefighters blood serum concentrations of perfluorooctane sulfonate (PFOS), 74-ng/mL, and perfluorohexane sulfonate (PFHxS), 33-ng/mL, were six to fifteen times higher and significantly different from the general populations, as seen in Figure 5.1a&b. The general populations of Australia and Canada had PFOS levels of 12-ng/mL and 6.8-ng/mL, respectively, and PFHxS levels of 3.2-ng/mL and 1.7-ng/mL, respectively. It should be noted that values for the Rotander study and Australian study are median values, while the values reported for the Canadian study are geometric means[8,330-332].

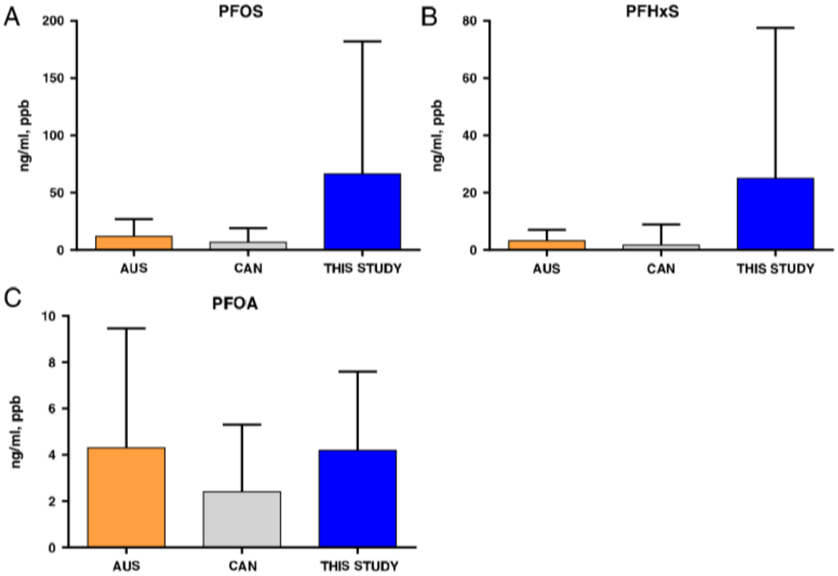


*Figure 5.1: Australian firefighters (a) PFOS, (b) PFHxS, and (c)PFOA blood serum concentrations compared to the Australian and Canadian national averages. Taken without permission from*[330]

These studies hypothesize this exposure to be from the use of aqueous film forming foams (AFFFs), which contain high levels of PFOS. The use of AFFFs began in the 1960's, from a collaboration between 3M and the USA Navy, to aid in the suppression of jet fuel (Class B) fires. In essence, the PFCs present in the solution create a film that sits on top of the burning material, making it difficult for the burning material to receive oxygen, thus smothering the fire. Since their creation, AFFFs have been utilized at airports (both commercial and private), firefighter training grounds, military training grounds, oil refineries/platforms, causing vast environmental contamination[8,10,47,48,53,63,73,75,330].

This study will not be considering AFFFs, instead it will be focusing on finding unused turnout gear from a 20-year range of service years, 2000-2020. The goal of this study it to determine the concentrations of PFOA and PFOS in these gear, using two of the validated textile extraction methods determined in Chapter 4. Due to the difficulty of finding older unused gear, i.e. from 2000-2009, this study was not restrictive in terms of the manufacturer or materials of the gear. Due to the National Fire Protection Association 1971 standard stating a 10-year use time of turnout gear and the EPA's Good Stewardship Program to eliminate PFOA and PFOS production and use by 2015, the desired range for gear falls into both of those categories. Although, understanding previous contamination for career firefighters would be necessary. Which is why the desired service years range is so wide. Unused gear was desired to understand the possible highest contamination concentrations, as it is hypothesized that older gear would lose PFC concentration due to wear, laundering, and fire exposure. Ultimately, this is a pilot study to be scaled up in future work.

## 5.3. Materials and Methods

### 5.3.1. Turnout Gear

Unused turnout gear from ten years, ranging from 2000 to 2020, was collected. Most of the gear was donated or bought to be used for alternative studies but ended up being excess and were never put into service or used in a fire service response. Some of the gear was developed in house and used for some years for display purposes. Altogether, none of the gear was worn in a fire exposure and, to the best of our knowledge, has never been washed or exposed to sunlight UV radiation, the amount of exposure to fluorescent or iridescent light is unknown. Information on material for each layer of the turnout jackets can be found in Table 5.2. Due to the difficulty of finding unused legacy gear, the study had to accept any turnout gear that could be found, despite the differences in manufacturer, and materials for the different layers. The variability was considered when data was analyzed. However, this limitation reflects the real-world variety of gear firefighters have worn or currently wear.

*Table 5.2: Trade name and common name for materials used in each layer of turnout coats*

| Year<br>Manufacturer | | 2003<br>A | 2004<br>A | 2006<br>** | 2007<br>A | 2008<br>B | 2010<br>C | 2014<br>B | 2015<br>B | 2016<br>B | 2017<br>B |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Outer shell (OS) | Trade Name | Nomex 7.5 oz | PBI/Kevlar Gold | ** | Millenia XT, Gold | PBI Matrix | ** | PBI Matrix | PBI Matrix Model: CSTM | PBI Matrix Model: CSTM | PBI Matrix |
| | Common Name | Nomex | 60% Kevlar® /40% PBI™ | ** | 60% Kevlar® /40% PBO | 60% Kevlar® /40% PBI™ | ** | 60% Kevlar® /40% PBI™ | 60% Kevlar® /40% PBI™ | 60% Kevlar® /40% PBI™ | 60% Kevlar® /40% PBI™ |
| Moisture Barrier (MB) | Trade Name | Comfortzone | Crosstech | ** | ChemPAK | Crosstech | Stedair 3000 | Crosstech | Crosstech | Crosstech | Crosstech |
| | Common Name | ** | Nomex/ePTFE Laminate† | ** | ** | Nomex/ePTFE Laminate† | Nomex/PTFE with Polyurethane coating Laminate | Nomex/ePTFE Laminate† | Nomex/ePTFE Laminate† | Nomex/ePTFE Laminate† | Nomex/ePTFE Laminate† |
| Thermal Liner (TL) | Trade Name | Aralite‡ | Caldura Aralite Quilt‡ | ** | Quantum 3D | Glide 2L Araflo E-89 (K) | 8 oz Q8 | Caldura SL | Caldura SL | Caldura SL | Caldura SL |
| | Face Cloth Common Name | Kevlar Filament /Kevlar/Nylon Blend‡ | Kevlar Filament /Kevlar/Nylon Blend‡ | ** | Kevlar Filament /Kevlar/Nylon Blend‡ | 60% Nomex filament/40% spun twill weave | Nomex/FR Modacrylic | Kevlar Filament /Kevlar/Nylon Blend† | Kevlar Filament /Kevlar/Nylon Blend† | Kevlar Filament /Kevlar/Nylon Blend† | Kevlar Filament /Kevlar/Nylon Blend† |
| | Batting Common Name | Meta-aramind 0.6 oz/yd$^2$ with meta-aramind nonwoven 2.3 oz/yd$^2$‡ | Meta-aramind 0.6 oz/yd$^2$ with meta-aramind nonwoven 2.3 oz/yd$^2$‡ | | Nomex/Kevlar blend non-woven spunlace‡ | Two layer durable water repellent Kevlar/Nomex spunlace | Aramid/FR Rayon needle punch, non-woven reprocessed batt | Two layers of Nomex non-woven spunlace† | Two layers of Nomex non-woven spunlace† | Two layers of Nomex non-woven spunlace† | Two layers of Nomex non-woven spunlace† |

**Data not able to be found

† These brands have many different subclasses to choose from. Information here is an average across the different types of subclasses.

‡ These trade materials have been discontinued, thus their common material names were assumed similar to current trade materials with similar names.

### 5.3.2. Extraction Methods

#### *5.3.2.1. Sonicator Method*

Nine Thermo Scientific EP 20-mL clear vials with polypropylene (PP) lids were labeled per jacket, and a vial weight was recorded. Three, 2 cm by 2 cm replicates were then cut from each layer, of each jacket. These samples were then placed in their respective vials, and a vial + fabric weight was recorded. Ultimately, there were 90 samples for the entire experiment: ten jackets, with nine samples per jacket.

Once vial + fabric weights were recorded, 2-mL of Fisher Chemical Optima® LC/MS-grade methanol (MeOH) was then placed in the vials using an Eppendorf Repeater® M4 pipette with a 10-mL tip, care was taken to ensure fabric was fully submerged. A vial + fabric + solvent weight was recorded, before sonication. Once all weights were recorded, the 20-mL vials were placed in a FisherBrand CPX 5800 Ultrasonic Bath 9.5L for 30 minutes on the sonication setting.

After the 30-minute sonication time, vials were removed, and water was thoroughly wiped off the vials. A vial + fabric + solvent weight was recorded, after sonication, to ensure no MeOH evaporated during the sonication time. Fabric was then carefully removed from each vial and a vial + solvent weight was recorded. Lastly, 100-μL of 5 ng/mL $C^{13}$PFOA/$C^{13}$PFOS mixture was placed in each vial, for quantitation purposes. Each $^{13}C$ labeled compound was bought as an analytical standard from Millapore Sigma.

Each sample was then filtered using Thermo Scientific Luer-Lock plastic syringes (3-mL) with a Thermo Scientific Choice 25-mm, 0.22-um nylon syringe tip into a Waters 12x32-mm glass screw neck, pre-slit HPLC-vial. Samples were then sent to be analyzed.

#### *5.3.2.2. Pressurized Solvent Extractor Method*

Nine Thermo Scientific EP 20-mL clear vials with PP lids were labeled per jacket. Three, 7.62 cm x 7.62 cm replicates were then cut from each layer, of each jacket. Pre-extraction weights were taken for each sample Ultimately, there were 90 samples for the entire experiment: ten jackets, with nine samples per jacket.

A BUCHI E-916 Speed Extractor pressurized solvent extractor (PSE) was utilized to extract the chemicals off the jackets. Two different methods were utilized for this study, one for the outer shells (OS) and thermal liners (TL) and another for the moisture barriers (MB). Two methods were used due to the issue with the melting point of the polyurethane laminates.

For outer shells and thermal liners, the PSE was set to pressurize the 10-mL extractor cells to 100 bar at 100°C for 3 cycles using MeOH for the solvent and 5.0 grade nitrogen as the carrier gas. Each cycle had a 1-minute heat-up, 5-minute hold, and 2-minute discharge. After discharge, the sample was flushed with solvent for one minute and gas for two minutes. Altogether, the extraction time was approximately45 minutes. When packing the cells, 8-9 grams of glass beads were used to fill the empty space of the 10-mL extractor cell to ensure less solvent was used during the extraction process.

Ultimately, the method for the moisture barriers was similar to the one for outer shells and thermal liners. The only change was to the temperature of the extractor cells, to prevent melting the polyurethane laminate. The PSE was set to pressurize the 10-mL extractor cells to 100 bar at 50°C for 3 cycles using MeOH for the solvent and 5.0 grade nitrogen as the gas. The rest of the method is the same at the method for the outer shells and thermal liners.

After extraction, all samples had less than 50 mL of solvent. This allowed a dilution to 50 mL, using Fisher Chemical Optima® LC/MS-grade MeOH in a 50-mL volumetric flask, to ensure constant volume across all samples for quantification. During dilution, 400 µL of 100 ng/µL $C^{13}$PFOA/$C^{13}$PFOS mixture was doped into each volumetric flask, to have a 5 ng/µL concentration in each solution for quantitation.

After dilution, each sample was then filtered using Thermo Scientific Luer-Lock plastic syringes (3-mL) with a Thermo Scientific Choice 25-mm, 0.22-um nylon syringe tip into a Waters 12x32-mm glass screw neck, preslit HPLC-vial. Sample were then sent to be analyzed. The rest of the diluent was stored in a refrigerator at 4°C for further analysis on later dates.

### 5.3.3. Chemical Analysis

Separation of PFOA/PFOS mixture was completed on a Waters ultra performance liquid chromatography (UPLC), equipped with an Acquity BEH C18 2.1 x 50 mm, 1.7 µm column with a vanguard precolumn attached. A gradient method was used (see Table 5.3) for a total run time of five minutes using 0.1% formic acid in water for mobile phase A and 0.1% formic acid in acetonitrile for mobile phase B. An injection volume of 7.0-µL was used, and the column temperature was set to 35°C.

*Table 5.3: Gradient method for PFC Analysis*

| Time | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0 | 0.4 | 90 | 10 |
| 1 | 0.4 | 90 | 10 |
| 2.5 | 0.4 | 5 | 95 |
| 3.5 | 0.4 | 5 | 95 |
| 3.51 | 0.4 | 90 | 10 |
| 5 | 0.4 | 90 | 10 |

Detection of compounds was completed on a Waters triple-quadrupole (QQQ) LC-MS system. Mass spectrometer instrument parameters can be seen in Table 5.4, while MS/MS parameters, used for quantitation, can be seen in Table 5.5.

*Table 5.4: Triple Quadrupole Mass Spectrometry Parameters for PFOA/PFOS Analysis*

| | |
|---|---|
| Capillary Voltage (kV) | 3.25 |
| Cone Voltage (V) | 55 |
| Desolvation Temperature (°C) | 200 |
| Desolvation Gas Flow (L/h) | 550 |
| Cone Gas (L/h) | 50 |
| Collision Energy (V) | 30 |
| Source Temperature (°C) | 150 |

*Table 5.5: Tandem Mass Spectrometry Parameters for PFOA/PFOS Quantitation Analysis*

| Compound | Parent (m/z) | Daughter (m/z) | Dwell (s) | Cone Voltage (V) | Collision Energy (V) |
|---|---|---|---|---|---|
| PFOA | 413.2 | 169 | 0.08 | 18 | 20 |
| $C^{13}$ PFOA | 421.2 | 172 | 0.08 | 16 | 16 |
| PFOS | 499.2 | 130 | 0.08 | 76 | 42 |
| $C^{13}$ PFOS | 507.2 | 80 | 0.08 | 78 | 44 |

The same analytical standards used to make the PFOA/PFOS mixture that doped the griege fabric earlier were utilized to make the calibration curve standards. The calibration curve ranged from 0.025 ng/mL to 1,000 ng/mL, over 20 concentrations: 0.025, 0.05, 0.1, 0.2, 0.3, 0.5, 2, 5, 10, 15, 20, 40, 75, 100, 250, 350, 500, 600, 750, 1,000. For quantitation purposes, $C^{13}$PFOA and $C^{13}$PFOS were used as an internal standard at concentrations of 5 ng/mL. All calibration curves were linear with an $R^2$ value of 0.99 or higher. The limit of detection (LOD) and limit of quantitation (LOQ) for PFOA and PFOS can be found in Table 5.6.

*Table 5.6: Limit of Detection and Limit of Quantitation for PFOA and PFOS for each different method.*

| | Instrument | | Sonication | | PSE | |
|---|---|---|---|---|---|---|
| Compound | LOD (ng/mL) | LOQ (ng/mL) | LOD (ng/cm$^2$) | LOQ (ng/cm$^2$) | LOD (ng/cm$^2$) | LOQ (ng/cm$^2$) |
| PFOA | 1.07 | 2.81 | 0.55 | 1.4 | 0.95 | 2.41 |
| PFOS | 0.91 | 2.24 | 0.46 | 1.1 | 0.78 | 1.89 |

## 5.4. Results and Discussion

### 5.4.1. Analysis of PFOA

Extraction using sonication (Figure 5.2) and PSE (Figure 5.3) indicate quantifiable values of PFOA from certain years of turnout gear. Values for concentrations, along with coefficients of variation, can be found in Table 5.7. For sonication, only the moisture barrier from 2003 and the outer shell and moisture barrier from 2008 had quantifiable values of PFOA, albeit low concentrations (< 10 ng/cm$^2$). Meanwhile, the 2003 outer shell, 2008 thermal liner, 2014 outer shell, and 2017 outer shell had concentrations of PFOA above the LOD, but below the LOQ. Thus, only allowing the study to determine PFOA is present but not allowing a quantifiable number to be reported.

Furthermore, there are a few years missing for the sonication extraction part of this study, i.e. 2006, 2007, and 2010. These turnout coats had not yet been collected by the time this part of the study was conducted. Due to extraneous circumstances, repeats of this study could not be conducted, with the missing turnout coats.

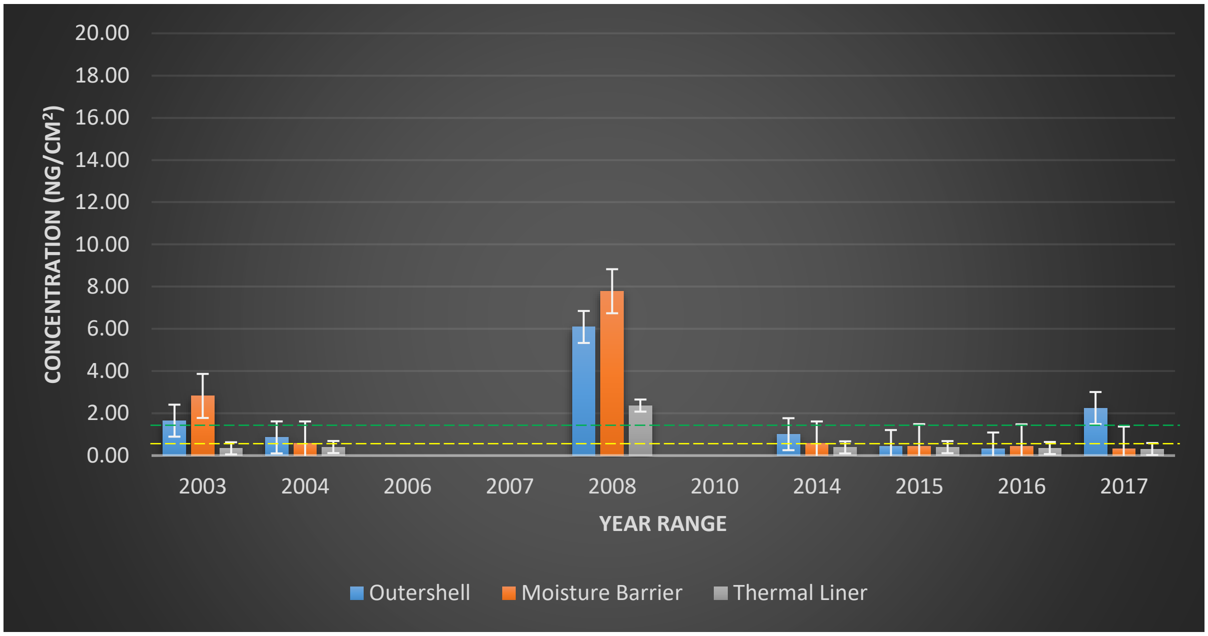


*Figure 5.2: Analysis of PFOA concentration (ng/cm$^2$) possibly present on seven-year range of firefighter turnout gear, 2003 to 2017, due to extraction in a sonicator. Yellow dashed line represents LOD (0.55 ng/cm$^2$) of analytical method. Green dashed line represents LOQ (1.4 ng/cm$^2$) of analytical method*

Extraction using the PSE (Figure 5.3) did produce differences in the concentration of PFOA extracted from the turnout coats. Most notably the thermal liner for 2008 had a seven-fold increase in the amount of PFOA detected from the sonication trial. This can also be seen in the 2004 thermal liner, albeit at a lesser fold increase. These notable increases could be due to running the PSE thermal liner extractions with the batting material. To increase the thermal protection of turnout gear, batting material is typically quilted to the thermal liner to increase the air layer between a firefighter and the fire.

The batting material was not run with the sonication trials due to an issue with absorption of the methanol used for extraction, only the face cloth or the side in contact with the skin was run. The batting material would soak up a majority of the 2-mL of methanol used for that method, leaving little to be used for instrumental analysis. Thus, the large increase for 2004 and 2008 could be due to the addition of the batting material being added to the PSE extraction method. Unfortunately, due to extraneous circumstances, additional trials could not be completed to confirm this theory. However, looking back at Table 5.2, it can be seen that the 2008 batting material had a DWR finish applied, which may account for the large increase in PFOA concentration between the sonication and PSE extraction methods.

Additionally, some data points are missing from the PSE extraction method: thermal liners for 2003, 2006, 2007, and 2010, along with the outer shell for 2010. Due to the increased heat of the PSE extraction method, the dyes/colorants in the fabrics were also extracted leading to a highly colored extract. The high color concentration, as seen in Figure 5.4, made it impossible to run on the LC/QQQ system because it would ruin the column and mass spec ionizer. Again, due to extraneous circumstances, the ability to run these samples on a GC system could not be completed to fill in these data points.

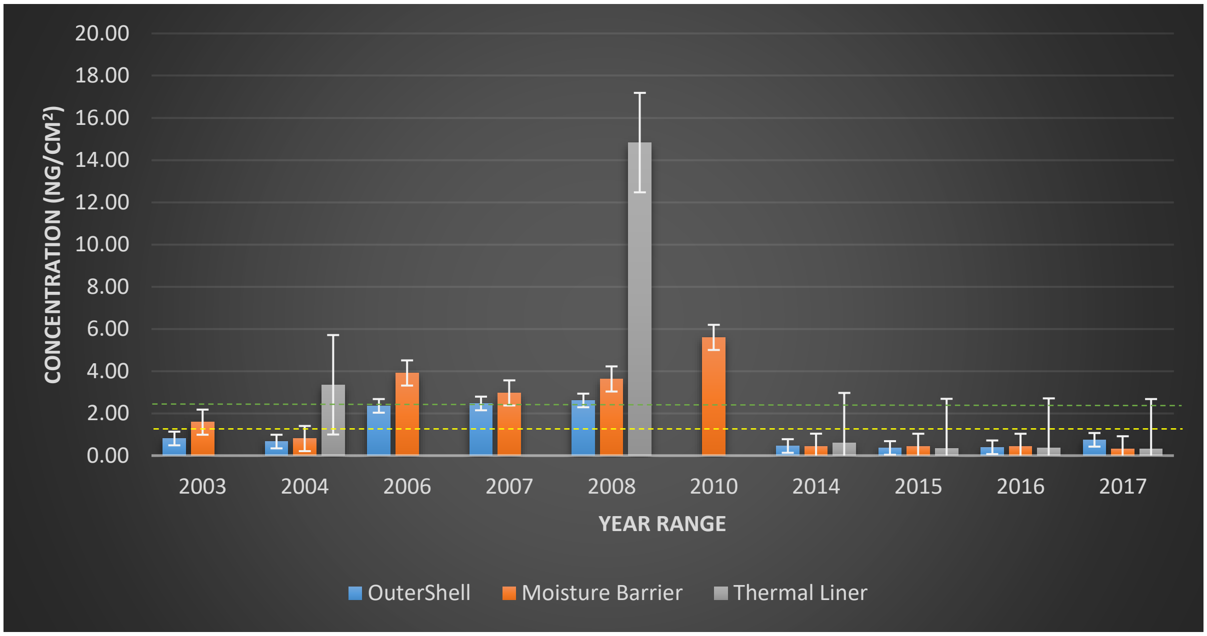


*Figure 5.3: Analysis of PFOA concentration (ng/cm$^2$) possibly present on ten-year range of firefighter turnout gear, 2003 to 2017, due to extraction in a pressurized solvent extractor. Yellow dashed line represents LOD (0.95 ng/cm$^2$) of analytical method. Green dashed line represents LOQ (2.41 ng/cm$^2$) of analytical method*

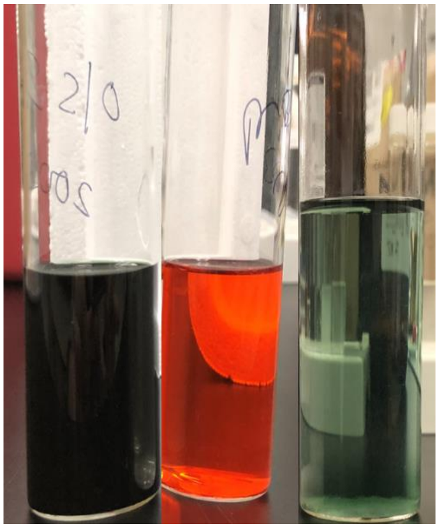


*Figure 5.4: Samples of colored eluent from pressurized solvent extractor from 20-year range of turnout coats.*

Other than GC analysis, there are some other possible ways to run such highly colored solutions. The easiest of which would be to continue diluting the solution until the color concentration was lower. Unfortunately, this method would greatly dilute any PFCs in the solution, hence reducing the ability to see the chemical during instrumental analysis. On the other hand, a solid phase extraction might work to separate out the colored portion of the solution. Although, without knowing what kind of dye was used and what kind of PFCs are in these solutions, a short pilot study would need to be conducted to see if this method could possibly work.

Due to the differences in material for each turnout and the missing data points between the different extraction methods it is difficult to make many comparisons between the two sets of data. For the few data points that can be compared, an ANOVA analysis was completed between the sonicator and PSE extraction methods. This analysis was only completed on layers that were above the LOD, for at least one of the extraction methods: outer shell and moisture barrier for 2003, thermal liner for 2004, all layers for 2008, outer shell for 2014, and outer shell for 2017.

On the whole, most of the layers indicate a significant difference between the two extraction methods (p-values in Table 5.7), which is a contradiction to the earlier study completed in Chapter 4. However, this could be due to multiple variables. As mentioned above, the batting was included in the PSE extractions and not in the sonicator extractions, which may explain the significant differences for the 2004 and 2008 thermal liners.

Additionally, the samples were cut from different portions of the jacket, which could be showing the variability of the textile processing of these garments. Unfortunately, the places where samples were cut from was not recorded and this hypothesis cannot be concluded definitively. That being said, the coefficients of variation were considerably higher compared to those reported in Chapter 4 for method development of these textile extraction methods. In essence, they followed the trends seen in Chapter 4, with the sonication extraction having lower CVs than the PSE extraction, but the increased variability could be due to variability in textile processing.

*Table 5.7: Concentration (ng/cm$^2$), p-values*, and Coefficient of Variation (%) values for PFOA extracted from ten-year age range of unused turnout gear.*

| Year | Extractor | | | | | | p-values* | | | Sonication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OS | CV | MB | CV | TL | CV | OS | MB | TL | OS | CV | MB | CV | TL | CV |
| 2003 | < LOD | 37.6 | 1.58 | 13.2 | - | - | - | 0.9 | - | 1.65 | 16.1 | 2.83 | 66.5 | < LOD | 6.2 |
| 2004 | < LOD | 23.3 | < LOD | 55.6 | 3.36 | 59.7 | - | 0.01 | 1E-4 | 0.86 | 12.4 | 0.57 | 24.9 | < LOD | 18.6 |
| 2006 | 2.36 | 61.5 | 3.91 | 20.8 | - | - | - | - | - | - | - | - | - | - | - |
| 2007 | 2.47 | 18.0 | 2.97 | 69.0 | - | - | - | - | - | - | - | - | - | - | - |
| 2008 | 2.61 | 8.9 | 3.63 | 14.4 | 14.83 | 52.2 | 0.03 | 0.02 | 1E-4 | 6.09 | 34.5 | 7.78 | 17.5 | 2.37 | 34.6 |
| 2010 | - | - | 5.60 | 33.7 | - | - | - | - | - | - | - | - | - | - | - |
| 2014 | < LOD | 29.2 | < LOD | 27.7 | < LOD | 40.8 | 0.1 | 0.23 | - | 1.01 | 21.4 | 0.57 | 14.5 | < LOD | 20.7 |
| 2015 | < LOD | 3.9 | < LOD | 18.1 | < LOD | 6.2 | - | - | - | < LOD | 26.3 | < LOD | 50.1 | < LOD | 5.0 |
| 2016 | < LOD | 20.4 | < LOD | 44.2 | < LOD | 13.7 | - | - | - | < LOD | 5.5 | < LOD | 24.5 | < LOD | 9.8 |
| 2017 | < LOD | 38.2 | < LOD | 10.0 | < LOD | 2.5 | 0.03 | - | - | 2.25 | 52.4 | < LOD | 9.9 | < LOD | 5.7 |

*p-value threshold set to 0.05

Furthermore, the sonication extraction uses 2 cm x 2 cm samples while the PSE extraction uses 7.62 cm x 7.62 cm samples. While a larger surface area would typically reduce the CV due to a more points of measurement, this could add to the increased CVs for the PSE extractions if there is a large variability of the textile processing, especially with the samples being cut from different locations of the turnout coats. That being said, the increased heat from the PSE extractions could add to more fluorinated chemical being removed with the durable water repellent finishes, and the higher CVs could indicate a large variation from the PSE instrument itself. Although, this doesn't account for the drop in concentration values seen in the outer shell and moisture barrier for 2008 and the outer shell for 2017.

Nevertheless, the values found in this study do not differ greatly from those reported in another study[12]. The Canadian Commission for Environmental Cooperation (CEC) looked at 137 textile consumer products in Mexico, America, and Canada for 31 different PFCs. Across the products tested a range of PFCs were found. In particular, a concentration range of 0.02-99 $\mu g/m^2$ for PFOA were found on 62 of the 137 products. Table 5.8 indicates the range of concentrations, in $\mu g/m^2$, for PFOA found in this study for all the layers of the turnout coats and for the two different extraction methods. For the most part, the range found in this study compares well the range from the Canadian CEC, with the exception of the 148.3 $\mu g/m^2$ outlier for the thermal liner in the PSE extraction method. Although, when compared to Gremmel and co-workers study looking at PFC concentration on outdoor jackets, this value falls within their range of 0.02-171 $\mu g/m^2$ [328].

*Table 5.8: PFOA concentration ($\mu g/m^2$) for each extraction method and each layer of turnout coats tested*

| PSE Extraction | | | Sonication Extraction | | |
|---|---|---|---|---|---|
| OS | MB | TL | OS | MB | TL |
| 3.6 – 26.1 | 3.2 – 56.0 | 3.2 – 148.3 | 1.9 – 35.4 | 1.9 – 45.2 | 1.8 – 13.8 |

### 5.4.2. Analysis of PFOS

Extraction using sonication (Figure 5.5) and a PSE (Figure 5.6) indicated no detectable amount of PFOS for any of the years of turnout gear, values can be seen in Table 5.9. The missing data points for sonication and PSE are for the same reasons as described above in the analysis of PFOA. However, unlike the PFOA data, the coefficient of variations for the PFOS values are well within the range for the Association of Official Analytical Chemists guidelines. Unfortunately, due to the concentrations being below the LOD, this is a moot point.

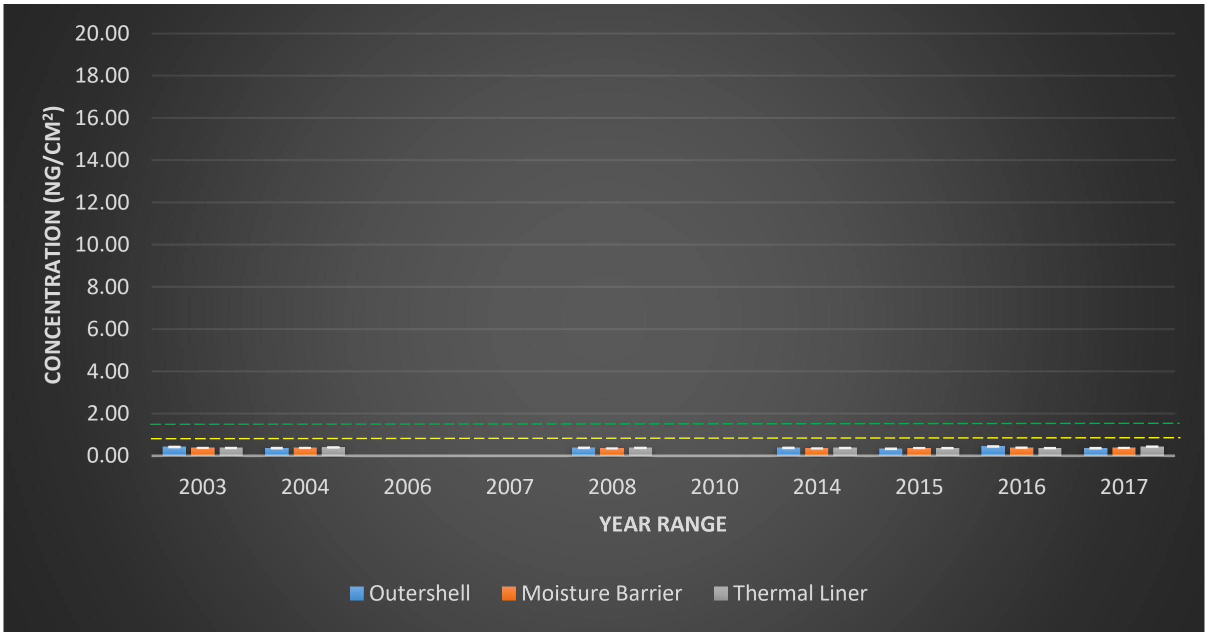


*Figure 5.5: Analysis of PFOS concentration (ng/cm$^2$) possibly present on seven-year range of firefighter turnout gear, 2003 to 2017, due to extraction in a sonicator. Yellow dashed line represents LOD (0.78 ng/cm$^2$) of analytical method. Green dashed line represents LOQ (1.89 ng/cm$^2$) of analytical method*

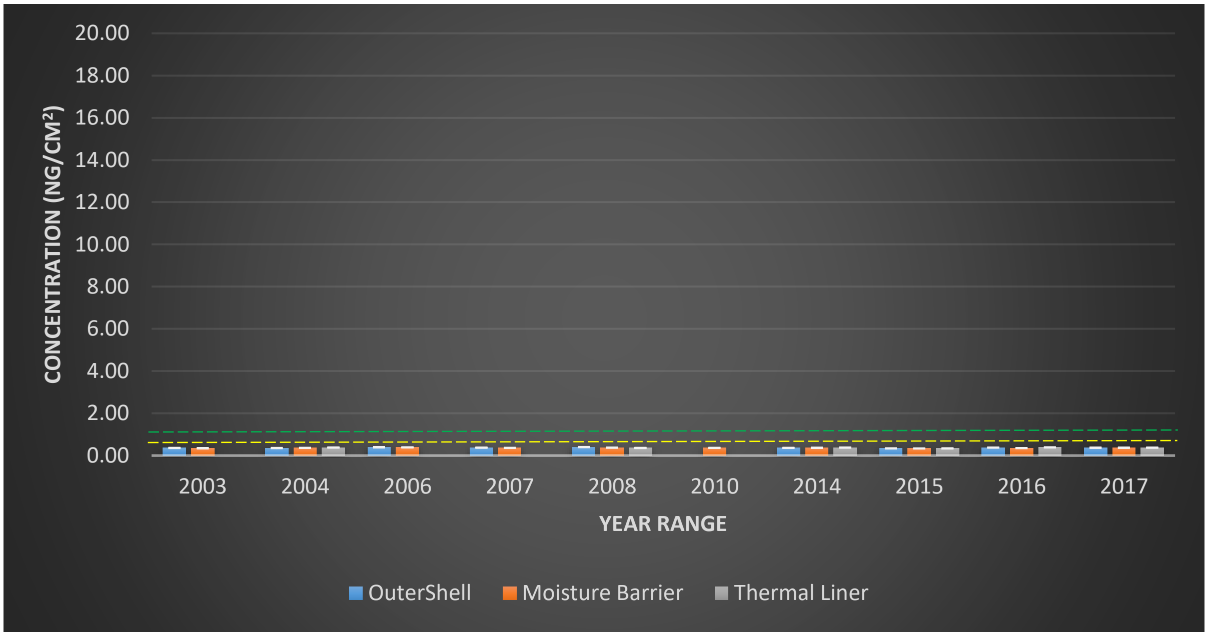


*Figure 5.6: Analysis of PFOs concentration (ng/cm$^2$) possibly present on ten-year range of firefighter turnout gear, 2003 to 2017, due to extraction in a pressurized solvent extractor. Yellow dashed line represents LOD (0.46 ng/cm$^2$) of analytical method. Green dashed line represents LOQ (1.1 ng/cm$^2$) of analytical method*

*Table 5.9: Concentration (ng/cm$^2$) and Coefficient of Variation (%) values for PFOS extracted from ten-year age range of unused turnout gear.*

| Year | Extractor | | | | | | Sonication | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | OS | CV | MB | CV | TL | CV | OS | CV | MB | CV | TL | CV |
| 2003 | < LOD | 11.6 | < LOD | 5.8 | - | - | < LOD | 2.4 | < LOD | 14.9 | < LOD | 15.0 |
| 2004 | < LOD | 4.9 | < LOD | 8.5 | < LOD | 12.5 | < LOD | 3.2 | < LOD | 7.8 | < LOD | 17.8 |
| 2006 | < LOD | 9.0 | < LOD | 13.8 | - | - | - | - | - | - | - | - |
| 2007 | < LOD | 8.2 | < LOD | 8.5 | - | - | - | - | - | - | - | - |
| 2008 | < LOD | 5.8 | < LOD | 13.3 | < LOD | 9.0 | < LOD | 7.1 | < LOD | 6.0 | < LOD | 3.1 |
| 2010 | < LOD | - | < LOD | 8.5 | - | - | - | - | - | - | - | - |
| 2014 | < LOD | 4.2 | < LOD | 8.2 | < LOD | 13.9 | < LOD | 14.9 | < LOD | 6.0 | < LOD | 9.6 |
| 2015 | < LOD | 9.5 | < LOD | 3.0 | < LOD | 6.8 | < LOD | 7.6 | < LOD | 7.3 | < LOD | 6.4 |
| 2016 | < LOD | 8.0 | < LOD | 9.2 | < LOD | 9.4 | < LOD | 11.3 | < LOD | 10.6 | < LOD | 1.6 |
| 2017 | < LOD | 8.8 | < LOD | 4.2 | < LOD | 8.7 | < LOD | 4.3 | < LOD | 5.6 | < LOD | 18.8 |

## 5.5. Conclusions

Consequently, this pilot study has indicated that there are some years of turnout gear that do show quantifiable levels of PFOA. However, these levels are no greater than those quantified from other studies on consumer textile goods, which may indicate limited occupational exposure from these chemicals present on turnout gear. In terms of PFOS, these levels may compare to those found in other studies, but due to the limitation of the LOD and LOQ of the method used no information can be definitively concluded about PFOS.

Further studies are needed to fully determine the occupational PFC exposure for firefighters. This study looked at an extremely small subset of turnout gear, from an array of different manufacturers with varying types of materials. Additionally, a study which uses a method to examine a wider range of PFCs would be beneficial, due to the size of the PFC class being greater than 4,000 chemicals.

# CHAPTER 6: Durability Assessment of Durable Water Repellent Finished Present on Firefighter Turnout Outershell Materials

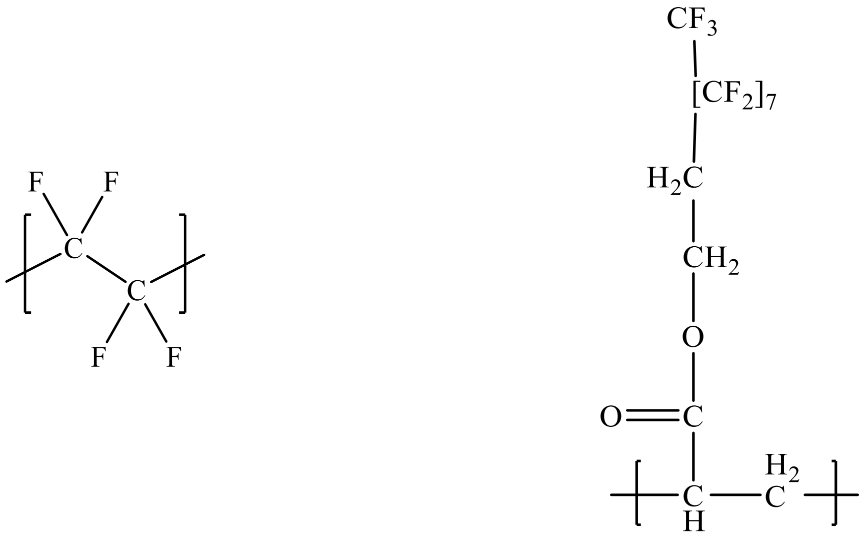
## 6.1. Abstract

Over the past few decades, environmental contamination due to water repellent finishes has become a growing issue. Recently, the firefighting community has been worried about their occupational exposure to water repellent finishes present on their gear. In these studies, the water repellency of unused firefighter outer shells manufactured from 2003 to 2017, ranging in type of material and water repellent finish, was tested after standard laundering and UV exposure, in comparison to two control materials. The overall results for the laundering study indicate that there is no significant decrease in the water repellency of the materials until after 50 standard launderings, with at most a 20% drop in repellency. Testing after UV exposure indicated no changes to the water repellency of outershell materials after 120 hours of exposure. Chemical analysis would need to be done in order to better understand the nature of the durable water repellent after washing and UV exposure. The objective of this study is to be a pilot study to set a framework for further investigation on durability of water repellent finishes on firefighter turnout gear.

## 6.2. Introduction

As the issue surrounding per- and polyfluorinated chemicals (PFCs) grows with concern to exposure to the general population, concerns also grow within the firefighting community. Several years ago, firefighters became weary of the gear they wear as they were misinformed that their gear was made of PFCs, which are known to cause a myriad of health effects. This is inaccurate, as their gear is made of high performance fibers such as Kevlar®, Nomex®, polybenzimidazole (PBI), or polybenzoxazole (PBO), which are able to withstand the high heats of the environments in which firefighters work[333,334].That being said, this does not mean that PFCs are not present on their gear.  The moisture barrier, which is middle layer of turnout composite, is typically made of polytetrafluoroethylene (PTFE) coated to a polyurethane-knit laminate[333,334].  The PTFE part of the moisture barrier is a polymeric-based PFC, with no known associated health effects.  However, small molecule PFCs, such as perfluorooctanoic acid (PFOA), have historically been used as a processing agent to polymerize PTFE[83], which would mean that there could be some available PFCs, with known health effects, on the gear.

Furthermore, National Fire Protection Association (NFPA) 1971: *Standard on Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting* requires firefighter turnout gear to pass a whole garment and ensemble liquid penetration test (Section 8.48)[25].  Based on NFPA 1971, liquid penetration should rely on the efficiency of the moisture barrier.  However, some fabric manufacturers have historically placed a durable water repellent (DWR) finish on the surface of the outer shell to increase the likelihood the garments pass the test outlined in Section 8.48. These DWR finishes are another polymeric PFC, but dissimilar to PTFE.  While PTFE is a polymer chain, in and of itself, the DWR finish is a fluorine vinyl group attached to the backbone of a polymer chain (see Figure 6.1). Nevertheless, the carbon-fluorine side chains could still pose an occupational exposure to firefighters.  Unfortunately, no current studies were found in the literature that evaluated the durability of these finishes on firefighter outer shell materials.



(a) (b)

*Figure 6.1: Repeat unit chemical structure for (a) PTFE and (b) a generic fluorine DWR finish.*

In order to investigate the durability of textile finishes, studies need to focus on the stressors those garments will undergo during wear: rubbing (also know as croc-fastness), color fastness, laundering, UV exposure, etc. For the purposes of this study the stressors of laundering and UV exposure will be examined.

Some studies have investigated the durability of the turnout coat materials to ultra-violet (UV) light exposure[15,335]. When each layer was exposed to UV light, there was a uniform drop in the physical strength of the materials, hinting at a change in either the crystalline structure of the fiber or degradation of the chemical structure of the materials[335,336]. Of note was the decline in physical strength of the PTFE moisture barriers, which could be considered a polymeric PFC. This is in direct contrast with UV studies conducted on non-polymeric PFCs, such as PFOA, which show no degradation due to UV exposure[16,83,337]. With no other studies conducted on DWR finishes, it is unknown whether DWR finishes may or may not degrade due to UV exposure.

On the other hand, there has been studies conducted on the laundering durability of DWR finishes, although it has not been with turnout gear material. These studies on DWR finishes have indicated a drop in water repellency across the board for an array of textiles: nylons, cotton, polyester, and cotton/polyester blends The papers utilized either AATCC Test Method 61: Colorfastness to Accelerated Laundering or a home laundering system to evaluate DWR finishes. Across the board, there was a 10-30% drop in water repellency. While these studies looked at PFCs (both polymeric and non-polymeric) and utilized differing laundering procedures, it can be hypothesized that laundering outer shells will reduce their water repellent properties[289,291,293,294,338,339]..

This manuscript contains two components: a durability assessment of DWR finishes to UV exposure and a durability assessment of DWR finishes to laundering. In the following studies, outer shell materials from unused gear between 2003-2017 and two control fabrics were exposed to four intervals of UV-exposure and five intervals of laundering cycles. These components are stand-alone studies. Ultimately, the studies presented in this paper are to be a pilot study, to set a framework for further investigation on PFCs present on firefighter gear.

## 6.3. Materials and Methods

### 6.3.1. Outershell Materials

For the purposes of the following durability experiments, only outer shell materials were considered. Only outer shell materials were utilized due to known DWR finish placed on their surface and for space constraints within the washer-extractor and weatherometer. These materials came from ten separate unused turnout coats, with manufacturing dates ranging from 2003-2017. The coats were collected from previous experiments and it is assumed the gear has not been laundered, used in the field, or been significantly exposed to sunlight. However, for some of the older coats, this cannot be definitively determined. Conversely, control materials from purchased rolls of outer shell fabrics were utilized. The trade name and common name of these materials can be found in Table 6.1, along with other details.

*Table 6.1: Outer shell Materials for Durability Studies*

| Year | Manufacturer | Trade Name | Common Name | Fabric Weight ($oz/yd^2$) |
|---|---|---|---|---|
| 2003 | A | Nomex® | Nomex® | 7.5 |
| 2004 | A | PBI/Kevlar Gold | 60% Kevlar®/ 40% PBI™ | 7.5 |
| 2006 | ** | ** | ** | ** |
| 2007 | A | Millenia XT, Gold | 60% Kevlar®/ 40% PBO | ** |
| 2008 | B | PBI Matrix | 60% Kevlar®/ 40% PBI™ | 7.3 |
| 2010 | C | ** | ** | ** |
| 2014 | B | PBI Matrix | 60% Kevlar®/ 40% PBI™ | 7.3 |
| 2015 | B | PBI Matrix Model: CSTM | 60% Kevlar®/ 40% PBI™ | 7.3 |
| 2016 | B | PBI Matrix Model: CSTM | 60% Kevlar®/ 40% PBI™ | 7.3 |
| 2017 | B | PBI Matrix | 60% Kevlar®/ 40% PBI™ | 7.3 |
| PBI Max | D | PBI Max | 70% “PBI Dominant” PBI/Kevlar® spun yarns, 30% Kevlar® Filament | 7.0 |
| Griege | D | Pioneer | Kevlar®/Nomex® | ** |

**Data not able to be found

### 6.3.2. Overall Sample Preparation

For the ten turnout coats, material types and brand names were noted, then outer shells were separated from the thermal liners and moisture barriers. As the two studies used different types of degradation, each outer shell jacket was cut in half. One half was utilized for laundering durability, while the other half was used for UV durability.

### 6.3.3. ISO 6530

For the following durability experiments, ISO 6530: *Test Method for Resistance of Materials to Penetration by Liquids* was utilized to measure the change of water repellency. This test method was utilized to give a measure to the water repellency that could more easily be compared to the different amounts of laundering cycles or UV exposure times. There are other metrics to measure repellency, but those metrics either utilize different solvents or are purely qualitative. As the purpose of the DWR finish is to add an additional layer of protection to the firefighter from steam burns, water was the desired solvent to study.

The ISO 6530 standard includes a test method which allows for a measurement of the amount of penetration, absorption, and repellency of a material against liquid chemicals. Penetration is defined as the process by which the test liquid moves through the macroscale structure of the test material (i.e., pores, apertures, essential openings, etc.). Absorption is the test liquid diffusing through the surface and defined in terms of permeation, which is the process by which the test liquid moves through the microscale of the test material (i.e. how it interacts with the molecular structure of the test material). Repellency is defined as the ability of the test material to resist the test liquid applied[340].

Ultimately, the standard is a mass balance of the before and after weights of the fabric, beaker, and filter/wax paper combination. Based on the recorded weights, one can achieve a percent of repellency ($I_R$, Equation 6.1), absorption ($I_P$, Equation 6.2), and penetration ($I_A$, Equation 6.3)[340].

$$I_R = \left(\frac{M_r}{M_t}\right) * 100 \tag{6.1}$$

$$I_P = \left(\frac{M_p}{M_t}\right) * 100 \tag{6.2}$$

$$I_A = \left(\frac{M_a}{M_t}\right) * 100 \tag{6.3}$$

Where, $M_t$ is the mass of the test liquid discharged on the test material (i.e. 10 grams, 1-mL/second*10 seconds), $M_r$ is the mass of the test liquid in the beaker, $M_a$ is the mass of the test liquid absorbed by the material, and $M_p$ is the mass of the test liquid absorbed by the filter/wax paper combination. To get these values, the standard requires specimens sizes to be 14.2” (360 mm) x 9.3” (235mm) and filter/wax paper combination sized to be 11.8” (300mm) x 9.3” (235 mm), which allows for 30 mm of the fabric to be rolled under to allow for an adequate channel for the test liquid to fall into the beaker. The standard also requires the needle, where the test liquid is ejected from, to be 10 cm above the test material. Apparatus requirements can be seen in Figure 6.2[340].

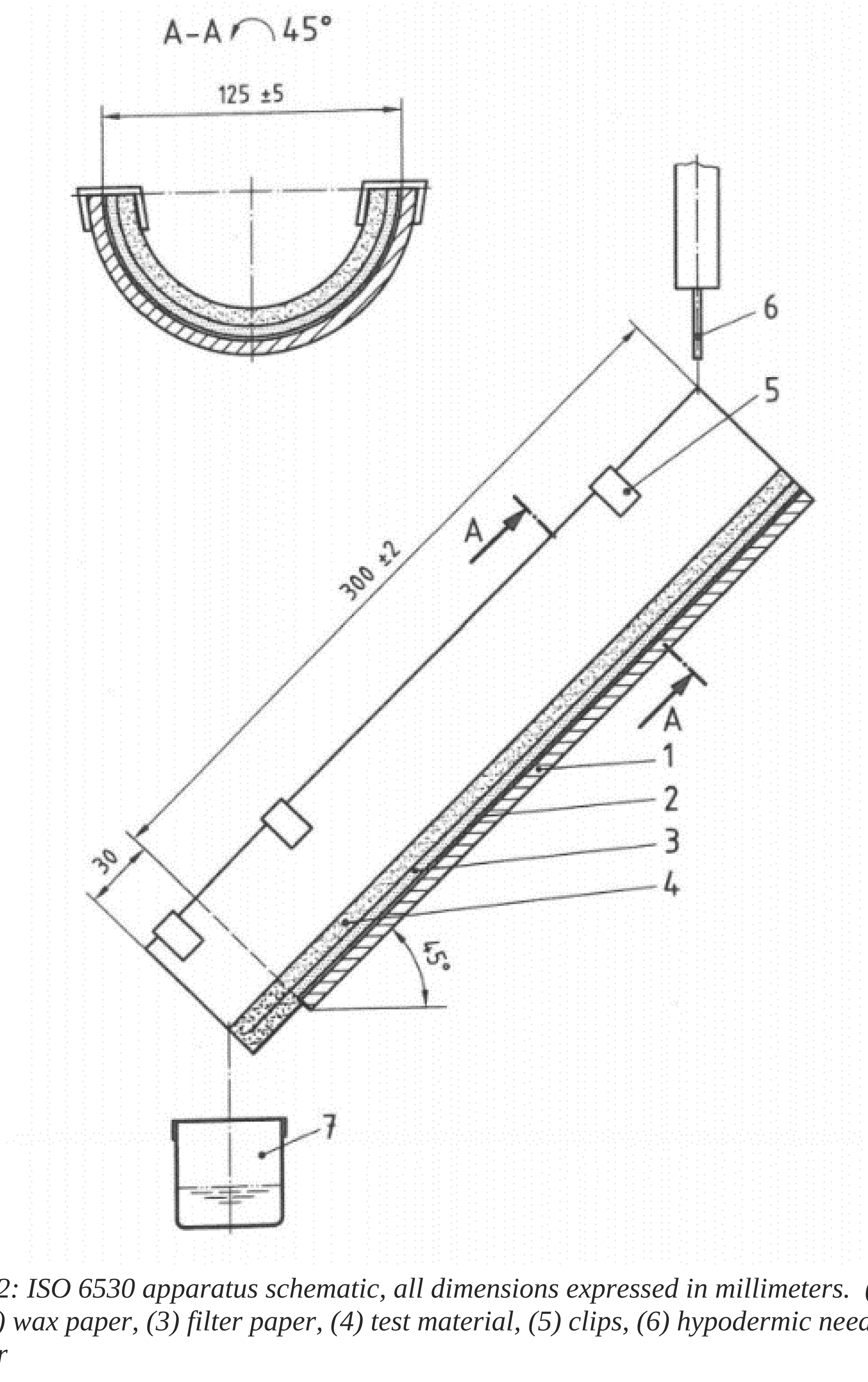


*Figure 6.2: ISO 6530 apparatus schematic, all dimensions expressed in millimeters. (1) gutter, (2) wax paper, (3) filter paper, (4) test material, (5) clips, (6) hypodermic needle, and (7) beaker*

The apparatus was calibrated to dispense 1-mL of water per second over 10 seconds at a height of 10 cm above the point of contact of the fabric specimen. The amount of water collected in a beaker at the bottom of the apparatus was weighed. The fabric specimen and the wax and filter paper were weighed separately and used in the calculation of percent repellency, absorption, and penetration.

There are other requirements based on the standard, but these are the main components to testing specimens. Furthermore, these are the parameters that had to be modified for the following durability experiments. Due to constraints of available material or apparatus mount size for degradation, ISO 6530 test material size had to be decreased. The specific modification for each study will be discussed in their respective sections.

### 6.3.4. UV Durability Study

#### *6..3.4.1. Sample Preparation*

Fabric specimens (5.625” (142.875 mm) x 2.5” (63.5 mm)) were cut from each half of the turnout coats. This specimen size was determined by the size of the rack panel specimen holders for the weatherometer used for the UV exposures. Eight specimens were taken out of each half of the turnout coat to have duplicates of each material at each time point. The same number of specimens for the two roll materials, were cut as well.

#### *6..3.4.2. Experimental Setup*

Four different times of UV Exposure were selected for durability purposes: 0, 30, 60, and 120 hours. In total, there were 96 specimens (24 for each time point) and the weatherometer can hold 60 specimens at a time. Meaning exposure times had to be staggered. The 120- and 30-hour specimens were put into the weatherometer, first, and exposed for the limiting time, i.e. 30 hours. At the end of this exposure time, the 30-hour specimens were removed, and the 60-hour specimens were put in the weatherometer. Both the 120- and 60-hours specimens were exposed for a further 60 hours. At the end of this exposure time, the 60-hour specimens were removed and the weatherometer was set to run the last 30 hours to get the 120-hour specimens to their full exposure.

#### *6..3.4.3. Ci 4000 Weatherometer Conditions*

UV exposure was completed in an Atlas Ci 4000 Weatherometer[341], using Arizona conditions based on the high irradiant condition and low humidity. The parameters for Arizona conditions are listed in Table 6.2, and are based on annual UV radiation, average summer temperatures, and average relative humidity.

*Table 6.2: Conditions set for Ci 4000 Weatherometer*

| | |
|---|---|
| Irradiance (W/m$^2$) | 0.55 |
| Rack Panel Temp (°C) | 63 |
| Chamber Temperature (°C) | 43 |
| Relative Humidity (%) | 30 |

#### *6..3.4.4. ISO 6530 Modifications*

Due to limitation of the specimen holder for the rack panel of the Ci 4000, the specimen size was altered to 5.625" (142.875 mm) x 2.5" (63.5 mm) rectangles. Wax paper and filter paper were also cut to 3.625" (92.075 mm) x 2.5" (63.5 mm) for testing, allowing for two-inches of the fabric specimen to be folded over at the bottom of the apparatus to allow an adequate channel for water to flow into the beaker. Furthermore, the pump apparatus was lowered so that the needle was 3 cm above the testing material. Each specimen was run in triplicate and air dried completely between replicates. A picture of the modification can be seen in Figure 6.3.

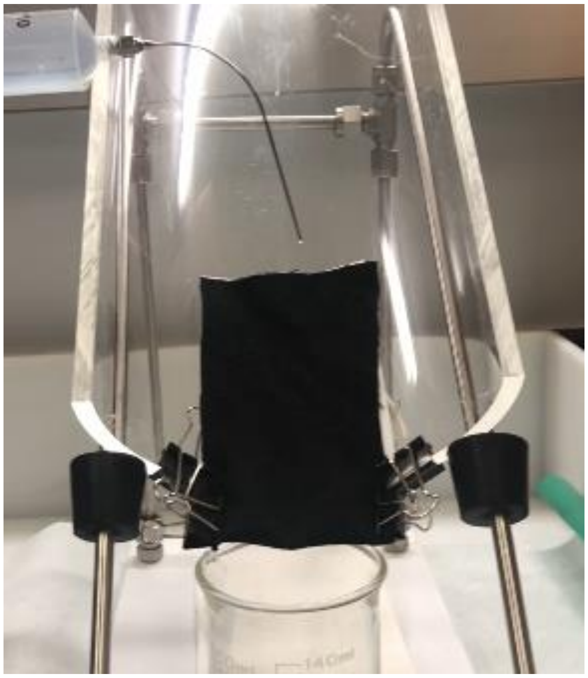


*Figure 6.3: ISO 6530 apparatus and specimen modification for testing of UV specimens.*

### *6..3.4.5. Contact Angle Method*

The specimens were analyzed on an FDS Corporation Dataphysics OCA 15 goniometer unit and SCA20_U software. Specimens were mounted to the stage via double-sided tape. A dosing syringe with a diameter of 0.52 mm was filled with deionized water, and any air bubbles were removed after the needle was attached. The syringe was secured to the instrument and adjusted to a height visible on the camera. The syringe dispense amount was set to 5-μL and the dosing rate was set to 0.5-μL/s. A drop was dispensed from the needle, and then the stage was brought up until the water drop was transferred to the surface of the specimen. A snapshot of the drop was taken, and the contact angle was measured using the software. Measurements were taken in triplicate and the process was then repeated for the remaining specimens.

### 6.3.5. Laundering Durability Study

#### *6..3.5.1. Sample Preparation*

To increase the available sampling area, pockets and reflective tapes were removed via seam ripping from the second half of the turnout jackets, as seen in Figure 6.4. Serging was completed, as necessary, to protect edges from fraying during laundering. For any hook and loop stripping that could not be removed, like the remaining long strip near the zipper in Figure 6.4b, the opposing hook and loop side was cut from a roll and placed over the strip, to keep it from fraying other materials during laundering. For the two roll materials in the study, PBI Max and a griege, a 1-yd x 1-yd square was cut and edges were serged. The control specimens (0 wash) were cut before laundering, and edges were serged.

(a) (b)

*Figure 6.4: (a) pre-seam ripping, (b) post-seam ripping*

### *6..3.5.2. Experimental Setup*

This study considered five different amounts of washing cycles for durability purposes: 0, 1, 25, 50, and 100. After each incremental washing, 10” (254 mm) x 4” (101.6 mm) specimens were cut, edges were serged where needed in order to prevent fraying during subsequent wash cycles, and specimens were left to dry before storing. On the whole, jackets were not dried between wash cycles, all cycles were consecutive. If needed, jackets were placed out to air dry during extended times where washer-extractor was dormant.

### *6..3.5.3. UNIMAC Washer-Extractor Conditions*

A UNIMAC 45-lb capacitance Washer-Extractor was utilized to wash all outer shell materials. Jackets and control materials were laundered according to NFPA 1851 standards. Table 6.3 shows the parameters set in NFPA 1851 and the conditions for this part of the study.

*Table 6.3: NFPA 1851 and Study Conditions*

| | NFPA 1851 | Current Study |
|---|---|---|
| Water Temperature (°C) | ≤ 40 | 40 |
| Detergent pH (Brand Name) | 6 – 10.5 | 6.5 (Citrosqueeze®) |
| G-Forces | ≤ 100 | 85 |

#### *6..3.5.4. ISO 6530 Modifications*

ISO 6530 was used to test the effect of standard washing on the specimens. Due to limited usable material on the jackets, the specimen size was altered to 10” (254 mm) x 4” (101.6 mm) rectangles. Wax paper and filter paper were also cut to 8” (203.2 mm) x 4” (101.6 mm) specimens for testing, allowing for two inches of the fabric specimen to be folded over at the bottom of the apparatus to allow an adequate channel for water to flow into the beaker. The pump apparatus height was not modified for testing of these specimens. The needle was positioned 10 cm above the testing material. Each specimen was run in triplicate and laid to air dry completely between replicates. A picture of the modification can be seen in Figure 6.5.

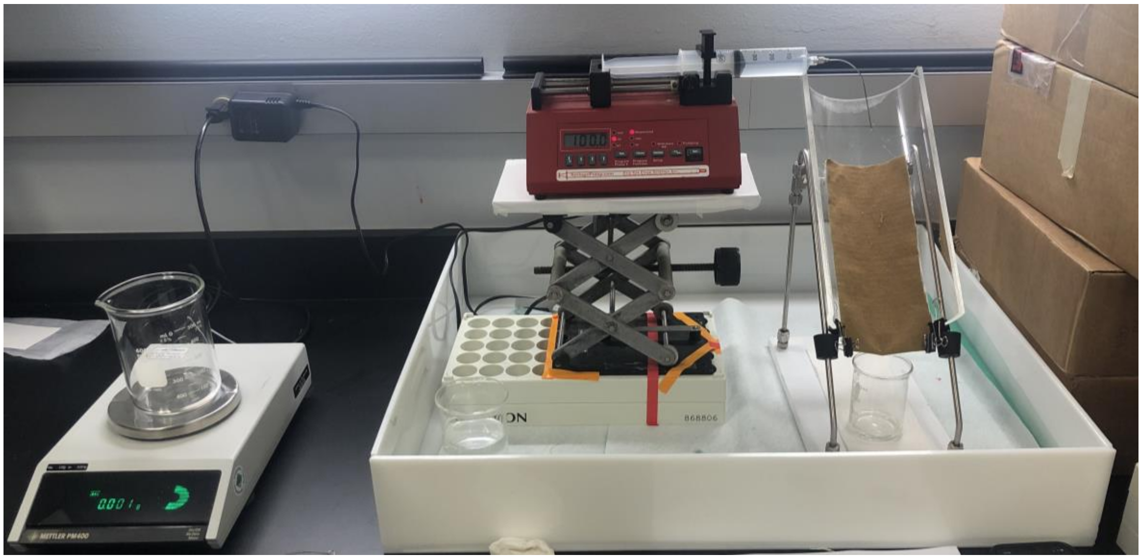


*Figure 6.5: ISO 6530 apparatus and specimen modification for testing of laundering specimens.*

## 6.4. Results and Discussion

### 6.4.1. UV Durability Study

It is widely known that sunlight exposure causes color change and fading of materials, so, as expected, the longer UV exposure times had a larger effect on color degradation of the turnout materials, as seen in Figure 6.6. The figure only shows a representative outer shell material from the 2010 turnout coat, but the trend was seen across all materials tested. It may be difficult to tell in these pictures, but close observation of the 120-hour exposure picture shows a faint discoloration in the center of the fabric specimen. This discoloration is in contrast to the pre-exposure picture and the dark outline of the specimen for the post-exposure picture. This dark outline is due to the Ci 4000 Weatherometer specimen holder covering that portion of the specimen. The 30- and 60-hour exposures indicate discoloration, as well, although their differences are more subtle in pictures than the 120-hour exposure specimen.

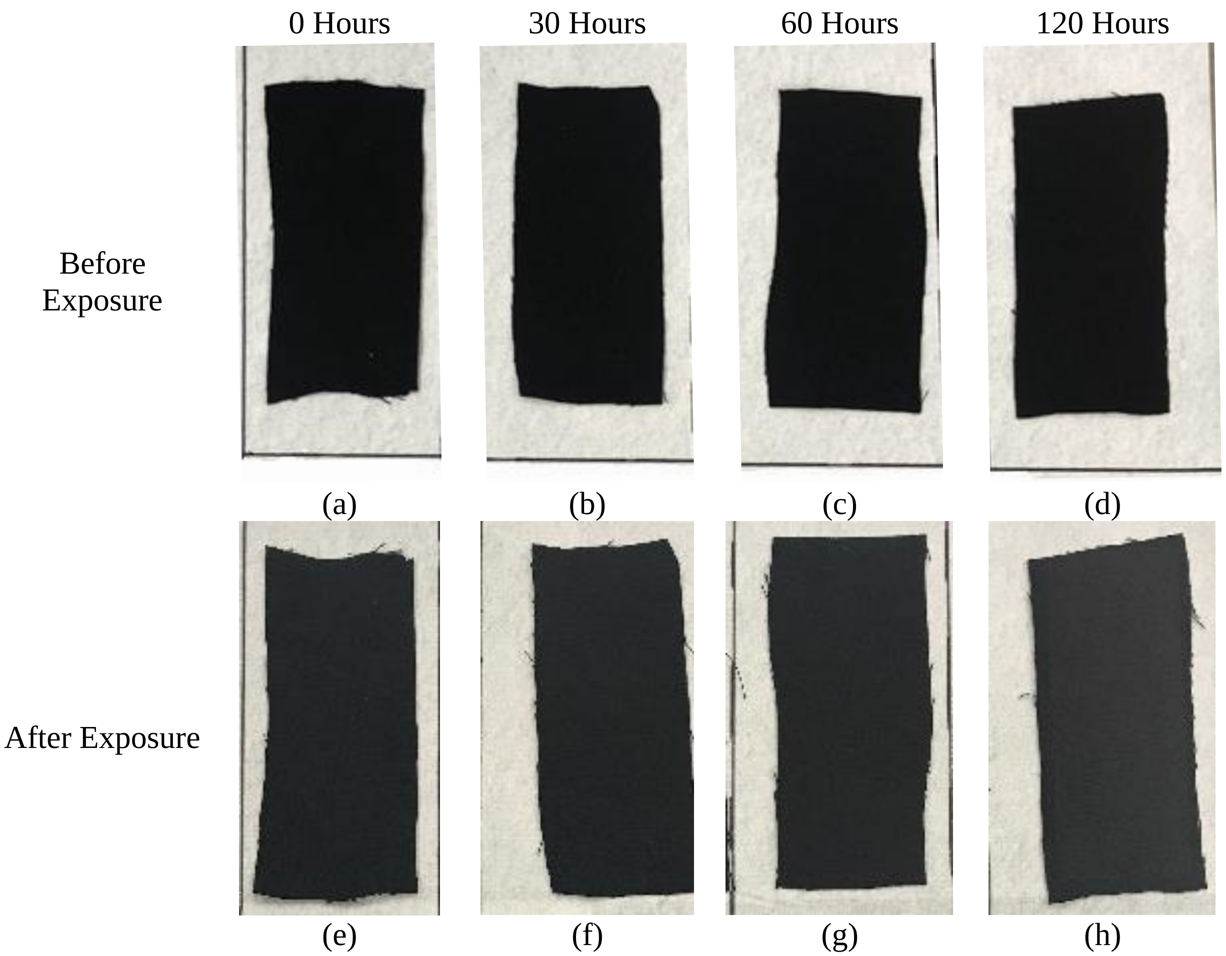


*Figure 6.6: Representative picture of degradation due to UV Exposure, before (a-d) and after (e-h) exposure, for the 2010 outershell for 0, 30 60, and 120 hours of exposure.*

As seen in Figure 6.7, each of the turnout materials had over 90% repellency at baseline, with the exception of the griege material. This was to be expected as the greige material had no DWR finish applied to the surface. However, unexpectedly, there was no difference in the water repellency across the different exposure times, as indicated by the trends shown in Figure 6.7. Which seems to be in direct contrast on other studies done on polymeric fluorocarbons, such as PTFE[16,83,337]. However, those studies were looking at the physical strength of the polymeric fluorocarbons, while this date only indicates that UV exposure does not affect the efficacy of the water repellent finishes present on the fabrics. The data shown here lacks the ability to indicate whether the UV exposure is affecting the chemical structure of these finishes or physical strength of these finishes. While these possible chemical changes don't affect the water repellency, they may affect the crock/rub fastness (i.e. their ability for color to withstand rubbing against other surfaces) or thermal durability.

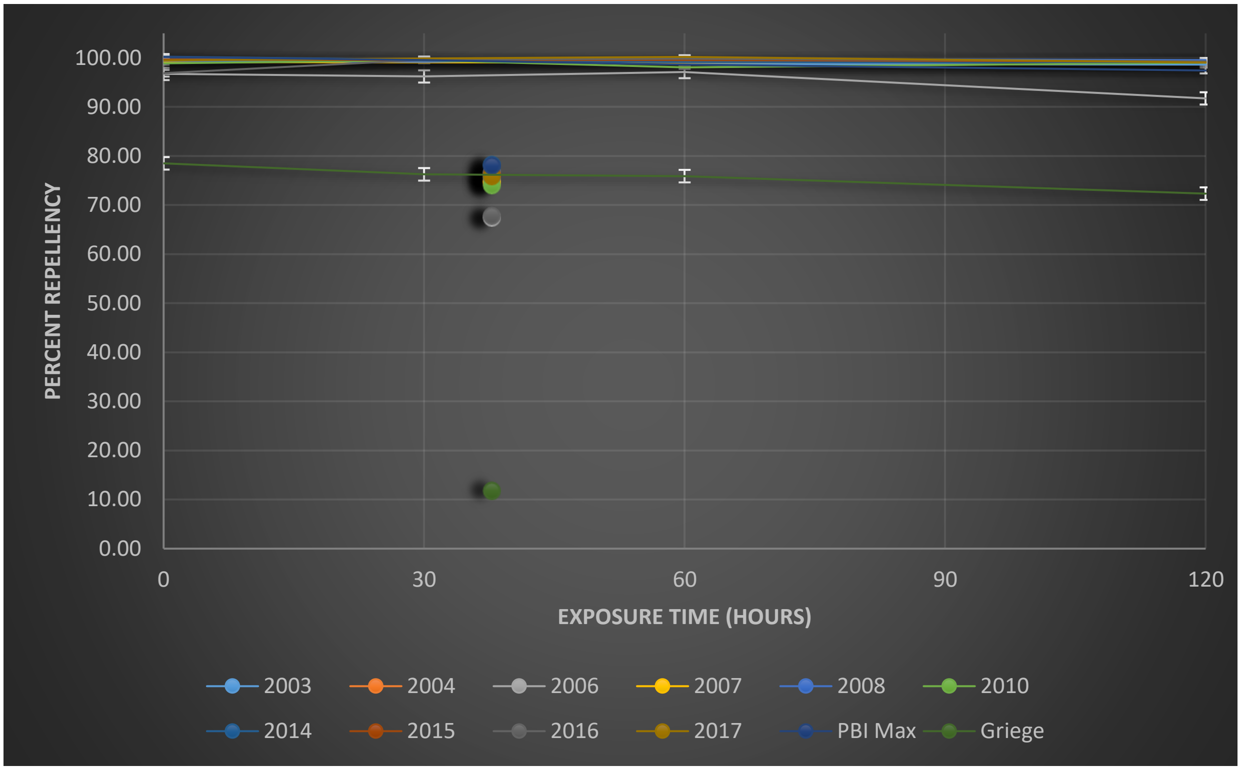


*Figure 6.7: ISO 6530 percent repellency trends for all study specimens for each time point of exposure*

What is interesting, is the UV exposure does not seem to have a huge effect on the water retention properties of the outershell materials, as indicated by the flat curve for the griege material. If other UV studies are to be believed, UV exposure effects the material strength of outershell materials, which means the light is probably changing the crystalline structure of the fibers in the material[14,335,342]. However, on a short time scale (such as the ISO 6530 testing), this crystalline degradation does not affect the water retention properties. On a longer time scale, where the fabric is allowed to reach equilibrium with its surrounding environment, the degradation to the crystalline structure may affect the moisture regain of a fabric, but the data cannot answer this question.

It is important to note that the 2006 turnout coat did have a slightly lower measure of water repellency, especially for the 120-hour exposure. It is unclear as to why this occurred. As this was a pilot study which relied on the use of unused gear happened to be accessible, it is difficult to know the whole exposure history of the gear used. The lower water repellency measurement could also have to do with a difference in the weight of the fabric, but this is not reflected with the other materials which had differences in weight.

Since ISO 6530 is a mass balance, it can only indicate no change or a decrease in repellency, it cannot indicate if there happens to be an increase in repellency. For this reason, 0- and 120-hour specimens were sent for testing on a goniometer for contact angle with water, to see if UV exposure happened to increase the water repellency of the gear. As revealed by the data in Table 6.4, overall, there was no tremendous increases or decreases in the contact angle between the two time intervals, across all specimens tested. The exception to this is the 2010 material, which had a 10 degree drop in contact angle. However, without a correlating drop in the ISO 6530 water repellency, it seems unlikely to have a huge effect on the material’s water repellency.

*Table 6.4: Goniometer data for contact angle with water droplets*

| Specimen | 0-hour (°) | 120-hour (°) | Difference (°) |
|---|---|---|---|
| 2003 | 148.36 | 141.52 | 6.84 |
| 2004 | 143.02 | 145.57 | -2.55 |
| 2006 | 141.46 | 138.65 | 2.81 |
| 2007 | 145.21 | 139.71 | 5.50 |
| 2008 | 141.37 | 140.09 | 1.28 |
| 2010 | 147.24 | 136.30 | 10.94 |
| 2014 | 141.57 | 145.02 | -3.45 |
| 2015 | 146.07 | 141.15 | 3.77 |
| 2016 | 144.97 | 141.15 | 3.82 |
| 2017 | 142.79 | 138.46 | 4.33 |
| PBI Max | 143.32 | 141.0 | 1.81 |

### 6.4.2. Laundering Durability Study

#### *6..4.2.1. Visual Effects of Laundering*

As expected, 100 wash cycles, following NFPA 1851 guidelines, turned out to be rough on the turnout coat outer shell materials, as seen in Figure 6.8. Figure 6.8a must be taken with a grain of salt, as much of the damage seen in this picture is due to, either, removing the 10” x 4” specimens at each sampling interval or from removing certain trimming. However, Figure 6.8b, zoomed in on the reflective trimming on the wrist cuff, truly shows the impact of 100 wash cycles following NFPA 1851 guidelines, in comparison to Figure 6.8c, the before wash picture. All of this damage is with less trimming and available hook and loop stripping than there would be for a typical wash cycle at a fire department, which may mean washing with regular gear could be harsher.

(a) (b) (c)

*Figure 6.8: (a) physical damage of 100 washes on turnout coat outershell material, (b) focus of damage on reflective trimming on wrist cuff, (c) reflective trimming before washing*

Furthermore, Figure 6.9 illustrates a visual discoloration of the turnout coat outer shell materials over the different wash cycles. This trend was seen for all materials tested in this part of the study. The discoloration could be from the severe wash conditions removing dye from the fibers. On the other hand, the discoloration might not be a chemical discoloration, but a physical discoloration. Due to the repeated agitation and spinning, the threads of the woven fabric could be fraying more and more during each wash cycle, causing light to interact with the surface of the material differently. Ultimately, this would make it look like the fabric changed color, but instead our eyes are perceiving a color that is different.  Unfortunately, due to extraneous circumstances, data was not able to be collected to confirm or deny these hypotheses.

Upon observation, there was a subjective change in the softness of each material. Additional laundering increased the flexibility of the material. When picked up, the 0-wash specimens would stay perfectly straight, while the 100-wash specimens would bend or drape. This could be due to either the loss of the polymeric DWR finish or from the loosening of the woven structure.

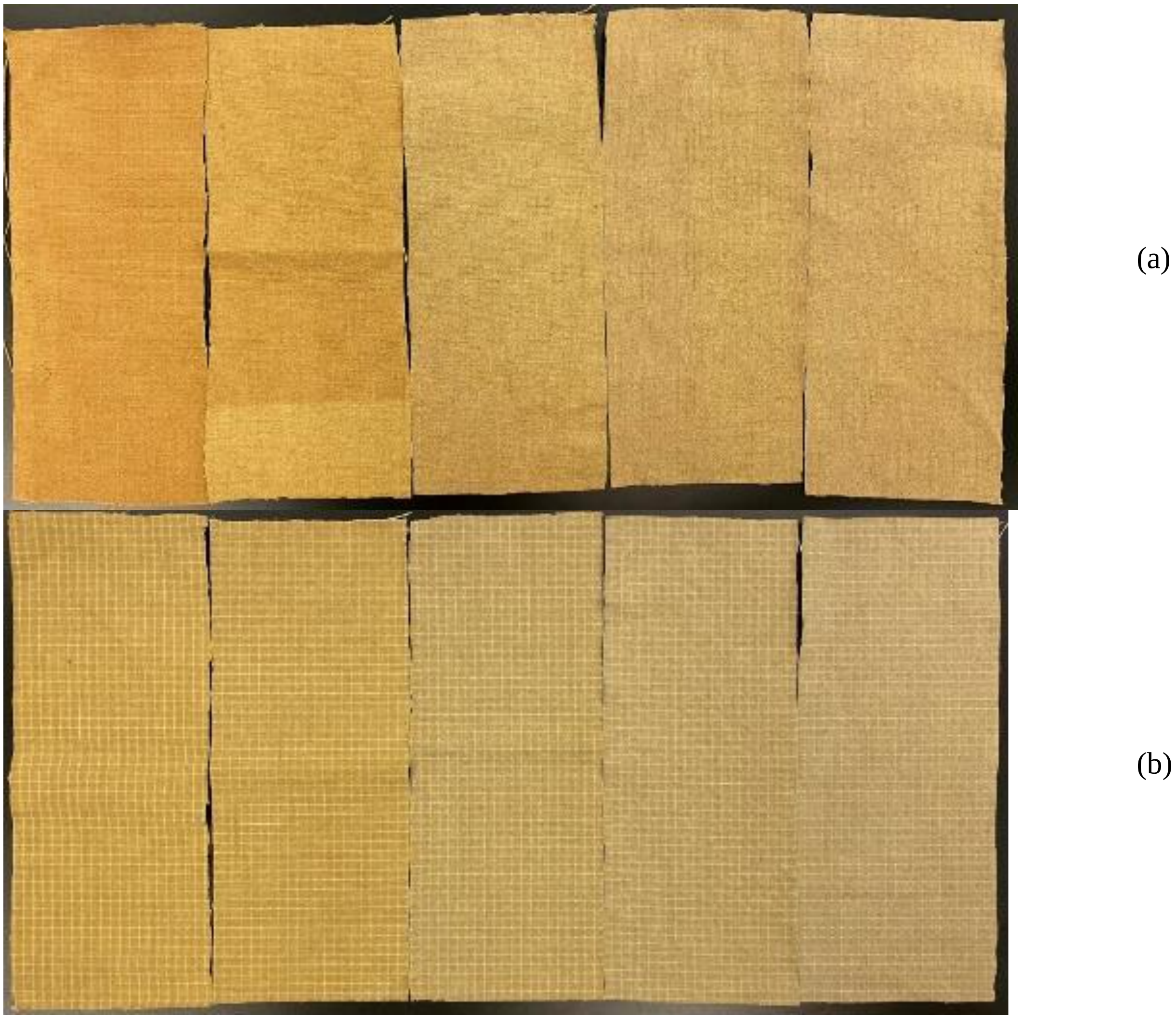


*Figure 6.9: Visual change of fabric from 0 wash (far left) to 100 washes (far right) for the (a) 2003 and (b) 2017 turnout materials.  From left to right in each picture the specimens are 0, 1, 25, 50, 100 wash cycles.*

### *6..4.2.2. Effects of Laundering on Water Repellency*

In relation to the water repellency of the gear after each increment of washing, there is a general trend, seen in Figure 6.10, which indicates a significant drop in repellency after 50 washes. Up to 50 washes, the water repellency of the outer shells seems to be consistent. The griege fabric is the exception to both of these statements, as its water repellency trends negative throughout the entire study. Since the griege material did not start out with a DWR finish, this data shows the incremental damage the NFPA 1851 guidelines are causing to the material. However, if requirements of Section 8.48.8.1 of NFPA 1971 are to be followed, then some specimens at the 50 wash cycles would fail the whole garment liquid penetration test. Section 8.48.8.1 states "Any evidence of liquid on the liquid-absorptive garment…shall constitute failure of the specimen"[25].

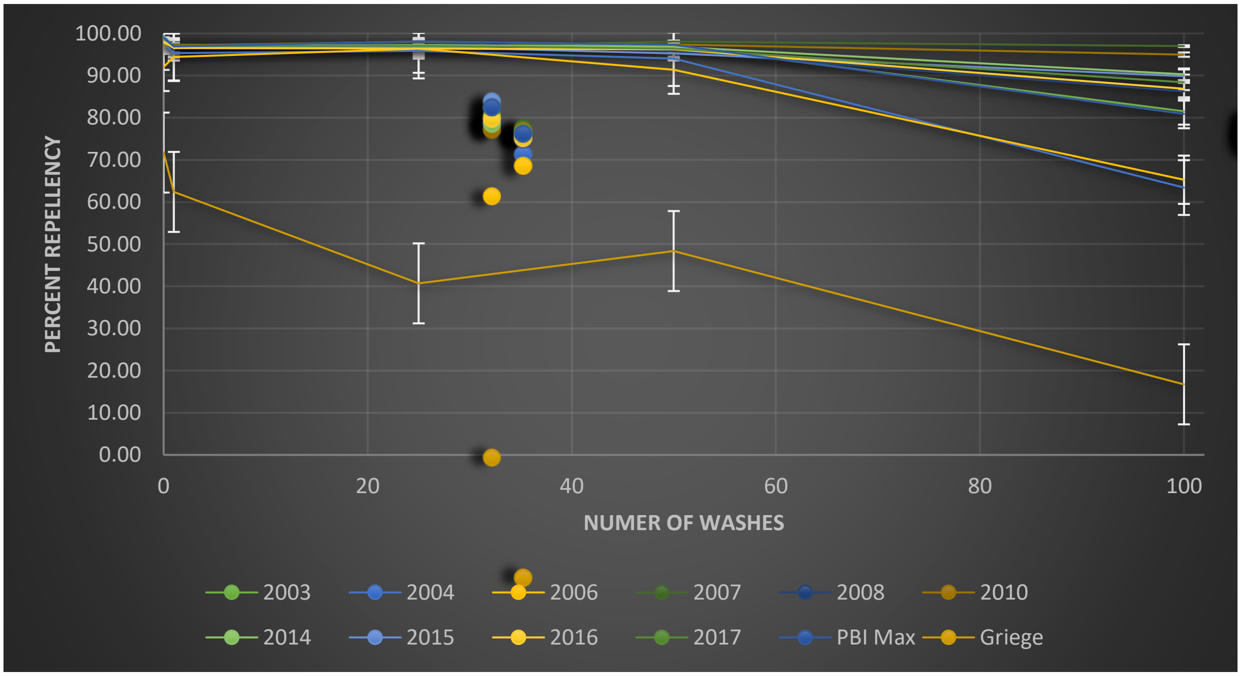


*Figure 6.10: Trend of wash cycles on ISO 6530 percent repellency for all turnout coat outershell materials tested in this study.*

While the biggest limitation to this study is the wide array of materials used, the trend seen in the griege fabric could be compared to the general trend seen with the other outershell materials.  It indicates that damage is occurring to the fabric, but the DWR finish applied to the surface is, generally, durable up to 50 washes before a significant decrease is seen in its efficacy. However, nothing more can be extrapolated since the kind of DWRs present on the outer shell materials are unknown and each manufacturer may have used different DWRs.

The significant difference between the repellency of 0-wash versus 100-wash can be seen in Figure 6.11.  Analysis of variance of the data, p-values in Table 6.5, indicates that every outer shell material has a significant difference between the 0 and 100 wash, with the exceptions of the 2007 and 2010 outershell materials.  This could be due to the type of fiber used in the material (which is unknown for 2010) or based on the type of DWR on the fabric.  Since these are both major limitations to this study, neither hypothesis can be definitively determined.

Similar trends have been seen for laundering studies on DWR finishes.  A majority of these studies used AATCC Method 61: Colorfastness to Accelerated Laundering, which is equivalent to five home launderings.  These studies looked at a wide array of PFCs from perfluorobutyric acid (PFBA), a non-DWR, to 1H, 1H, 2H, 2H-nonafluorohexyl-1-acrylate, a commercial DWR.  Despite the large difference in PFCs every study reported a change from their controls to the laundered specimens.  There were differences in the durability of the different finishes, which is to be expected, but the general trends are similar to those seen in this study[289,291,293,294,338,339].

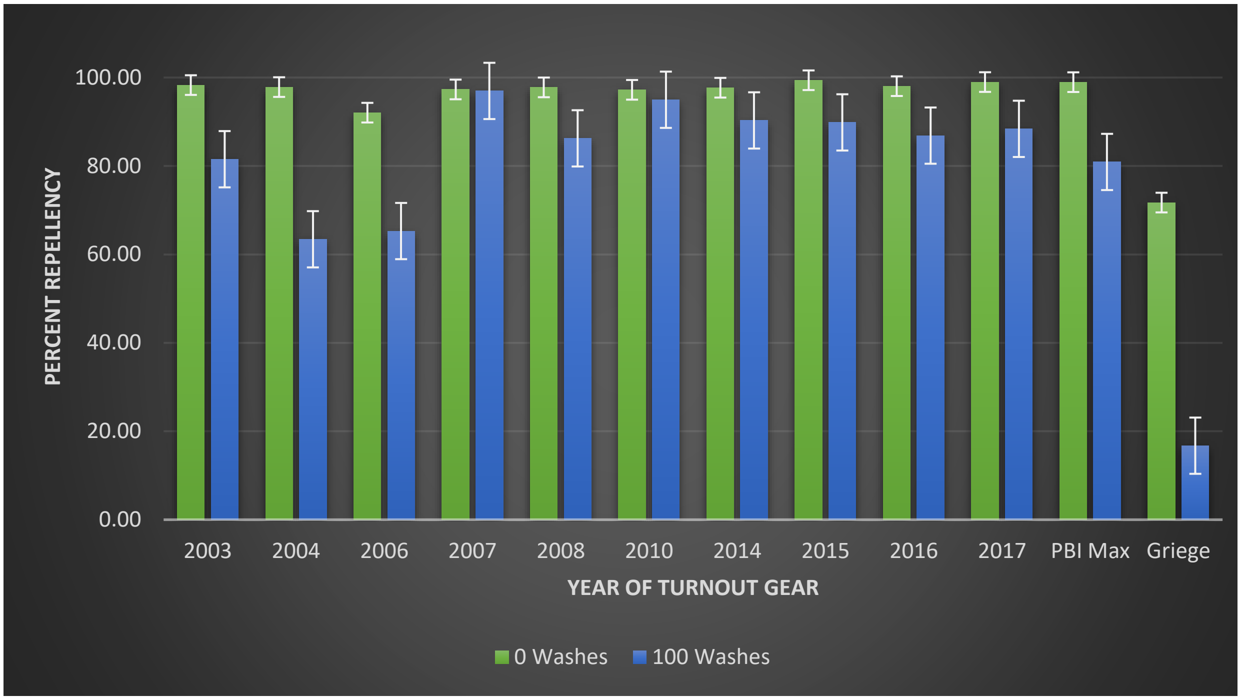


*Figure 6.11: ISO 6530 percent repellency for 0 wash vs 100 wash for all outershell materials tested.*

*Table 6.5: ANOVA p-values for water repellency of 0 wash vs. 100 wash*

| Year | p-Value* | Year | p-Value* |
|---|---|---|---|
| 2003 | << 0.01 | 2014 | << 0.01 |
| 2004 | < 0.01 | 2015 | 0.01 |
| 2006 | < 0.01 | 2016 | < 0.01 |
| 2007 | 0.77 | 2017 | 0.01 |
| 2008 | << 0.01 | PBI Max | << 0.01 |
| 2010 | 0.20 | Griege | 0.01 |

*p-value threshold set to 0.05

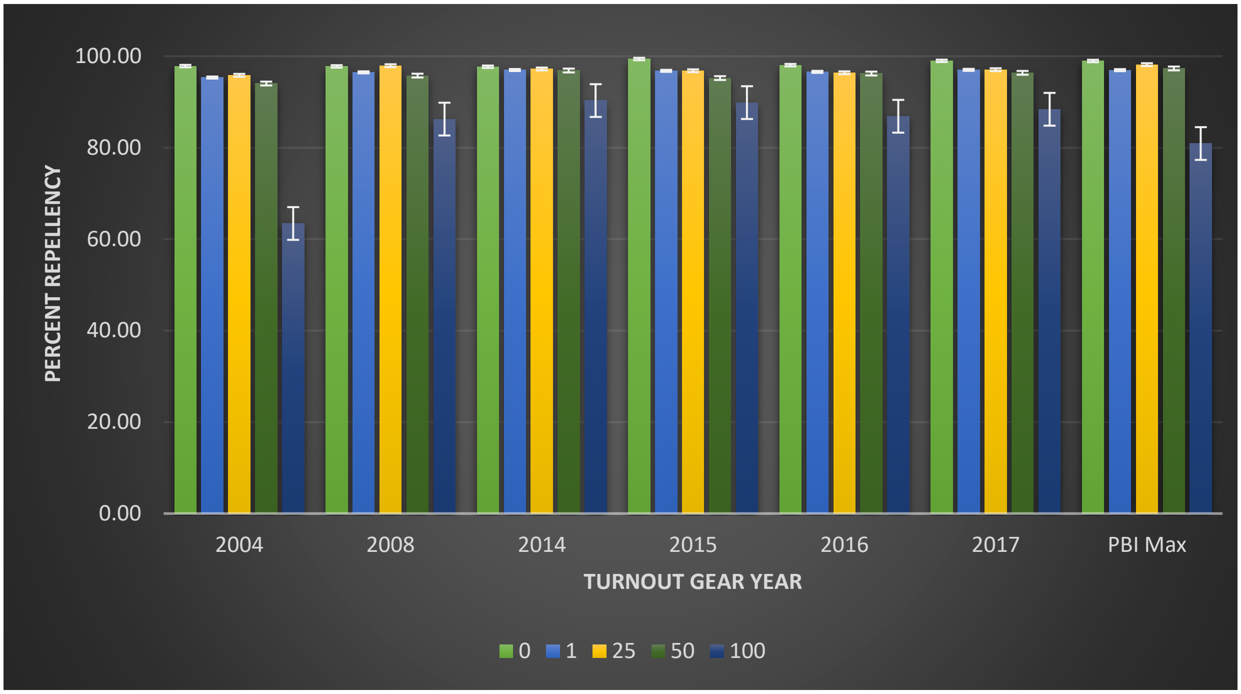


*Figure 6.12: Trend of wash cycles on ISO 6530 percent repellency for only Kevlar/PBI materials.*

While the materials behaved similarly for percent repellency, when the other two parameters of ISO 6530 are considered, differences between the materials can be elucidated, as seen for 100 wash data in Figure 6.13. As Table 6.6 illustrates, there is a significant difference when all data points are considered. However, on the whole, when the 2004 and PBI Max data are subtracted, there is no longer a significant difference, i.e. the 2008, 2014, 2015, 2016, and 2017 outer shell materials are statistically similar. This could be explained via three possible routes: differing DWR finishes, differing weight of fabrics, or a combination of both factors.

As the type of DWR is unknown, it is impossible to tell whether this is the factor which caused a higher absorption and penetration. It should also be considered that the 2004 turnout coat is 16 years-old, which may also be a reason why the DWR finish degraded in laundering so easily, regardless of what type DWR that was used. Although, it may also be due to developments in the type of DWR used on turnout coats.

In relation to the weight of the fabric, seen up in Table 6.1, this may have an effect, although the data does not correlate as would be expected for this factor. However, the 2004 (7.5 oz/yd$^2$) somehow ended up having both a larger amount of penetration and absorption compared to the PBI Max (7.0 oz/yd$^2$). There is an expectation for lighter fabrics to be penetrative and heavier fabrics to be absorptive. Yet again, this may also be due to the age of the 2004 turnout coat and DWR degradation compared to the newer PBI Max material.

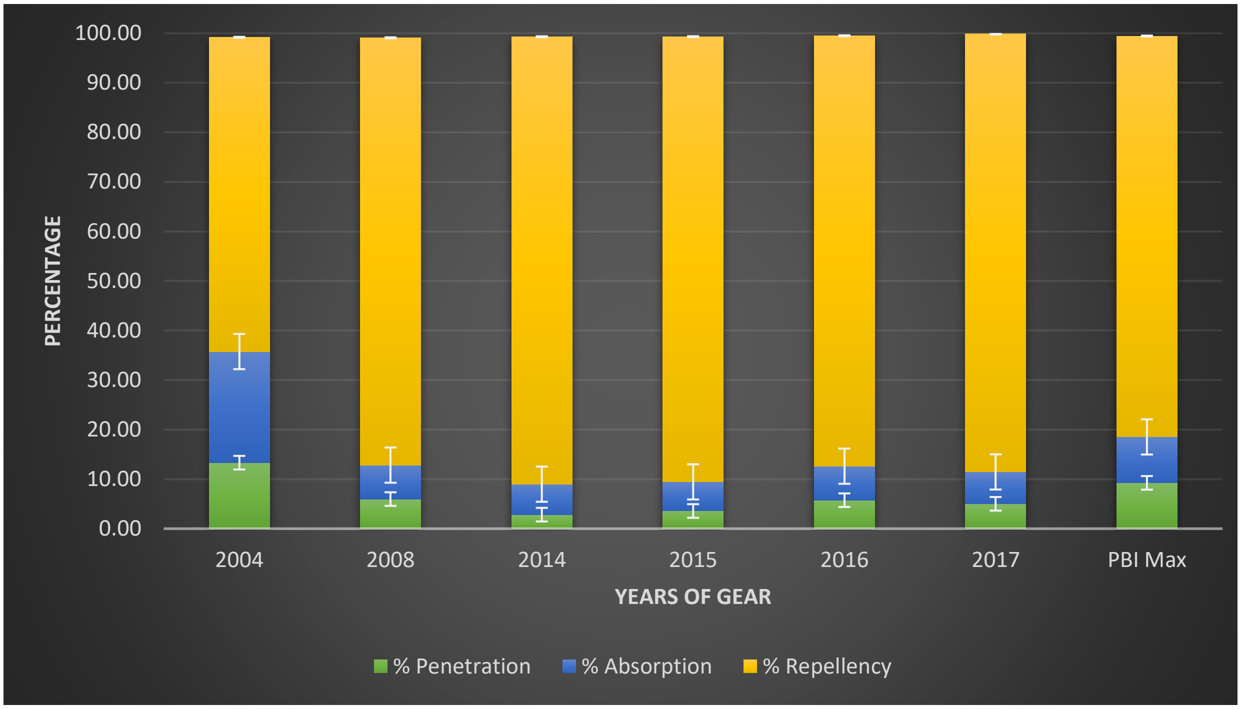


*Figure 6.13: ISO 6530 percent repellency, absorption, and penetration for only Kevlar/PBI materials at 100 washes.*

*Table 6.6: ANOVA p-values for all ISO 6530 parameters for Kevlar/PBI Materials*

| | All Data | Minus 2004 | Minus PBI Max | Minus '04 and Max |
|---|---|---|---|---|
| % Repellency | << 0.01 | 0.01 | << 0.01 | 0.33 |
| % Penetration | < 0.01 | 0.07 | < 0.01 | 0.46 |
| % Absorption | << 0.01 | < 0.01 | << 0.01 | 0.51 |

*p-value threshold set to 0.05

Coincidentally, all the Kevlar®/PBI material all came from the same manufacturer, Manufacturer C. Unfortunately, this indicates that this manufacturer kept their materials and

DWR chemistry and application consistent over these production years. Figure 6.14 and 6.15 show a comparison of different materials from Manufacturer A, indicating that materials from the same manufacturer do not perform in a similar manner. The performance of the material is an intrinsic property to the material, rather than an extrinsic property of where the material came from. An important note is that the Kevlar®/PBI data was averaged across all the Kevlar®/PBI materials. Additionally, the data for the Kevlar®/Nomex (i.e. the griege fabric) is greatly skewed, compared to the other materials, due to its lack of a DWR finish.

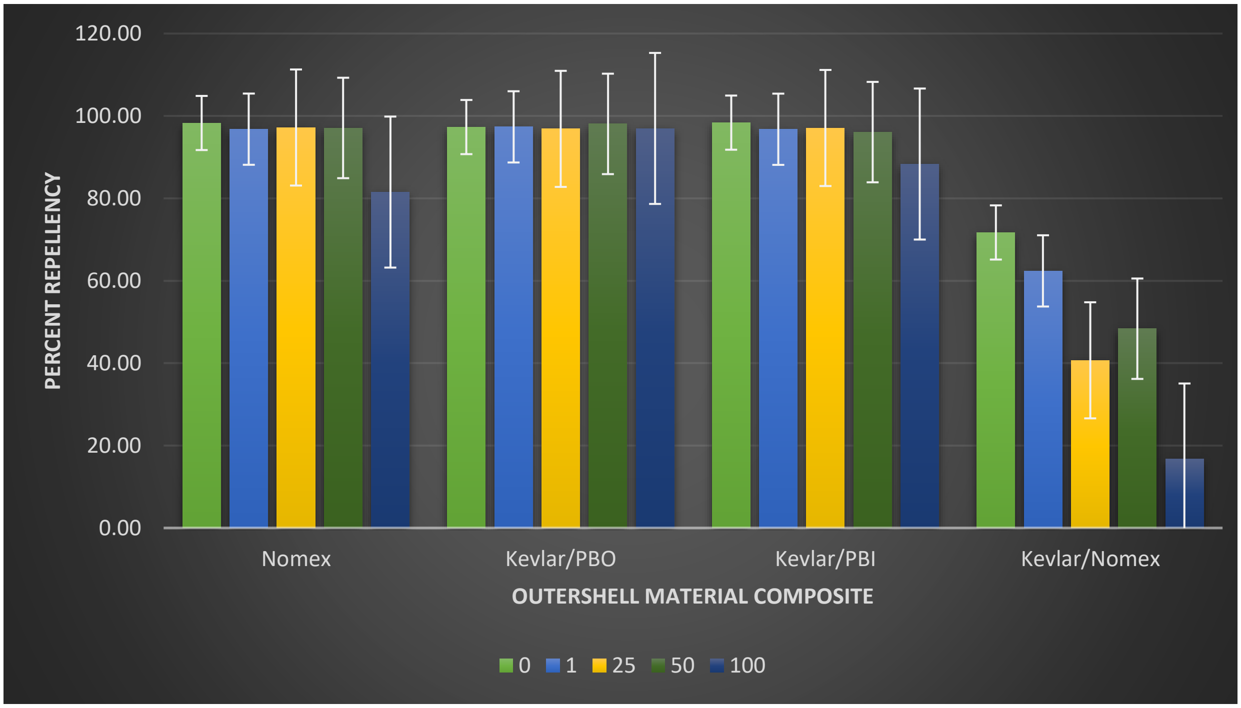


*Figure 6.14: Comparison of ISO 6530 percent repellency of the different outershell composites tested.*

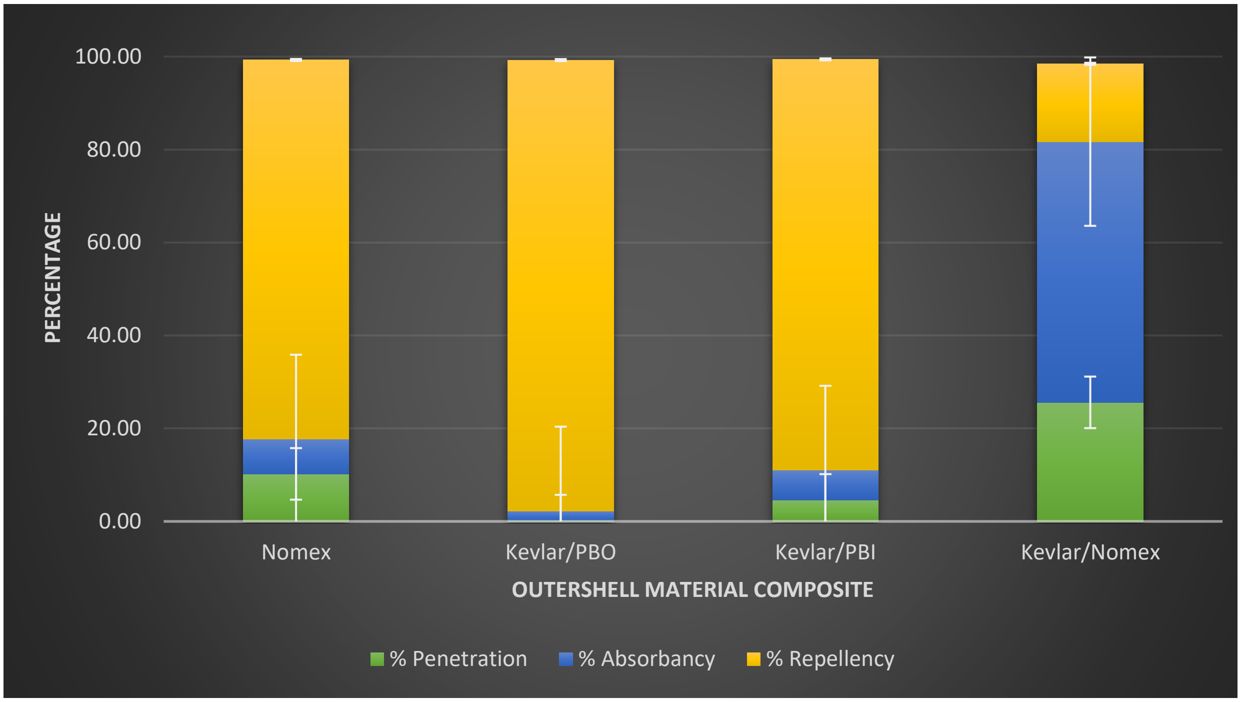


*Figure 6.15: ISO 6530 percent repellency, absorption, and penetration for the different outershell composite materials at 100 washes.*

Furthermore, the term “absorption” used for the ISO 6530 standard should be taken into consideration for this study. The term absorption refers to the interaction of two chemicals on a molecular level where the two species assimilate together. In contrast, there is adsorption where the liquid accumulates on the surface of the material and interacts on a macro-molecular scale. This is all to say that the term of percent absorption for ISO 6530 may not be completely correct for the use of water on the fabrics here. As seen in Figure 6.16, the structures of the different fibers indicate few functional groups which can make hydrogen bonds with water. Additionally, all of these fibers are high performance fibers, typically with high percentages of crystallinity[343,344].

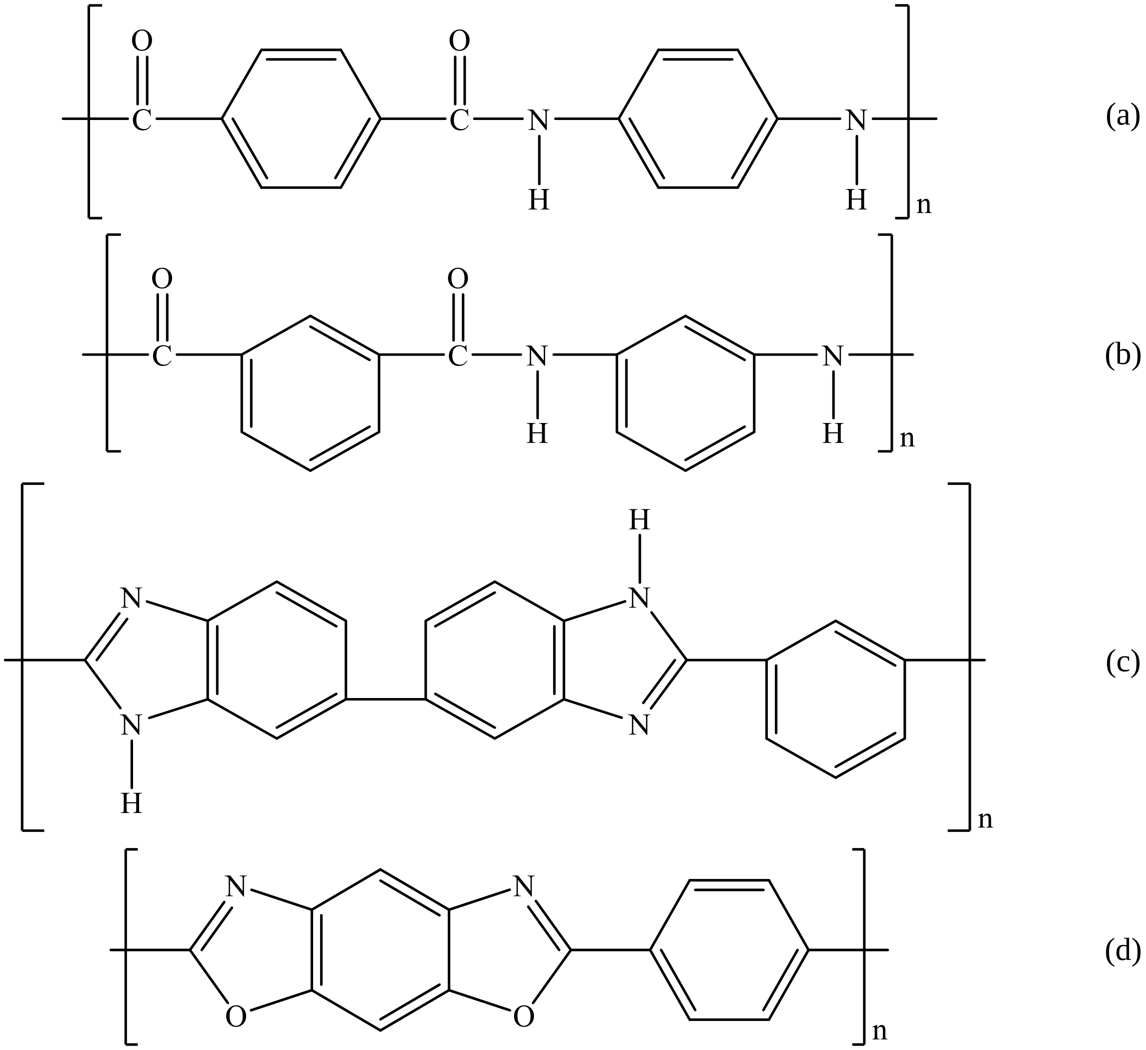


*Figure 6.16: Repeat unit chemical structures of (a) Kevlar, (b) Nomex, (c) PBI, and (d) PBO*

Ultimately, the increase in percent absorption in earlier figures, may actually be an increase in percent adsorption. As the fabrics were subjected to further wash cycles, more damage was done to the yarns in the material, possibly causing small fibers to come out of the yarns, which could increase the space between yarns, and, ultimately, loosening the woven structure. Interestingly, most PBI yarns are actually a mixture of PBI and Kevlar with staple Kevlar fibers, rather than filament Kevlar fibers[345]. Due to the use of staple fibers, the increase in space is probable, especially for the PBI/Kevlar materials.

Due to the increase in space within the material, water could fall into these crevices making it look like the material is actually absorbing more water. In reality, the water was most likely sitting on top of the thread or fiber surfaces, not interacting with the molecular structure of the fibers. Unfortunately, due to extenuating circumstances, microscopy could not be completed, making all of this speculation.

### 6.5. Conclusions

As the issue surrounding firefighter's occupational exposure to PFCs grows, ever increasing research will need to be completed. The studies presented in this paper were pilot scale studies, done to create a framework for further investigations. The UV exposure study indicated no change in water repellency after 120 hours of UV exposure, at Arizona conditions, in a weatherometer. Additionally, this amount of exposure had no increase in the water repellency, either, based on the contact angle data. However, future work may want to look at longer time scales, e.g. 240 hours or beyond, as other studies looking at outer shell durability were exposed for such extended periods of time.

The findings from the laundering study mimic those seen from other studies. Laundering has an impact on the water repellency of the fabric. For this study, significant decreases in water repellency did not occur until after 50 washes following NFPA 1851 guidelines. Again, due to the scale of this research, it is unclear whether the drop was from the removal of possible DWR finishes present on the turnout gear. Although, the data does illustrate that the performance of a material depends on the type of material rather than which manufacturer it comes from.

Overall, the ISO 6530 method was beneficial for the ability to give a number to the repellency, penetration, and absorption of the materials. However, it lacked the ability to assess the DWR finishes. Future work is necessary to complete a chemical analysis of each time/wash interval from the UV and laundering studies, respectively. Both a targeted and non-targeted approach will allow for proper quantitation of chemicals present and qualification of any degradation species produced.

# CHAPTER 7: Risk Assessment and Future Work

## 7.1. Risk Assessment

The United States Environmental Protection Agency (EPA) defines risk as the possibility of harmful effects towards human health or ecological systems, which also happen to be the two broad categories of risk assessment. Ultimately, risk assessment depends on three factors: the concentration of a present chemical, the amount of exposure to that chemical, and the chemicals toxicity. Human health and ecological risk assessment go about answering those question somewhat different. For the purposes of the data described in the above dissertation, only human health risk assessment will be discussed here[346].

The human health risk assessment can be broken down into four process, outlined in Figure 7.1. The first step is to plan and scope extent of the issue. This includes asking who, what and where the risk is, what is the environmental hazard, where do they come from, how does exposure occur, etc[346]. For the purposes of this dissertation these answers were determined by the community afflicted by the hazard, the firefighting community. They were concerned with their exposure to per- and polyfluorinated chemicals (PFCs), mainly from the gear they wear daily. Ultimately, this would mean that the most likely pathway for exposure was dermally. This compounds on the fact that there is a plethora of research indicating the occupation of firefighting as possibly carcinogenic[31].

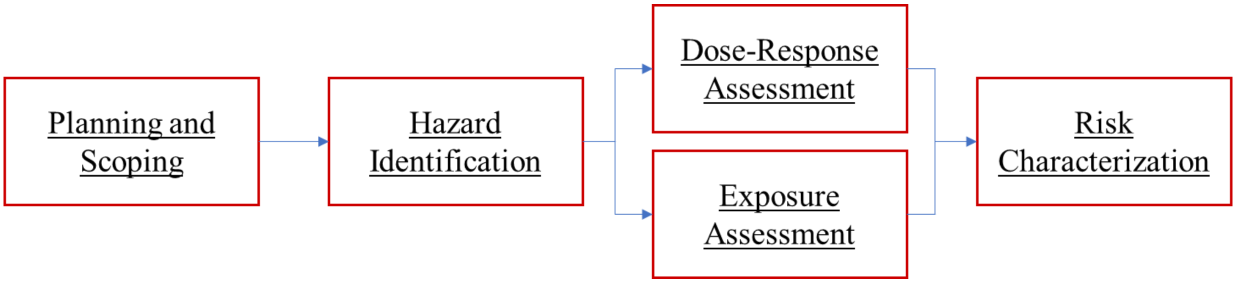


*Figure 7.1: The United States Environmental Protech Agency human health risk assessment outline.*

Once the plan is set, hazard identification can begin in earnest, which is the determination of whether the chemicals of interest do cause adverse health effects in humans. The literature review presented in Chapters 2 and 3 of this dissertation aimed to answer this question. The literature search indicated a plethora of research on animals and epidemiologic data on humans showing adverse health effects. Most notably were the six probable links the C8 Science Panel determined from studying the Mid-Ohio Valley regions drinking water exposure: testicular and kidney cancer, pregnancy induced hypertension/preeclampsia, thyroid disease, high cholesterol, and ulcerative colitis[1-5]. Although, these may not be the only health effects due to PFC exposure.

Once adverse health effects are known for a specific hazard, the next two steps can happen in parallel: dose-response assessment and exposure assessment. The dose-response assessment takes into account the exposure route to determine a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL). For the purposes of this dissertation, the dose-response assessment was also answered by the literature review. None of the literature that this dissertation found discovered an NOAEL for PFCs, in which case the LOAELs had to be utilized. The LOAELs were in the concentration range of part-per-billions, which may indicate the high toxicity of these chemicals.

Simultaneously, the exposure assessment can be completed, which measures/estimates the magnitude, frequency, and duration of human exposure. This assessment entails multiple factors: different exposure pathways, range of exposure, and quantifying exposure. The exposure pathway can be determined from dermal exposure data, which found very little amount of PFC can penetrate skin[260,262]. As the firefighting community typically work on 24 hour shift, they would have a large range of exposure. Meanwhile, the Chapters 4-6 were able to come together in quantifying the exposure firefighters have from PFCs.

Ultimately, this can all come together to make a risk characterization of firefighters exposure. While PFCs can cause adverse health effect, especially with chronic exposure, these were all based off ingestion exposure. Dermal exposure indicated very littler PFC can penetrate the skin. When the highest amount of PFC found on unused turnout gear (0.017-µg/mL) is considered in comparison to the lowest dose tested in the literature (14- µg/mL), dermal exposure is highly unlikely. The level found on gear would be more of a concern if the firefighters were using their gear as napkins or if they were licking their gear. Furthermore, the durability assessment of the durable water repellent (DWR) finish present on the gear indicates a high level of durability to both UV and laundering exposures. Thus, compounding on the dermal exposure data making exposure even more unlikely.

## 7.2. Future Work

Unfortunately, the risk characterization made here should be taken with a grain of salt. For starters, much of the EPA risk assessment outline could not be completed in house and had to be extrapolated from literature data, some of which there was very little to extrapolate from. That being said, there is a plethora of future work to be conducted to increase the depth of the risk assessment.

Due to extenuating circumstances, a chemical analysis was unable to be completed on the UV and laundering durability samples. Based on the ISO 6530 data, it could be hypothesized that the PFC concentration may decrease over wash cycles. However, for the UV exposure, the DWR may have changed chemical structure, making more PFC available for human exposure. Additionally, an increased timeframe of UV exposure may be necessary to see effects.

Furthermore, a thermal durability assessment should be conducted. As firefighters routinely enter high heat environments, the DWR finish may degrade more or off-gas due to the temperatures they are exposed. This could be studied both in lab scale situation using a thermal protective performance apparatus or head-space sampler attached to a GC/MS or in firefighting training exercises. The lab scale test would indicate if effects can be seen at long time scales and at different temperatures. The training exercise would determine if lab scale effects are seen in a real-world scenario.

This dissertation mainly focused on outershell materials from a limited number of manufactures for a limited number of years of service. A larger study could be conducted to look at the concentration of a wide range of PFCs across multiple different outer shells, moisture barriers, and thermal liners, possibly from a wide range of years if the gear could be found. If gear cannot be found, the risk assessment can be conducted for current materials and extrapolated to characterize risk for individuals wearing older gear.

The larger study could also take into account firefighter gear that has been retired (typically after the NFPA 1971 10-year requirement). This may make it difficult to analyze the data, as there will be a plethora of other chemicals present on the gear, but it would speak to the real-world scenario firefighter find themselves.

On a toxicological front, contact transfer tests or flow-through diffusion test could be conducted using turnout fabric. The extraction methods presented in this dissertation are assumed to removal all PFCs on the fabric. This may mean that some of these chemicals aren't actually dermally available, especially if they are engulfed in the fiber matrix of a yarn. Doing contact transfer or flow-through diffusion testing may speak to the actual exposure firefighter have by touching their gear.

Along those lines, a non-destructive sampling method could be determined to sample PFCs on gear overtime. This may include using wipes or swabs to sample the surface of the firefighters gear, firetruck cab, or living quarter in the fire station. This would allow for a more in-depth analysis of firefighters true exposure to these chemicals and their migration through the different aspects of the firefighters job.

# REFERENCES

1. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Pregnancy Induced Hypertension and Preeclampsia. **2011**.

2. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation for Heart Disease (Including High Blood Pressure, High Cholesterol, Coronary Artery Disease). **2012**.

3. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Autoimmune Disease. **2012**.

4. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Cancer. **2012**.

5. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Thyroid Disease. **2012**.

6. Dobraca, D.; Israel, L.; McNeel, S.; Voss, R.; Wang, M.; Gajek, R.; Park, J.; Harwani, S.; Barley, F.; She, J.; Das, R. Biomonitoring in California Firefighters: Metals and Perfluorinated Chemicals. *Journal of Occupational and Environmental Medicine* **2015**, *57*, 88-97.

7. Rotander, A.; Toms, L. L.; Aylward, L.; Kay, M.; Mueller, J. F. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film-forming foam (AFFF). *Environment International* **2015**, *82*, 28-34.

8. Rotander, A.; Kärrman, A.; Toms, L. L.; Kay, M.; Mueller, J. F.; Gómez Ramos, M. J. Novel Fluorinated Surfactants Tentatively Identified in Firefighters Using Liquid Chromatography Quadrupole Time-of-Flight Tandem Mass Spectrometry and a Case-Control Approach. *Environmental science & technology* **2015**, *49*, 2434-2442.

9. Shaw, S. D.; Berger, M. L.; Harris, J. H.; Hun Yun, S.; Wu, Q.; Liao, C.; Blum, A.; Stefani, A.; Kannan, K. Persistent organic pollutants including polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans in firefighter from Northern California. *Chemosphere* **2013**, *91*, 1386-1394.

10. Tao, L.; Kannan, K.; Aldous, K. M.; Mauer, M. P.; Eadon, G. A. Biomonitoring of Perfluorochemicals in Plasma of New York State Personnel Responding to the World Trade Center Disaster. *Environmental science & technology* **2008**, *42*, 3472-3478.

11. AnonymousFire extinguishers: Class of Fires. https://www.marsden-fire-safety.co.uk/resources/fire-extinguishers (accessed 09/06/19, .

12. AnonymousFurthering the Understanding of the Migration of Chemicals from Consumer Products: A Study of Per- and Polyfluoroalkyl Substancs (PFASs) in Clothing, Apparel, and Children's Items. *Commission for Environmental Cooperation* **2017**, 201.

13. Trojanowicz, M.; Koc, M. Recent developments in methods for analysis of perfluorinated persistent pollutants. *Microchim Acta* **2013**, *180*, 957-971.

14. Shonali Nazaré; Rick D. Davis; Jyun-Siang Peng; Joannie Chin Accelerated Weathering of Firefighter Protective Clothing: Delineating the Impact of Thermal, Moisture, and Ultraviolet Light Exposures.

15. Arrieta, C.; David, E.; Dolez, P.; Vu-Khanh, T. Hydrolytic and Photochemical Aging Studies of a Kevler-PBI Blend. *Polymer degradation and stability* **1979**.

16. Rahman, M. F.; Peldszus, S.; Anderson, W. B. Behaviour and fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in drinking water treatment: A review. *Water Research* **2014**, *50*, 318-340.

17. Anonymous *Firefighter's handbook : essentials of firefighting and emergency response;* Albany, N.Y. : Delmar ; London : International Thomson, 2000: 2000; .

18. Anonymous *Dennis Smith's History of firefighting in America : 300 years of courage;* New York : Dial Press, 1978]: 1978; .

19. Layman, L. *Fundamentals of Fire Fighting Tactics;* Magruder publishing Company: 1940; .

20. Layman, L. *Attacking and Extinguishing Interior Fires;* National Fire Protection Association: 1967; .

21. Kerber, S. 20 Tactical Considerations from Firefighting Research. **2014**.

22. Carlton, N. P. Effective Methods for the Evaluation of Thermal Protective Performance of Firefighter Protective Hoods , North Carolina State University, 2019.

23. Loflin, M. E. NFPA 1500 Standard For a Fire Department OSH Program. *Professional Safety* **1989**, *34*, 15.

24. AnonymousNFPA 1500: Standard on Fire Department Occupational Safety, Health, and Wellness Program. *NFPA* **2018**.

25. AnonymousNFPA 1971: Standard on Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting. *NFPA* **2018**.

26. AnonymousTurnout Gear Outer Shells. *https://globe.msasafety.com/materials/outer-shell* (accessed 8/16/19, .

27. AnonymousTurnout Gear Moisture Barriers. *https://globe.msasafety.com/materials/moisture-barrier* (accessed 8/16/19, .

28. AnonymousTurnout Gear Thermal Liners. *https://globe.msasafety.com/materials/thermal-liner* (accessed 8/16/19, .

29. By, B. T. Y. Demystifying Turnout Gear Terminology. *https://www.fireengineering.com/articles/print/volume-162/issue-4/departments/technology-today/demystifying-turnout-gear-terminology.html* (accessed Sep 1, 2019).

30. Supply, C. Fire-Dex. *https://chiefsupply.com/collections/fire-dex* (accessed Sep 1, 2019).

31. AnonymousIARC Monographs on the Evaluation of Carcinogenic Risks to Humans - Volume 98: Painting, Firefighting, and Shiftwork. *International Agency for Research on Cancer* **2010**.

32. LeMasters, G.; Genaidy, A.; Succop, P.; Deddens, J.; Sobeih, T.; Barriera-Viruet, H.; Dunning, K.; Lockey, J. Cancer Risk Among Firefighters: A Review and Meta-analysis of 32 Studies. *Journal of Occupational and Environmental Medicine* **2006**, *48*, 1189-1202.

33. Daniels, R. D.; Kubale, T. L.; Yiin, J. H.; Dahm, M. M.; Hales, T. R.; Baris, D.; Zahm, S. H.; Beaumont, J. J.; Waters, K. M.; Pinkerton, L. E. Mortality and cancer incidence in a pooled cohort of US firefighters from San Francisco, Chicago and Philadelphia (1950–2009). *Occupational and Environmental Medicine* **2014**, *71*, 388-397.

34. Demers, P. A.; Checkoway, H.; Vaughan, T. L.; Weiss, N. S.; Heyer, N. J.; Rosenstock, L. Cancer Incidence among Firefighters in Seattle and Tacoma, Washington (United States). *Cancer Causes & Control* **1994**, *5*, 129-135.

35. Kang, D.; Davis, L. K.; Hunt, P.; Kriebel, D. Cancer incidence among male Massachusetts firefighters, 1987–2003. *American Journal of Industrial Medicine* **2008**, *51*, 329-335.

36. Ma, F.; Fleming, L. E.; Lee, D. J.; Trapido, E.; Gerace, T. A. Cancer Incidence in Florida Professional Firefighters, 1981-1999. *Journal of Occupational and Environmental Medicine* **2006**, *48*, 883-888.

37. Tsai, R. J.; Luckhaupt, S. E.; Schumacher, P.; Cress, R. D.; Deapen, D. M.; Calvert, G. M. Risk of cancer among firefighters in California, 1988–2007. *American Journal of Industrial Medicine* **2015**, *58*, 715-729.

38. Shaw, S. D.; Berger, M. L.; Zahm, S. H.; Kannan, K. Is Firefighting Carcinogenic? Chemical Exposure and Cancer Risk Among Fire Fighters. **2018**.

39. Stec, A. A.; Dickens, K. E.; Salden, M.; Hewitt, F. E.; Watts, D. P.; Houldsworth, P. E.; Martin, F. L. Occupational Exposure to Polycyclic Aromatic Hydrocarbons and Elevated Cancer Incidence in Firefighters. *Scientific reports* **2018**, *8*, 2476-8.

40. Heus, R. Exposure to smoke. *Instituut Rysieke Veiligheid* **2018**, 50.

41. Fabian, T.; Borgerson, J. L.; Kerber, S. I.; Gandhi, P. D.; Baxter, C. S.; Ross, C. S.; Lockey, J. E. Firefighter Exposure to Smoke Particulates. *Underwriters Laboratories* **2010**.

42. Bečanová, J.; Melymuk, L.; Vojta, Š; Komprdová, K.; Klánová, J. Screening for perfluoroalkyl acids in consumer products, building materials and wastes. *Chemosphere* **2016**, *164*, 322-329.

43. AnonymousCOMPARISON OF MODERN AND LEGACY HOME FURNISHINGS. *https://ulfirefightersafety.org/research-projects/comparison-of-modern-and-legacy-home-furnishings.html* (accessed 8/23/19, .

44. AnonymousGlossary of Firefighting Terms. *https://www.translationdirectory.com/glossaries/glossary093.htm#f* (accessed 8/23/19, .

45. Stull, J. O.; Stull, G. G. Firefighter research shows PPE exposure risk. **2015**https://www.firerescue1.com/firefighter-safety/articles/2154096-Firefighter-research-shows-PPE-exposure-risk/.

46. Harrison, T. R.; Muhamad, J. W.; Yang, F.; Morgan, S. E.; Talavera, E.; Caban-Martinez, A.; Kobetz, E. Firefighter attitudes, norms, beliefs, barriers, and behaviors toward post-fire decontamination processes in an era of increased cancer risk. *Journal of Occupational and Environmental Hygiene* **2018**, *15*, 279-284.

47. Lerner, S.; Mackey, D.; Thomason, J. Part 6 - Poisoning the Well - Toxic Firefighting Foam has Contaminated U.S. Drinking Water. *https://theintercept.com/2015/12/16/toxic-firefighting-foam-has-contaminated-u-s-drinking-water-with-pfcs/* (accessed May 18, 2018).

48. Lerner, S. Part 15 - The U.S. Military is Spending Millions to Replace Toxic Firefighting Foam with Toxic Firefighting Foam. *https://theintercept.com/2018/02/10/firefighting-foam-afff-pfos-pfoa-epa/* (accessed May 18, 2018).

49. Schaefer, T.; Dlugogorski, B.; Kennedy, E. Sealability Properties of Fluorine-Free Fire-Fighting Foams (FfreeF). *Fire Technol* **2008**, *44*, 297-309https://search.proquest.com/docview/217449155.

50. Taniyasu, S.; Kannan, K.; So, M. K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *Journal of Chromatography A* **2005**, *1093*, 89-97.

51. Taylor, P. H.; Yamada, T.; Striebich, R. C.; Graham, J. L.; Giraud, R. J. Investigation of waster incineration of fluorotelomer-based polymers as a potential source of PFOA in the environment. *Chemosphere* **2014**, *110*, 17-22.

52. Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A. Fluorotelomer Alcohol Biodegradation Yields Poly- and Perfluorinated Acids. *Environmental science & technology* **2004**, *38*, 2857-2864.

53. Laitinen, J. A.; Koponen, J.; Koikkalainen, J.; Kiviranta, H. Firefighters' exposure to perfluoroalkyl acids and 2-butoxyethanol present in firefighting foams. *Toxicology Letters* **2014**, *231*, 227-232.

54. Hatton, J.; Holton, C.; DiGuiseppi, B. Occurrence and behavior of per- and polyfluoroalkyl substances from aqueous film-forming foam in groundwater systems. *Remediation Journal* **2018**, *28*, 89-99.

55. McGuire, M. E.; Schaefer, C.; Richards, T.; Backe, W. J.; Field, J. A.; Houtz, E.; Sedlak, D. L.; Guelfo, J. L.; Wunsch, A.; Higgins, C. P. Evidence of Remediation-Induced Alteration of Subsurface Poly- and Perfluoroalkyl Substance Distribution at a Former Firefighter Training Area. *Environmental science & technology* **2014**, *48*, 6644-6652.

56. Moody, C. A.; Field, J. A. Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environmental Science & Technology* **2000**, *34*, 3864-3870.

57. Moody, C. A.; Field, J. A. Perfluorinated Surfactants and the Environmental Implications of Their Use in Fire-Fighting Foams. *Environmental Science & Technology* **2000**, *34*, 3864-3870.

58. AnonymousFact Sheet on AFFF Fire Fighting Agents. **2017**.

59. Allcorn, M.; Bluteau, T.; Corfield, J.; Day, G.; Cornelsen, M.; Holmes, N. J. C.; Klein, R. A.; McDowall, J. G.; Olsen, K. T.; Ramsden, N.; Ross, I.; Schaefer, T. H.; Weber, R.; Whitehead, K. FLUORINE-FREE FIREFIGHTING FOAMS (3F) – VIABLE ALTERNATIVES TO FLUORINATED AQUEOUS FILM-FORMING FOAMS (AFFF). *IPEN* **2018**.

60. Hu, X. C.; Andrews, D. Q.; Lindstrom, A. B.; Bruton, T. A.; Schaider, L. A.; Grandjean, P.; Lohmann, R.; Carignan, C. C.; Blum, A.; Balan, S. A.; Higgins, C. P.; Sunderland, E. M. Detection of Poly- and Perfluoroalkyl Substances (PFASs) in U.S. Drinking Water Linked to Industrial Sites, Military Fire Training Areas, and Wastewater Treatment Plants. *Environmental science & technology letters* **2016**, *3*, 344-350.

61. Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environmental Science & Technology* **1999**, *33*, 2800-2806.

62. Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A. Monitoring Perfluorinated Surfactants in Biota and Surface Water Samples Following an Accidental Release of Fire-Fighting Foam into Etobicoke Creek. *Environmental science & technology* **2002**, *36*, 545-551.

63. Dauchy, X.; Boiteux, V.; Bach, C.; Rosin, C.; Munoz, J. Per- and polyfluoroalkyl substances in firefighting foam concentrates and water samples collected near sites impacted by the use of these foams. *Chemosphere* **2017**, *183*, 53-61.

64. Shaw, S. D.; Berger, M., L.; Harris, J. H.; Yun Hun, S.; Wu, Q.; Liao, C.; Blum, A.; Stefani, A.; Kannan, K. Corrigendum to: "Persistent organic pollutants including polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans in firefighter from Northern California". *Chemosphere* **2014**, *102*, 87.

65. Calafat, A. M.; Wong, L.; Kuklenyik, Z.; Reidy, J. A.; Needham, L. L. Polyfluoroalkyl Chemicals in the U.S. Population: Data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and Comparisons with NHANES 1999-2000. *Environmental Health Perspectives* **2007**, *115*, 1596-1602.

66. Coakley, J.; Bridgen, P.; Mueller, J.; Douwes, J.; 't Mannetje, A. Polybrominated diphenyl ethers and perfluorinated alkyl substances in blood serum of New Zealand adults, 2011–2013. *Chemosphere* **2018**, *208*, 382-389.

67. de la Torre, A.; Navarro, I.; Sanz, P.; de los Angeles Martinex, Maria Occurrence and human exposure assessment of perfluorinated substances in house dust from European countries. *Science of the Total Environment* **2019**, *685*.

68. Karásková, P.; Venier, M.; Melymuk, L.; Bečanová, J.; Vojta, Š; Prokeš, R.; Diamond, M. L.; Klánová, J. Perfluorinated alkyl substances (PFASs) in household dust in Central Europe and North America. *Environment International* **2016**, *94*, 315-324.

69. Tian, Z.; Kim, S.; Shoeib, M.; Oh, J.; Park, J. Human exposure to per- and polyfluoroalkyl substances (PFASs) via house dust in Korea: Implication to exposure pathway. *Science of the Total Environment* **2016**, *553*, 266-275.

70. Wu, N.; Cai, D.; Guo, M.; Li, M.; Li, X. Per- and polyfluorinated compounds in saleswomen's urine linked to indoor dust in clothing shops. *Science of the Total Environment* **2019**, *667*, 594-600.

71. Yao, Y.; Sun, H.; Gan, Z.; Hu, H.; Zhao, Y.; Chang, S.; Zhou, Q. Nationwide Distribution of Per- and Polyfluoroalkyl Substances in Outdoor Dust in Mainland China From Eastern to Western Areas. *Environmental science & technology* **2016**, *50*, 3676-3685.

72. Yao, Y.; Zhao, Y.; Sun, H.; Chang, S.; Zhu, L.; Alder, A. C.; Kannan, K. Per- and Polyfluoroalkyl Substances (PFASs) in Indoor Air and Dust from Homes and Various Microenvironments in China: Implications for Human Exposure. *Environmental science & technology* **2018**, *52*, 3156-3166.

73. Shaw, S. D.; Berger, M. L.; Zahm Shelia, H.; Kannan, K. Is Firefighting Carcinogenic? Chemical Exposure and Cancer Risk Among Fire Fighters. **2018**.

74. Jin, C.; Sun, Y.; Islam, A.; Qian, Y.; Ducatman, A. Perfluoroalkyl Acids Including Perfluorooctane Sulfonate and Perfluorohexane Sulfonate in Firefighters. *Journal of Occupational and Environmental Medicine* **2011**, *53*, 324-328.

75. AnonymousNovel Fluorinated Compounds Found in Firefighters Blood. **2015**, 36.

76. Michaels, D.; Monforton, C. Manufacturing Uncertainty: Contested Science and the Protection of the Public's Health and Environment. *American Journal of Public Health* **2005**, *95*, S39-S48.

77. Richter, L.; Cordner, A.; Brown, P. Non-stick science: Sixty years of research and (in)action on fluorinated compounds. *Social Studies of Science* **2018**, *48*, 691-714.

78. Gould, K. A. Slowing the nanotechnology treadmill: impact science versus production science for sustainable technological development. *Environmental Sociology* **2015**, *1*, 143-151.

79. Gibbs, L. Citizen Activism for Environmental Health: The Growth of a Powerful New Grassroots Health Movement. *The Annals of the American Academy of Political and Social Science* **2002**, *584*, 97-109.

80. Carson, R. *Silent spring;* Houghton Mifflin,: Boston, 1962; , pp 378.

81. Roy, A. *Toxic Substances Control Act;* 2012; Vol. 2, pp 922-923.

82. Lerner, S.; Kofman, A.; McNeill, S. Part 3 - How DuPont Slipped Past the EPA. *https://theintercept.com/2015/08/20/teflon-toxin-dupont-slipped-past-epa/* (accessed May 18, 2018).

83. Lindstrom, A. B.; Strynar, M. J.; Libelo, E. L. Polyfluorinated Compounds: Past, Present, and Future. *Environmental science & technology* **2011**, *45*, 7954-7961.

84. AnonymousRoy J. Plunkett. *https://www.sciencehistory.org/historical-profile/roy-j-plunkett* (accessed Jan 15, 2019).

85. Frisbee, S. J.; Brooks, A. P.; Maher, A.; Flensborg, P.; Arnold, S.; Fletcher, T.; Steenland, K.; Shankar, A.; Knox, S. S.; Pollard, C.; Halverson, J. A.; Vieira, V. M.; Jin, C.; Leyden, K. M.; Ducatman, A. The C8 Health Project: Design, Methods, and Participants. *Environmental Health Perspectives* **2009**, *117*, 1873-1882.

86. Lerner, S.; Brown, A.; Gold, H.; McNeill, S. Part 1 - DuPont and the Chemistry of Deception. *https://theintercept.com/2015/08/11/dupont-chemistry-deception/* (accessed May 18, 2018).

87. Andrew B Lindstrom; Mark J Strynar; E Laurence Libelo Polyfluorinated compounds: past, present, and future. *Environmental science & technology* **2011**, *45*, 7954-7961.

88. Taves, D. R. Evidence That There are Two Forms of Fluoride in Human Serum. *Nature* **1968**, *217*, 1050-1051.

89. Guy, W. S.; Taves, D. R.; Brey Jr, W. S. In *In Organic Fluorocompounds in Human Plasma: Prevalence and Characterization;* American Chemical Soc: Washington, DC, 1976; Vol. 28, pp 117-134.

90. Lerner, S. Part 5 - DuPont Found Liable in Teflon Toxin Trial. *https://theintercept.com/2015/10/08/dupont-found-liable-in-c8-trial/* (accessed May 18, 2018).

91. Lerner, S.; Brown, A.; Gold, H.; McNeill, S. Part 2 - The Case Against DuPont. *https://theintercept.com/2015/08/17/teflon-toxin-case-against-dupont/* (accessed May 18, 2018).

92. US EPA, O. Reference News Release: EPA Settles PFOA Case Against DuPont for Largest Environmental Administrative Penalty in Agency History. *https://www.epa.gov/enforcement/reference-news-release-epa-settles-pfoa-case-against-dupont-largest-environmental* (accessed Feb 5, 2019).

93. Savan, L. Teflon Is Forever. https://www.motherjones.com/environment/2007/05/teflon-forever/.

94. AnonymousDuPont Heritage Timeline. *http://www2.dupont.com/Phoenix_Heritage/en_US/1935_a_detail.html* (accessed Feb 7, 2019).

95. David Michaels Waiting for the Body Count: Corporate Decision Making and Bladder Cancer in the U. S. Dye Industry. *Medical Anthropology Quarterly* **1988**, *2*, 215-232.

96. Lerner, S. Part 4 - The Teflon Toxin goes to Court. *https://theintercept.com/2015/09/19/teflon-toxin-goes-to-court/* (accessed May 18, 2018).

97. Vieira, V.; Hoffman, K.; Fletcher, T. Assessing the Spatial Distribution of Perfluorooctanoic Acid Exposure via Public Drinking Water Pipes Using Geographic Information Systems. *Environmental health and toxicology* **2013**, *28*, e2013009.

98. Lerner, S. Part 13 - Citizen Group Will Sue DuPont and Chemours for Contaminating Drinking Water in North Carolina. *https://theintercept.com/2017/08/08/citizen-groups-will-sue-dupont-and-chemours-for-contaminating-drinking-water-in-north-carolina/* (accessed May 18, 2018).

99. Lerner, S. Part 14 - EPA Orders Testing for GenX Contamination Near Chemours Plant in West Virginia. *https://theintercept.com/2018/01/25/epa-genx-chemours-west-virginia/* (accessed May 18, 2018).

100. Velez, M. P.; Arbuckle, T. E.; Fraser, W. D. Maternal Exposure to Perfluorinated Chemicals and Reduced Fecundity: the MIREC Study. *Human Reproduction* **2015**, *30*, 701-709.

101. Olsen, G.; Zobel, L. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *Int Arch Occup Environ Health* **2007**, *81*, 231-246.

102. Olsen, G. W.; Gilliland, F. D.; Burlew, M. M.; Burris, J. M.; Mandel, J. S.; Mandel, J. H. An Epidemiologic Investigation of Reproductive Hormones in Men with Occupational Exposure to Perfluorooctanoic Acid. *Journal of Occupational and Environmental Medicine* **1998**, *40*, 614-622.

103. Olsen, G. W.; Burris, J. W.; Burlew, M. M.; Mandel, J. H. Epidemiologic Assessment of Worker Serum Perfluorooctanesulfonate (PFOS) and Perfluorooctanoae (PFOA) Concentrations and Medical Surveillance Examinations. *Journal of Occupational and Environmental Medicine* **2003**, *45*, 260-270.

104. Olsen, G. W.; Burris, J. M.; Burlew, M. M.; Mandel, J. H. Epidemiologic assessment of worker serum PFOS and PFOA concentrations and medical surveillance examinations. *Journal of Occupation and Environmental Medicine* **2003**, *45*, 260-270.

105. Butenhoff, J.; Costa, G.; Elcombe, C.; Farrar, D.; Hansen, K.; Iwai, H.; Jung, R.; Kennedy, G.; Lieder, P.; Olsen, G.; Thomford, P. Toxicity of Ammonium Perfluorooctanoate in Male Cynomolgus Monkeys after Oral Dosing for 6 Months. *Toxicological sciences : an official journal of the Society of Toxicology* **2002**, *69*, 244-257.

106. US EPA, O. Fact Sheet: 2010/2015 PFOA Stewardship Program. *https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program* (accessed Jul 17, 2019).

107. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Birth Defects. **2011**.

108. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Miscarriage and Stillbirths. **2011**.

109. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Preterm Birth and Birthweight. **2011**.

110. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation for Chronic Kidney Disease. **2012**.

111. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation for Non-infectious Lung Disease (Asthma and Chronic Obstructive Pulmonary Disease - COPD). **2012**.

112. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation for Parkinson's Disease. **2012**.

113. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Diabetes. **2012**.

114. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Infectious Disease. **2012**.

115. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Liver Disease. **2012**.

116. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Neurodevelopmental Disorder in Children. **2012**.

117. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation of Stroke. **2012**.

118. Fletcher, T.; Steenland, K.; Savitz, D. Probable Link Evaluation or Osteoarthritis. **2012**.

119. AnonymousGuidelines for carcinogen risk assessment. **2005**, 166http://purl.access.gpo.gov/GPO/LPS62197.

120. AnonymousWhat Is Cancer? *https://www.cancer.org/cancer/cancer-basics/what-is-cancer.html* (accessed Aug 14, 2019).

121. AnonymousCancer Facts & Figures 2019. *American Cancer Society* **2019**, 76.

122. AnonymousCancer Facts & Figures 2019. **2019**.

123. AnonymousDraft risk assessment of the potential human health effects associated with exposure to perfluorooctanoic acid and its salts. **2005**, 1-120http://purl.access.gpo.gov/GPO/LPS110663.

124. Hardisty, J. F.; Willson, G. A.; Brown, W. R.; McConnell, E. E.; Frame, S. R.; Gaylor, D. W.; Kennedy, G. L.; Butenhoff, J. L. Pathology Working Group review and evaluation of proliferative lesions of mammary gland tissues in female rats fed ammonium perfluorooctanoate (APFO) in the diet for 2 years. *Drug and Chemical Toxicology* **2010**, *33*, 131-137.

125. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings. *Toxicological Sciences* **2007**, *99*, 366-394.

126. DeWitt, J. C.; Shnyra, A.; Badr, M. Z.; Loveless, S. E.; Hoban, D.; Frame, S. R.; Cunard, R.; Anderson, S. E.; Meade, B. J.; Peden-Adams, M. M.; Luebke, R. W.; Luster, M. I. Immunotoxicity of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and the Role of Peroxisome Proliferator-Activated Receptor Alpha. *Critical Reviews in Toxicology* **2009**, *39*, 76-94.

127. Leonard, R. C.; Kreckmann, K. H.; Sakr, C. J.; Symons, J. M. Retrospective Cohort Mortality Study of Workers in a Polymer Production Plant Including a Reference Population of Regional Workers. *Annals of Epidemiology* **2008**, *18*, 15-22.

128. Lundin, J. I.; Alexander, B. H.; Olsen, G. W.; Church, T. R. Ammonium Perfluorooctanoate Production and Occupational Mortality. *Epidemiology* **2009**, *20*, 921-928.

129. Eriksen, K. T.; Sørensen, M.; McLaughlin, J. K.; Lipworth, L.; Tjønneland, A.; Overvad, K.; Raaschou-Nielsen, O. Perfluorooctanoate and Perfluorooctanesulfonate Plasma Levels and Risk of Cancer in the General Danish Population. *Journal of the National Cancer Institute* **2009**, *101*, 605-609.

130. Bonefeld-Jorgensen, E. C.; Long, M.; Bossi, R.; Ayotte, P.; Asmund, G.; Krüger, T.; Ghisari, M.; Mulvad, G.; Kern, P.; Nzulumiki, P.; Dewailly, E. Perfluorinated compounds are related to breast cancer risk in greenlandic inuit: A case control study. *Environmental Health* **2011**, *10*, 88.

131. Steenland, K.; Woskie, S. Cohort Mortality Study of Workers Exposed to Perfluorooctanoic Acid. *American Journal of Epidemiology* **2012**, *176*, 909-917.

132. Vieira, V. M.; Hoffman, K.; Shin, H.; Weinberg, J. M.; Webster, T. F.; Fletcher, T. Perfluorooctanoic Acid Exposure and Cancer Outcomes in a Contaminated Community: A Geographic Analysis. *Environmental health perspectives* **2013**, *121*, 318-323.

133. Barry, V.; Winquist, A.; Steenland, K. Perfluorooctanoic Acid (PFOA) Exposures and Incident Cancers among Adults Living Near a Chemical Plant. *Environmental health perspectives* **2013**, *121*, 1313-1318.

134. Hanauer, S. B. Inflammatory bowel disease: Epidemiology, pathogenesis, and therapeutic opportunities. *Inflammatory Bowel Diseases* **2006**, *12*, S3-S9.

135. Cooper, G. S.; Bynum, M. L. K.; Somers, E. C. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. *Journal of Autoimmunity* **2009**, *33*, 197-207.

136. Sellin, J. H.; Shah, R. R. The Promise and Pitfalls of Seroloic Testing in Inflammatory Bowel Disease. *Gastroenterology Clinics of North America* **2012**, *41*, 463-482.

137. AnonymousUlcerative Colitis vs Crohn's Disease. *https://www.uclahealth.org/gastro/ibd/ulcerative-colitis-vs-crohns-disease* (accessed 8/14/19, .

138. Miller, F. W.; Alfredsson, L.; Costenbader, K. H.; Kamen, D. L.; Nelson, L. M.; Norris, J. M.; De Roos, A. J. Epidemiology of environmental exposures and human autoimmune diseases: Findings from a National Institute of Environmental Health Sciences Expert Panel Workshop. *Journal of Autoimmunity* **2012**, *39*, 259-271.

139. AnonymousControl Your Cholesterol. *https://www.heart.org/en/health-topics/cholesterol/about-cholesterol* (accessed May 17, 2019).

140. staff, f. o. e. High Cholesterol: Symptoms, Causes and Treatment. *https://familydoctor.org/condition/cholesterol/* (accessed May 17, 2019).

141. Publishing, H. H. Sliding scale for LDL: How low should you go? *https://www.health.harvard.edu/heart-health/sliding-scale-for-ldl-how-low-should-you-go* (accessed May 17, 2019).

142. AnonymousHigh cholesterol - Symptoms and causes. *https://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/symptoms-causes/syc-20350800* (accessed May 17, 2019).

143. Anonymous2018 Cholesterol Guidelines for Heart Health Announced. *https://www.hopkinsmedicine.org/news/newsroom/news-releases/2018-cholesterol-guidelines-for-heart-health-announced* (accessed May 17, 2019).

144. Grundy, S. M.; Stone, N. J.; Bailey, A. J. Cholesterol Management Guide. *American Heart Association* **2018**, 27.

145. Lau, C.; Anitole, K.; Hodes, C.; Lai, D.; Pfahles-Hutchens, A.; Seed, J. Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings. *Toxicological Sciences* **2007**, *99*, 366-394.

146. Martin, M. T.; Brennan, R. J.; Hu, W.; Ayanoglu, E.; Lau, C.; Ren, H.; Wood, C. R.; Corton, J. C.; Kavlock, R. J.; Dix, D. J. Toxicogenomic Study of Triazole Fungicides and Perfluoroalkyl Acids in Rat Livers Predicts Toxicity and Categorizes Chemicals Based on Mechanisms of Toxicity. *Toxicological Sciences* **2007**, *97*, 595-613.

147. Fitz-Simon, N.; Fletcher, T.; Luster, M. I.; Steenland, K.; Calafat, A. M.; Kato, K.; Armstrong, B. Reductions in Serum Lipids with a 4-year Decline in Serum Perfluorooctanoic Acid and Perfluorooctanesulfonic Acid. *Epidemiology* **2013**, *24*, 569-576.

148. Fletcher, T.; Galloway, T. S.; Melzer, D.; Holcroft, P.; Cipelli, R.; Pilling, L. C.; Mondal, D.; Luster, M.; Harries, L. W. Association between PFOA, PFOS and changes in the expression of genes involved in cholesterol metabolism in humans. *Environment international* **2013**, *57-58*, 2-10.

149. Shin, H.; Vieira, V. M.; Ryan, P. B.; Detwiler, R.; Sanders, B.; Steenland, K.; Bartell, S. M. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. *Environmental science & technology* **2011**, *45*, 1435-1442.

150. Shin, H.; Vieira, V. M.; Ryan, P. B.; Detwiler, R.; Sanders, B.; Steenland, K.; Bartell, S. M. Environmental Fate and Transport Modeling for Perfluorooctanoic Acid Emitted from the Washington Works Facility in West Virginia. *Environmental science & technology* **2011**, *45*, 1435-1442.

151. Shin, H.; Ryan, P. B.; Vieira, V. M.; Bartell, S. P. Modeling the air-soil transport pathway of perfluorooctanoic acid in the mid-Ohio Valley using linked air dispersion and vadose zone models. *Atmospheric environment* **2012**, *51*, 67-74.

152. Shin, H.; Ryan, P. B.; Vieira, V. M.; Bartell, S. M. Modeling the air-soil transport pathway of perfluorooctanoic acid in the mid-Ohio Valley using linked air dispersion and vadose zone models. *Atmospheric environment* **2012**, *51*, 67-74.

153. Costa, G.; Sartori, S.; Consonni, D. Thirty Years of Medical Surveillance in Perfluorooctanoic Acid Production Workers. *Journal of Occupational and Environmental Medicine* **2009**, *51*, 364-372.

154. Olsen, G. W.; Burris, J. M.; Burlew, M. M.; Mandel, J. H. Plasma Cholecystokinin and Hepatic Enzymes, Cholesterol and Lipoproteins in Ammonium Perfluorooctanoate Production Workers. *Drug and Chemical Toxicology* **2000**, *23*, 603-620.

155. Nelson, J. W.; Hatch, E. E.; Webster, T. F. Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General U.S. Population. *Environmental Health Perspectives* **2010**, *118*, 197-202.

156. Frisbee, S. J.; Shankar, A.; Knox, S. S.; Steenland, K.; Savitz, D. A.; Fletcher, T.; Ducatman, A. M. Perfluorooctanoic Acid, Perfluorooctanesulfonate, and Serum Lipids in Children and Adolescents: Results From the C8 Health Project. *Archives of Pediatrics & Adolescent Medicine* **2010**, *164*, 860-869.

157. Emmett, E. A. Community Exposure to Perfluorooctanoate: Relationships between Serum Levels and Certain Health Parameters. *Journal of Occupational and Environmental Medicine* **2006**, *48*, 771-779.

158. Steenland, K.; Tinker, S.; Frisbee, S.; Ducatman, A.; Vaccarino, V. Association of Perfluorooctanoic Acid (PFOA) and Perfluoroctanesulfonate (PFOS) with Serum Lipids Among Adults Living Near a Chemical Plant. *Epidemiology* **2009**, *20*, S236.

159. Sakr, C. J.; Kreckmann, K. H.; Green, J. W.; Gillies, P. J.; Reynolds, J. L.; Leonard, R. C. Cross-Sectional Study of Lipids and Liver Enzymes Related to a Serum Biomarker of Exposure (ammonium perfluorooctanoate or APFO) as Part of a General Health Survey in a Cohort of Occupationally Exposed Workers. *Journal of Occupational and Environmental Medicine* **2007**, *49*, 1086-1096.

160. Philadelphia, The Children's Hospital of Gestational Hypertension. *https://www.chop.edu/conditions-diseases/gestational-hypertension* (accessed Apr 24, 2019).

161. Nolan, J. M.; Nolan, L. A.; Shofer, F. S.; Rodway, N. V.; Emmett, E. A. Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water. *Reproductive Toxicology* **2010**, *29*, 147-155.

162. David A. Savitz; Cheryl R. Stein; Beth Elston; Gregory A. Wellenius; Scott M. Bartell; Hyeong-Moo Shin; Veronica M. Vieira; Tony Fletcher Relationship of Perfluorooctanoic Acid Exposure to Pregnancy Outcome Based on Birth Records in the Mid-Ohio Valley. *Environmental Health Perspectives* **2012**, *120*, 1201-1207.

163. Darrow, L. A.; Stein, C. R.; Steenland, K. Serum Perfluorooctanoic Acid and Perfluorooctane Sulfonate Concentrations in Relation to Birth Outcomes in the Mid-Ohio Valley, 2005–2010. *Environmental health perspectives* **2013**, *121*, 1207-1213.

164. Stein, C. R.; Savitz, D. A.; Dougan, M. Serum Levels of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and Pregnancy Outcome. *American Journal of Epidemiology* **2009**, *170*, 837-846.

165. Shin, H.; Vieira, V. M.; Ryan, P. B.; Steenland, K.; Bartell, S. M. Retrospective Exposure Estimation and Predicted versus Observed Serum Perfluorooctanoic Acid Concentrations for Participants in the C8 Health Project. *Environmental Health Perspectives* **2013**, *121*, 1760-1766.

166. David A. Savitz; Cheryl R. Stein; Scott M. Bartell; Beth Elston; Jian Gong; Hyeong-Moo Shin; Gregory A. Wellenius Perfluorooctanoic Acid Exposure and Pregnancy Outcome in a Highly Exposed Community. *Epidemiology* **2012**, *23*, 386-392.

167. AnonymousAn Overview of the Pituitary Gland. *https://www.endocrineweb.com/endocrinology/overview-pituitary-gland* (accessed Aug 31, 2019).

168. AnonymousThe Endocrine System and Glands of the Human Body. *https://www.webmd.com/diabetes/endocrine-system-facts* (accessed Aug 31, 2019).

169. AnonymousList: Excerpts From My Son’s 6th Grade Report on the Endocrine System. *https://www.mcsweeneys.net/articles/excerpts-from-my-sons-6th-grade-report-on-the-endocrine-system* (accessed Sep 1, 2019).

170. Adin, D. Cardiology Drugs with Renal Implications. **2018**.

171. Mishra, S. Cholinergic Agonists and Antagonists. **2018**.

172. AnonymousHPA Axis Dysfunction: Genes and Environment - Genetic Lifehacks. **2019**https://www.geneticlifehacks.com/hpa-axis-dysfunction-genes-and-environment/.

173. AnonymousCommon Thyroid Gland Diseases and Problems to Watch For. *https://www.healthline.com/health/common-thyroid-disorders* (accessed Aug 31, 2019).

174. AnonymousThyroid Diseases. *https://www.health.harvard.edu/topics/thyroid-diseases* (accessed Aug 31, 2019).

175. AnonymousCommon Thyroid Gland Diseases and Problems to Watch For. *https://www.healthline.com/health/common-thyroid-disorders* (accessed Aug 31, 2019).

176. Lopez-Espinosa, M. J.; Mondal, D.; Armstrong, B.; Bloom, M. S.; Fletcher, T. Thyroid Function and Perfluoroalkyl Acids in Children Living Near a Chemical Plant. **2012**.

177. Lopez-Espinosa, M.; Fitz-Simon, N.; Bloom, M. S.; Calafat, A. M.; Fletcher, T. Comparison between free serum thyroxine levels, measured by analog and dialysis methods, in the presence of perfluorooctane sulfonate and perfluorooctanoate. *Reproductive Toxicology* **2012**, *33*, 552-555.

178. Kato, K.; Wong, L.; Jia, L. T.; Kuklenyik, Z.; Calafat, A. M. Trends in Exposure to Polyfluoroalkyl Chemicals in the U.S. Population: 1999−2008. *Environmental science & technology* **2011**, *45*, 8037-8045.

179. Melzer, D.; Rice, N.; Depledge, M. H.; Henley, W. E.; Galloway, T. S. Association between Serum Perfluorooctanoic Acid (PFOA) and Thyroid Disease in the U.S. National Health and Nutrition Examination Survey. *Environmental Health Perspectives* **2010**, *118*, 686-692.

180. Chan, E.; Burstyn, I.; Cherry, N.; Bamforth, F.; Martin, J. W. Perfluorinated Acids and Hypothyroxinemia in Pregnant Women. *Environmental Research* **2011**, *111*, 559-564.

181. Bloom, M. S.; Kannan, K.; Spliethoff, H. M.; Tao, L.; Aldous, K. M.; Vena, J. E. Exploratory Assessment of Perfluorinated Compounds and Human Thyroid Function. *Physiology and Behavior* **2010**, *99*, 240-245.

182. Emmett, E. A.; Zhang, H.; Shofer, F. S.; Freeman, D.; Rodway, N. V.; Desai, C.; Shaw, L. M. Community Exposure to Perfluorooctanoate: Relationships Between Serum Levels and Certain Health Parameters. *Journal or Occupational and Environmental Medicine* **2006**, *48*, 771-779.

183. Knox, S. S.; Jackson, T.; Frisbee, S. J.; Javins, B.; Ducatman, A. M. Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project. *The Journal of Toxicological Sciences* **2011**, *36*, 403-410.

184. Olsen, G.; Zobel, L. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. *Int Arch Occup Environ Health* **2007**, *81*, 231-246.

185. Martin, M. T.; Brennan, R. J.; Hu, W.; Ayanoglu, E.; Lau, C.; Ren, H.; Wood, C. R.; Corton, J. C.; Kavlock, R. J.; Dix, D. J. Toxicogenomic Study of Triazole Fungicides and Perfluoroalkyl Acids in Rat Livers Predicts Toxicity and Categorizes Chemicals Based on Mechanisms of Toxicity. *Toxicological Sciences* **2007**, *97*, 595-613.

186. Kotthoff, M.; Bücking, M. Four Chemical Trends Will Shape the Next Decade's Directions in Perfluoroalkyl and Polyfluoroalkyl Substances Research. *Frontiers in chemistry* **2018**, *6*, 103.

187. Krafft, M. P.; Riess, J. G. Selected physicochemical aspects of poly- and perfluoroalkylated substances relevant to performance, environment and sustainability—Part one. *Chemosphere* **2015**, *129*, 4-19.

188. Cooke, M. TECHNICAL FACT SHEET – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA). **2017**.

189. Kissa, E. *Fluorinated surfactants and repellents;* New York : Marcel Dekker, 2001]: 2001; .

190. Lehmler, H. Synthesis of environmentally relevant fluorinated surfactants—a review. *Chemosphere* **2005**, *58*, 1471-1496.

191. Vieira, V.; Webster, T.; Bartell, S.; Steenland, K.; Savitz, D.; Fletcher, T. PFOA community health studies: exposure via drinking water contaminated by a Teflon manufacturing facility. *Organohalogen Compounds* **2008**, *70*, 730-732.

192. Vieira, V.; Hoffman, K.; Fletcher, T. Assessing the Spatial Distribution of Perfluorooctanoic Acid Exposure via Public Drinking Water Pipes Using Geographic Information Systems. *Environmental health and toxicology* **2013**, *28*, e2013009.

193. Butt, C. M.; Muir, D. C. G.; Stirling, I.; Kwan, M.; Mabury, S. A. Rapid Response of Arctic Ringed Seals to Changes in Perfluoroalkyl Production. *Environmental science & technology* **2007**, *41*, 42-49.

194. Furdui, V. I.; Helm, P. A.; Crozier, P. W.; Lucaciu, C.; Reiner, E. I.; Marvin, C. H.; Whittle, D. M.; Mabury, S. A.; Tomy, G. T. Temporal Trends of Perfluoroalkyl Compounds with Isomer Analysis in Lake Trout from Lake Ontario (1979−2004). *Environmental science & technology* **2008**, *42*, 4739-4744.

195. Hart, K.; Kannan, K.; Isobe, T.; Takahashi, S.; Yamada, T. K.; Miyazaki, N.; Tanabe, S. Time Trends and Transplacental Transfer of Perfluorinated Compounds in Melon-Headed Whales Stranded Along the Japanese Coast in 1982, 2001/2002, and 2006. *Environmental science & technology* **2008**, *42*, 7132-7137.

196. Christensen, K. Y.; Raymond, M.; Blackowicz, M.; Liu, Y.; Thompson, B. A.; Anderson, H. A.; Turyk, M. Perfluoroalkyl Substances and Fish Consumption. *Environmental Researh* **2017**, *154*, 145-151.

197. Oliaei, F.; Weber, R.; Watson, A. PBDE contamination in Minnesota Landfills, waste water treatment plants and sediments as PBDE sources and reservoir. *Organohalogen Compounds* **2010**, *72*, 1346-1349.

198. Oliaei, F.; Kriens, D.; Kessler, K. INVESTIGATION OF PERFLUOROCHEMICAL (PFC) CONTAMINATION IN MINNESOTA PHASE ONE. *Report to Senate Environment Committee* **2006**.

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

200. Vecitis, C. D.; Park, H.; Cheng, J.; Mader, B. T.; Hoffmann, M. R. Treatment Technologies for Aqueous Perfluorooctanesulfonate (PFOS) and Perfluorooctanoate (PFOA). *Environmental Science & Engineering* **2009**, *3*, 129-151.

201. Hill, P. J.; Taylor, M.; Goswami, P.; Blackburn, R. S. Substitution of PFAS chemistry in outdoor apparel and the impact on repellency performance. *Chemosphere* **2017**, *181*, 500-507.

202. Herzke, D.; Olsson, E.; Posner, S. Perfluoroalkyl and polyfluoroalkyl substances (PFASs) in consumer products in Norway – A pilot study. *Chemosphere* **2012**, *88*, 980-987.

203. Kotthoff, M.; Müller, J.; Jürling, H.; Schlummer, M.; Fiedler, D. Perfluoroalkyl and polyfluoroalkyl substances in consumer products. *Environ Sci Pollut Res* **2015**, *22*, 14546-14559.

204. Ye, F.; Zushi, Y.; Masunaga, S. Survey of perfluoroalkyl acids (PFAAs) and their precursors present in Japanese consumer products. *Chemosphere* **2015**, *127*, 262-268.

205. Fujii, Y.; Harada, K. H.; Koizumi, A. Occurrence of perfluorinated carboxylic acids (PFCAs) in personal care products and compounding agents. *Chemosphere* **2013**, *93*, 538-544.

206. Favreau, P.; Poncioni-Rothlisberger, C.; Place, B. J.; Bouchex-Bellomie, H.; Weber, A.; Tremp, J.; Field, J. A.; Kohler, M. Multianalyte profiling of per- and polyfluoroalkyl substances (PFASs) in liquid commercial products. *Chemosphere* **2017**, *171*, 491-501.

207. Vestergren, R.; Herzke, D.; Wang, T.; Cousins, I. T. Are imported consumer products an important diffuse source of PFASs to the Norwegian environment? *Environmental pollution* **2015**, *198*, 223-230.

208. Jiang, W.; Zhang, Y.; Yang, L.; Chu, X.; Zhu, L. Perfluoroalkyl acids (PFAAs) with isomer analysis in the commercial PFOS and PFOA products in China. *Chemosphere* **2015**, *127*, 180-187.

209. Robel, A. E.; Marshall, K.; Dickinson, M.; Lunderberg, D.; Butt, C.; Peaslee, G.; Stapleton, H. M.; Field, J. A. Closing the Mass Balance on Fluorine on Papers and Textiles. *Environmental science & technology* **2017**, *51*, 9022-9032.

210. Schultz, M. M.; Barofsky, D. F.; Field, J. A. Fluorinated Alkyl Surfactants. *Environmental Engineering Science* **2003**, *20*, 487-501.

211. van der Putte, I.; Murin, M.; van Velthoven, M.; Affourtit, F. Analysis of the risks arising from the industrial use of Perfuorooctanoic acid (PFOA) and Ammonium Perfluorooctanoate (APFO) and from their use in consumer articles. Evaluation of the risk reduction measures for potential restrictions on the manufacture, placing on the market and use of PFOA and APFO. **2010**.

212. Still, M.; Schlummer, M.; Gruber, L.; Fiedler, D.; Wolz, G. Impact of Industrial Production and Packaging Processes on the Concentration of Per- and Polyfluorinated Compounds in Milk and Dairy Products. *Journal of agricultural and food chemistry* **2013**, *61*, 9052-9062.

213. Schaider, L. A.; Balan, S. A.; Blum, A.; Andrews, D. Q.; Strynar, M. J.; Dickinson, M. E.; Lunderberg, D. M.; Lang, J. R.; Peaslee, G. F. Fluorinated Compounds in U.S. Fast Food Packaging. *Environmental science & technology letters* **2017**, *4*, 105-111.

214. Trier, X.; Granby, K.; Christensen, J. Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging. *Environ Sci Pollut Res* **2011**, *18*, 1108-1120.

215. AnonymousPerfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA Journal* **2012**, *10*, n/a.

216. Lerner, S. Part 9 - Teflon Toxin Contamination has Spread Throughout the World. *https://theintercept.com/2016/04/19/teflon-toxin-contamination-has-spread-throughout-the-world/* (accessed May 18, 2018).

217. Lerner, S.; Liu, C. Part 11 - Under DuPont Bridge - The Teflon Toxin Goes to China. *https://theintercept.com/2016/09/15/the-teflon-toxin-goes-to-china/* (accessed May 18, 2018).

218. Li, Y.; Fletcher, T.; Mucs, D.; Scott, K.; Lindh, C. H.; Tallving, P.; Jakobsson, K. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occupational and environmental medicine* **2018**, *75*, 46-51.

219. Fan, H.; Ducatman, A.; Zhang, J. Perfluorocarbons and Gilbert syndrome (phenotype) in the C8 Health Study Population. *Environmental Research* **2014**, *135*, 70-75.

220. Weber, R.; Watson, A.; Forter, M.; Oliaei, F. Persistent organic pollutants and landfills - a review of past experiences and future challenges. *Waste Management & Research* **2011**, *29*, 107-121.

221. Busch, J. Analysis of poly- and perfluorinated compounds (PFCs) in landfill effluent using HPLC-MS/MS. **2009**.

222. Busch, J.; Ahrens, L.; Sturm, R.; Ebinghaus, R. Polyfluoroalkyl Compounds in Landfill Leachates. *Environmental pollution* **2010**, *158*, 1467-1471.

223. Bossi, R.; Strand, J.; Sortkjær, O.; Larsen, M. M. Perfluoroalkyl compounds in Danish wastewater treatment plants and aquatic environments. *Environment International* **2008**, *34*, 443-450.

224. Woldegiorgis, A.; Andersson, J.; Remberger, M.; Kaj, L.; Ekheden, Y.; Blom, L.; Brorstrom-Luden, E.; Borgen, A.; Dye, C.; Schlabach, M. Results from the Swedish National Screening Programme 2005. Perfluorinated alkylated substances (PFAS). *Swedish Environmental Research Institute* **2006**, *1698*http://libris.kb.se/resource/bib/10311745.

225. Taylor, P. H.; Yamada, T.; Striebich, R. C.; Grahm, J. L.; Giraud, R. J. Investigation of waste incineration of fluorotelomer-based polymer as a potential source of PFOA in the environment. **2014**, *110*, 17-2.

226. Jahnke, A.; Berger, U. Trace analysis of per- and polyfluorinated alkyl substances in various matrices—How do current methods perform? *Journal of Chromatography A* **2009**, *1216*, 410-421.

227. Villagrasa, M.; López de Alda, M.; Barceló, D. Environmental analysis of fluorinated alkyl substances by liquid chromatography–(tandem) mass spectrometry: a review. *Anal Bioanal Chem* **2006**, *386*, 953-972.

228. Ohya, T.; Kudo, N.; Suzuki, E.; Kawashima, Y. Determination of perfluorinated carboxylic acids in biological samples by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications* **1998**, *720*, 1-7.

229. Onghena, M.; Moliner-Martinez, Y.; Picó, Y.; Campíns-Falcó, P.; Barceló, D. Analysis of 18 perfluorinated compounds in river waters: comparison of high performance liquid chromatography-tandem mass spectrometry, ultra-high-performance liquid chromatography-tandem mass spectrometry and capillary liquid chromatography-mass spectrometry. *Journal of chromatography. A* **2012**, *1244*, 88.

230. Schröder, H. F. Determination of fluorinated surfactants and their metabolites in sewage sludge samples by liquid chromatography with mass spectrometry and tandem mass spectrometry after pressurised liquid extraction and separation on fluorine-modified reversed-phase sorbents. *Journal of Chromatography A* **2003**, *1020*, 131-151.

231. Berger, U.; Langlois, I.; Oehme, M.; Kallenborn, R. Comparison of Three Types of Mass Spectrometer for High-Performance Liquid Chromatography/Mass Spectrometry Analysis of Perfluoroalkylated Substances and Fluorotelomer Alcohols. *European Journal of Mass Spectrometry* **2004**, *10*, 579-588.

232. Voogt, P. d.; Sáez, M. Analytical chemistry of perfluoroalkylated substances. *Trends in Analytical Chemistry* **2006**, *25*, 326-342.

233. Takino, M.; Saishima, S.; Nakahara, T. Determination of perfluorooctane sulfonate in river water by liquid chromatography/atmospheric pressure photoionization mass spectrometry by automated on-line extraction using turbulent flow chromatography. *Rapid Communication in Mass Spectrometry* **2003**, *17*, 383-390.

234. Yamashita, N.; Kannan, K.; Taniyasu, S.; Horii, Y.; Okazawa, T.; Petrick, G.; Gamo, T. Analysis of Perfluorinated Acids at Parts-Per-Quadrillion Levels in Seawater Using Liquid Chromatography-Tandem Mass Spectrometry. *Environmental science & technology* **2004**, *38*, 5522-5528.

235. Taniyasu, S.; Kannan, K.; So, M. K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N. Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *Journal of Chromatography A* **2005**, *1093*, 89-97.

236. Martin, J. W.; Muir, D. C. G.; Moody, C. A.; Ellis, D. A.; Kwan, W. C.; Solomon, K. R.; Mabury, S. A. Collection of Airborne Fluorinated Organics and Analysis by Gas Chromatography/Chemical Ionization Mass Spectrometry. *Analytical chemistry* **2002**, *74*, 584-590.

237. Stock, N. L.; Lau, F. K.; Ellis, D. A.; Martin, J. W.; Muir, D. C. G.; Mabury, S. A. Polyfluorinated Telomer Alcohols and Sulfonamides in the North American Troposphere. *Environmental science & technology* **2004**, *38*, 991-996.

238. Dinglasan, M. J. A.; Ye, Y.; Edwards, E. A.; Mabury, S. A. Fluorotelomer Alcohol Biodegradation Yields Poly- and Perfluorinated Acids. *Environmental science & technology* **2004**, *38*, 2857-2864.

239. Shoeib, M.; Harner, T.; Vlahos, P. Perfluorinated Chemicals in the Arctic Atmosphere. *Environmental science & technology* **2006**, *40*, 7577-7583.

240. Shoeib, M.; Harner, T.; Wilford, B. H.; Jones, K. C.; Zhu, J. Perfluorinated Sulfonamides in Indoor and Outdoor Air and Indoor Dust:  Occurrence, Partitioning, and Human Exposure. *Environmental science & technology* **2005**, *39*, 6599-6606.

241. Dreyer, A.; Temme, C.; Sturm, R.; Ebinghaus, R. Optimized method avoiding solvent-induced response enhancement in the analysis of volatile and semi-volatile polyfluorinated alkylated compounds using gas chromatography–mass spectrometry. *Journal of Chromatography A* **2008**, *1178*, 199-205.

242. Jahnke, A.; Ahrens, L.; Ebinghaus, R.; Berger, U.; Barber, J. L.; Temme, C. An improved method for the analysis of volatile polyfluorinated alkyl substances in environmental air samples. *Anal. Bioanal. Chem* **2007**, *387*, 965-975.

243. Gołębiowski, M.; Siedlecka, E.; Paszkiewicz, M.; Brzozowski, K.; Stepnowski, P. Perfluorocarboxylic acids in cell growth media and technologically treated waters: Determination with GC and GC–MS. *Journal of Pharmaceutical and Biomedical Analysis* **2011**, *54*, 577-581.

244. Ylinen, M.; Hanhijarvi, H.; Peura, P.; Ramo, O. Quantitative Gas Chromatographic Determination of Perfluorooctanoic Acid as the Benzyl Ester in Plasma and Urine. *Archives of Environmental Contamination and Toxicology* **1985**, *14*, 713-717.

245. Belisle, J.; Hagen, D. F. A method for the determination of perfluorooctanoic acid in blood and other biological samples. *Analytical Biochemistry* **1980**, *101*, 369-376.

246. Dufková, V.; Čabala, R.; Maradová, D.; Štícha, M. A fast derivatization procedure for gas chromatographic analysis of perfluorinated organic acids. *Journal of Chromatography A* **2009**, *1216*, 8659-8664.

247. Kudo, N.; Bandai, N.; Kawashima, Y. Determination of perfluorocarboxylic acids by gas-liquid chromatography in rat tissues. *Toxicology Letters* **1998**, *99*, 183-190.

248. Adejare, A. Chemistry of organic fluorine compounds II : a critical review. *Journal of Medicinal Chemistry* **1996**, *39*, 3601.

249. Alzaga, R.; Salgado-Petinal, C.; Jover, E.; Bayona, J. M. Development of a procedure for the determination of perfluorocarboxylic acids in sediments by pressurised fluid extraction, headspace solid-phase microextraction followed by gas chromatographic–mass spectrometric determination. *Journal of Chromatography A* **2005**, *1083*, 1-6.

250. Alzaga, R.; Bayona, J. M. Determination of perfluorocarboxylic acids in aqueous matrices by ion-pair solid-phase microextraction–in-port derivatization–gas chromatography–negative ion chemical ionization mass spectrometry. *Journal of Chromatography A* **2004**, *1042*, 155-162.

251. Moody, C. A.; Field, J. A. Determination of Perfluorocarboxylates in Groundwater Impacted by Fire-Fighting Activity. *Environmental Science & Technology* **1999**, *33*, 2800-2806.

252. Yamamoto, G.; Yoshitake, K.; Sato, T.; Kimura, T.; Ando, T. Distribution and forms of fluorine in whole blood of human male. *Analytical Biochemistry* **1989**, *182*, 371-376.

253. March, J. *Advanced Organic Chemistry;* Wiley: New York, 1985; .

254. Kuehl, D. W.; Rozynov, B. Chromatographic and mass spectral studies of perfluorooctanesulfonate and three perfluorooctanesulfonamides. *Rapid Communication in Mass Spectrometry* **2003**, *17*, 2364-2369.

255. Sodhi, R. N. S. Time-of-flight secondary ion mass spectrometry (TOF-SIMS):— versatility in chemical and imaging surface analysis. *Analyst* **2004**, *129*, 483-487.

256. Batts, G. N.; Paul, A. J. A Study of the Competitive Adsorption of a Fluorosurfactant at the Gelatin-Air Interface Using Time-of-Flight Secondary Ion Mass Spectrometry. *Langmuir* **1994**, *10*, 218-224.

257. Hoshi, T.; Tozu, M.; Oiwa, R.; Zhanping, L.; Kudo, M. Secondary Ion Emissions from Fluorolubricants Under Several Primary Beam Conditions by ToF SIMS. *Applied Surface Science* **1997**, *121/122*, 146-151.

258. Torrisi, A.; Tuccitto, N.; Maccarrone, G.; Licciardello, A. ToF-SIMS Characterization of Fluoroalkyl Derivatives of Possible Interest as Water Repellent for Stone Protection. *Applied Surface Science* **2008**, *255*, 1527-1530.

259. Torrisi, A.; Tuccitto, N.; Licciardello, A. Effects of the substrate on ToF-SIMS spectra of thin films of some fluorinated compounds deposited on calcarenite. *International Journal of Mass Spectrometry* **2009**, *281*, 157-166.

260. Fasano, W. J.; Kennedy, G. L.; Szostek, B.; Farrar, D. G.; Ward, R. J.; Haroun, L.; Hinderliter, P. M. PEnetration of Ammonium Perfluorooctanoate Through Rat and Human Skin In Vitro. *Drug and Chemical Toxicology* **2005**, *1*, 79-90.

261. Fasano, W. J.; Carpenter, S. C.; Gannon, S. A.; Snow, T. A.; Stadler, J. C.; Kennedy, G. L.; Buck, R. C.; Korzeniowski, S. H.; Hinderliter, P. M.; Kemper, R. A. Absorption, Distribution, Metabolism, and Elimination of 8-2 Fluorotelomer Alcohol in the Rat. *Toxicological sciences : an official journal of the Society of Toxicology* **2006**, *91*, 341-355.

262. Franko, J.; Meade, B. J.; Frasch, H. F.; Barbero, A. M.; Anderson, S. E. Dermal Penetration Potential of Perfluorooctanoic Acid (PFOA) in Human and Mouse Skin. *Journal of Toxicology and Environmental Health, Part A* **2012**, *75*, 50-62.

263. Kennedy Jr., G. L. Dermal toxicity of ammonium perfluorooctanoate. *Toxicology and Applied Pharmacology* **1985**, *81*, 348-355.

264. Kudo, N.; Kawashima, Y. Toxicity and Toxicokinetics of Perfluorooctanoic Acid in Humans and Animals. *The Journal of Toxicological Sciences* **2003**, *28*, 49-57.

265. Baynes, R. E. Porcine skin flow-through diffusion cell system. *Current protocols in toxicology* **2001**, *Chapter 5*, Unit5.5.

266. AnonymousAnatomy and Physiology - 5.1: Layers of the Skin. *https://opentextbc.ca/anatomyandphysiology/chapter/5-1-layers-of-the-skin/* (accessed 08/26/19, .

267. AnonymousThe Layers of Your Skin. *https://www.aad.org/public/kids/skin/the-layers-of-your-skin* (accessed 08/26/19, .

268. Hoffman, M. The Skin (Human Anatomy): Picture, Definition, Function, and Skin Conditions. *https://www.webmd.com/skin-problems-and-treatments/picture-of-the-skin* (accessed Sep 1, 2019).

269. Baynes, R. Principles of Drug Absorption & Distribution . **2017**.

270. AnonymousHow Do Toxics Enter Our Bodies? *https://www.ursamajorvt.com/blogs/the-blog-cabin/37974081-how-do-toxics-enter-our-bodies* (accessed Sep 1, 2019).

271. Roberts, M.; Grice, J.; Hungerford, N.; Liang, X.; Liu, X. A Critical Review of Pharmacokinetic Modelling of PFOS and PFOA to Assist in Establishing HGBVs for these Chemicals. **2016**.

272. Scott, R. C.; Walker, M.; Dugard, P. H. A comparison of the in vitro permeability properties of human and some laboratory animal skins. *International journal of cosmetic science* **1986**, *8*, 189-194.

273. Shore, P. A.; Brodie, B. B.; Hogben, C. A. M. THE GASTRIC SECRETION OF DRUGS: A pH PARTITION HYPOTHESIS. *Journal of Pharmacology and Experimental Therapeutics* **1957**, *119*, 361-369.

274. Monteiro-Riviere, N. A.; Baynes, R. E.; Riviere, J. E. Animal Skin Morphology and Dermal Absorption. **2008**.

275. Bronaugh, R. L. In Vitro Percutaneous Absorption Models.

276. Bronaugh, R. L. Methods for In vitro Percutaneous Absorption. *Marzulli and Maibach s Dermatotoxicology Seventh Edition* **2001**.

277. van de Sandt, J. J. M; van Burgsteden, J. A.; Cage, S.; Carmichael, P. L.; Dick, I.; Kenyon, S.; Korinth, G.; Larese, F.; Limasset, J. C.; Maas, W. J. M.; Montomoli, L.; Nielsen, J. B.; Payan, J. -.; Robinson, E.; Sartorelli, P.; Schaller, K. H.; Wilkinson, S. C.; Williams, F. M. In vitro predictions of skin absorption of caffeine, testosterone, and benzoic acid: a multi-centre comparison study. *Regulatory Toxicology and Pharmacology* **2004**, *39*, 271-281.

278. Roux, L. N.; Brooks, J. D.; Yeatts, J. L.; Baynes, R. E. Skin absorption of six performance amines used in metalworking fluids. *Journal of Applied Toxicology* **2015**, *35*, 520-528.

279. Wester, R. C.; Maibach, H. I. Percutaneous Absorption: Short-Term Exposure, Lag Time, Multiple Exposures, Model Variations, and Absorption from Clothing. *Marzulli and Maibach s Dermatotoxicology Seventh Edition* **2008**.

280. Zhong, W.; Xing, M. M. Q.; Pan, N.; Maibach, H. I. Textiles and Human skin, Microclimate, Cutaneous Reactions: Overview. **2008**.

281. Baynes, R. E.; Brooks, J. D.; Riviere, J. E. Membrane Transport of Naphthalene and Dodecane in Jet Fuel Mixtures. *Toxicology and Industrial Health* **2000**, *16*, 225-238.

282. Baynes, R. E.; Monteiro-Riviere, N. A.; Riviere, J. E. Pyridostigmine Bromide Modulates the Dermal Disposition of [14C]Permethrin. *Toxicology and Applied Pharmacology* **2002**, *181*, 164-173.

283. Haskell, W. E.; King, S. J. NFPA 1851: Standard on Selection, Care, and Maintenance of Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting. **2014**.

284. Owen, K. NFPA 1851: How Far We've Come. *https://www.fireengineering.com/articles/print/volume-171/issue-1/supplement/nfpa-1851-how-far-we-ve-come.html* (accessed Aug 28, 2019).

285. Baxter, C. M. Firefighter Exposure: Assessing and Minimizing Dermal Risk. **2019**, 28-31.

286. AnonymousISO 105-C06: 2010, TEXTILES -- TESTS FOR COLOUR FASTNESS -- PART C06: COLOUR FASTNESS TO DOMESTIC AND COMMERCIAL LAUNDERING. **2010**.

287. AnonymousASTM E3162-18: Standard Practice Measuring the Durability of Antibacterial Agents Applied to Textiles under Simulated Home Laundering Conditions. **2018**.

288. AnonymousAATCC LP1-2018, Home Laundering: Machine Washing. **2018**.

289. Deng, B.; Cai, R.; Yu, Y.; Jiang, H.; Wang, C.; Li, J.; Li, L.; Yu, M.; Li, J.; Xie, L.; Huang, Q.; Fan, C. Laundering Durability of Superhydrophobic Cotton Fabric. *Advanced Materials* **2010**, *22*, 5473-5477.

290. Arunyadej, S.; Mitchell, R.; Walton, J.; Carr, C. M. An Investigation into the Effect of Laundering on the Repellency Behaviour of a Fluorochemical-treated Cotton Fabric. *The Journal of The Textile Institute* **1998**, *89*, 696-702.

291. Shaw, A.; Lin, Y. Effects of Laundering upon the Removal of Atrazine and Metolachlor from Cotton, Cotton/Polyester, and Polyester Fabrics Treated with Fluorochemical Finishes. *Bull. Environ. Contam. Toxicol* **2001**, *66*, 319-325.

292. Rhee, H.; Young, R. A.; Sarmadi, A. M. The Effect of Functional Finishes and Laundering on Textile Materials Part I: Surface Characteristics. *The Journal of The Textile Institute* **1993**, *84*, 394-405.

293. Midha, V.; Vashisht, R.; Midha, V. Durability of fluoropolymer and antibacterial finishes on woven surgical gown fabrics. *Fashion and Textiles* **2014**, *1*, 1-12.

294. Juhué, D.; Gayon, A. C.; Corpart, J. M. Washing Durability of Cotton Coated with a Fluorinated Resin: An AFM, XPS, and Low Frequency Mechanical Spectroscopy Study. *Textile Research Journal* **2002**, *72*, 832-843.

295. Supreeyasunthorn, P.; Boontanon, S. K.; Boontanon, N. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) contamination from textiles. *Journal of Environmental Science and Health, Part A* **2016**, *51*, 472-477.

296. Bradham, A.; Thompson, D.; Barker, R.; Morton-Aslanis, J.; Deaton, A. S. A Method for Determining Off-Gassing of Protective Fabrics Exposed to Simulated Flash Fire Conditions. *AATCC Review* **2013**, 57-65.

297. Budnik, L. T.; Austel, N.; Gadau, S.; Kloth, S.; Schubert, J.; Jungnickel, H.; Luch, A. Experimental outgassing of toxic chemicals to simulate the characteristics of hazards tainting globally shipped products. *PloS one* **2017**, *12*, e0177363.

298. Fent, K. W.; Eisenberg, J.; Snawder, J.; Sammons, D.; Pleil, J. D.; Stiegel, M. A.; Mueller, C.; Horn, G. P.; Dalton, J. Systemic Exposure to PAHs and Benzene in Firefighters Suppressing Controlled Structure Fires. *The Annals of occupational hygiene* **2014**, *58*, 830-845.

299. Fent, K. W.; Evans, D. E.; Booher, D.; Pleil, J. D.; Stiegel, M. A.; Horn, G. P.; Dalton, J. Volatile Organic Compounds Off-gassing from Firefighters' Personal Protective Equipment Ensembles after Use. *Journal of Occupational and Environmental Hygiene* **2015**, *12*, 404-414.

300. Kirk, K. M.; Logan, M. B. Structural Fire Fighting Ensembles: Accumulation and Off-gassing of Combustion Products. *Journal of Occupational and Environmental Hygiene* **2015**, *12*, 376-383.

301. Kallonen, R.; von Wright, A.; Tikkanen, L.; Kastia, K. THE TOXICITY OF FIRE EFFLUENTS FROM TEXTILES AND UPHOLSTERY MATERIALS. *Journal of Fire Sciences* **1985**, *3*, 145-160.

302. Svedberg, U.; Johanson, G. Work Inside Ocean Freight Containers—Personal Exposure to Off-Gassing Chemicals. *The Annals of occupational hygiene* **2013**, *57*, 1128-1137.

303. Shinde, A.; Ormond, R. B. Analysis of Fireground Contaminants from Retired Firefighting Turnout Jackets using Headspace sampling-Gas Chromatogrpahy- Mass Spectrometry (HS-GC-MS). *Fire and Materials Journal* **In Print**.

304. Zabaleta, I.; Negreira, N.; Bizkarguenaga, E.; Prieto, A.; Covaci, A.; Zuloaga, O. Screening and identification of per- and polyfluoroalkyl substances in microwave popcorn bags. *Food Chemistry* **2017**, *230*, 497-506.

305. Moreta, C.; Tena, M. T. Determination of perfluorinated alkyl acids in corn, popcorn and popcorn bags before and after cooking by focused ultrasound solid–liquid extraction, liquid chromatography and quadrupole-time of flight mass spectrometry. *Journal of Chromatography A* **2014**, *1355*, 211-218.

306. Rosati, J. A.; Krebs, K. A.; Liu, X. Emissions from Cooking Microwave Popcorn. *Critical Reviews in Food Science and Nutrition* **2007**, *47*, 701-709.

307. Sinclair, E.; Kim, S. K.; Akinleye, H. B.; Kannan, K. Quantitation of Gas-Phase Perfluoroalkyl Surfactants and Fluorotelomer Alcohols Released from Nonstick Cookware and Microwave Popcorn Bags. *Environmental science & technology* **2007**, *41*, 1180-1185.

308. Watanabe, N.; Takemine, S.; Yamamoto, K.; Haga, Y.; Takata, M. Residual organic fluorinated compounds from thermal treatment of PFOA, PFHxA and PFOS adsorbed onto granular activated carbon (GAC). *J Mater Cycles Waste Manag* **2016**, *18*, 625-630.

309. Horn, G. P.; Kesler, R. M.; Kerber, S.; Fent, K. W.; Schroeder, T. J.; Scott, W. S.; Fehling, P. C.; Fernhall, B.; Smith, D. L. Thermal response to firefighting activities in residential structure fires: impact of job assignment and suppression tactic. *Ergonomics* **2018**, *61*, 404-419.

310. Henry, B. J.; Carlin, J. P.; Hammerschmidt, J. A.; Buck, R. C.; Buxton, L. W.; Fiedler, H.; Seed, J.; Hernandez, O. A critical review of the application of polymer of low concern and regulatory criteria to fluoropolymers. *Integrated Environmental Assessment and Management* **2018**, *14*, 316-334.

311. Arrieta, C.; David, É; Dolez, P.; Vu-Khanh, T. Hydrolytic and Photochemical Aging Studies of a Kevlar(R)-PBI Blend. *Polymer Degradation and Stability* **2011**, *96*, 1411-1419.

312. Davis, R.; Chin, J.; Lin, C.; Petit, S. Accelerated weathering of polyaramid and polybenzimidazole firefighter protective clothing fabrics. *Polymer Degradation and Stability* **2010**, *95*, 1642-1654.

313. Nazaré, S.; Davis, R. D.; Peng, J.; Chin, J. NIST Technical Note 1746: Accelerated Weathering of Firefighter Protective Clothing: Delineating the Impact of Thermal, Moisture, and Ultraviolet Light Exposures. **2012**.

314. Aidani, R. E.; Nguyen-Tri, P.; Malajati, Y.; Lara, J.; Vu-Khanh, T. Photochemical aging of an e-PTFE/NOMEX.sup.[R] membrane used in firefighter protective clothing. *Polymer Degradation and Stability* **2013**, *98*.

315. Bentel, M. J.; Yu, Y.; Xu, L.; Li, Z.; Wong, B. M.; Men, Y.; Liu, J. Defluorination of Per- and Polyfluoroalkyl Substances (PFASs) with Hydrated Electrons: Structural Dependence and Implications to PFAS Remediation and Management. *Environmental science & technology* **2019**, *53*, 3718-3728.

316. Anumol, T.; Dagnino, S.; Vandervort, D. R.; Snyder, S. A. Transformation of Polyfluorinated compounds in natural waters by advanced oxidation processes. *Chemosphere* **2016**, *144*, 1780-1787.

317. Gomez-Ruiz Beatriz; Ribao, P.; Diban, N.; Rivero, M. J.; Ortiz, I.; Urtiaga, A. Photocatalytic degradation and mineralization of perfluorooctanoic acid (PFOA) using a composite TiO2-rGO catalyst. *Journal of Hazardous Materials* **2018**, *344*, 950-957.

318. Rahman, M. F.; Peldszus, S.; Anderson, W. B. Behaviour and fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in drinking water treatment: A review. *Water Research* **2014**, *50*, 318-340.

319. Luedtke, E. R. Drying and Curing of Coated and Laminated Fabrics. *Journal of Industrial Textiles* **1997**, *27*, 115-125.

320. Franko, J.; Meade, B. J.; Frasch, H. F.; Barbero, A. M.; Anderson, S. E. Dermal Penetration Potential of Perfluorooctanoic Acid (PFOA) in Human and Mouse Skin. *Journal of Toxicology and Environmental Health, Part A* **2012**, *75*, 50-62.

321. AnonymousSafety Components. http://www.safetycomponents.com/.

322. AnonymousMillapore Sigma. https://www.sigmaaldrich.com/united-states.html.

323. AnonymousFisher Scientific. https://www.fishersci.com/us/en/home.html.

324. AnonymousThermo Scientific. https://www.thermofisher.com/us/en/home.html.

325. AnonymousWaters . https://www.waters.com/nextgen/us/en.html.

326. AnonymousAOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals. **2002**.

327. C. Diane The REAL Cancer is in Your Gear. https://www.firefighterclosecalls.com/the-real-cancer-in-your-gear/ (accessed May 10, 2018).

328. Gremmel, C.; Frömel, T.; Knepper, T. P. Systematic determination of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in outdoor jackets. *Chemosphere* **2016**, *160*, 173-180.

329. Hanssen, L.; Herzke, D. Investigation of outdoor textiles and gear with respect to determine the content of ionic perfluorinated substances (PFASs). Evaluation of results. **2015**http://hdl.handle.net/11250/2383338.

330. Rotander, A.; Toms, L. L.; Aylward, L.; Kay, M.; Mueller, J. F. Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF). *Environment international* **2015**, *82*, 28-34.

331. Anonymous *Second Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 2 (2009-2011);* Health Canada: 2013; .

332. Aylward, L. L.; Green, E.; Porta, M.; Toms, L.; Den Hond, E.; Schulz, C.; Gasull, M.; Pumarega, J.; Conrad, A.; Kolossa-Gehring, M.; Schoeters, G.; Mueller, J. F. Population variation in biomonitoring data for persistent organic pollutants (POPs): An examination of multiple population-based datasets for application to Australian pooled biomonitoring data. *Environment International* **2014**, *68*, 127-138.

333. Rossi René, M.; Song Guowen; Mandal Sumit. Development of High Performance Thermal Protective Clothing. In *Thermal Protective Clothing for Firefighters*Elsevier: 2017; pp 1.

334. Watkins, S. M., 1943- Clothing : the portable environment. **1995**https://catalog.hathitrust.org/Record/100962713?signon=swle:urn:mace:incommon:ncsu.edu.

335. FINAL REPORT BY: R. Bryan Ormond; William J. Gabler Non-Destructive Assessment of Outer Shell Degradation for Firefighter Turnouts. **2017**.

336. Aidani, R. E.; Nguyen-Tri, P.; Malajati, Y.; Lara, J.; Vu-Khanh, T. Polymer Degradation and Stability: Photochemical aging of an e-PTFE/Nomex membrane used in firefighter protective clothing. *Cellular Polymers* **2013**, *32*, 384.

337. Anumol, T.; Dagnino, S.; Vandervort, D. R.; Snyder, S. A. Transformation of Polyfluorinated compounds in natural waters by advanced oxidation processes. *Chemosphere* **2016**, *144*, 1780-1787.

338. Arunyadej, S.; Mitchell, R.; Walton, J.; Carr, C. M. An Investigation into the Effect of Laundering on the Repellency Behaviour of a Fluorochemical-treated Cotton Fabric. *The Journal of The Textile Institute* **1998**, *89*, 696-702.

339. Brooks, J. R. Durability of Water-Repellent Finishes to Accelerated Laundering, University of Rhode Island, 2015.

340. AnonymousISO 6530: Protective Clothing - Protection against liquid chemicals - Test Method for Resistance of Materials to Penetration by Liquids. **2005**.

341. AnonymousAtlas-Metek. *https://www.atlas-mts.com/*.

342. Davis, R.; Chin, J.; Lin, C.; Petit, S. Accelerated weathering of polyaramid and polybenzimidazole firefighter protective clothing fabrics. *Polymer Degradation and Stability* **2010**, *95*, 1642-1654.

343. Song, G. Thermal protective clothing for firefighters. **2017**https://proxying.lib.ncsu.edu/index.php?url=https://www.sciencedirect.com/science/book/9780081012857
https://proxying.lib.ncsu.edu/index.php?url=https://app.knovel.com/web/toc.v/cid:kpTPCF0011/viewerType:toc/root_slug:thermal-protective-clothing?kpromoter=federation.

344. Watkins, S. M., 1943- Clothing : the portable environment. **1995**https://catalog.hathitrust.org/Record/100962713?signon=swle:urn:mace:incommon:ncsu.edu.

345. AnonymousPBI Max. *https://pbiproducts.com/fabrics/product/pbi-max/*.

346. Environmental Protection Agency Risk Assessment. *https://www.epa.gov/risk/about-risk-assessment* (accessed 05/07/, 2020).