ABSTRACT

PROBERT, CHANDLER AUSTIN. Development of a Contact Transfer Test Method for Firefighter Turnout Gear Contaminants (Under the direction of Dr. R. Bryan Ormond).

In their line of work, firefighters are exposed to thermal and chemical hazards far more frequently than their first responder counterparts. Repeated exposures to combustion byproducts, can have serious health implications. Studies have shown that firefighters are 1.3 - 2.0 times more likely for various types of cancer, including skin, prostate, and testicular cancers. As of 2010 the International Agency for Research on Cancer deemed firefighting as "possibly carcinogenic". With more studies establishing a clearer link between firefighting and cancer, awareness of the chemical hazards within the fire service industry has ironically spread like wildfire. New focus has been shifted towards characterizing, monitoring, mitigating, and preventing firefighter's exposure to hazardous contaminants.

Firefighter turnout gear is designed to protect against thermal hazards, some even say it over protects. However, turnout gear is not designed to protect against the carcinogenic vapors and particulate matter. Firefighter exposure studies have found carcinogenic chemicals like asbestos, benzene, benzo[a]pyrene, formaldehyde, polycyclic aromatic hydrocarbons (PAHs), phenols and phthalates present at fire scenes. The two primary exposure pathways firefighters must protect against are inhalation and dermal absorption of combustion byproducts. Concerns about exposure through the inhalation pathway can be minimized because firefighter are mandated to wear self-contained breathing apparatuses (SCBA) as a piece of their turnout ensemble. When all the proper personal protective equipment (PPE) is worn dermal exposure becomes the primary route of exposure. A study done at RTI discovered the interfaces of turnout gear allow openings for particles to deposit directly on the skin. Wipe samples collected after fire training exercises and

fire exposure confirms the presence of fireground contaminants on the skin in the susceptible interface areas of the turnout gear such as the hands, neck and calves.

While doffing their equipment and gear, firefighters instinctively remove their gloves first and remove the remainder of their gear with bare hands. Handling contaminated firefighter gear without protection increases firefighters' risk of dermal absorption for any hazardous chemicals that may have been deposited on the gear or equipment. Even though there are several studies on firefighters' exposure to combustion products there is minimal research on the transfer rate of fire scene contaminants from turnout gear to human skin.

This research aims to develop a contact transfer test method to examine the transfer of PAHs from firefighter turnout gear to the skin. SynDaver™ skin had comparable rates of absorption and increased permeability for naphthalene compared to porcine skin and was used as a human skin surrogate in the contact transfer test method. Pressurized solvent extraction and high-performance liquid chromatography analysis methods were optimized to create a reliable method to recover the PAH compounds from all materials used in the contact transfer test method. Early contact transfer test method trials found that liquid PAHs were unlikely to transfer from outer shell material to the skin. Although minimal amounts of PAHs transferred, modifications to the contact transfer test method such as using particulate PAHs and implementing dynamic motion to simulate frictional forces would create a better representation of the transfer of chemicals when firefighters handle contaminated gear.

Development of a Contact Transfer Test Method for Firefighter Turnout Gear Contaminants

by
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DEDICATION

I would like to dedicate this work to my parents, Margie and Charlie Probert, who have continuously supported my efforts towards obtaining a degree in higher education. The life lessons my parents have taught me have given me the ability to face the stresses and workload of graduate school. My mother taught me the importance of discipline. Although some days are better than others my mother has always worked hard day after day to provide for her family. Her work ethic has shown me that you cannot reach the top of a mountain without taking many small steps in the right direction. In order to reach your highest aspirations continuous progress no matter how small will ultimately help you reach the mountain peak. My father has taught me to think big to see the entire picture but to also think small and understand how and why things work the way they do. Growing up I always thought my father was the smartest person in the world. Being knowledgeable in numerous subjects he would always have an answer for every question I had but was also to explain why or how things worked in terms I could understand. Because of his vast understanding across numerous subjects he has inspired me to learn much as I can every day. Throughout my life my parents have provided me the tools and resources needed for me to succeed and for that I am truly grateful and dedicate this work to them.

BIOGRAPHY

Chandler Probert was brought home on the hottest day of the summer of 1995 to his parents, Margie and Charlie Probert. Throughout his childhood, he was a natural athlete and competitor, striving to be the best at baseball, football, soccer, track and field, swimming, and basketball. After taking his first steps on the red brick of North Carolina State University he embarked on a journey to explore new opportunities, make new friends and learn. During his undergraduate career Chandler participated in several intramural sports, served as vice president of Phi Sigma Pi National Honor Fraternity, and mentored a group of freshman in the Exploratory Studies Village. At the end of his undergraduate career and four years of lectures, projects, and homework, Chandler was still unsure what to do next. It wasn't until he started graduate school and began working on firefighters' occupational chemical exposures, did Chandler discover a field that would challenge him to critically think, experiment new methodology and have an opportunity to help those who serve their communities.

ACKNOWLEDGMENTS

I would like to acknowledge and thank the Federal Emergency Management Agency for the funding for me to conduct this research. I would also like to thank Dr. R. Bryan Ormond for acting as my committee head, advisor, and mentor to me. Without the encouragement, inspiration, and guidance my graduate school career would not have been the same. Finally, I would like to acknowledge and thank Dr. Thompson, Dr. Barker, and Dr. Baynes for their guidance and teachings throughout my master's degree.

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LIST OF ABBREVIATIONS AND DEFINITIONS

- Bioavailability – the proportion of a drug or other substance which enters the circulation when introduced into the body and is able to have an active effect.
- Bunker gear or Turnout Gear – firefighter's full protective ensemble, which includes coat, pants, protective hood, boots, gloves, SCBA, helmet, and PASS device
- Carcinogen – a substance capable of causing cancer in living tissue
- CDC – Center for Disease Control and Prevention
- Contaminant – a polluting or toxic substance that makes something impure
- Coke – a fuel source with high carbon content made by a destructive distillation process
- DNA Adduct – a segment of DNA bound to a cancer-causing chemical
- Dosing Vehicle - the media that a chemical is in when it comes into contact with skin
- IAFF – International Association of Fire Fighters
- mCi/mmol – a unit of measure describing the radioactivity of a substance based on disintegrations per second or minute
- NFPA – National Fire Protection Association
- Oncotic pressure – another term for "osmotic pressure," specifically generated by colloids and proteins in blood capillaries
- OSHA – Occupational Safety and Health Administration
- Overhaul – a post-fire process where firefighters search areas in the home where residual fire could still be burning and evaluate the source for reignition.
- PAH(s) – Polycyclic Aromatic Hydrocarbon(s)
- SCBA – Self-Contained Breathing Apparatus
- Skin Surrogate – a material used to mimic the physical or chemical properties of human skin

- VO_2 Max – Maximum consumption rate of oxygen

Chapter 1: Purpose of Research and Research Objectives

1.1. Purpose of Research

Firefighting is a hazardous occupation where individuals volunteer to put themselves in life-threatening scenarios to serve and protect the people of their communities. In 2017 the United States Fire Administration reported 33 career, 48 volunteer, and 6 wildland firefighters died in the line of duty [1]. In addition to the line of duty fatalities, chronic exposure to toxic combustion products like polycyclic aromatic hydrocarbons (PAHs) has established cancer as the leading cause of death for firefighters [2]. Several health studies on firefighters have repeatedly found increased incident rates of esophageal, kidney, prostate, brain, and colon cancer [3] [4] [5] [6]. Other cancers and diseases such as respiratory cancer, testicular cancer, breast cancer, multiple myeloma, and Non-Hodgkin's lymphoma have been reported to have increased incidence in at least one firefighter health study [3, 7, 4, 6, 5, 8].

Chronic exposure to toxic combustion products during fire response, post-fire activities, and exposures at the fire station all attribute to firefighters increased risk of cancer. While responding to fire emergencies, firefighters get covered in soot and are exposed to combustion products created from burning materials such as wood, furniture, insulation, plastics, and other synthetic products. The most apparent combustion products are soot and ash because of the dark black and gray colors as well as their abundance at a fire scene. Soot particles can range from 2.5 – 10 micrometers in diameter. These soot particles pose serious health risks. Smaller particles ($\leq$ 2.5 μm) can reach the lower depths of the lungs if inhaled or absorbed into the body through the skin [9]. Furthermore, these soot particles act as vehicles for the toxic combustion chemicals produced during the fire which may adsorb onto the surface of these particulates. Toxic chemicals such as polycyclic aromatic hydrocarbons (PAHs), carbon monoxide (CO), hydrogen

cyanide (HCN), nitrogen dioxide (NO_2), formaldehyde, and several other organic and inorganic compounds have been found in smoke and soot at fire scenes. Moreover, the previously mentioned compounds have been found on the skin or equipment of structural firefighters [10], [11], [12], [13]. Other contaminants such as plasticizers and flame retardants commonly added to household products have been found on the skin or gear of firefighters after exposure to a structural fire [14], [15]. Unfortunately, the threat of exposure to toxic chemicals is inevitable for firefighters.

A firefighter's amount of contamination after leaving the scene is dependent on their assigned duty, whether it be fire attack, search and rescue, overhaul, etc. [10]. Firefighters tasked with fire attack may have to crawl on the floor to maneuver through the structure or move burning materials with their hands. The movement of charred materials can send soot and particulate matter into the air, increasing contamination and risk of exposure. After fire response, firefighters can again be exposed to any chemicals in the soot when they remove their contaminated turnout gear. Upon extinguishing a fire, firefighters may remove their gear to relieve the burden of the extra weight, despite the continued threat of exposure from off-gassing chemicals and particulates. Highly volatile compounds evaporate off the turnout gear because of their low boiling points and can enter the lungs if firefighters have removed their self-contained breathing apparatus (SCBA). It is recommended that the SCBA be worn throughout post-fire activities such as overhaul [16].

When removing their turnout gear, firefighters instinctively remove their gloves first to increase their dexterity and have greater precision with their bare hands. When handling contaminated turnout gear the chemicals may transfer to the skin of firefighters and be absorbed into the body, thus increasing their chemical exposure. To illustrate the potential of cross-

contamination of chemicals that an individual can spread to themselves, other firefighters, gear, and equipment, firefighters have used shaving cream in training exercises [17].

Luckily as of recent years, firefighters' awareness of their occupational exposures has led to improved decontamination procedures and standards. As a result of firefighters' increased awareness of particulate matter and combustion products, more significant efforts are being made to reduce their exposure to contaminants. Gross decontamination is a practice that is becoming more common across fire departments across the world. The objective of on-scene gross decontamination is to remove as many contaminants from the gear and equipment before doffing the gear and returning to the fire station ultimately minimizing cross-contamination. Unfortunately, not all firefighters follow these decontamination protocols nor feel confident in their ability to clean their gear properly [18].

Currently, there is an insufficient amount of literature available on the transfer of fireground contaminants to the skin and the absorption rate of those contaminants. Few to not studies have investigated the threat of post-fire dermal exposures when firefighters come into contact with contaminated turnout gear. The scope of this research is to develop a contact transfer test method for fireground contaminants to assess the threat of dermal absorption when firefighters come into contact with or handle contaminated gear.

1.2. Research Objectives

1.2.1. Development of Analytical Methods for Contact Transfer Test Method

There are extraction methods in the literature that have been utilized for PAH extraction. These extraction methods include Soxhlet extraction, liquid extraction, pressurized solvent extraction, microwave extraction, and a few others. Soxhlet extraction remains the gold standard for PAH extraction but is rather time intensive and tedious. Alternative extraction methods such

as pressurized solvent extraction have been shown to obtain similar extraction results and are significantly less time-intensive [19]. This type of extractor is currently available in the Textile Protection and Comfort Center's Chemical Protection and Analytical Laboratory, saving cost and time searching for an extractor.

Extraction samples will then be separated and analyzed using high-performance liquid chromatography (HPLC). HPLC is an analytical method used for separating chemicals found in a mixture. The chemicals are separated based on their affinity of a stationary phase, which can be either polar or non-polar, in this work I will be using a non-polar PAH Zorbax Eclipse column from Agilent Technologies. HPLC will be used to separate, identify, and quantify the 16 PAH mixture that will be used to contaminated lab samples. Additionally, this analytical technique can be used to quantify PAH contamination on used firefighter gear.

1.2.2. Comparison of Dermal Absorption Properties of Skin Surrogate to Human Skin

A proper skin surrogate needs to be specified and employed to create a contact transfer test method for fireground contaminants that realistically represents the percutaneous absorption properties of human skin. Ideally, the skin surrogate would have an absorption rate of polycyclic aromatic hydrocarbons that is comparable to actual skin. If the absorption is similar, the skin surrogate could be used to evaluate the absorption of any fireground chemical that transfers from the outer shell of turnout gear. There are several materials used to represent frictional, mechanical, thermal properties, and other properties of human skin, however, few studies compare the absorption rates of these materials. SynDaver™ tissue plates, one of the candidate surrogates for this study, are designed to mimic many of human skin's physical properties and

therefore, have been used for intradermal injection training for nurses and other medical professions.

This skin surrogate will be tested to determine if it is suitable for use in the contact transfer test method. *In vitro* flow-through diffusion cell systems are frequently used to measure the diffusion or penetration capability of drug formulations, toxic chemicals, or mixtures through a membrane, substrate, or skin [20]. These systems use several types of skin for testing including, human, monkey, rat, and porcine/pig skin. Pig skin is the most similar to human skin and relatively accessible. Human skin can be challenging to obtain as it can only be obtained from a cadaver or donated from cosmetic surgeries. For these reasons porcine skin will be used in the flow-through diffusion cell experiments.

1.2.3. Development of Contact Transfer Test Methods

Firefighter turnout gear is contaminated with PAHs, semi-volatile organic compounds, fire retardants, plasticizers, and several other contaminants. Upon exiting the fire scene, firefighters are still at risk of absorbing these chemicals into their body. When doffing their personal protection equipment and turnout gear the soot and particulate matter can transfer to their bodies. When doffing their personal protective equipment (PPE) and turnout gear, the soot and particulate matter can transfer to their skin. The soot and particulate matter at fire scenes act as vehicles for toxic combustion products such as PAHs. Using field-contaminated turnout gear and a skin surrogate, the transfer of fireground contaminants can be simulated in a lab. Ultimately, this contact transfer test method will simulate the contact made when firefighters remove their gear after firefighting activities. This test method will also serve to evaluate the potential threat of contaminants transferring from their turnout gear to their skin.

1.3. Research Questions

1. Do PAHs easily transfer from the outer shell of turnout gear material to human skin?
2. If any PAHs do transfer to the skin, what amount of PAH transferred to human skin penetrates the skin?
3. Are there any currently available skin surrogates that can be used to simulate dermal absorption of human skin?

1.4. Limitations

Limitations of this work include:

1. Skin surrogates do not have identical dermal absorption properties as human skin. For example, pig skin and polymer-based surrogates can overestimate the dermal absorption of a chemical. The skin surrogates used in this work were selected based on price, availability, and reproducibility.
2. Turnout gear used in this work was donated from local fire station and may not have equivalent amounts of exposure to fire conditions.
3. Laboratory contamination methods may not represent structural fire exposures in the field.

Chapter 2: Review of Literature

2.1. Occupational Firefighting

Firefighting is a well-respected career where professional and volunteer firefighters put themselves in life-threatening situations to serve and protect the general public. Firefighters are one of the first responders to any 911 emergency. Typical duties include extinguishing or controlling fires, personnel rescue, minimizing property damage, vehicle crash response, medical treatment, hazardous material removal or clean up, and conducting fire inspections. It is well known that firefighting is a dangerous occupation, and every year there are about 100 line-of-duty deaths [1]. In addition to the line-of-duty dangers, firefighters are routinely exposed to combustion products while extinguishing fires. Chronic exposure to combustion products has been linked to increased cancer incidence [21]. Cancer is now the leading cause of death for firefighters. Several health studies indicate that firefighters can be two times more likely to develop various types of cancers such as mesothelioma and prostate cancer than the general public [3] [4] [5] [6]. In 2018, Congress passed the Firefighter Cancer Registry Act which requires the CDC to develop and maintain a voluntary registry of firefighters to collect history and occupational information that can be used to determine the incidence of cancer among firefighters [22].

2.1.1. Current Personal Protective Equipment and Ensembles

The earliest known firefighters were groups of individuals equipped with buckets and axes who served to protect the city of Rome in 60 A.D. These individuals had no form of protection compared to modern-day firefighters. The firefighting methods of these early firemen were extremely simplistic, forming bucket brigades to pour water on the fire. When the fire

would become too dangerous, they would use hooks and tools to tear down the structure to prevent the spread of the fire [23].

The equipment and gear modern firefighters wear are vastly different compared to the tools and equipment used by the earliest firefighters. Modern firefighters have several layers of protection from the hazards they encounter during a fire. As technology and new materials were developed firefighters have been able to carry out their responsibilities in much safer ways.

Current firefighter protective ensembles include a helmet, face mask, turnout coat, turnout trousers, protective hood, boots, gloves, a self-contained breathing apparatus (SCBA), and a personal alert safety system (PASS) device, seen in Figure 1. The current firefighting helmet is an advancement of the early traditional helmet designed by Henry T. Gratacap [24]. Gratacap's design was a reinforced dome-shaped leather helmet with a front shield and brim to protect firefighters from falling debris. The addition of the facemask and SCBA did not come around until 1863, when James Braidwood used canvas, rubber, and hosing to create his first prototype [24]. This early design was reinvented many times leading to the development of the modern SCBA with an air tank to provide clean air to breathe while inside a burning structure.

Figure 1: Diagram of Firefighter Turnout Gear [25]

Early turnout gear and boots were made of rubber to protect against the heat and falling embers and lined with cotton or wool to reduce the thermal hazard. However, the rubber material limited the range of motion and lacked thermal comfort [24]. The PASS device was added to firefighter's turnout gear in the 1980s to help signal when a firefighter became unconscious, injured, or unable to move [24]. When a firefighter remains still for too long, a loud alarm goes off to signal that they could be in trouble. The particulate-blocking protective hood has been the most recent addition to the firefighting turnout ensemble, as it was introduced in late 2015. These hoods started as knit hoods to provide extra protection against thermal hazards and the dermal deposition of soot and particulates.

In 2015, the International Association of Fire Fighters (IAFF) commissioned experiments to evaluate particle penetration of firefighter ensembles [26]. Full turnout gear worn in conjunction with an SCBA was donned by a test subject and placed in a chamber filled with

particulates. The test subject was exposed to high-level concentration of silica powder particles tagged with fluorescent tracers having particle sizes ranging from 0.1 – 10 μm [26]. After exposure, the test subject was photographed with ultraviolet lights to observe particle contamination on the skin. Upon photographic analysis, depicted in Figure 2, a significant amount of particle contamination was seen around the neck, wrist, thigh, and calf areas. These contaminated areas of the body correspond to interfaces between the different pieces of the turnout ensemble. Figure 2 picture B, shows a clear line where the SCBA would sit while being worn. The face has little to no particle contamination, which reinforces the protection an SCBA provides while being worn, indicating that dermal exposure is the most popular pathway for combustion products and other fire scene contaminants. This study demonstrated the need for change to increase protection against chemical exposure and particulate deposition for firefighters. Subsequently, traditional hoods were redesigned to implement a particulate-blocking layer, and particulate-blocking turnout ensembles were developed [27].

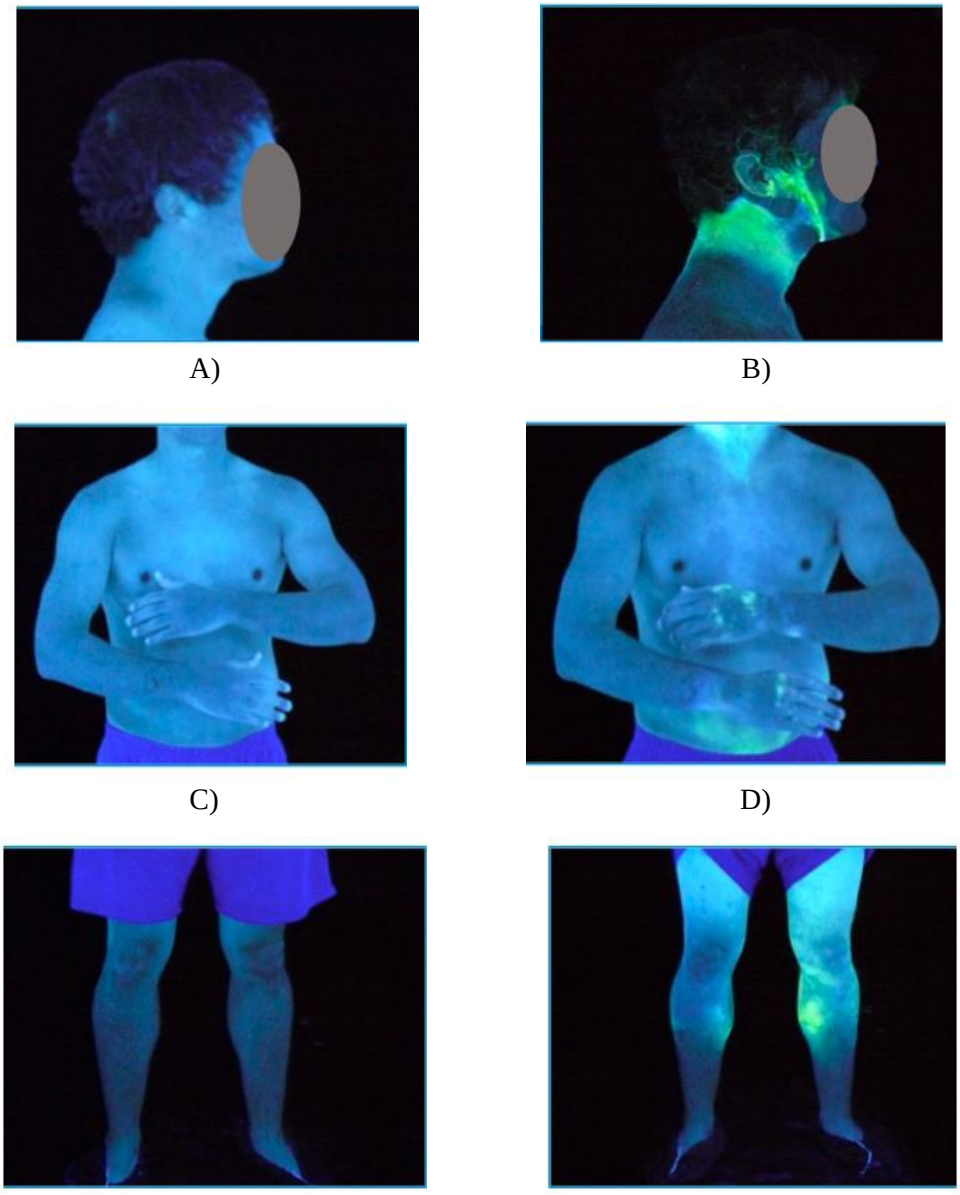


Figure 2: Fluorescent particle deposition on the skin after fluorescent particle exposure while wearing full turnout gear [26]. Part A and B show the before and after of the head area. Part C and D show the before and after of the torso area. Part E and F show the before and after of the lower body area.

To ensure the equipment provides sufficient protection, turnout gear and equipment must pass performance requirements set by the National Fire Protection Association (NFPA). To meet NFPA certifications modern turnout gear is required by NFPA 1971 to have three layers: an outer shell, moisture barrier and thermal liner [28]. Figure 3 depicts the arrangement of

materials, with the outer shell on top facing the environment, the thermal liner closest to the skin, and the moisture barrier between these two layers. Each layer of the turnout ensemble has a specific function; the thermal liner provides insulation and thermal protection, the moisture barrier prevents moisture from penetrating the ensemble preventing burns, and the outer shell protects against cuts, abrasions, liquid penetration and flame resistant. The outer shell is commonly made out of polybenzimidazole (PBI), Nomex®, Kevlar®, or a combination of these materials. The moisture barrier contains either a woven or nonwoven fabric with a polytetrafluoroethylene (PTFE)-based membrane film laminated onto fabric. The thermal liner itself has two layers, the face cloth, which is closest to the skin, and a batting, closest to the moisture barrier.

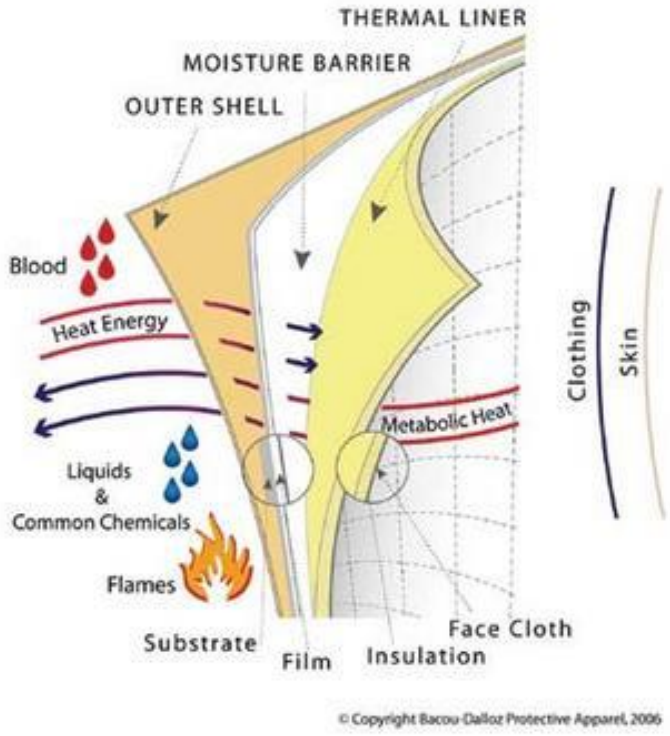


Figure 3: Diagram of the layers in turnout gear coats and pants [29]

2.1.2. Firefighter Exposures

The primary exposure hazards that firefighters frequently encounter are thermal and chemical exposures. Fires have three ways of transferring heat to their environment and the firefighter that is battling them. The transfer of heat can occur by conduction, convection, and radiation. Conduction is heat transfer within solids or between contacting solids, convection is heat transfer by the movement of liquids or gases, and radiation is heat transfer by electromagnetic waves [30]. Fires can be broken down into three stages: growth, fully developed, and declining. During the growth stage the rate of heat released from the fire increases as the fire spreads and burns more fuel. The fire grows until it reaches the fully developed stage where flashover is likely to occur. Flashover transpires when flammable sources like gases or smoke instantaneously ignite and the fire spreads rapidly across the ceiling or floor [31]. Flashover conditions can be seen in Figure 4. Firefighters are extremely cautious when there is a threat of flashover as it is the most dangerous stage of a fire, and temperatures can exceed 600°C [30], [31]. The final stage, declining, occurs when fuel sources begin to deplete and heat output decreases. The intensity and threat of the fire decline ultimately extinguishing itself.

A study conducted by Underwriter Laboratories in 2005 compared the effects of materials found in modern and legacy homes on the time for a room to reach flashover conditions [32]. Both rooms were filled with a sofa, a throw, lamp and shade, books or magazines, coffee table, TV, and toys. The objects in the modern room were made from synthetic materials such as polyurethane, polyester, or other plastics. Conversely, the objects in the legacy room were made from natural resources like cotton or wood. Both rooms were ignited by placing a lit candle on the right side of the sofa and monitored until flashover conditions were met. The modern room reached flashover in 3.5 minutes while the legacy room reached flashover

in 29.5 minutes [32]. The findings in this study show that materials inside a structure or home can greatly impact the growth of a fire. Modern-day fires burn roughly eight times faster and as a result produce greater amounts of combustion products that increase firefighters' chemical exposure.

Figure 4: Images from a time-lapse demonstrating the time to reach flashover conditions of a room filled with legacy vs modern room furnishings [32]

Luckily, modern turnout gear is designed to provide ample protection against extreme temperatures. If certified by the NFPA, the material composite is rated to provide approximately seventeen and a half seconds of protection until a second-degree burn occurs in flashover conditions [33]. This amount of protection is often more than sufficient as flashover conditions last about 7-10 seconds [34]. The materials and gear used by modern firefighters provide more than adequate protection against thermal hazards. However, there is a tradeoff to the thermal protection provided by the turnout gear. In addition to the extraordinary stresses put on the body during fire response activities, the gear is heavy, provides insufficient heat loss, and restricts movement, thus, resulting in dangerously high core body temperatures and elevated heart rates. Firefighters' core temperature and heart rates have been recorded as high as nearly 40°C and 200 bpm, respectively [35]. Elevated core temperatures and increased heart rates are natural physiological responses of increased physical activity and if elevated for an extended period, it can be fatal.

The threat of chemical exposure and the long-term effects are far less understood. Previously it was seen as a badge of honor to wear dirty turnout gear, as it displayed an individual's masculinity and experience. Unfortunately, it is now known that dirty turnout gear may contain numerous toxic chemicals, resulting in adverse health effects if worn throughout a firefighter's career. It has only been due to the recent increase of cancer incidence in the fire service industry that researchers have begun investigating firefighter's occupational exposure during an active-fire response, post-fire activities, and exposures at the fire station. A single fire can produce abundant amounts of smoke and soot, as well as thousands of combustion products, some of which are carcinogenic, as a result of the incomplete pyrolysis during a fire.

Common materials in modern homes include wood, furniture upholstery, insulation, plastics, paints, adhesives, electronics, cleaning products and synthetic products. When these materials are burned they produce various toxic combustion products, seen in Table 1. Conventional combustion products include: PAHs, HCN, CO, HCl, NO_2, SO_2, and many other organic and inorganic compounds [10], [11], [12], [13].

Table 1: List of toxic combustion products produced from the burning of common materials found in modern homes

Household Applications	Materials	Combustion Products	Health Effects
Paints Detergents	Resins Adhesives	Benzene	Cognitive Impairment, Irregular Heartbeat, Unconsciousness, Leukemia
Building Construction	Natural Polymers Synthetic Polymers	Carbon Monoxide	Cognitive impairment, Compromised immune System, Death
Electronic Devices Furniture Appliances	Metals Synthetic Finishes	Flame Retardants	Heart Disease, Neurological Effects
Cleaners	Chemicals	Hydrochloric Acid	Cancer, Neurological Effects
Insulation Carpets Appliances	Nylon Polyurethane Acrylonitrile	Hydrogen Cyanide	Bronchitis, Dermatitis, Death
Upholstery Fiberglass	Wood Nylon Acrylonitrile Butadiene Styrene	Nitrogen Dioxide	Neurological Effects, Skin Irritation
Toys Furniture	Poly(vinyl) Chloride Plasticizers	Phthalate Esters	Respiratory Effects
Paints Insulation Upholstery	Polytetrafluoroethylene Resins Rubbers	Polycyclic Aromatic Hydrocarbon	Reproduction Effects
Electrical Wiring Electronic Devices	Wood Copper Lead	Sodium Dioxide	Tissue and Eye Damage with contact

Upon exiting a fire, the gear and skin of firefighters can be covered with soot and particulates. Particulate matter includes particles such as dust, dirt, soot, or smoke that is large or dark enough to be seen with the naked eye [9]. Particles that are 10 micrometers in diameter can be inhaled into the lungs and reach the bloodstream causing severe health effects. Finer particles, less than 2.5 micrometers in diameters, can pose even greater health risks [9]. These particles are an issue due to the adsorption of toxic combustion chemicals onto their surface, which increases a firefighter's exposure to toxic chemicals as the particulate matter is inhaled, ingested, or absorbed into the body through the skin.

Bostald-Johnson and coworkers examined the air quality during overhaul and fire training scenarios for harmful chemicals. They found levels of acrolein, carbon monoxide, formaldehyde, benzene, nitrogen dioxide, sulfur dioxide, and PAHs that had exceeded either published ceiling values, short term exposure limits, or NIOSH recommended exposure limits in the air [16].

Additionally, PAHs, plasticizers, flame retardants, and several other combustion products have been found on the skin or gear of firefighters after fire responses [14] [15] [10, 36]. Ultimately, a single fire can produce thousands of potentially harmful combustion products from burning materials found in modern homes. The chemicals produced during a fire can remain in the air available for inhalation. In contrast, others adsorb onto the surface of particles which can then come into contact with firefighter's skin.

2.1.3. Firefighter Exposure Pathways to Chemical Hazards

There are three main pathways in which firefighters can be exposed to chemicals: inhalation, dermal, and ingestion, although the ingestion pathway is often neglected. Inhalation is the primary exposure pathway of concern for combustion products for firefighters and other

occupations, but this pathway can be significantly mitigated if an SCBA is worn properly, as previously shown in Figure 1. For each firefighter, a fit-test is conducted to ensure a tight seal between the facemask and the face to prevent any environmental air, particulates, or gases from entering.

An SCBA operates by a positive pressure system that pressurizes the interior of the mask and activates flow when the pressure difference is reduced from taking a breath. The positive pressure system is preferred over a negative pressure system because in the case of an air leak the positive pressure system will push air through the mask into the atmosphere and prevent particulate matter and hazardous chemicals from entering the mask. However, inward leakage can occur if the SCBA is "over-breathed" [37]. Over-breathing occurs when the user rapidly inhales, making the SCBA unable to provide the necessary air-flow to prevent the facepiece pressure from going negative [37].

The second most susceptible exposure pathway is dermal exposure. It is the most difficult exposure to assess because of its variability between individuals and the ability to remove the contaminant of interest from the skin. The role a firefighter has, either attack, search and rescue, ventilation, or hose operator, can influence their chemical exposure and skin contamination. Firefighters who enter a structure and use their hands to move and clear debris will have higher amounts of contaminants on their gear and skin than those who are outside the structure, such as the engine operator [10].

As previously discussed, the 2015 study commissioned by the IAFF demonstrated that current firefighter turnout gear is not properly equipped to prevent particulate deposition on the skin [26].

Polycyclic aromatic hydrocarbons have been found inside firefighting instructors' ensembles during training exercises. Australian firefighting instructor exposures were measured during training exercises to characterize PAH deposition onto the outer and inner layers of firefighting ensembles during a fire [13]. Typical turnout gear was worn by the firefighting instructor and the fire was created by burning Class A fire materials, i.e. wood and hay. The deposition of PAHs on the structural firefighting ensemble was sampled by a swatch of outer shell material attached to the front of the turnout coat. PAH concentrations on the ensemble swatches ranged from 69 – 290 ng/cm^2 across five evolutions with four compounds (phenanthrene, fluoranthene, pyrene, and benzo[a]anthracene) detected on all swatches [38]. Air samples collected from the outside of the turnout ensemble had PAH concentration ranged from 4.4 – 63 µg/m^3 and 0.6 – 17 µg/m^3 inside the turnout ensemble [38]. The PAH concentrations found on the ensemble swatches and in the air samples are below than the permissible exposure limit for PAHs set by OSHA, which is 0.2 mg/ m^3 based on an 8-hour work shift. However, even though these concentrations are below the exposure limit set by OSHA it again shows that particulates and smoke contaminate the external and internal layers of firefighter turnout gear.

2.1.4. Current Firefighter Decontamination Practices

Firefighters are becoming ever more aware of the chemicals and contaminants they encounter on a day-to-day basis. Fire stations across the country have begun implementing decontamination procedures both at the fire scene and back at the fire station. Most importantly, the old mentality that "dirty bunker gear is a badge of honor" is shifting towards routinely cleaning equipment and gear, reflecting a more educated industry and user.

Current firefighter decontamination practices are based on the level of hazards that were encountered at a fire scene. There are six different levels: A for light hazards, B for medium

hazards, C for extreme hazards, D for dry contamination for water-reactive and certain dry substances, E for etiologic agents and certain dry pesticides, and R for radioactive materials [39]. Decontamination procedures for E and R level exposures will not be included in this review as they do not pertain to the particulate and smoke exposure discussed in this literature review.

Depending on the severity of the exposure, firefighters may either conduct on-scene cleaning with a single or combination of decontamination procedures or wait until returning to the fire station. Level A threats require a wash down of protective clothing that can be done after returning to the fire station. Threat levels B and C require an assistant to spray another fire fighter on-scene to remove bulk contaminants from the helmet, mask, SCBA, and other parts of the turnout gear before additional cleaning is done at the fire station [39]. If needed, scrub stations are set-up near the burn structure equipped with brushes, soap and water to clean each firefighter upon exiting the fire.

As part of implementing on-scene decontamination methods firefighters have begun placing their contaminated gear in an air-tight container or a garbage bag to prevent cross-contamination [40, 39]. Upon returning to the fire station, it is recommended that firefighters first take a shower and use soap and water to wash away any contaminants on their skin. Afterward, they clean the individual parts of the protective ensemble that was taken to the fire scene. The NFPA also recommends that personnel have a second set of gear to allow firefighters to respond to multiple calls without cleaning gear in between responses. However, providing multiple sets of gear to firefighters is costly and most rural and volunteer departments do not have sufficient funding to purchase additional sets of equipment. Any vehicles that were driven in contaminated environments are also washed and the air filters replaced after returning to the fire station [39].

Figure 5 illustrates one method of firefighter on-scene decontamination after responding to a structural fire. Fire departments may use various types of cleaning methods such as brush, airbrush, wet, or wet-soap. All cleaning methods have been found to remove contaminants from the turnout gear [10]. Wet-soap decon has been found to remove 40-100% of PAH contamination and is the best method of decontamination compared to dry brush and air-based decontamination methods [10].

Figure 5: Los Angeles County firefighters demonstrate the "On-Scene Decontamination" process [41]

Unfortunately, firefighters do not always participate in on-scene decontamination procedures, remaining stuck in the old cultural mindset that being dirty is a badge of honor. A 2017 survey interviewed roughly 480 firefighters from South Florida fire departments to better understand firefighter beliefs, norms, barriers, and behaviors toward post-fire decontamination procedures [18]. Overall, firefighters reported positive attitudes towards clean turnout gear and believed that cleaning their gear will reduce their risk of cancer; however, lower norms for gear cleaning behavior were observed. Even though firefighter beliefs and attitudes towards clean gear were positive overall, 63.5% of firefighter responses showed that showering within the hour of exposure was the only frequent method of decontamination [18]. Firefighters were concerned about the effects of wet gear impacting their ability to perform their job while others expressed concerns about the time required to clean turnout gear. These concerns can result in fewer firefighters practicing on-scene or routine decontamination methods. Surprisingly, nearly 95% of firefighters claimed they never or only occasionally bagged their gear before returning to the fire station [18]. Few firefighters, less than 20%, reported they frequently or always cleaned their gear before leaving the fire-scene or used decontamination wipes. This study predicted that peer influence and routine practice could increase the frequency of decontamination methods [18].

Additionally, a second Florida study questioned firefighters about their decontamination practices, knowledge, confidence and showering practices [42]. A web-based survey with eight questions was filled out by 250 of 873 firefighters (28.6% response rate) who were sent invitations to participate in the study. Roughly 150 firefighters said they had cleaned their turnout gear within the last year and had responded to a fire within the same time. Sixty-one firefighters said they had not cleaned their gear, and they had responded to a fire within the last year [42]. Slightly over half of the firefighters, 51.3%, clean their gear at the fire station, and

26.6% of firefighters said they clean their gear at home most frequently [42]. The practice of cleaning turnout gear at home goes against the guidelines of NFPA 1851 further proving that firefighters do not always follow guidelines. Interestingly, this study found that firefighters who were not confident in their cleaning abilities cleaned their gear most frequently. Although there are several limitations of this study, such as sample size, non-response bias, and method of data collection, it provides insight into the routine decontamination practices of firefighters.

Decontamination wipes provide a possible means of increasing firefighters' usage of some form of decontamination to remove combustion products from the skin. Wipes are easy to use, store, and transport, addressing several worries mentioned in the 2017 study on South Florida firefighters [42]. Baby wipes are most prominently used among firefighters, although there are several substitutes advertised towards firefighters. Wipe samples have been used in several studies to evaluate the amount of contaminants on the skin of firefighters. Later in this literature review the term "decontamination wipes" will be generalized into wipes used for wipe sampling because they are intended to remove particulate matter and chemicals from the surface of the skin. More information on wipe sampling and decontamination wipes will be discussed later in section 2.4 Wipe Sampling.

2.1.5. Health Studies on Firefighters

Firefighters are repeatedly exposed to chemical and thermal hazards during fire response. There have been several health studies on firefighters in response to thermal hazards, such as determining physiological responses in elevated temperature environments. However, health studies in response to chemical hazards are more difficult to conduct. These studies require large sample sizes, detailed exposure documentation, and can continue for years sometimes across decades. Despite these challenges there has been a drastic increase in the number of studies that

have investigated cancer incidence and risk in firefighters. American studies have examined cancer incidence rates of firefighters in California, Florida, Illinois, Massachusetts, Philadelphia, and Washington to name a few [3] [5] [7] [8]. Not every health study will be discussed in this literature review. Only a handful of American studies will be reviewed to provide insight into American firefighters' cancer risks.

In Washington State, a study followed firefighters and police officers for 16 years (1974 through 1989) and compared incidence rates of cancer between the study population and the general public. police officers were selected as a comparison population because of their similar socioeconomic status, access to healthcare, retirement benefits, and physical entry requirements. The incidence of cancer of 2,500 male firefighters was compared to 1,800 police officers from the same cities. An elevated risk of prostate cancer was observed in firefighters compared to the general population but was less elevated compared to police officers. The risk of colon cancer appeared to increase as the duration of employment increased but was only slightly elevated compared to the general public and police officers [7].

A Florida study examined the cancer risk associated with firefighters during the years 1981 to 1999 (18 years). The study population included roughly 37,000 professional firefighters, 35,000 male and 2,000 female firefighters. Increased incidence of thyroid cancer was found in both the male and female populations. Male firefighters were found to have an increased incidence of bladder and testicular cancer. In contrast, female firefighters had an excess of overall cancer incidence and site-specific cancers like thyroid, cervical, and Hodgkin disease [8].

A Massachusetts study examined firefighters' risk of cancer by comparing cancer incidence of white, male firefighters to all other occupations in the Massachusetts Cancer Registry from 1986 to 2003 (17 years). Of the 250,000 eligible cancer cases, only 2,125 cases

were firefighters. Within the study population a moderately elevated risk of colon and brain cancer was found, and weaker evidence of increased risk was found for bladder and kidney cancer as well as Hodgkin's lymphoma [6].

A California study examined firefighters' risk of cancer and the influence of race/ethnicity on cancer incidence by using California Cancer Registry data from 1988 to 2007. The study population was found by searching firefighter related occupation codes and excluded females, homemakers, military, and cases with benign tumors, which resulted in almost 4,000 firefighters. Firefighters were found to have a significantly elevated risk for melanoma, multiple myeloma, acute myeloid leukemia and cancers of the esophagus, prostate, brain, and kidney [5].

In 2006 a meta-analysis of 32 studies sought to quantitatively and qualitatively determine firefighters' cancer risk. Twenty-one cancers were assessed to be "not likely," "possible," or "probable" given a three criteria assessment: "pattern of meta-relative risk association," "study type," and "consistency." The results of this study indicated that firefighters had a probable cancer risk for multiple myeloma, non-Hodgkin lymphoma, and prostate cancer, while testicular cancer was evaluated to be probable after having the highest summary risk estimate. Additional cancers that were evaluated to be "possible," included buccal, stomach, colon, rectum, skin, testicular, brain cancer, malignant melanoma and leukemia [4].

One of the most extensive studies on cancer incidence in firefighters was done in 2014 and included nearly 30,000 career firefighters. The study population included individuals who were of any race and employed for at least one day in fire departments in San Francisco, Chicago, or Philadelphia from 1950 to 2009. All cancer incidence was slightly above expectation. Cancers with significant excess included esophagus, large intestine, kidney, and

lung cancer and additional excess included buccal, laryngeal, and pharynx cancers and malignant mesothelioma [3].

In 2010 the International Agency for Research on Cancer (IARC) released a report that examined all health studies investigating cancer incidence in firefighters. This report found three types of cancer to show significant summary risk estimates. The incidence of testicular cancer was roughly 50% in excess based on six studies and about 150 cases. The incidence of prostate cancer was about 30% in excess based on 17 studies and approximately 1800 cases. Lastly, the incidence of non-Hodgkin lymphoma was 20% excess based on several studies and more than 300 cases [21]. Overall, this report declared that firefighting to be likely carcinogenic. The abundant amount of data has persuaded the American government to recognize the inherent risks that are now associated with firefighting. A new law, the Firefighter Cancer Registry Act of 2018, requires the CDC to maintain a database of the firefighters who get cancer [22].

The previously mentioned studies have found several connections, of varying degrees, between firefighting and an increased risk of various cancers and diseases, found in Table 2. However, even though multiple studies have found correlations between firefighting and cancer, the findings may underestimate the risk of cancer in the fire service industry. Multiple studies used cancer registries or databases to identify their study population [7, 5, 8, 6, 3]. One issue when using registries or databases to identify a study population is an under or over-estimation of the number of people to include in the study. People may go untested, misdiagnosed, or miscategorized, which may ultimately lower or inflate the total numbers, which in these studies influences the number of cancer cases.

Additionally, nearly all studies compared firefighters' cancer incidence to that of the general public [7, 5, 8, 6, 3]. Now generally, firefighters are healthy individuals because of their

training and the physical requirements of the job. Health comparisons between firefighters and the general public can be skewed based on the healthy worker effect. The healthy worker effect may reduce the death rate among workers by 70-80% relative to the general population. The magnitude of this effect may be even more significant in firefighters because of the rigorous fitness demands of the profession. Despite this fact, firefighters are found to have an increased incidence of cancer than the general public, which may speak to the severity of the chemical exposure experienced during their work.

One surprising difference between the Tsai and coworkers' study and all other studies reviewed is the focus on minority groups. All studies included white male firefighters in their study population. When including firefighters of different races and ethnicities, 12 cancers (tongue, melanoma, prostate, testicular, bladder, kidney, brain, non-Hodgkin, multiple myeloma, overall leukemia, CLL, and CML) were found to have significantly elevated risk. In contrast, only six cancers were significantly elevated among white firefighters [5]. Similarly, the study by Ma and coworkers included female firefighters in their study population, where they found a significantly elevated risk of overall cancer, cervical cancer, and thyroid cancer, as well as Hodgkin disease in female firefighters [8]. All of these, except for thyroid cancer, were not found in male firefighters. These findings demonstrate the need of further research on the impacts of race and gender on firefighter cancer rates.

Table 2: Summary of previous firefighter health studies

Study	Location	Length of Study	Study Population	Measurement Parameter	Results of the Study
Cancer incidence among firefighters in Seattle and Tacoma, Washington (United States) [7]	Seattle and Tacoma, Washington	1974 – 1989	2500 male firefighters	SIR Standardized Incidence Ratio	Elevated risk of prostate cancer compared to general population (SIR=1.4). Slightly elevated risk of colon cancer compared to general population (SIR=1.1). Risk of colon cancer increased with duration of employment.
Cancer Incidence Among Male Massachusetts Firefighters, 1987 – 2003 [6]	Massachusetts	1986 – 2003	2200 white male firefighters	SMOR Standardized Morbidity Odds Ratio	Moderately elevated risk of colon cancer (SMOR = 1.36) and brain cancer (SMOR = 1.90). Weaker evidence of increased risk was observed for bladder cancer (SMOR=1.22), kidney cancer (SMOR=1.34) and Hodgkin's lymphoma (SMOR=1.81)
Cancer Incidence in Florida Professional Firefighters, 1981 to 1999 [8]	Florida	1981 – 1999	35000 male and 2000 female firefighters	SIR Standardized Incidence Ratio	970 male and 52 female cases of cancer were identified. Male firefighters had significantly increased risk of bladder (SIR=1.29), testicular (SIR=1.60), and thyroid cancers (SIR=1.77). Female firefighters had significantly increased risk of overall cancers (SIR=1.63), cervical cancer (SIR=5.24), and Thyroid cancer (SIR=3.97) and Hodgkin disease (SIR=6.25).
Risk of Cancer Among Firefighters in California 1988 – 2007 [5]	California	1988- 2007	4000 male firefighters	Odds Ratio	Significantly elevated melanoma, prostate and brain cancer for all firefighters. Three cancers were significantly elevated among all firefighters and white firefighters: esophagus, lung cancers, and acute myeloid leukemia. Three cancers were significantly elevated among all firefighters combined and firefighters of other race/ethnicity : kidney cancer, multiple myeloma, and overall leukemia.

Table 2: (continued)					
Mortality and cancer incidence in a pooled cohort of firefighters from San Francisco, Chicago and Philadelphia (1950 – 2009) [3]	Philadelphia, Chicago, and California	1950 – 2009	30000 male firefighters	SMR Standardized Mortality Ratio and SIR Standardized Incidence Ratio	Excess cancer mortality and incidence for digestive (SMR=1.26, SIR=1.17), respiratory (SMR=1.10, SIR=1.16) cancers, malignant mesothelioma (SMR=2.00, SIR=1.16). However, evidence of excess lymphatic or haemopoietic cancers were lacking.
Cancer Risk Among Firefighters: A Review and Meta-Analysis of 32 Studies [4]	N/A	N/A	32 previous studies on firefighters	SRE Summary Risk Estimate	Probable cancer risk for multiple myeloma (SRE=1.53), non-Hodgkin lymphoma (SRE=1.51), prostate cancer (SRE=1.28), and testicular (SRE=2.02). Possible risk of skin cancer (SRE=1.39), Malignant Melanoma (SRE=1.32), Brain cancer (SRE=1.32), Rectum cancer (SRE=1.29), Buccal Cavity and Pharynx cancers (SRE=1.23), Stomach cancer (SRE=1.22), Colon cancer (SRE=1.21), Leukemia (SRE=1.14),
IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 98 Painting, Firefighting, and Shiftwork [21]	N/A	N/A	Previous studies on firefighters	N/A	There is limited evidence in humans for the carcinogenicity of occupational exposure as a firefighter. Occupational exposure as a firefighter is possibly carcinogenic to humans (group 2B)

2.2. Polycyclic Aromatic Hydrocarbons

2.2.1. What are Polycyclic Aromatic Hydrocarbons?

Polycyclic aromatic hydrocarbons (PAHs), also referred to as polynuclear aromatic hydrocarbons, are a class of organic chemicals that contains over 100 compounds [43]. For this literature review, "polycyclic aromatic hydrocarbons" will be utilized from here on out. These compounds are strictly composed of carbon and hydrogen atoms arranged in either linear, cluster, or angular arrangements of aromatic rings. Figure 6 illustrates the 16 PAHs the Environmental Protection Agency (EPA) has deemed as priority chemicals. Priority chemicals are evaluated by the EPA based on the chemical's abundance, toxicity, and potential for exposure.

Polycyclic aromatic hydrocarbons are primarily produced as complex mixtures via the incomplete combustion of materials including crude oil, motor fuel, wood, and manufacturing processes such as the distillation process of coal into coke or coal tar and crude oil maturation [43]. This class of chemicals is used in several applications: medicines, pharmaceuticals, dyes, pigments, plastics, and pesticides [43]. Due to the widespread use of these chemicals, and the possibility to be produced naturally, they are a persistent environmental contaminant found throughout the world and can have adverse health effects in acute and chronic exposures.

Polycyclic aromatic hydrocarbons have been found in: air with concentrations ranging 1-20 ng/m^3 in Europe and 1 ng/m^3 in the USA [44], soil and sediments samples ranging from 0.1 – 2225.5 µg/kg in El Paso, Texas [45], drinking water ranging from 15-844 ng/L in Henan Province, China [46]; and food where people were consuming 3µg/day/person of PAHs [47].

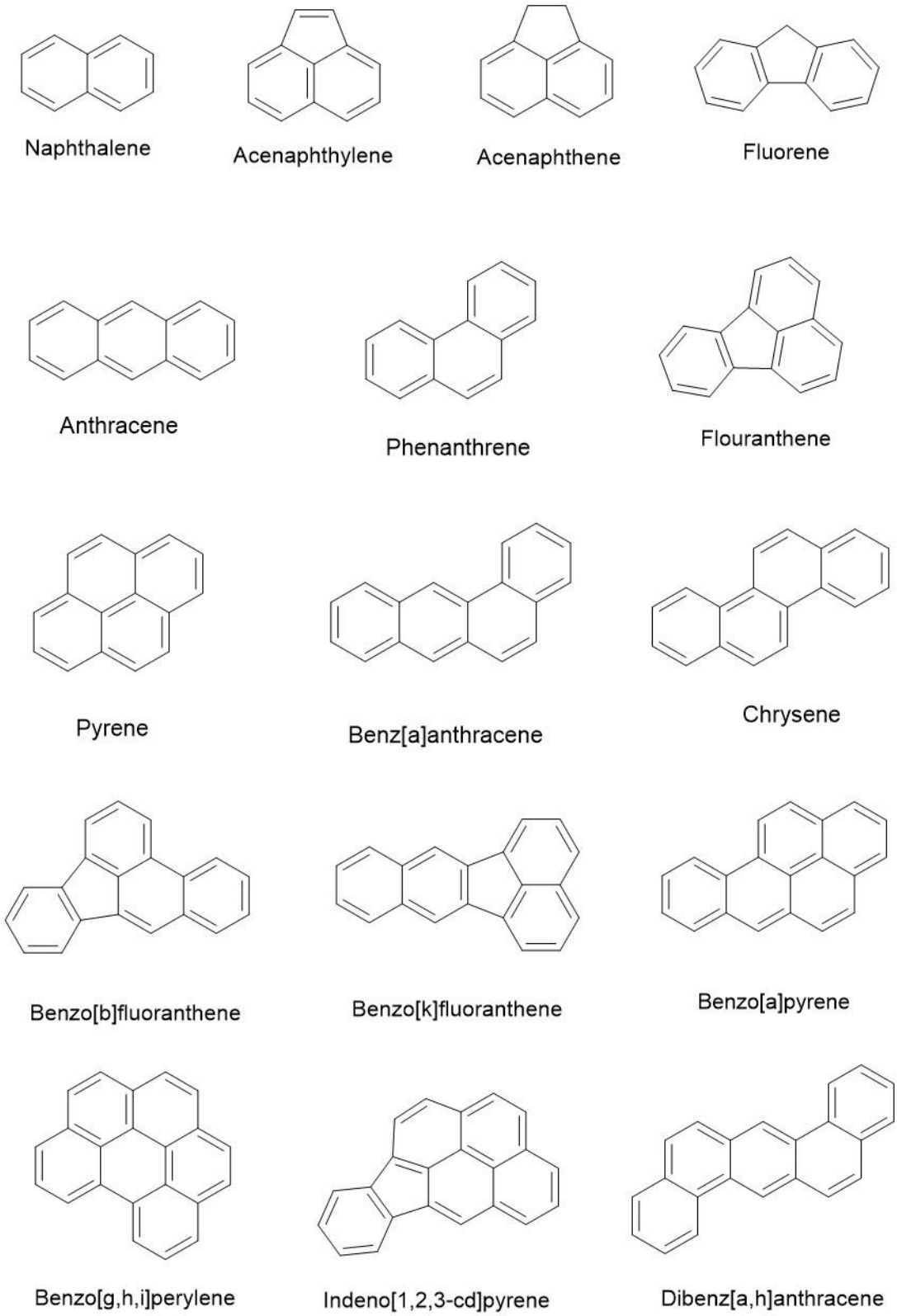


Figure 6: Chemical structures of the 16 priority EPA polycyclic aromatic hydrocarbons

2.2.2. Chemical and Physical Properties

As pure chemicals, PAHs are mostly colorless, white, or pale yellow-green solids that can have a faint pleasant odor [48]. The class of PAHs is often split into two categories; low molecular weight, PAHs with less than four aromatic rings, and high molecular weight, those with four or more aromatic rings [49]. These two categories influence the chemical properties seen in Table 3. Smaller molecular weight PAHs tend to have low vapor pressures and low aqueous solubility, and, as PAHs increase in molecular weight these properties tend to decrease, unlike boiling point which increases [43]. Due to their inherent non-polar nature, PAHs are more soluble in organic solvents, corresponding to high octanol-water partition coefficients (K_{ow} or Log P). High molecular weight PAHs, such as benzo[a]pyrene, are known to fluoresce, which can be beneficial for analysis purposes.

Table 3: Chemical Properties of the Environmental Protection Agency's 16 Priority PAHs

Compound	Molecular Weight (g/mol)	Number of Aromatic Rings	Boiling Point (°C)	Vapor Pressure (mmHg @ 25°C)	logP	IARC Class	CAS Number
Naphthalene	128.17	2	218.0	8.50e-2	3.30	2B	91-20-3
Acenaphthylene	152.19	2	280.0	4.80e-3	3.94	3	208-96-8
Acenaphthene	154.20	2	279.0	2.20e-3	3.92	3	83-32-9
Fluorene	166.22	2	295.0	6.00e-4	4.18	3	86-73-7
Phenanthrene	178.23	3	340.0	1.21e-4	4.46	3	85-01-8
Anthracene	178.23	3	340.0	6.53e-6	4.45	3	120-21-7
Fluoranthene	202.26	3	384.0	9.22e-6	5.16	3	206-44-0
Pyrene	202.25	4	404.0	4.50e-6	4.88	3	129-00-0
Benz[a]anthracene	228.29	4	438.0	2.10e-7	5.76	2B	56-55-3
Chrysene	228.30	4	448.0	6.23e-9	5.81	2B	218-01-9
Benzo[a]pyrene	252.31	5	495.0	5.49e-9	6.13	1	50-32-8
Benzo[b]fluoranthene	252.32	4	481.0	5.00e-7	5.78	2B	205-99-2
Benzo[k]fluoranthene	252.32	4	480.0	9.65e-10	6.11	2B	207-08-9
Benzo[g,h,i]perylene	276.33	6	550.0	1.00e-10	6.63	3	191-24-2
Dibenz[a,h]anthracene	278.35	5	524.0	9.55e-10	6.75	2A	53-70-3
Indeno[1,2,3-c,d]pyrene	276.33	6	536.0	1.25e-10	6.70	2B	193-39-5

2.2.3. Polycyclic Aromatic Hydrocarbon Analytical Techniques

To quantify the risk of these contaminants, analytical techniques have been developed to measure contamination in the various media that PAHs are commonly found. Due to the EPA's involvement in identifying the current 16 priority PAHs back in 1976, high-performance liquid chromatography (HPLC) and gas chromatography (GC) methods are used to analyze PAH compounds. These analytical techniques require the PAH analytes to be extracted from solid matrices such as particulate matter [44], sediment/soils [45] [50] [51], water [46] [52] [53], food [47] [54], and biological samples [55] [56] using solvent or thermal extraction [57]. Soxhlet extraction has been the traditional technique since the 1970s for solid matrix extraction. However, alternative methods have been developed to reduce solvent requirements and run-time. There are several alternative extraction methods, one example being, pressurized solvent extraction.

HPLC initially provided several advantages over GC methods in the 1970s because of the improved separation that HPLC offered using C_{18} non-polar columns [57]. Table 4 shows current HPLC columns used for PAH separation, with a majority of the columns being polymeric C_{18} columns with various particle sizes (μm), pore size (Å) and surface area (m^2). HPLC detection of PAHs is done either by fluorescence or ultraviolet (UV) absorption using a diode array detector (DAD). Fluorescence analysis provides greater sensitivity than DAD analysis. However, large concentrations can "overload" the detection system resulting in unusable data. Furthermore, only the high molecular weight PAHs are known to fluoresce, thus limiting its analytical capability. DAD analysis can analyze all PAHs in a single run but does require samples to be relatively clean to provide the necessary quantification [57]. The best HPLC analytical methods for PAHs

use a combination of UV detection and fluorescence to provide the most sensitive method for analyzing the 16 priority PAHs.

Table 4: Liquid Chromatography Columns made specifically for PAH analysis **[57]**

Column Name	Manufacturer	Phase Type	Particle Size (µm)	Pore Size (Å)	Comments
BlueOrchid PAH	Knauer	Polymeric	3 , 5	120	
Brownlee Analytical PAH	Perkin Elmer	Polymeric	5	110	End capped
ChromSphere 3-PAH	Perkin Elmer	Polymeric	3		
Cosmosil C18 AR	Nacalai USA, Inc	Polymeric	3, 5 , 15	120	End capped; pH 2-9
Enviro-Sep PP	Phenomenex	Polymeric	5		
Hypersil Green PAH 5	Thermo	Polymeric	3, 5	120	End capped; pH 2-9
LiChrospher PAH	Merck Millipore	Polymeric	5	150	pH 2-7.5
Nucleodur C18 PAH	Macherey Nagel	Polymeric	1.8, 3	110	Type B silica; Separation of EPA PAH in <3min
Nucleosil 100 PAH	Macherey Nagel	Polymeric	5	100	Type A silica
Pinnacle II PAH	Restek	Polymeric	4	110	Not end capped; pH 2.5-8; temperature limit 80°C
Pursuit PAH	Agilent	Polymeric	3, 5	200	
Supelcosil LC-PAH	Supelco	Polymeric	3, 5	120	
Ultisil PAH	Welch Materials	Polymeric	3, 5, 10	20, 200, 300	Stable pH 1.5-10
Vydac 201TP	Grace	Polymeric	3, 5, 10	300	
YMC PAH	YMC	Polymeric	3, 5		
Zorbax Eclipse PAH	Agilent	Polymeric	1.8, 3.5, 5	95	

2.2.4. Health Effects and Toxicity of Polycyclic Aromatic Hydrocarbons

In 2015 the Agency for Toxic Substances and Disease Registry (ATSDR) ranked the entire class of PAHs 9th on their Priority List of Hazardous Substances (PLHS). Meanwhile, seven individual PAHs ranked in the top 100 chemicals of the 2017 National Priorities List (NPL), which is set by the EPA. Benzo(a)pyrene ranked 8th, benzo(b)fluoranthene ranked 10th, dibenzo(a,h)anthracene ranked 15th, benzo(a)anthracene ranked 38th, benzo(k)fluoranthene

ranked 61st, benzo(b)fluoranthene ranked 73rd, and naphthalene ranked 80th. The order of these chemicals was based on the chemical's frequency, toxicity, and potential for human exposure at Superfund: National Priority List (NPL) sites [49].

Additionally, the EPA has identified 16 priority PAHs; which include: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene, and dibenz[a,h]anthracene. Of those 16 priority compounds benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, and indeno(1,2,3-c,d)pyrene are known to cause tumors in laboratory animals after inhalation, ingestion, and dermal exposure, and have been classified probable carcinogens to humans [58]. Acenaphthylene, anthracene, benzo(g,h,i)perylene, fluoranthene, fluorene, phenanthrene, and pyrene are not classifiable as to human carcinogenicity. However, this does not mean that these chemicals are not carcinogenic. Compounds listed in the not classifiable as to human carcinogenicity category are subject to change as more testing is conducting and additional data are collected.

Table 5 shows the carcinogenicity of PAH compounds according to the US Department of Health and Human Services (HHS), International Agency for Research on Cancer (IARC), and U.S. Environmental Protection Agency (EPA). Even though each agency has their own classification system, there is a consensus that benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, chrysene, dibenzo[a,h]anthracene, and indeno[1,2,3-c,d]pyrene are of concern for carcinogenicity.

Table 5: PAH Carcinogenicity according to the U.S. Department of Health and Human services, International Agency for Research on Cancer (IARC), U.S. Environmental Protection Agency (EPA) [59]

Agency	PAH Compound(s)	Carcinogenic Classification
U.S. Department of Health and Human Services	Benz(a)anthracene	Known Animal Carcinogen
	Benzo(b)fluoranthene	
	Benzo(a)pyrene	
	Dibenz(a,h)anthracene	
	Indeno(1,2,3-c,d)pyrene	
International Agency for Research on Cancer (IARC)	Benz(a)anthracene	Probably Carcinogenic to Humans
	Benzo(a)pyrene	
	Benzo(a)flouranthene	Possibly Carcinogenic to Humans
	Benzo(k)fluoranthene	
	Indeno(1,2,3-c,d)pyrene	
	Anthracene	Not Classifiable as to their Carcinogenicity to Humans
	Benzo(g,h,i)perylene	
	Benzo(e)pyrene	
	Chrysene	
	Fluoranthene	
	Fluorene	
	Phenanthrene	
	Pyrene	
U.S. Environmental Protection Agency (EPA)	Benz(a)anthracene	Probable Human Carcinogen
	Benzo(a)pyrene	
	Benzo(b)fluoranthene	
	Benzo(k)fluoranthene	
	Chrysene	
	Dibenzo(a,h)anthracene	
	Indeno(1,2,3-c,d)pyrene	
	Acenaphthylene	Not Classifiable as to Human Carcinogenicity
	Anthracene	
	Benzo(g,h,i)perylene	
	Fluoranthene	
	Fluorene	
	Phenanthrene	
	Pyrene	

Several studies have indicated that PAHs are toxic and induce serious health effects in both acute and chronic exposures. Acute effects depend on the time of exposure, concentration or

mixture of chemicals, and route of exposure [59]. Reports have shown PAHs to cause impaired lung function in asthmatics and thrombotic effects in people affected by coronary heart disease [59]. Occupational exposures to high levels of individual PAHs have resulted in symptoms such as eye irritation, nausea, vomiting, diarrhea, and confusion, while mixtures of PAHs are known to cause skin irritation and inflammation [60]. The acute health effects of PAHs are essential to consider, but chronic toxicity is more relevant to firefighter health and will be further explored.

Studies on chronic exposure to PAHs have indicated a plethora of health effects. Some studies have shown that the exposure pathway is a key component when considering which type of cancer forms, in both human and animals [58]. Animal studies have shown adverse reproductive and developmental effects from PAH exposure, but similar effects have not been found in humans [61].

Toxicological studies done by ATSDR have evaluated the carcinogenicity of each exposure pathway of the 16 priority EPA PAHs along with benzo[e]pyrene. Inhalation studies on animals exposed to benzo[a]pyrene found dose-dependent relationships in producing lung tumors. Oral exposure studies found that high doses of benz[a]anthracene, benzo[a]pyrene, and dibenzo[a,h]anthracene are carcinogenic to rodents. Dermal exposure studies have displayed the capability of benz[a]anthracene, benzo[b]flouranthene, benzo[j]fluoranthene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene to induce skin tumors in laboratory animals [58].

Surprisingly, the PAH compounds themselves are not carcinogenic but rather the metabolite byproduct produced when PAHs are metabolized by the body. Of the 16 priority PAHs, the metabolism of benzo(a)pyrene will be highlighted as it is used as an exposure marker for the assessment of the carcinogenicity of PAH mixtures [62]. When benzo[a]pyrene is

absorbed into the body, it is metabolized by three known major pathways: 1) CYPA1/1B1 and Epoxide Hydrolase pathway, 2) CYP Peroxidase pathway, and 3) Aldo-Keto Reductases pathway [63]. The CYPA1/1B1 and Epoxide Hydrolase pathway will be highlighted in this literature review and is shown in Figure 7.

When PAHs, like benzo(a)pyrene, enter the body they bind to aryl hydrocarbon receptors (AHRs) found in common cells. When a PAH binds to an AHR it induces production of metabolic enzymes, cytochrome P450s (CYPs), to metabolize the PAH [64]. Cytochrome P450s are a group of enzymes in the body that react with drugs, xenobiotics, and other compounds to increase the hydrophilicity of a compound by adding reactive groups [64]. In the CYPA1/1B1 and Epoxide Hydrolase pathway, CYPs will add an epoxide group to benzo[a]pyrene, which is then catalyzed by epoxide hydrolase to create hydroxyl groups on benzo[a]pyrene, forming benzo(a)pyrene-7,8-diol [64]. Epoxide and hydroxyl groups are extremely reactive and capable of reacting with cells and DNA. The metabolite, benzo(a)pyrene-7,8-diol, can react with CYPs enzymes again to form the ultimate carcinogen benzo(a)pyrene-7,8-diol-9,10-epoxide [64]. Once in this form, the benzo[a]pyrene metabolite can easily react with DNA producing DNA adducts that cause cancer. Benzo(a)pyrene can undertake multiple metabolic pathways creating various metabolites. Out of these, the isomer (+)-benzo(a)pyrene 7,8-diol-9,10 epoxide is extremely reactive with significant carcinogenic potential and is referred to as the “ultimate carcinogen” [65].

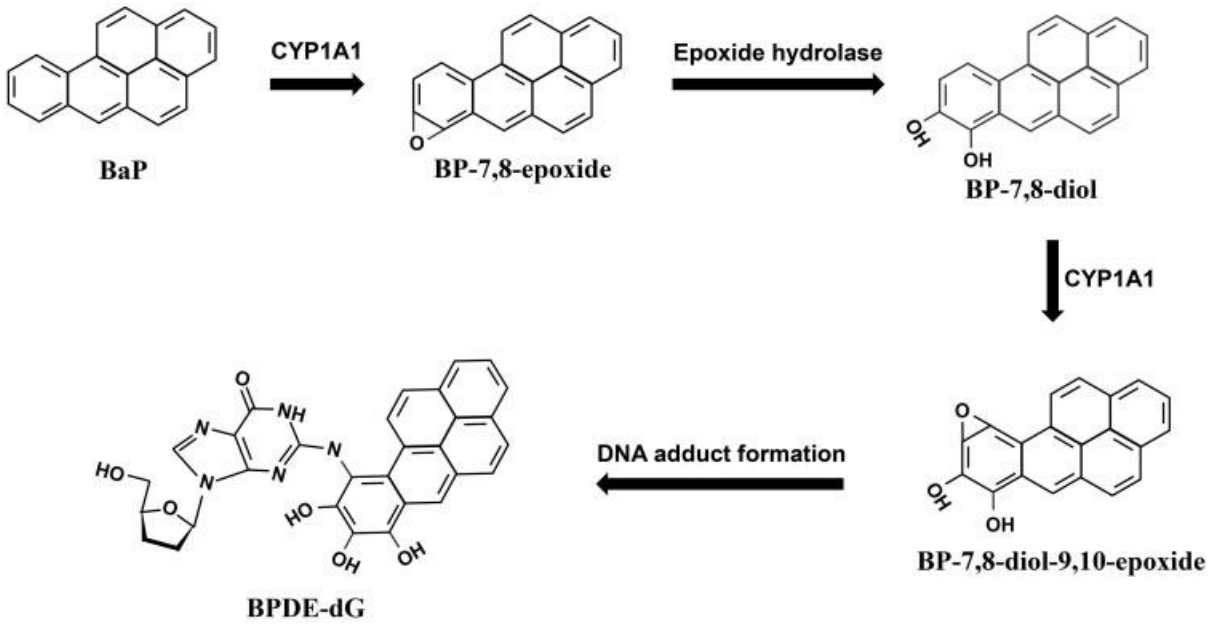


Figure 7: Illustration of the metabolic reaction of Benzo[a]pyrene into Benzo[a]pyrene-7,8-diol-9,10-epoxide via the CYPA1/1B1 pathway [63]. The parent compound benzo[a]pyrene is metabolized by cytochrome P450s, which will add an epoxide group to benzo[a]pyrene to increase its hydrophilicity to remove the chemical from the body, forming BP-7,8-epoxide. BP-7,8-epoxide is then catalyzed by epoxide hydrolase to create hydroxyl groups in place of the epoxide group. BP-7,8-diol can react with the CYPs a second time, again adding an epoxide group. The final product BP-7,8-diol-9,10-epoxide is extremely reactive and can react with DNA creating DNA adducts [64].

2.2.5. Occupational Exposures and Exposure Limits

Dermal exposure is a topic of concern for several occupations who regularly work with or work near chemicals, such as painters, farmers, and firefighters. Dermal absorption of harmful chemicals was first realized when Sir Percivall Pott discovered that children who worked as chimney sweeps were developing scrotal cancer in the late 1700s [66]. Since first realizing the threat of dermal absorption as an occupational hazard numerous studies have provided a wealth of knowledge for the dermal absorption of thousands of chemical groups and classes. This section will focus on occupations that have the highest PAH exposure and who are most frequently exposed to these chemicals.

Occupational exposures to PAHs are often based on a person's exposure to benzo(a)pyrene because it is used as an exposure marker for the assessment of the carcinogenicity of PAH mixtures [62]. To prevent individuals from getting cancer, occupational exposures are set to limit the exposure of PAHs and other harmful chemicals. OSHA's Permissible Exposure Limit (PEL) for PAHs in the workplace is 0.2 mg/m^3 based on an 8-hour work shift [67]. The National Institute for Occupational Safety and Health (NIOSH) recommends that the average workplace air levels for coal tar products not exceed 0.1 mg/m^3 for a 10-hour work day in a 40-hour workweek [67] [68]. Although OSHA and NIOSH have set exposure limits for PAHs, several occupations that work closely to fires and carbon matter products struggle at limiting their exposure and firefighters are no exception.

Several occupations like aluminum production, tar distillation, shale oil extraction, roofers, road pavers and maintenance, carbon electrode production, coke production, and coke oven workers who repeatedly work with carbon matter products, continuously burning fires, or near carbon combustion products have the highest exposure rates to PAHs [69]. These occupations with high PAH exposure rates were also found to have excess risk of bladder, digestive, lung, kidney, prostate, scrotal, and skin cancer(s) depending on the occupation of the worker [69]. The amount of PAHs an individual may be exposed to depends on two factors: the raw material being burned and availability of the PAHs in proximity to the individual, which is primarily determined by a person's occupation. Firefighting is now an occupation included in the long list of professions with high exposure rates to PAHs. Polycyclic aromatic hydrocarbons are frequently produced in complex mixtures making it challenging to determine the exact exposure to each PAH, especially in a dynamic workplace such as firefighting.

A study comparing benzo(a)pyrene exposure of Eastern European coke production workers to Western European and US coke production workers found Eastern European coke production workers had benzo(a)pyrene exposures as high as 112 μg/m^3, while Western European and US workers had concentrations lower by an order of magnitude [69]. Roofers and asphalt workers are exposed to PAHs from fumes formed during the heating of bitumen, a black viscous mixture of hydrocarbons often a residual product from petroleum distillation. These workers' PAH exposures ranged from 1-100mg/m^3 [69].

2.2.6. Polycyclic Aromatic Hydrocarbon Dermal Absorption Studies

The dermal absorption of PAHs has been thoroughly studied. Studies have tested the absorption across several laboratory animal species, including mice, rats, monkeys, and guinea pigs, as well as humans in occupational exposure studies [70, 71, 72, 73, 74, 75]. These studies have examined individual or complex mixtures of PAHs to assess the penetration capabilities or effects of mixtures on dermal absorption. Percutaneous absorption of PAHs appears to be rapid in both humans and animals. However, the extent of absorption is variable among PAH compounds and may be affected by the dosing vehicle used to expose the test subject [58]. Additional factors, including the anatomical site of application, animal species, skin temperature, hydration of the skin, and physicochemical properties of the chemical, can influence dermal absorption [76]. Further detail will be given later in section 2.3 Human Skin.

Dermal absorption studies on mice, rats, monkeys, and guinea pigs have shown the percutaneous absorption of [^{14}C]-benzo[a]pyrene to be quick and high [73], [74], [75]. A study by Ng and coworkers tested the dermal absorption of pyrene and benzo[a]pyrene *in vivo* and in high and low dose amounts *in vitro* using hairless guinea pigs [75]. *In vivo* percutaneous absorption results indicated that benzo[a]pyrene penetrated the skin slower than pyrene, where

34% of the dose was absorbed and eliminated in twenty-four hours and 73% of the dose absorbed and eliminated in seven days [75]. *In vitro* testing demonstrated similar results with 67% absorption of the administered dose in twenty-four hours [75]. A noticeable finding was when the dose was increased five-fold, it produced double the of metabolites [75]. The ultimate carcinogen metabolite of benzo[a]pyrene accounted for 2.56% of the dose in the low dose group and 0.27% in the high dose group [75].

Few studies examined the effects of the dosing vehicle on the dermal absorption of benzo[a]pyrene. Wester and coworkers compared the effects of dosing vehicles on percutaneous absorption of benzo[a]pyrene using an acetone solutions and soil [73]. Using a flow-through diffusion cell, the percutaneous absorption of benzo[a]pyrene in the different dosing vehicles was assess on human cadaver skin. Benzo[a]pyrene was found to readily penetrate human skin in the acetone vehicle compared to the soil vehicle, which had significantly less benzo[a]pyrene penetrate the skin [73]. A 10 ng/mL dose of benzo[a]pyrene applied to the skin of rhesus monkeys reported 51 ± 22% average absorption with an acetone vehicle compared to 13.2 ± 4% average absorption with a soil vehicle [73]. The increased absorption of benzo[a]pyrene in the acetone vehicle is to be expected as acetone can increase the absorption of several chemicals as it is known to damage the skin cells, thus reducing the effectiveness of the skin barrier [77]. However, it should be noted that the analyte of interest excluded the analysis of feces which could affect the absorption results [73].

A similar study by Yang and coworkers compared the dosing vehicle effects on percutaneous absorption of benzo[a]pyrene using rats [78]. A 1 ng/mL aqueous dose of benzo[a]pyrene was applied to the skin and absorbed four to five times more of the dose compared to a soil vehicle. The low absorption of benzo[a]pyrene in the soil vehicle was due to

the reduced concentration of the compound that contacted the skin, indicating that soil binding of PAHs reduces dermal absorption. An earlier study by Yang and coworkers investigated the percutaneous absorption of benzo[a]pyrene *in vivo* and *in vitro* using rats [74]. *In vitro* percutaneous absorption using a Franz-type diffusion cell with rat skin evaluated methods to enhance dermal penetration of lipophilic compounds. *In vitro* results showed that 2.1% of the dose (9-10 $\mu g/cm^2$) diffused into the receptor fluid over five days. The liquid dose vehicles resulted in greater absorption through the skin compared to the soil vehicles, which had significantly less absorption.

Nowadays, human subject studies are rare. However, previous studies dating back in the 1990s included human subjects. A dermal absorption study done by Storer and coworkers applied 2% coal tar mixture to the skin of humans for 8-hour periods for two consecutive days and found detectable levels of phenanthrene, anthracene, pyrene and flouranthene in the blood [79]. Surprisingly, benzo[a]pyrene was not detected in the blood but was present in the coal tar mixture. However, during the time that this study took place biomonitoring techniques were not capable of analyzing the benzo[a]pyrene metabolites that were likely present in the blood. Thus, allowing benzo[a]pyrene to go undetected.

Another study, by Van Rooij and coworkers, applied coal tar to the skin of volunteer at various anatomical sites examined the surface disappearance of PAHs, and monitored the excretion of PAH metabolites to determine PAH absorption at different anatomical sites [70]. The study reported low but significant differences in dermal absorption between anatomical sites: shoulder > forehead, forearm, groin > ankle, hand by monitoring the surface disappearance [70]. Differences in absorption based on anatomical site is to be expected as skin thickness is not consistent across the body. Areas of the body subject to repeated abrasion, such as the soles of

the feet and palms of the hand, have the thickest skin. Areas that contain sweat glands, hair follicles, or sebaceous glands typically have thin skin [80].

Another occupational exposure study done by Van Rooij and coworkers examined the dermal uptake of pyrene in twelve coke plant workers [81]. Exposure pads were placed at the jaw/neck, shoulder, upper arm, wrist, groin, and ankle to record the skin contamination of pyrene during five consecutive 8-hour shifts. The contamination of the exposure pads ranged from 21 µg to 166 µg per day, and biological samples reported dermal uptake to be 4 – 34 µg/day, roughly 20% of the pyrene contamination on the skin [81]. Personal air samples had an average concentration of pyrene of 0.1 – 5.4 µg/m^3 and the average respiratory uptake was estimated to be 0.5 – 32.2 µg/day [81]. Based on the estimated dermal and inhalation exposure the study concluded that an average of 75% of the total absorbed amount of pyrene to be attributed to dermal absorption [81].

These studies show the importance of how and where penetrating chemicals encounter human skin. Wester and coworkers showed that penetrating chemicals in organic solvents, such as acetone, can have elevated absorption compared to chemicals adsorbed onto soil [73]. This finding is reiterated by Yang and coworkers who found that an aqueous dose of benzo[a]pyrene absorbed four to five times more of the dose compared to a soil-based dose [78]. Van Rooij and coworkers found that different anatomical sites have varying absorption of the chemical. The differences in absorption are due to the differences in epidermal skin thickness of each area of the body. The hands and feet have thicker skin because of the increased use and number of surfaces they encounter. An occupation's exposure to PAHs was well demonstrated in the Van Rooij study with coke plant workers, where they found that workers absorbed roughly 20% of pyrene that was found on their skin [81]. This study exemplified how people are at risk of dermal

absorption and inhaling PAHs and other chemicals. Firefighters should be less concerned with inhalation exposure as long as they wear their SCBAs.

However, the data collected from dermal absorption studies that use animal skin can only estimate human dermal absorption. Depending on the species of animal used the absorption of a chemical can be either over or underestimated. Rodent skin is the most commonly used animal model but often overestimates the dermal absorption because the skin morphology is different from human skin [82]. Porcine skin is the most similar animal model to human skin. However, porcine skin also overestimates the dermal absorption of chemicals but to a lesser degree than other animal skin models [82]. Ultimately, animal skin should be used to determine if chemicals have the potential to penetrate human skin. Without sufficient animal testing the quickest way to determine a chemicals ability to penetrate human skin is to use human skin. However, it is difficult to gather viable human skin for dermal absorption studies.

2.2.7. Firefighter Exposure to Polycyclic Aromatic Hydrocarbons

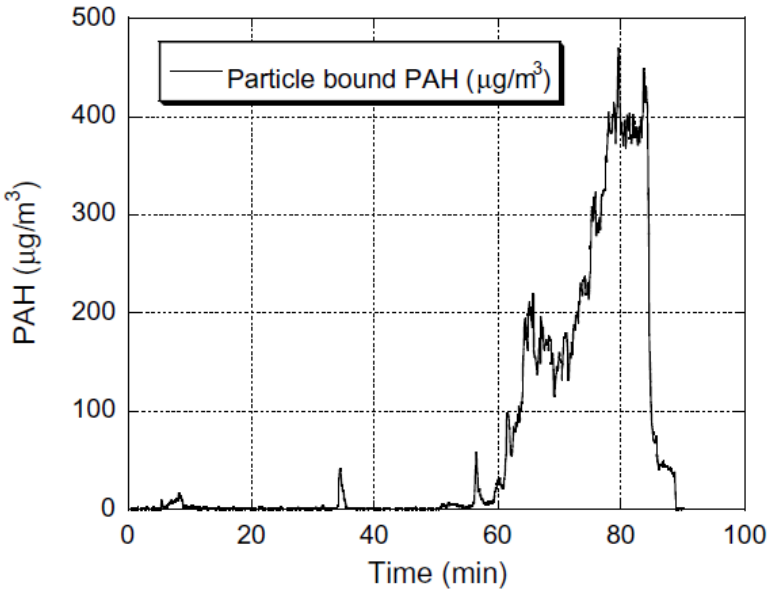
Firefighting is an occupation that has similar exposure rates to those occupations with the highest exposure rate to PAHs. Like most occupations that work closely with combustion materials, exposure to PAHs is most common through either inhalation or dermal exposure through contact with contaminated clothing or chemicals deposited on the skin.

A study done by the City of Phoenix Fire Department aimed to characterize firefighter exposure during fire suppression collected air samples during the overhaul phase from 25 structural fires. Across these 25 fires published ceiling values were exceeded by carbon monoxide at five fires, formaldehyde at 22 fires, and glutaraldehyde at five fires. Published short-term exposure limit values were exceeded by benzene at two fires, NO_2 at two fires, SO_2 at five fires. Out of the 88 total PAH samples three compounds: acenaphthylene (n=34),

naphthalene (n=28), and phenanthrene (n=13), were consistently found to be above the limit of detection. All other PAHs had five or fewer samples above the limit of detection. However, when summed together the total PAH concentration exceeded the NIOSH recommended exposure limits (0.1 mg/m^3) for coal tar pitch volatiles at two fires [16]. This study perfectly exemplifies the variability between each fire, as no two fires were found to have the same concentration of chemicals.

A Swedish study investigated the chemicals in the emissions produced during automobile fires. A vehicle from 1998 was burned in a concrete pool where the water used to extinguish the car fire and gases produced during the fire were analyzed. Inorganic compounds, volatile organic compounds, isocyanates, and PAHs were all found in the gases produced during the fire. Using an adsorbent sampling method particle bound and gaseous PAH species were identified with a time-resolved measurement method. Particle bound PAHs totaled 10.1 grams, and the total amount of PAH found for the duration of the test series was 119 grams, where smaller PAHs like naphthalene and acenaphthene are most abundant [83]. Additionally, it was found that the production of particle-bound PAHs greatly increased after 60 minutes, seen in Figure 8 part A. A surprising finding was the drastic increase of total PAH production during the extinguishment of the automobile found in the third test, seen in Figure 8 part B. This indicates that firefighting methods and materials can increase the production of PAHs during fire response.

A)



B)

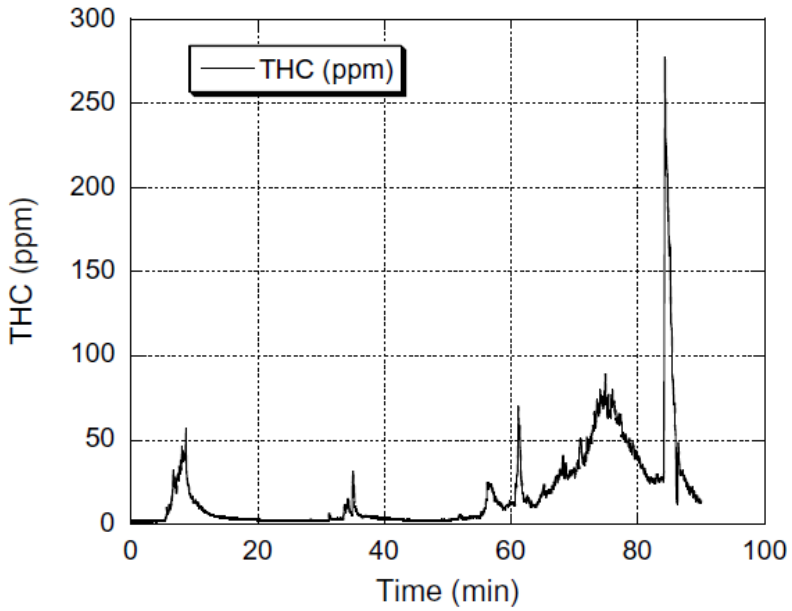


Figure 8: Part A shows the production of particle bound PAHs during automobile fires collected from the smoke gas duct. Part B shows the Total PAH concentration during the automobile fires collected from the smoke gas duct [83].

A firefighter instructor exposure study done in Australia measured the atmospheric concentrations of PAHs outside and inside the structural firefighting ensembles worn by instructors, during five live fire training cycles [38]. In between each evolution all personnel showered and changed their clothing to minimize contamination. Air sampling equipment was attached to the outside and inside of the structural firefighting ensemble to measure the amount of contamination. Total PAH concentrations ranged from 430 $\mu g/m^3$ – 2700 $\mu g/m^3$ outside the instructors' firefighting ensemble and from 32 $\mu g/m^3$ – 355 $\mu g/m^3$ inside the ensemble. Benzo[a]pyrene concentrations ranged from 4.4 $\mu g/m^3$ – 63 $\mu g/m^3$ and 0.6 $\mu g/m^3$ – 17 $\mu g/m^3$, outside and inside the instructor firefighting ensemble, respectively [38].

One study done in tandem with the Cincinnati Fire Department collected skin samples in addition to air samples during overhaul events, in fire stations, and university. Naphthalene was detected in samples collected from one of the firehouses. Naphthalene, acenaphthylene, and benzofluoranthene (a mixture of b, j, and k isomers) were detected in overhaul samples. No PAHs were found in the university location samples. Out of the 20 skin wipes collected from 10 firefighters following 5 fire events benzofluoranthene was found in 13 samples and pyrene was found in 6 samples. Other PAHs: benz[a]anthracene, chrysene, fluoranthene, phenanthrene, and benzo[a]pyrene were detected in at least one sample [84]. Surprisingly, benzo[b,j,k]fluoranthene was found in all samples where any PAH was found above the limit of detection, thus hinting to its consistent presence on the skin.

A study conducted at the University of Illinois Fire Service Institute with collaboration from NIOSH and Underwriters Laboratories Firefighter Safety Research Institute examined firefighter PPE, skin contamination, and the effectiveness of firefighter decontamination procedures. Wipe samples were used on firefighter skin and firefighter PPE to measure levels of

contamination. Wipe samples collected from firefighters assigned with different tasks had different quantities of PAHs on their turnout jackets. Inside attack and inside search had significantly more PAH contamination than overhaul or outside assignments after a single fire exposure [10]. PAH levels on turnout gear increased with each fire response showing that PAHs can accumulate on firefighter gear.

Even within the same occupation exposures can vary. For example, the PAH exposure of aluminum production workers differs depending on workers' duties. Those who are associated with Soderberg electrolysis have about ten times higher PAH exposures than those in other departments [69]. This variability with the job duty remains true for firefighters as well. The job assignment of a firefighter can affect their proximity to the smoke produced and, ultimately, the level of PAH contamination they can encounter. Fent and coworkers showed that firefighters tasked with going inside a structure during fire response had higher levels of PAH contamination than firefighters who never entered the burning structure [10].

Overall, the studies that collected air samples of firefighters found increased concentrations of the smaller PAHs: naphthalene, acenaphthylene and phenanthrene [16], [83], [38]. This result is to be expected as the smaller PAHs have lower vapor pressure and boiling points. The study by Lonnermark and Blomqvist shows the production of particle-bound PAHs began after 60 minutes of continuous burning, as well as, that fire extinguishment can drastically increase the production of PAHs, refer to Figure 8 [83]. As the time to reach flashover in modern structures occurs in less than four minutes, it is not irrational to believe that particle-bound PAH production occurs faster in structural fires. The study by Kirk and Logan found concentrations of PAHs inside the turnout jacket of firefighter instructors [38], thus reinforcing the fact that small

particles can penetrate firefighter turnout gear and deposit on the skin, further demonstrating the need for blocking layers in turnout gear.

There are significantly fewer skin sampling studies than air monitoring studies that measure firefighter chemical exposure. A recent 2017 study by Fent and coworkers collected wipe samples from firefighter gear and the skin. The wipes (Allegro® 1001) used in the study had no known collection efficiency for PAHs, which is consistent among several wipes that have been used to collect PAHs from the skin of people. If the collection efficiency is low, then all reported findings for skin samples could be low, ultimately underestimating the dermal exposure firefighters have to PAHs and other combustion products. The study by Baxter and coworkers provided no information on how skin wipe samples were collected and what wipes were used [84]. One consistency in both studies was that samples were collected from the neck and hands, which were shown to be primary areas of concern in the 2015 fluorescent particulate study.

The lack of knowledge on wipe efficacy and proper wipe sampling methods for PAHs on the skin are a result of the few studies. A wipe sampling method would address skin contamination and absorption of fireground contaminants for firefighters. This wipe sampling method could be developed in conjunction with a contact transfer test method that uses a proper skin surrogates for human skin.

2.3. Human Skin

To create the most realistic simulation of contact made between firefighter turnout gear and human skin a skin surrogate needs to have similar properties as human skin. The ideal skin surrogate needs to be representative of the complex functionality of human skin, possessing similar physical properties and dermal absorption properties. The layers and functionality of the skin may not be able to be reconstructed using a synthetic surrogate, but if the permeability of

the surrogate is similar to that of human skin then it would be a viable option for the contact transfer test method.

2.3.1. Structure and Function of Human Skin

Human skin is the largest organ of the human body, which acts as a barrier to the environment. The skin's primary functions are to protect the body against foreign microorganisms, toxic agents, ultraviolet radiation, and maintain homeostasis by regulating the transport of water, electrolytes, and heat [85]. The skin is divided into three layers shown in Figure 9, the most external layer being the epidermis followed by an underlying dermis layer and the innermost layer being the hypodermis layer.

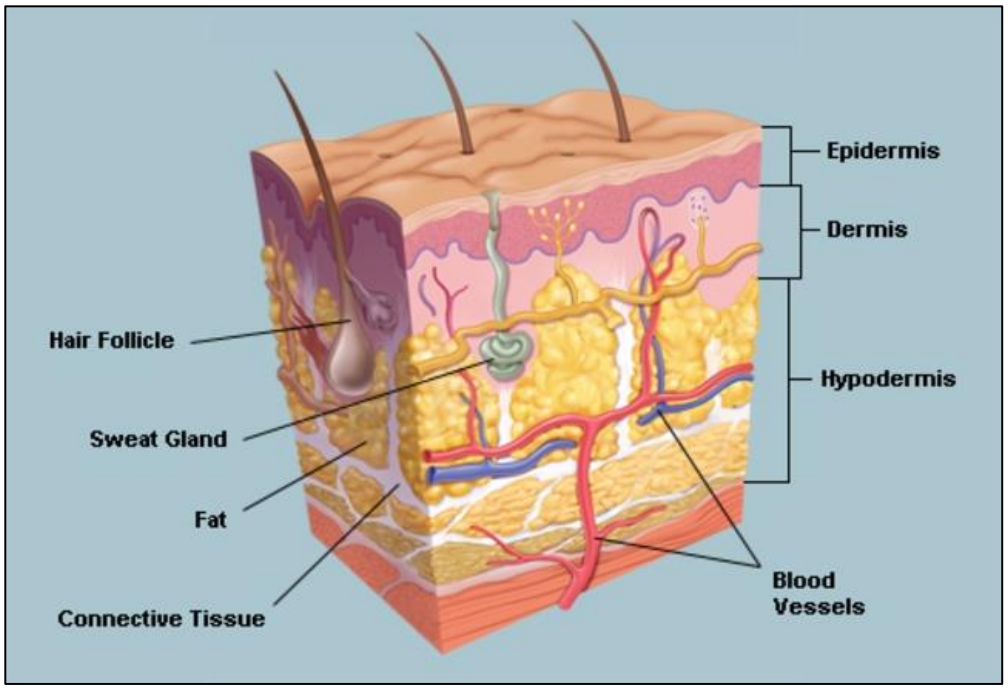


Figure 9: Structure of Human Skin detailing the Epidermis, Dermis, and Hypodermis layers [86]

The epidermis is further broken down into four layers: stratum corneum, stratum granulosum, stratum spinosum, and the stratum basal layers. All together the layers of the epidermis are responsible for protecting the body against xenobiotics, ultraviolet radiation, chemical compounds, while also maintaining skin hydration, and providing mechanical resistance to minor abrasions and cuts [87] [82]. The primary barrier to the environment is the stratum corneum (SC), which is the outermost layer of the epidermis composed of dead cells called corneocytes that are stacked 10 – 25 layers thick which is about 10 – 40 μm [87]. The dead cells of the SC are shed and replaced regularly to maintain the thickness of the stratum corneum. The dead cells of the stratum corneum are held together by lipids, giving the stratum corneum its lipophilic properties [82, 88]. The structure of the stratum corneum can be analogous to a brick wall, the corneocytes analogous to bricks, surrounded and held together by a lipid-rich matrix, similar to a brick and mortar structure, seen in Figure 10 [82].

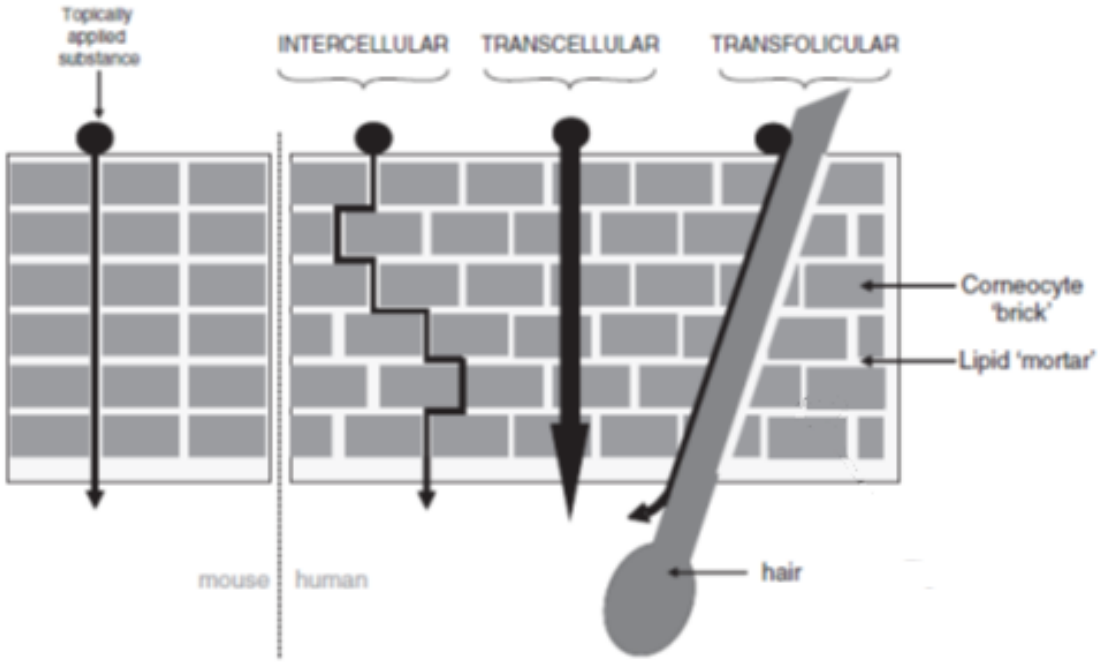


Figure 10: Schematic representation of the "Brick and Mortar" arrangement of corneocytes in mouse and human stratum corneum. The arrangement of the corneocytes create a long path for intercellular route, while transcellular and transfolicular routes are also shown [82].

Although the epidermis acts as a protective barrier, there are some areas of the body more susceptible to chemical absorption. The thickness of the epidermis is a crucial factor in preventing chemicals from penetrating the skin. Anatomical regions that are subject to repeated abrasion, such as the palms of the hands and the soles of the feet, have increased epidermal thickness [82]. Other areas of the body, such as the cheek, groin, and back, have similar epidermal thickness but have different skin permeability to nerve agent VX, seen in Figure 11. Furthermore, numerous studies have demonstrated that skin permeability is influenced by the anatomical region [89] [90] [73]. In the case of firefighter skin contamination, the neck, hands, calf, and upper leg area were found to be most susceptible to particulate deposition. Additionally,

firefighters can spread the contaminants to vulnerable areas of the body with their hands after handling contaminated gear.

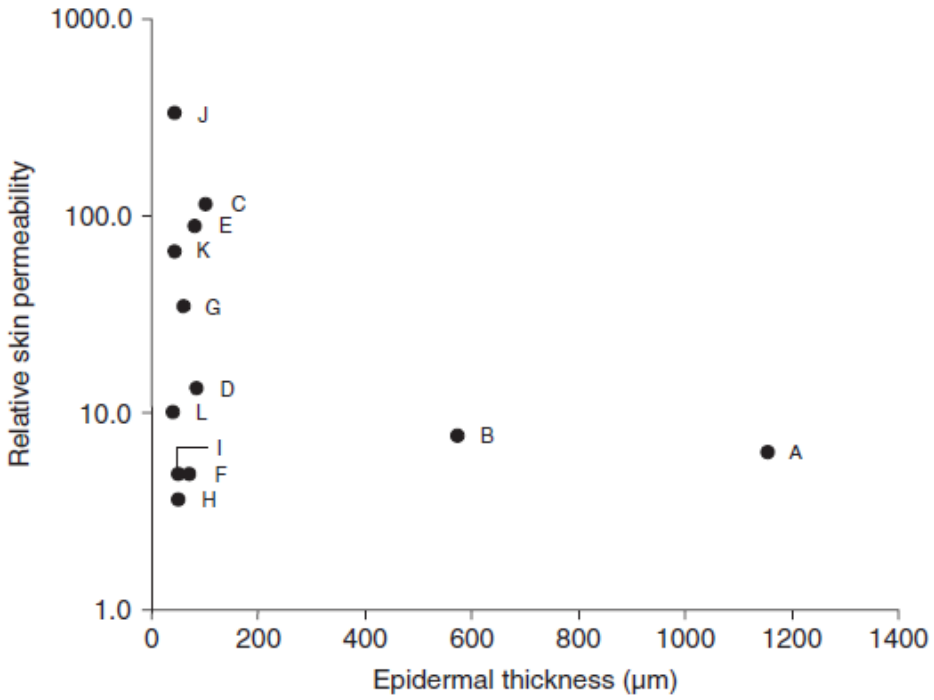


Figure 11: Skin permeability as a function of skin thickness measured in human volunteers to nerve agent VX (0-ethyl-S-[diisopropylamino)ethyl] methylphosphonothioate). Anatomical regions in order of thickest to thinnest: A = plantar; B = palmar; C = cheek; D = nape of neck; E = forehead; F = back; G = groin; H = underside of the forearm; I = topside of the forearm; J = scrotum; K = armpit; L = abdomen [82].

The dermis, below the epidermis, provides the skin with elasticity, flexibility, plasticity, structural support, and tensile strength, while also providing nutrients and immunological support to the epidermis [82]. This layer is highly vascular and provides ample opportunity for absorption into the body [91]. Finally, the subcutaneous hypodermis is the deepest layer of the skin and is made of loose connective tissue and fat, accounting for roughly 50% of a person's body fat. This layer provides insulation, energy metabolism, padding, and lubricant [82].

2.3.2. Mechanism of Dermal Absorption

Although the stratum corneum is an excellent barrier to several chemicals because of its inertness and structure, there are three primary pathways for chemicals to penetrate the stratum corneum and become bioavailable for systemic absorption. These three pathways are the intercellular route, transcellular route, and interfollicular/transfolicular route, as shown in Figure 10 [82] [87]

The intercellular route is the most tortuous as penetrating compounds must diffuse through the lipids in between the corneocytes of the stratum corneum [91]. The transcellular route is the passage through the skin barrier by diffusing through both corneocytes and the lipid [87]. Both the intercellular and transcellular routes are known as bulk pathways [87]. The final pathway is the interfollicular also known as the transfolicular routes where a compound travels down shunts provided by hair follicles, sweat glands, and sebaceous glands into the dermis [87]. This pathway is often regarded as a minor pathway because of the minimal area the hair follicles, sweat glands, and sebaceous glands occupy in relation to the skin [91] [87]. The absorption that occurs due to the interfollicular pathway could be described by draining an Olympic size pool using a handful of plastic straws.

The three previously mentioned routes apply to healthy skin, additional routes can be created when the skin is damaged. In an event that damages or kills the skin cells, xenobiotic or chemical will have fewer layers of skin to penetrate, resulting in decreased resistance. Examples include, sunburn, lacerations, abrasions, and puncture wounds. This work will focus on dermal absorption through healthy full-thickness skin; however, it is important to note that absorption can be increased if the skin is impaired.

The process of dermal absorption can be simplified into a three-stage process. Before a chemical can be absorbed into the body by dermal absorption, it must encounter the skin. The penetrating chemical may come in many forms: a pure compound either solid or liquid, in a lotion or cream, or in the case of firefighters, adsorbed onto a particle that is deposited on the skin or individuals come into contact with a vapor. The media that the contaminant is in that allows the chemical to encounter the skin is called the dosing vehicle. When the dosing vehicle containing a chemical meets the skin, the first stage of dermal absorption begins.

The first step in dermal absorption from a vehicle is the partitioning of the chemical from the dosing vehicle into the stratum corneum. This process will only occur if the penetrating chemical has a higher affinity for the skin than the dosing vehicle [82]. Afterward, the chemical will encounter the corneocytes of the stratum corneum and can diffuse through the barrier via any of the pathways previously mentioned. Finally, if the chemical has penetrated the stratum corneum, it then begins to diffuse into the underlying dermis. Once the chemical has penetrated the underlying dermis it will be absorbed into the body by the capillary loops where the epidermis and dermis layers meet [91].

2.3.3. Chemical Properties that Influence Dermal Penetration

The physical and physicochemical properties of a penetrating chemical can aid in its ability to navigate the lipophilic stratum corneum and hydrophilic dermis to enter the blood stream and absorb it into the body. In general, lipophilic compounds are more likely than hydrophilic compounds to penetrate the skin as they easily penetrate the lipid bilayers and cell membranes of the corneocytes in the skin.

When a compound is trying to penetrate the layers of the skin, lipophilic compounds quickly pass through the lipids between the corneocytes but struggle to penetrate the hydrophilic

layers of the dermis. Conversely hydrophilic compounds quickly pass through the hydrophilic layers of skin but struggle to penetrate the lipophilic stratum corneum of the skin, reinforcing why the brick and mortar structure provides excellent protection against a range of penetrants.

Furthermore, the size of the penetrating chemical can also limit its ability to permeate the skin. Bos and Meindardi propose that compounds with a molecular weight less than 500 Dalton can easily pass through the stratum corneum, but as compounds increase in molecular weight above 500 Dalton their absorption rapidly declines [92]. The 500 Dalton Rule is based on three primary arguments: 1) virtually all compounds that cause allergic reactions of the skin are under 500 Dalton whereas large molecules are not known to cause these allergic reactions; 2) most commonly used active ingredients used in dermal treatments, such as creams and lotions, are all under 500 Dalton; and 3) all known topical drugs used in transdermal drug-delivery systems are under 500 Dalton [92]. In terms of occupational exposure to PAHs, the largest PAH of the 16 priority compounds is 276 Dalton, ultimately meaning they could all be dermally absorbed through the skin.

Another property that influences a chemicals ability to penetrate the skin is whether the penetrating chemical is charged. Ionized chemical species have a difficult time penetrating the skin. The presence of proteins gives the SC with both positive and negative charge groups. However, there is a greater presence of negatively charged groups resulting in a net negative charge [82]. Hence, positively charged ions are more likely to penetrate the SC than negatively charged ions but are still less likely than neutral compounds. Neutral compounds are the most likely to penetrate the skin because dermal absorption is a passive process. Meaning there is no energy that will help transport charged species across the SC barrier. Comparative studies between ionized chemical species and their non-ionized counterparts found that the permeability

coefficient for non-ionized compounds is frequently one to two orders of magnitude larger than the ionized forms of the chemical [93]. The difference in dermal penetration between ionized and non-ionized chemicals is more severe for lipophilic species than hydrophilic species [87]. In dermal absorption studies, the dosing vehicle should be chosen carefully to favor the non-ionized form of the chemical species.

However, the importance of the dosing vehicle should not go overlooked. Essentially, the dosing vehicle must provide the maximum amount of transfer from the vehicle to the skin. The lipophilicity, pH, and solvent ratio can all influence the dosing vehicles ability to maximize the amount of penetrating chemicals available for absorption. For occupational dermal exposure assessment, the dosing vehicle should be similar to that found in real-world exposures.

Lipophilic dosing vehicles can reduce dermal absorption when dosing a lipophilic compound compared to hydrophilic vehicles [94, 95, 96]. This phenomenon is explained by the octanol/water partition coefficient, a physicochemical property of a chemical which is based on its affinity for either hydrophilic or lipophilic environments. If a lipophilic chemical is in a lipophilic dosing vehicle, it has a higher affinity for the vehicle and will not partition into the skin. Conversely, if a lipophilic chemical is dosed using a hydrophilic dosing vehicle, then the chemical will partition into a more lipophilic material. In order to maximize the amount of the penetrating chemical available for dermal absorption, the properties of the dosing chemical and vehicle need to be known.

However, there are additional factors that need to be considered when formulating the dosing vehicle. The pH of the dosing vehicle can ionize the penetrating compound, ultimately reducing its potential to penetrate the SC. Furthermore, using harsh solvents in the dosing

vehicle, such as acetone, can increase dermal absorption because it damages the cells in the skin, making it easier for the chemical to penetrate the SC.

2.3.4. Dermal Absorption Models

Dermal absorption is a passive process where chemicals pass through the skin layers via diffusion. Theoretical equations and models have been developed to predict a chemicals ability to penetrate the skin. These theoretical predictions are based on Fick's laws of diffusion. Fick described the diffusion of a compound across a membrane by transforming Fourier's Law of Thermal Diffusion, a thermodynamic model of the transfer of heat by conduction, into a model of an infinite dose, seen in Equation 1. Where J is the rate of transfer per unit area or flux, δC is the change in concentration, δx is the change over a distance and D is the diffusion coefficient. The negative sign indicates that the transfer of the penetrant into the skin [82].

Equation 1 Fick's Law of Diffusion for Infinite Dose [87]

$$J = -D\frac{\delta C}{\delta x} \quad (1)$$

In most dermal absorption experiments the chemical of interest is applied in a dosing vehicle to a skin sample. Initially the chemical has the highest concentration in the dosing vehicle (C_v) and over time the penetrating chemical permeates into the skin represented by the distribution coefficient (K_m). As mentioned previously, the thickness of the skin is not consistent across the body and inherently affects dermal absorption. For a membrane of thickness h, the flux at a steady-state J_{ss} is given in Equation 2, where D is the diffusion coefficient, K_m is the distribution coefficient, C_v is the concentration of the chemical in the dosing vehicle. The steady-

state flux across a membrane can be written in an alternate form in terms of the permeability coefficient (K_p), shown in Equation 3.

Equation 2 Flux at steady state for a membrane:

$$J_{ss} = D * K_m * C_V/h \quad (2)$$

Equation 3: Alternate form of Flux at steady state for a membrane:

$$J_{ss} = K_p * C_v \quad (3)$$

Typically, the steady-state flux (J_{ss}) and permeability coefficient (K_p) are determined from *in vitro* experiments where an infinite dose (i.e. the concentration of the penetrating compound is maintained) is applied to the test membrane or skin and a receiving well under the skin is constantly renewed with fresh receiving fluid acting as a sink. Over time, the flux approaches a steady-state and produces a linear curve. The time until steady-state conditions are met is known as the lag time, as seen in Figure 12, which illustrates the relationship between the cumulative mass penetrating a membrane area M_{out}/A and the steady state flux J_{ss}, permeability coefficient K_p, and lag time t_{lag} [87].

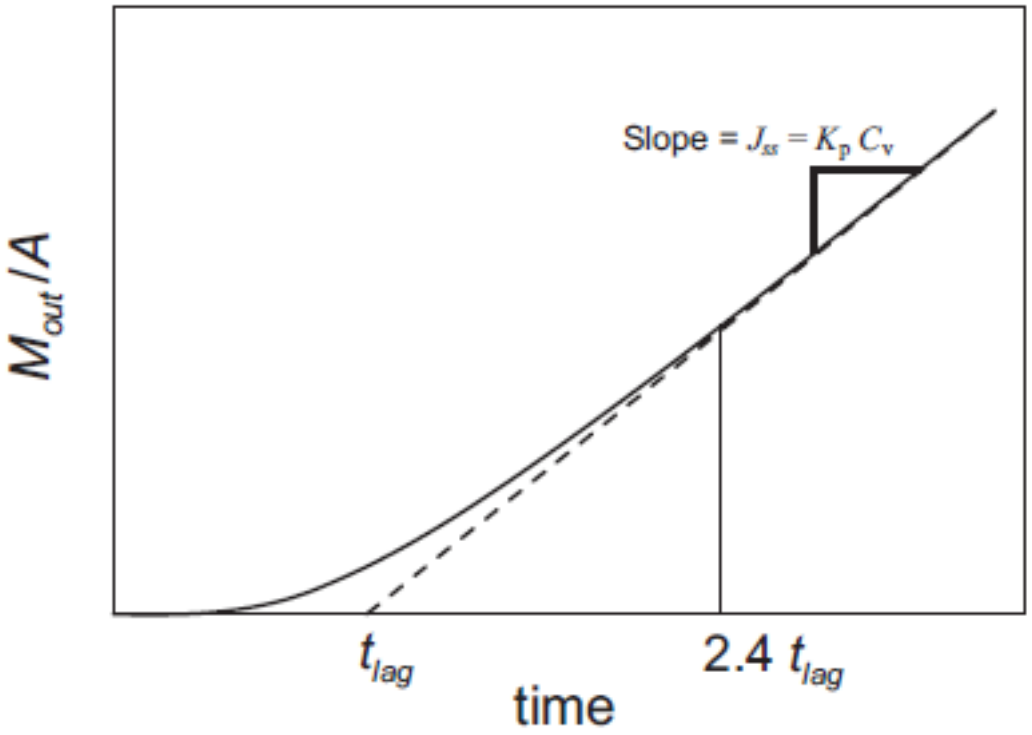


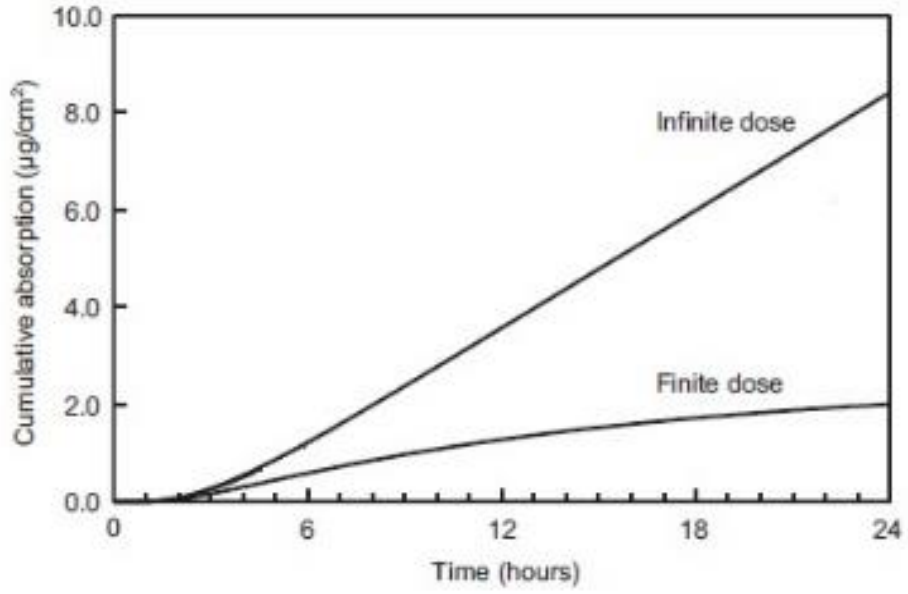
Figure 12: Illustration of the relationship between the cumulative mass penetrating a membrane area M_{out}/A and the steady-state flux J_{ss}, permeability coefficient K_p, and lag time t_{lag} [87]

Infinite dose experiments rarely represent occupational exposures scenarios because occupational exposures are limited by the work shift of a worker. These exposures may occur in swimming pools, bathing water, or any other scenario where the concentration of the penetrate cannot be depleted [87]. Infinite dose experiments are beneficial for determining the capability of a compound to penetrate the skin, determining any potential concern.

Conversely, finite dose experiments are more representative of occupational exposures and can predict maximal absorption rate/exposure and total absorption/exposure. However, the concentration of the penetrant in the dosing vehicle can change as it is absorbed into the skin, or the dosing vehicle is absorbed or evaporates, which can be difficult to predict for firefighter

exposure. Figure 13 depicts the differences between infinite dose and finite dose flux. In Figure 13A the infinite dose shows an increase in cumulative absorption as time goes on compared to the finite dose that levels out as the entire dose is absorbed. Inversely, Figure 13B shows the difference in concentration between the infinite and finite doses across time. The infinite dose is maintained constant while the finite dose decreases as it is absorbed into the skin.

A)



B)

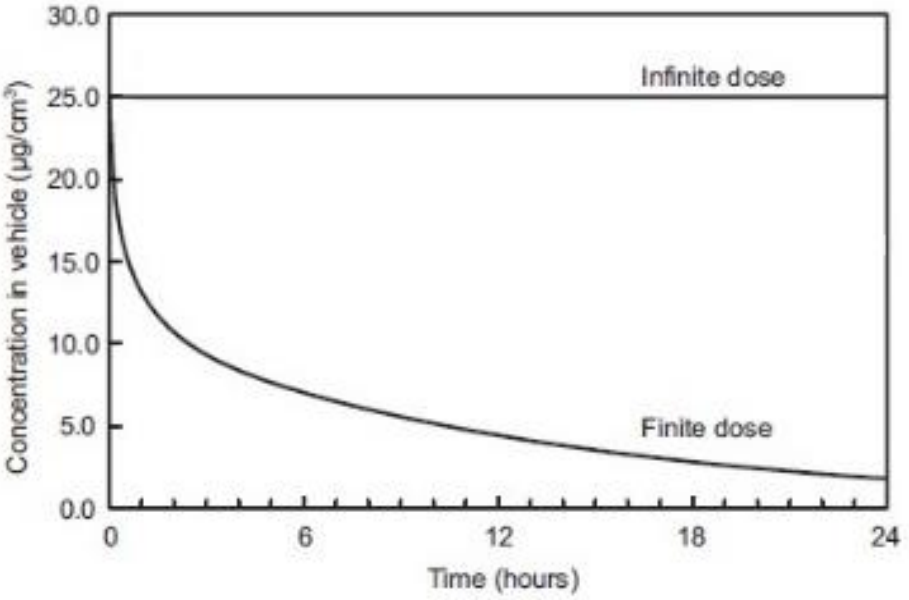


Figure 13: Infinite and Finite Dose Experiment Comparisons [94]. A) shows the cumulative absorption of the penetrant into the skin and B) shows the concentration of the dose over time [82]

2.3.5. Skin Models

Human skin is rather unique, and difficult to replicate. Ideally, human skin should be used for drug formulations and chemical testing; however, it is unethical to test on human subjects. Human cadaver skin or excess skin from surgical procedures are the best alternatives to human subject testing, but these materials are not easily accessible. When those materials are not available, animal skin or synthetic materials can be used as a human skin model.

2.3.5.1. Animal skin models

When using animal skin models, there is a difference in hair or fur density between animals and humans. An animals' fur/hair changes their skin's morphology and function while also providing extra protection from their environment [97]. To prevent the effect of fur/hair on dermal absorption, animal subjects are shaved, and the skin is washed and cleaned [20]. Then the animal skin is cut using a dermatome to a thickness similar to that of human skin, about 200-300μm [98].

No single animal species has the exact same morphology and physiology of human skin, but pig, or porcine, skin is the most relevant animal model and has been reviewed and validated over many years [97]. Porcine skin is most analogous to human skin because of its similar stratum corneum structure and skin morphology to human skin [97]. For most compounds tested, pig skin was similar to human skin absorption ranging 50 – 150% [97]. Other animals like rodents (rats or mice), rabbits, and monkeys have been used in dermatoxicological studies [97] [72].

The other popular animal skin model, rodent skin, is often used because of its availability and low cost. However, rodent skin is more permeable than human skin and often overestimates dermal absorption because their skin cells are arranged in columns, unlike the "brick and mortar"

structure of human skin, providing a more direct route to the underlying skin layers [82]. Rodent skin is 40 to 1600 times more permeable than human skin for a charged herbicide paraquat [99], while porcine skin was similar to human skin [100]. For nicorandil, a drug to treat chest pain, permeability differences were most significant when comparing human skin with rodent skin [97]. Rodent models have been repeatedly found to be orders of magnitude different to human skin, making it a better preliminary assessment of dermal absorption of drugs and chemicals than a model for human skin [97].

2.3.5.2. Synthetic skin models

Synthetic skin models could be more advantageous than animal skin models because of their low cost, ease of storage, and better control over physical properties [101]. Because of these characteristics, synthetic skin models avoid the controversy associated with animal testing and can have better reproducibility and reliability [101]. Several synthetic materials have been used to model the sweating, surface, mechanical, acoustic, optical, and thermal properties of human skin [101]. Table 6 shows the various model types used to simulate specific properties of human skin. These models include liquid suspensions, gelatinous substances, elastomers, epoxy resin and textiles.

Table 6: Synthetic materials used to simulate various properties of human skin [101]

Surface properties	**Mechanical properties**	**Optical properties**	**Thermal properties**	**Sweating**
Gelatinous Substances	Gelatinous Substances	Gelatinous Substances	Gelatinous Substances	Metals
Elastomers	Elastomers	Elastomers	Elastomers	Textiles
Textiles		Liquid Suspensions	Metals	
		Epoxy resin	Textiles	

A study by Uchida and coworkers evaluated the ability of a silicone membrane as an alternative to human skin for determining skin permeation parameters of chemical compounds [102]. Using a two-chamber diffusion cell the permeability coefficient was calculated for 15 test compounds with respective molecular weight and log K_{ow} values ranging from 188.2 – 244.3 and -1.51 – 3.86, respectively. The permeability coefficients were calculated for silicone membranes and compared to human and hairless rat skin. The permeation coefficient of hydrophilic compounds was similar for the silicone membrane. The permeation coefficient of amphiphilic compounds, compounds with both lipophilic and hydrophilic parts, were ten times higher in silicone membrane than human and hairless rat skin. Furthermore, the permeation coefficient of lipophilic compounds was 100 times higher in the silicone membrane than human and hairless rat skin. Even though the silicone membrane was not similar to human or hairless rat skin, there was a significant correlation observed between the partition coefficient values in human skin and silicone (r=0.869) and in hairless rat skin and silicone (r=0.823) [103]. Therefore, this study concluded that the permeation coefficient through human or hairless rat skin could be predicted using log K_{ow} in the silicone membrane.

Strat-M® is a synthetic membrane model for transdermal diffusion intended to predict the diffusion of test compounds in human skin. It has been used as a screening tool for active pharmaceutical ingredients, cosmetics, formulations, personal care products, pesticides, and chemicals [104]. The Strat-M® membrane is a multilayered structure with a tight top layer to resemble the stratum corneum, two layers of polyethersulfone to resemble the dermis of human skin, and polyolefin non-woven fabric support to resemble the subcutaneous tissue in human skin, seen in Figure 14.

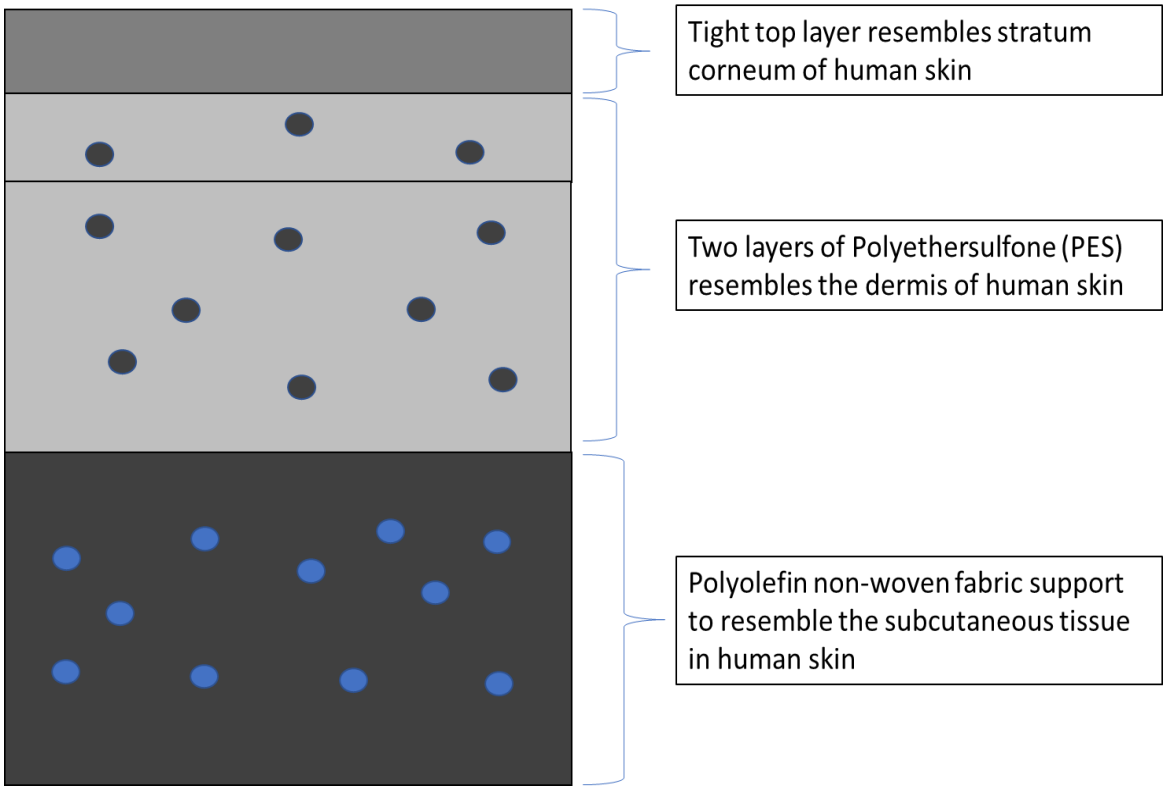


Figure 14: Illustration of the multilayered structure of Strat-M® membrane [105]

Uchida and coworkers examined the ability of Strat-M® membrane as an alternative to human skin by determining skin permeation values of chemical compounds [106]. The permeability coefficient of test compounds was found for the Strat-M® membrane, hairless rat skin and human skin using a Franz diffusion cell. There was a strong correlation between log P values for Strat-M® membrane and hairless rat skin (r=0.970) and for Strat-M® membrane and human skin (r=0.929). Another finding was that the more lipophilic compounds had higher partition coefficient values through each membrane tested. Ultimately, this study determined that the permeability coefficients using Strat-M® membrane can be used to predict those for human and rat skin, especially for chemical compounds with molecular weights between 151 and 288 and log K_{ow} between -0.90 and 3.53 [106].

The effects of penetration enhancers have been evaluated using the Strat-M® membrane and compared to human cadaver skin [107]. Although the Strat-M® membrane was more permeable and allowed more test compound to pass through the enhancement factor for all five test formulations were similar for the Strat-M® membrane and human skin. Furthermore, the correlation of flux between the Strat-M® membrane and human cadaver skin was relatively high with a correlation valuation of 0.99 [107].

Polyurethanes are polymers that can easily change their properties by changing the ratio of monomers and additives, such as, reinforcing particles [101]. Polyurethanes can be used as mechanical skin model, simulating the surface friction behavior of human skin [101]. A skin model based on polyurethanes was considered for the intradermal injection training system by Graham and Sabelman [101].

SynDaver™ is a company that manufactures sophisticated synthetic models of the entire human body or specific body parts such as muscles, tendons, veins, arteries, organs, and skin.

SynDaver™ tissue plates have been used for training intradermal injections and skin surgeries in the medical field. There are few studies that have compared the permeation between SynDaver™ tissue plates and human skin, but mechanical and deformation behavior comparisons have been made [108]. SynDaver™ tissue plates were found to undergo less deformation under equivalent loads compared to human skin and decreased in friction under wet conditions compared to human skin. The permeation of SynDaver™ tissue plates has yet to be tested.

2.3.6. Techniques used to Evaluate Skin surrogates

One characteristic the skin surrogate should be able to mimic is the absorption of chemicals of human skin. Both animal skin models and synthetic membranes have been used to assess the permeation of chemicals of toxicological interest. Skin surrogates are tested using either Franz diffusion cells or flow-through diffusion cells to obtain the permeability coefficient, partition coefficient, and diffusion coefficient [106]. These values are critical for predicting the dermal absorption of a chemical and can change based on the dosing vehicle [96, 94]. The permeation values of a chemical in animal and synthetic models can be compared against human skin and determined the model's ability to predict human skin absorption.

In vivo studies are the gold standard to determine chemical absorption, however it is extremely difficult to perform *in vivo* studies because of ethical controversy. I*n vitro* skin absorption studies are more frequently used because they are more economical, minimize or eliminate the use of animals, and can directly measure test compounds absorbed into and through the skin [98]. Two *in vitro* methods, static or Franz diffusion cell system and flow-through diffusion cell systems, are frequently used. These systems can be either one or two-chamber systems. The one-chamber system has a receptor chamber below the skin and is open to the

environment above the skin to simulate desired exposure conditions [20]. Two-chambered systems are separated by the membrane with an infinite dose maintained above the membrane.

Overall, the goal of the static and flow-through diffusion cell experiments is to dose a test chemical on the surface of the skin or membrane, collect perfusate samples throughout the experiment, and evaluate the depth of penetration at the end of the experiment [109]. Sometimes the skin or membrane can behave like a reservoir for the test chemical. To accurately determine systematic skin absorption in this case, both skin and receptor fluid should be measured for the test chemical [20]. When working with volatile compounds, the recovery of the test compounds can be lower than non-volatile compounds, which should have recoveries of at least 90% [20].

2.3.6.1. Static Diffusion Cell Experiment

The static diffusion cell systems are simpler in design compared to the flow-through diffusion cell system and are based on the Franz diffusion cell [20]. Figure 15 is an illustration of a typical set up of a static diffusion cell experiment. The skin or membrane sits atop the receptor chamber and is open to the environment [110]. The donor chamber is applied to the top of the skin or membrane to apply the test compounds. The receptor fluid is collected in a chamber bellow the skin or membrane and is continuously stirred with a magnetic stir bar. Periodically aliquots of the receptor fluid are collected through a side arm for analysis [20]. The static diffusion cell experiment remains relevant because of its relatively low cost and customizability to change the size of the opening, which can allow studies with transdermal devices [20].

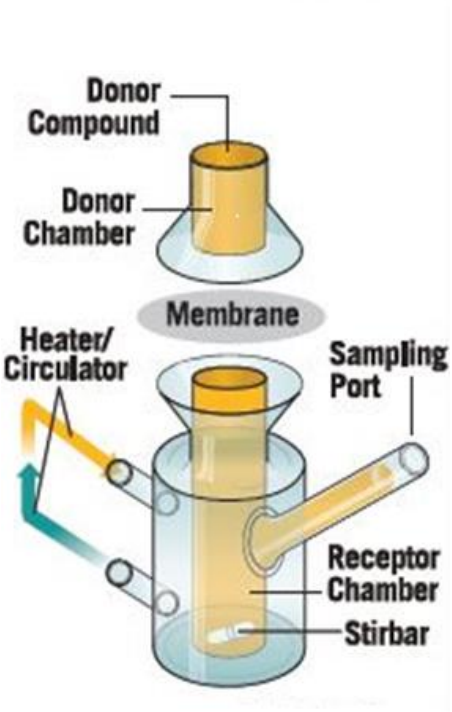


Figure 15: Illustration of a Static One-Chambered Diffusion Cell [111]

2.3.6.2. Flow-Through Diffusion Cell Experiments

The flow-through diffusion cell is a more complex system than the static diffusion cell system. There are three major components in the flow-through cell diffusion test: the heating blocks, peristaltic pump and tubing, and the sample collecting apparatus. The heating blocks are to keep the skin or membrane samples at a set temperature often similar to the human body. The peristaltic pump and tubing pumps the dissolved chemical onto the surface of the skin samples. The receptor fluids can be a saline solution or other solvent systems, but formulations should aim to represent *in vivo* systems. The artificial medium in the sampling apparatus may vary but should be physiochemically similar to blood to best mimic oncotic pressure *in vivo* [100]. A typical set up of a flow-through diffusion cell experiment can be seen in the illustration found in Figure 16. These experiments typically run from 8-12 hours to simulate worker exposure [109] but can run for 24 hours [20]. Flow-through diffusion cell experiments are better suited to

evaluate dermal absorption than static diffusion cell systems because they better mimic environmental conditions while not excessively hydrating the skin [109].

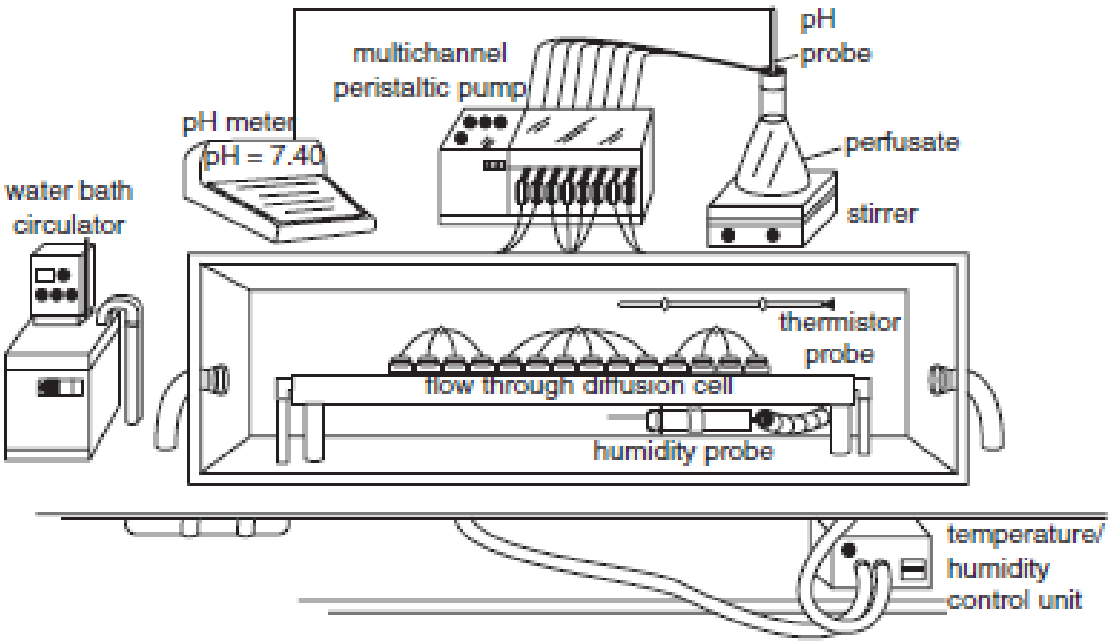


Figure 16: Illustration of the flow-through diffusion cell system in an environmentally controlled chamber [109]

2.4. Extraction Techniques for PAH Analysis

To create a contact transfer test method for the analysis of fireground contaminant transferring from firefighter turnout gear, effective and efficient extraction techniques are necessary. Extraction methods are used to remove the analytes of interest from the materials used in the test method. Firefighter turnout gear and synthetic skin are made from vastly different materials; however, the ideal extraction method will provide adequate and repeatable extraction of PAHs. Current extraction methods used to remove PAHs from media are primarily liquid extraction techniques, so those will be focused on in this literature review.

2.4.1. Liquid Extraction

Liquid extraction is a separation process that transfers an analyte from a solid to a solvent or from one solvent to another [112]. The solvents used in liquid extraction can include nonpolar organic liquids, polar organic liquids, or a mixture of solvents. In all extraction processes there consists of a mixing step to allow the extraction solvent to remove the intended analyte, followed by a separation step. Extraction is a process based on equilibrium, which is dependent on the analyte's affinity for one solvent or matrix over another. Equation 4 shows how the distribution coefficient is calculated, which is a measure of equilibrium. C_1 and C_2 are the equilibrium concentration of the analyte in the sample media and the extraction solvent [112]. The extraction efficiency can be increased by manipulating variables of the extraction procedure such as temperature, solvent, and time of extraction, all of which can change the distribution coefficient.

Equation 4 Distribution Coefficient K

$$K = \frac{C_1}{C_2} \tag{4}$$

For a mixture of PAHs to be analyzed by chromatographic analytical instruments, the chemical mixture needs to be separated from the matrices to which they are bound. Extraction techniques include: Soxhlet extraction [19], supercritical fluid extraction [113, 19], microwave-assisted extraction [19], ultrasound-assisted pressurized solvent extraction [114], solid-phase extraction [115], and accelerated/pressurized solvent extraction [113, 116, 19, 51]. Soxhlet extraction is traditionally viewed as the gold standard for PAH extraction; however, it uses high amounts of solvents, requires a great deal of labor, and is time-intensive. Several alternative extraction techniques have been developed. These alternative methods provide similar, if not

equal, or better extraction efficiencies. Pressurized solvent extraction is one alternative that provides similar extraction efficiencies to Soxhlet extraction, while using less solvent and reducing run time [51].

2.4.2. Pressurized Solvent Extraction

Pressurized solvent extraction (PSE) utilizes organic solvents, high temperatures, and high pressures. Figure 17 illustrates the increased operating area PSE has over classic solvent extraction techniques. As the temperature of the system increases, solvents will transition into the gas phase. The elevated pressure maintains the solvent in the liquid phase preventing it from evaporating at the higher temperatures [51]. The high pressures force the solvent through the sample and into areas of the matrix where the analytes may be trapped. Additionally, the high-pressure aids in the solubilization of air bubbles in the system. The reduction of air bubbles exposes more of the sample to the extraction solvent. The elevated temperature increases the solubility capacity of the solvent, aids to disrupt strong analyte-matrix interactions, increase diffusion rates, and reduces solvent viscosity and surface tension [51]. This alternative method to Soxhlet has been shown to have equal or better extraction efficiencies [47]. However, there are cases where the extraction efficiencies of these alternative methods have been reported to be lower [19, 113].

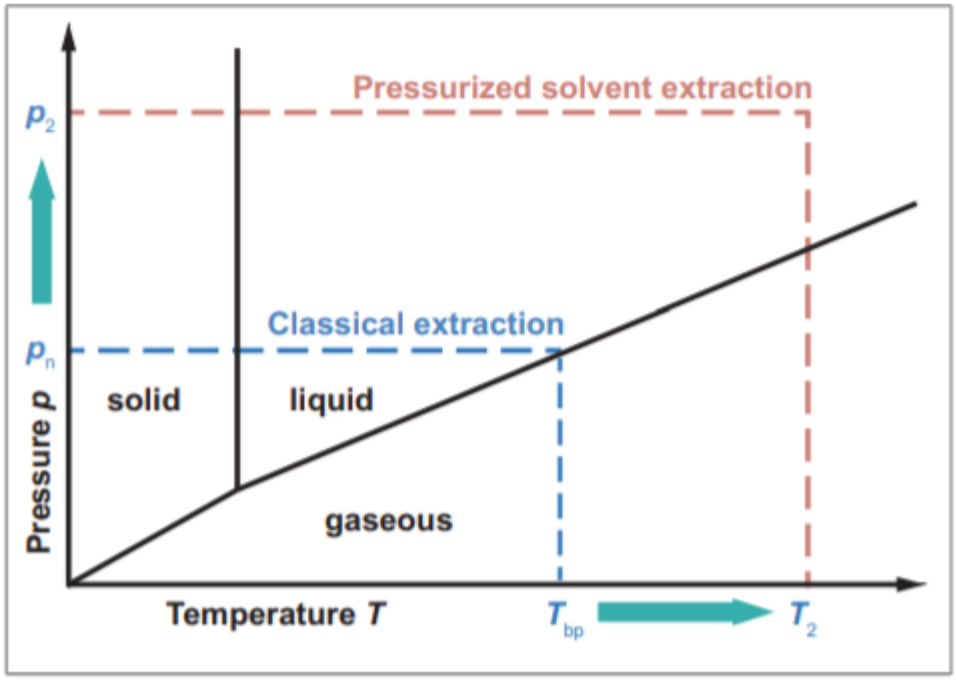


Figure 17: Illustration of increased effective area of pressurized solvent extraction compared to classical extraction. Pressurized solvent extraction can use high temperatures and pressures to increase the effectiveness range for various extraction media.

Pressurized liquid extraction is a popular extraction technique for measuring PAH contamination in environmental media like soils, soot, or ash. Kenny and Olesik, compared extraction techniques: supercritical fluid extraction (SFE), enhanced-fluidity solvents, and accelerated solvent extraction (ASE) to analyze PAH contamination of lignite coal fly ash collected from an electrostatic precipitator in North Dakota [113]. The size of the fly ash particles were found to be 6 – 16 µm, 45 – 210 µm, and 1 – 40 µm in diameter tested by Combustion Engineering, US Standard Testing Sieves, and ASTM Method F 662 respectively. Sixteen PAHs were used and separated into three groups: 1) low-molecular-weight, 2) medium-molecular-weight, and 3) high-molecular-weight PAHs. The low-molecular-weight species were best recovered using supercritical extraction. The recovery of the medium-molecular-weight

PAHs were similar across all extraction techniques. The high-molecular-weight species seemed to be equally recovered using all of the extraction conditions, seen in Table 7 [113].

Table 7: Results of the extraction efficiencies from the different extraction procedures used on the lignite coal fly ash in the study by Kenny and Olesik [113]

Compound	**Soxhlet**	**CO_2 90°C, 238 atm**	**90% CO_2 10% methanol @ 70°C, 238atm**	**60% CO_2 40% methanol @ 70°C, 238atm**	**Methanol Accelerated Solvent Extraction @ 150°C, 136 atm**	**Methylene Chloride Accelerated Solvent Extraction @ 150°C, 136 atm**
Naphthalene	7 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acenaphthylene	50 (5)	21 (8)	47 (17)	30 (14)	28 (1)	21 (9)
Acenaphthene	59 (4)	29 (9)	56 (20)	23 (10)	31 (3)	26 (10)
Fluorene	69 (5)	60 (10)	74 (13)	50 (8)	45 (2)	42 (14)
Phenanthrene	82 (4)	105 (5)	93 (6)	96 (3)	83 (7)	73 (27)
Anthracene	72 (5)	108 (5)	84 (6)	105 (6)	97 (10)	75 (30)
Fluoranthene	89 (3)	133 (1)	84 (6)	105 (6)	102 (9)	71 (23)
Pyrene	87 (4)	104 (5)	70 (8)	98 (5)	102 (8)	93 (27)
Benzo[a,h]anthracene	83 (5)	129 (6)	93 (6)	104 (70	96 (4)	69 (22)
Chrysene	76 (5)	62 (1)	86 (5)	112 (6)	125 (9)	81 (30)
Benzofluoranthenes	85 (3)	102 (8)	79 (7)	120 (12)	92 (3)	71 (21)
Benzo[a]pyrene	82 (3)	85 (1)	58 (6)	86 (5)	83 (2)	67 (21)
Indeno[1,2,3-c,d]pyrene	93 (2)	81 (4)	57 (5)	89 (2)	84 (10)	89 (31)
Dibenzo[a,h]anthracene	90 (4)	110 (6)	95 (12)	97 (2)	103 (10)	98 (24)
Benzo[g,h,i]perylene	87 (2)	100 (6)	94 (14)	98 (3)	94 (7)	93 (27)

Lundstedt and coworkers tested the reliability and efficiency of PSE for extracting PAHs from contaminated soils [51]. Soil samples were extracted using a Dionex ASE 200 Accelerated Solvent Extractor and analyzed using a GC/MS [51]. The extraction parameters such as sample load, cycle time, temperature, and solvent. were varied to find the optimal extraction conditions. The best results were obtained when mixing the soil with a bulk material before extraction, using multiple shorter static extraction cycles followed by rinsing with 100% of the extraction cell volume, and using elevated temperatures between 100°C – 150°C. Higher extraction temperatures than 150°C can be used if the equipment will not be harmed with repeated use. Generally, higher temperatures are known to shorten the lifetime of the equipment. High-molecular-mass PAHs recovery increased as the sample load decreased. Alternatively, low-molecular-mass PAHs were extracted to a similar extent across a range of sample loads but were more efficiently extracted at high temperatures [51]. The study concluded that the sample load and the volume of extraction solvent had the most significant effect on the extraction efficiency of aged contaminated soil samples [51]. The solvent used and the ratio of the solvent mixture had a minimal effect on extraction efficiency. Various binary solvents give similar extraction results. However, acetone-hexane (50:50 v/v) was the best performing solvent mixture.

Chapter 3: Development of Extraction Methods for PAH Analysis

Abstract

In order to create a contact transfer test method for fireground contaminants, sufficient extraction and analysis methods need to be developed for quantifiable PAH detection of a potential transfer from the outer shell of firefighter turnout gear. Several extraction parameters were tested in this study, including the number of extraction cycles, the use of glass beads, and the selection of extraction solvent. After extraction method optimization, the best extraction method for outer shell material was determined to include three extraction cycles, adding glass beads to the extraction cells, and using either acetonitrile or methanol as the extraction solvent. The same extraction parameters could also be used for extracting SynDaver™ skin, except for the extraction solvent. Acetonitrile generally had higher PAH recovery compared to methanol but exceeded the AOAC recovery limits of 80-115% for some PAH compounds. Meanwhile, methanol recovered at most 80% of PAHs, indicating that the extraction method for the outer shell would have to be modified for extractions of SynDaver™ skin when using methanol. The differences between the outer shell and SynDaver™ skin extraction indicate a relationship between extraction material and the extraction solvent. This study found that pressurized solvent extraction is a suitable extraction method for the extraction of PAH compounds from outer shell materials. Alternative materials like SynDaver™ skin still require further optimization.

3.1. Introduction and Background

Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring in coal, crude oil, and gasoline. At fire scenes, PAHs are most commonly found in the particulate matter of soot and smoke, produced due to the incomplete combustion of organic material. The particulate matter that makes up smoke and soot is typically small enough to penetrate the interfaces of firefighter turnout gear easily. When these small particles infiltrate the turnout gear, they deposit on the skin of firefighters, causing a possible occupational exposure to chemicals present on the particles. Meanwhile, the large particulates deposit on the surface of firefighter turnout gear, unable to make it through the interfaces. Studies have confirmed the presence of PAHs on the skin and turnout gear [84] [10] [117] [38]. Total PAH concentrations have been found to exceed the recommended exposure limits during overhaul at some fire scenes studied by Bostald-Johnson and coworkers [16].

Personal air samples collected by individual firefighters trained by researchers recorded PAH air concentrations ranging from 430 – 2700 $\mu g/m^3$ outside firefighting ensembles and 32 – 355 $\mu g/m^3$ inside firefighting ensembles [38]. Furthermore, PAH concentrations on a 10 cm x 10 cm outer shell swatch were 630 ng/cm^2 after a 50-minute exposure to a Class A fire [118]. PAHs have been found on the hands and necks of firefighters with concentrations as high as 313 $\mu g/m^2$ and 152 $\mu g/m^2$, respectively [10]. Even though these concentrations are below the Occupational Safety and Health Administration (OSHA) Permissible Exposure Limits (PEL) for PAHs of 0.2 mg/m^3 for an eight-hour workday and the National Institute for Occupational Safety and Health (NIOSH) exposure recommendation of 0.1 mg/m^2 for a ten-hour workday they show that firefighters are exposed to these PAHs routinely at fire scenes [119, 68].

To date, there have been few studies investigating the extraction or transfer of PAHs from turnout gear. However, PAH extraction methods have been developed for soil, tissue, food and water samples, but these methods have not been used for extracting textile materials. Current extraction methods could be adapted for the extraction of a substrate as specific as firefighter turnout gear. Chromatography coupled with mass spectrometry has been the primary method of analysis for PAHs since the 1970s.

Out of the many PAH extraction methods, Soxhlet extraction is the current gold standard for solid matrices. However, this method of extraction is time-consuming, requires an abundant amount of solvent, and requires a great deal of labor. Alternative extraction methods such as microwave-assisted, ultrasonic, pressurized solvent, supercritical fluid, are equally, or more, efficient than Soxhlet extraction, although in some cases, they have been reported to be less efficient [51, 19].

Alternatively, pressurized solvent extraction (PSE) could be a promising method for the extraction of PAHs from firefighter turnout gear. The main advantages of PSE feature high pressures and temperatures. High pressures ensure the solvent remains in the liquid state as the temperature of the system increases above the solvent's boiling point. Furthermore, the high pressures of PSE drive equilibrium in the system, forcing solvent into microporous areas, such as the space between the fibers in the yarns of turnout gear. Moreover, the high pressure prevents the creation of air bubbles, thus exposing the sample to more extraction solvent. The high temperatures increase the capacity of the solvent to dissolve sizeable molecular weight analytes, while also reducing the viscosity and surface tension, thus improving the contact between the analytes and the extraction solvent [51]. Lastly, liquid extractions can utilize multiple organic

solvents like methanol, hexane, and cyclohexane by themselves or in a mixture to maximize the extraction PAHs.

This study evaluated PSE as a potential extraction method for PAHs from firefighter turnout gear. Extraction variables, including the number of cycles, use of glass beads, and extraction solvent, were tested and compared to create the best extraction method for firefighter turnout gear. Not all of the layers of firefighter turnout gear are contaminated equally. Therefore, not all layers were included in this study; only the outer shell material of turnout composite was extracted because as the outer layer, it is the most contaminated layer of gear according to firefighter exposure studies. After the extraction parameters were set, SynDaver™ tissue plates were extracted to confirm the adequate recovery of PAHs.

3.2. Materials and Methods

Fabric

Firefighter outer shell samples were prepared using PBI Max 7.0 obtained from Safety Components Fabric Technologies Inc. The PBI Max 7.0 material is comprised of 70% PBI/para-aramid spun yarns and 30% 600 denier para-aramid filament woven together [120].

Skin Surrogate

The skin surrogate SynDaver™ basic tissue plate was purchased from SynDaver™ [121]. Little information was provided on the composition of the basic tissue plate. According to manufacturer specifications the tissue plate consisted of water, salt, and fiber, most likely polyurethane.

Chemicals and Solvents

A standard PAH mixture Certified Reference Material 31011/ SV Calibration Mix #5 was supplied by RESTEK (Bellefonte, PA, USA). The exact measurements of the PAH stock can be

seen in Table 8, calculations were based on each PAH having a concentration of 2,000 µg/mL. A separate 300 µg/µL PAH stock used to dope samples was created by diluting the SV calibration mix in methanol.

Table 8: Concentration and carcinogenicity of PAH compounds in SV calibration mixture

PAH Compound	Actual Concentration (µg/mL)	IARC Classification
Naphthalene	2018	2B
Acenaphthylene	2002	3
Acenaphthene	2009	3
Fluorene	2009	3
Phenanthrene	2016	3
Anthracene	2019	3
Fluoranthene	2011	3
Pyrene	2002	3
Benzo(a)anthracene	2014	2B
Chrysene	2017	2B
Benzo(b)fluoranthene	2006	2B
Benzo(k)fluoranthene	2010	2B
Benzo(a)pyrene	2009	1
Indeno(1,2,3,-c,d)pyrene	2002	2B
Dibenz(a,h)anthracene	2019	2A
Benzo(g,h,i)perylene	2008	3
*IARC Classifications: 1 = known carcinogen , 2A = probably carcinogenic to humans, 2B = possibly carcinogenic to humans, 3 = carcinogenicity not classifiable, 4 = probably not carcinogenic to humans		

Doping Procedure

Outer shell samples were created by cutting bulk material into 7.6 cm x 7.6 cm square samples. Afterward, each sample was spiked with 100 µL of a 300 µg/µL PAH stock solution, applying roughly 30,000 µg of each PAH onto the surface of the sample. Droplets were applied in a linear array, seen in Figure 18. Samples were then given 20 minutes to allow the PAH solvent to dry. Once the solvent evaporated, leaving the PAHs on the fabric surface, the samples were transferred to the extraction cells of the Buchi Speed E-916 extractor. Skin surrogate samples were created and doped in the same manner as the outer shell samples.

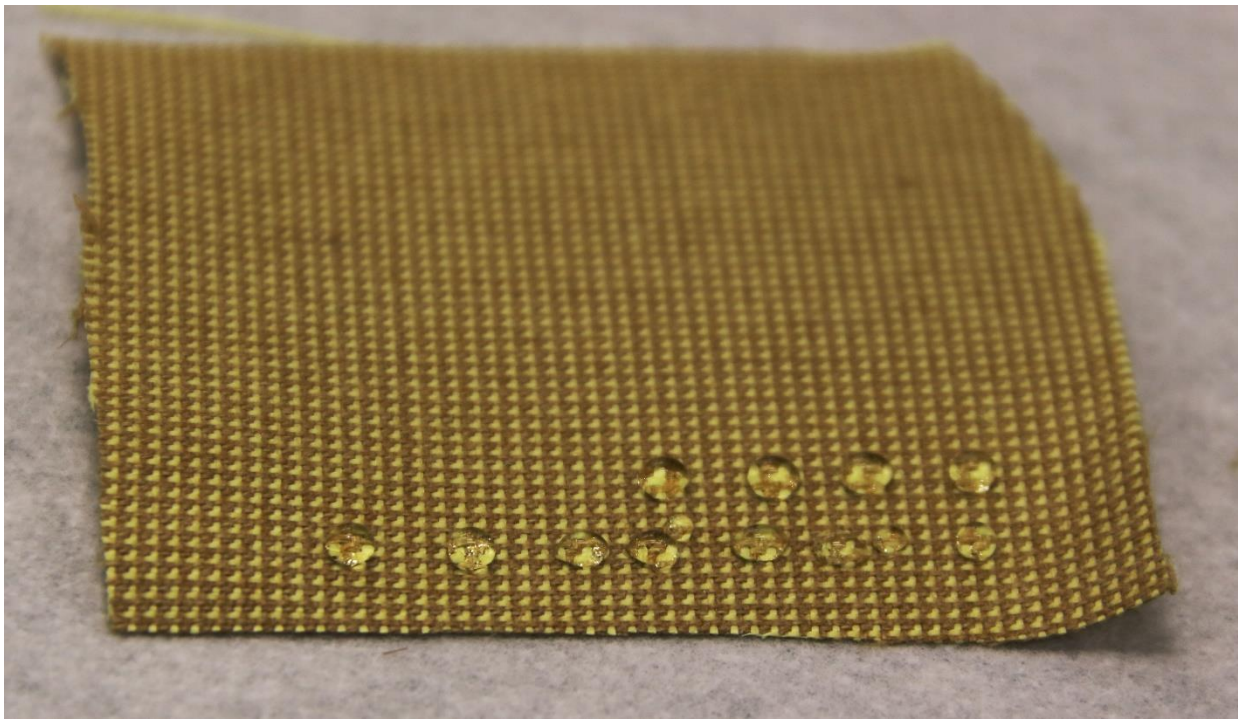


Figure 18: Image of the row and column arrangement pattern droplets were added to the outer shell sample

Pressurized Speed Extractor Method

Outer shell material and SynDaver™ skin samples were extracted using a Buchi Speed Extractor E-916, seen in Figure 19, equipped with 10 mL stainless steel extraction cells. The extraction process is a combination of dynamic and static steps. The static conditions of the extraction method hold the cells at a constant temperature, pressure, and volume of solvent for a specified time. After the static hold period, the solvent is flushed into scintillation vials. The static hold period can be repeated, resulting in multiple extraction cycles.

Figure 19: Image of Buchi E-916 speed extractor

Extraction cells were prepared by placing a small cellulosic filter at the bottom of the cell before the addition of contaminated samples. Contaminated samples were rolled to fit in the extraction cell; care was taken not to allow cross-contamination between samples. After the samples were added to the extraction cell, a large cellulosic filter was placed at the top of the cell. The cellulose filters were added to the top and bottom of the extraction cell to prevent any debris from clogging the condensing coils of the extractor.

Due to several extraction parameters being tested during this study, parameters that remained consistent among all extraction are displayed in Table 9.

Table 9: Pressurized solvent extraction parameters that remained constant throughout this study

Pressure	100 bar
Temperature	100°C
Gas	N_2
Cell Size	10 mL
Vial Size	60 mL
Solvent Flush Time	1 minute
Gas Flush Time	3 minute

Upon completion of the extraction each sample was diluted using a volumetric flask, to either 10 mL or 25 mL depending on the amount of solvent eluted during extraction, to create equitable dilutions for each sample before High Performance Liquid Chromatography (HPLC) analysis. Subsequently, 2 mL of extract solution was transferred into HPLC vials through 0.22 µm PTFE filters (Foxx Life Sciences). The filters were to prevent any lose fibers, particulates, or potential obstructions created during extraction from damaging the HPLC system used for analysis.

Chemical Analysis

Liquid chromatography analyses were performed on an Agilent 1260 Infinity II LC system coupled with Infinity Lab LC/MSD along with the following modules: Agilent 1260 Infinity Binary Pump (G1312B), Agilent 1260 Infinity Autosampler (G1392B), Agilent 1260 Infinity Diode Array Detector (G4212B) with 10 mm Max-Light flow cell (Agilent Technologies). Chromatographic separation was done using a PAH Zorbax Eclipse column (1.5 x 150mm 3µm pore size; Agilent Technologies). The HPLC operated under the following conditions: column temperature - 35°C; mobile phase - gradient method shown in Table 10; five µL injection volume; diode array detector was set to wavelengths 220nm, 254nm, 270nm,

285nm; fluorescence detector was set to emission λ: 425nm; excitation λ 340nm. Data were analyzed with Open Lab CDS Chemstation (Agilent Technologies).

Table 10: HPLC gradient method for PAH analysis

Time min	Flow mL/min	% A (Water)	% B (Acetonitrile)
0	0.5	60	40
0.66	0.5	60	40
20	0.5	0	100
25	0.5	0	100
27	0.5	60	40
30	0.5	60	40

3.3. Results and Discussion

Effect of the number of extraction cycles on PAH recovery

Extractions on the outer shell were done using three or five extraction cycles followed by a flush cycle. Optimization of the number of cycles was necessary to maximize recovery of PAHs, while minimizing time and solvent. Upon analysis, three extraction cycles were found to be better than five extraction cycles, recovering more PAHs from outer shell material, seen in Figure 20. Generally, the five-extraction cycle method had a lower recovery, ranging from 80% - 100%, except for naphthalene. The three-extraction cycle method had recoveries ranging from 95% - 127%, except for naphthalene. The general lower recovery in the five-extraction cycle method was surprising. The increased usage of solvent would expectedly lower sensitivity, as the sample would be diluted; however, the recovery of PAHs was decreased. Further investigation is required to confirm the findings of this abnormality.

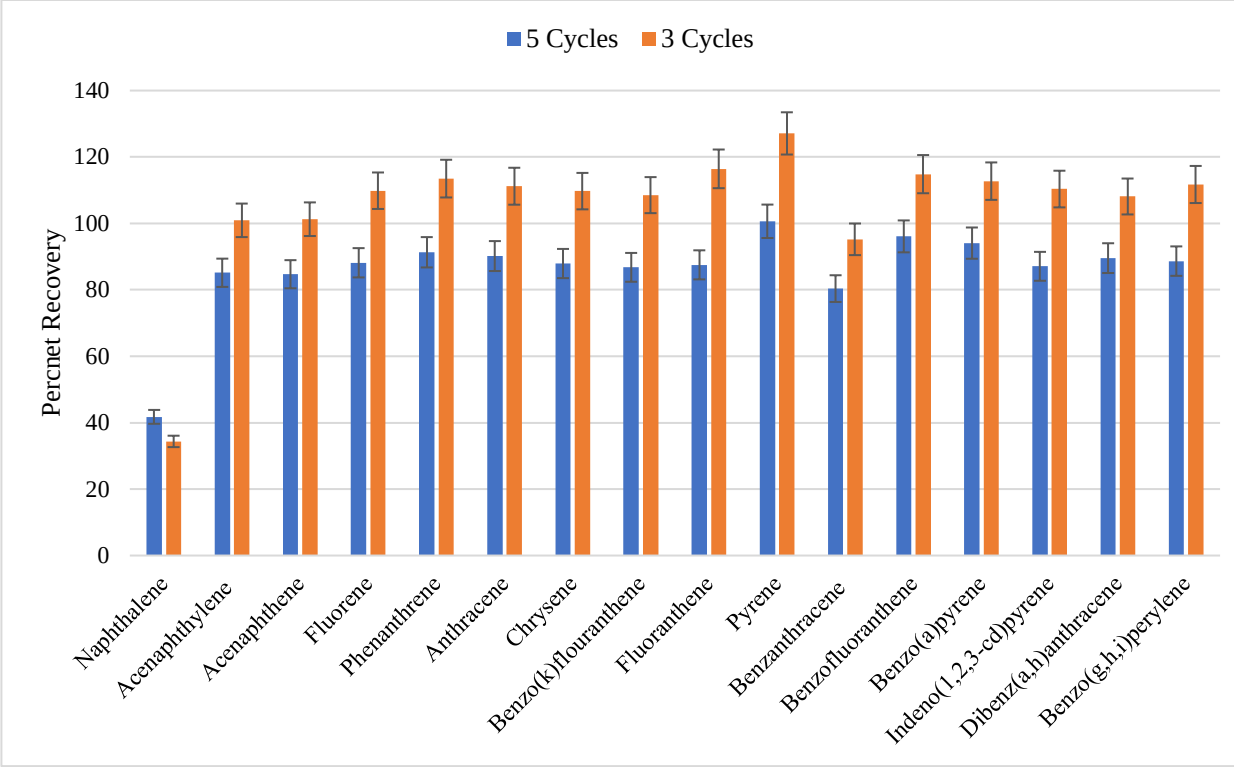


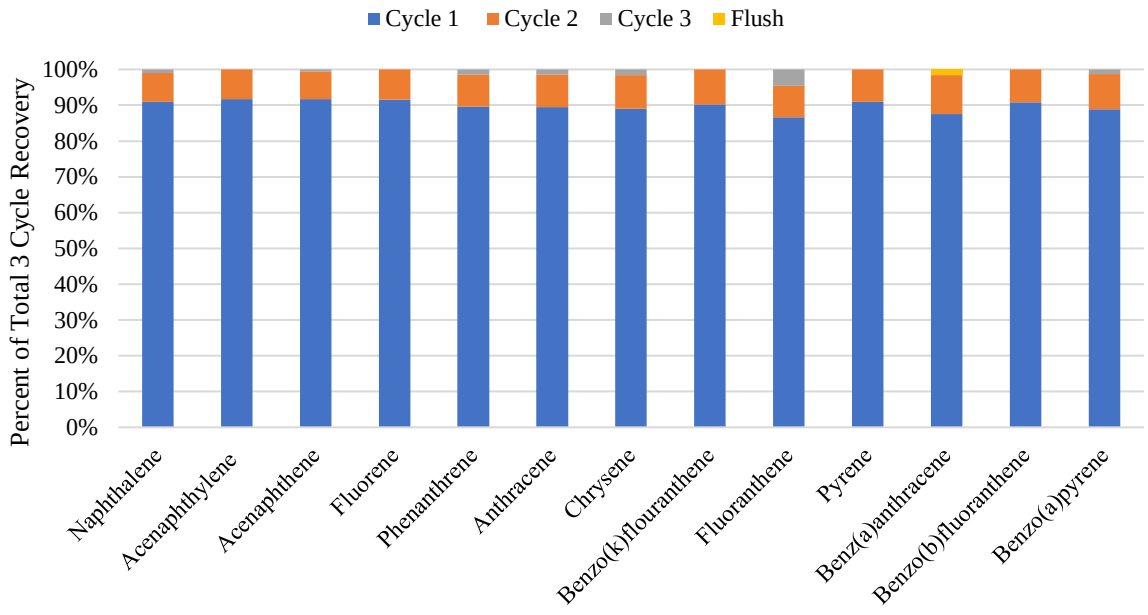
Figure 20: Cycle optimization results for pressurized solvent extraction method

Figure 21 demonstrates the individual cycle efficiency for the three and five-extraction cycle methods separately. Since this is done using a stacked bar graph it shows the percent of total recovery for each cycle relative to the number of extraction cycles. However, both graphs show that the majority of each PAH is extracted in the first cycle, roughly 80 – 90% of the total recovery. After the second extraction cycle, there is little PAH left to recover. The extraction method would likely have similar results if using two extraction cycles or three extraction cycles.

Naphthalene had a relatively low recovery, less than 50%, in both three and five cycle extractions. The low recovery of naphthalene could be explained by the compound volatilize within the extractor or during the doping of samples. Shorter condensing coils were installed in the speed extractor to prevent precipitants from forming, which leads to increased system and solvent temperatures, unintendingly creating a favorable scenario for naphthalene to volatilize. During the doping steps time was given to allow the methyl chloride solvent to evaporate,

naphthalene could have also evaporated during this time. The low recovery of naphthalene will be seen repeatedly throughout all extraction experiments.

(A)



(B)

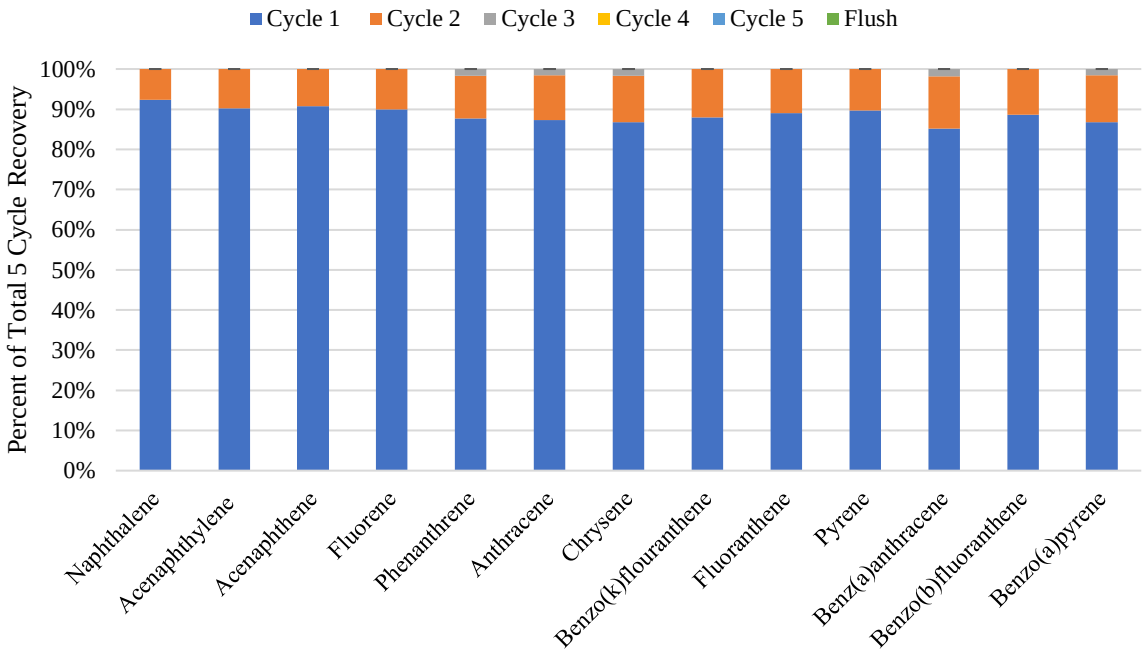


Figure 21: Pressurized solvent extraction method optimization efficiency of each extraction cycle. (A) Cycle efficiency using three extraction cycles. (B) Cycle efficiency using five extraction cycles.

Effect of glass beads on recovery of PAHs from outer shell material

Five grams of 4mm glass beads were added to each extraction cell to occupy any void space to reduce the solvent needed to fill the extraction cell, ultimately reducing the solvent required per extraction. The addition of glass beads did not significantly affect the recovery of twelve of the sixteen PAHs from outer shell material ($p<0.05$), seen in Figure 22. Naphthalene ($p=0.0004$), acenaphthylene ($p=0.002$), acenaphthene ($p=0.02$), and benzo(g,h,i)perylene ($p=0.02$) did have significantly different recoveries ($p<0.05$) with the addition of glass beads. Even though the extraction of most PAHs was not significantly affected by the addition of glass beads, there were four PAHs that had increased recovery. Thus, the addition of glass beads either had no effect on PAH recovery or increased the recovery.

Another benefit of the addition of glass beads to the extraction cell was the reduction of extraction solvent used. The amount of solvent used was reduced to less than 10 mL, a noticeable reduction from the 18 mL of solvent used when glass beads were not added. The extractor can run up to six samples at a time, and when accounting for the reduction of solvent used for each sample and each extraction, the amount of solvent saved becomes significant. Within a single extraction, nearly 50 mL of solvent will be saved, and after 20 extractions, the amount of solvent saved equates to a liter of solvent. Perhaps more importantly, using minimal amounts of solvent will prevent the dilution of samples, which may affect the limit of detection. In order to reduce the use of extraction solvent, glass beads will be used in the extraction method.

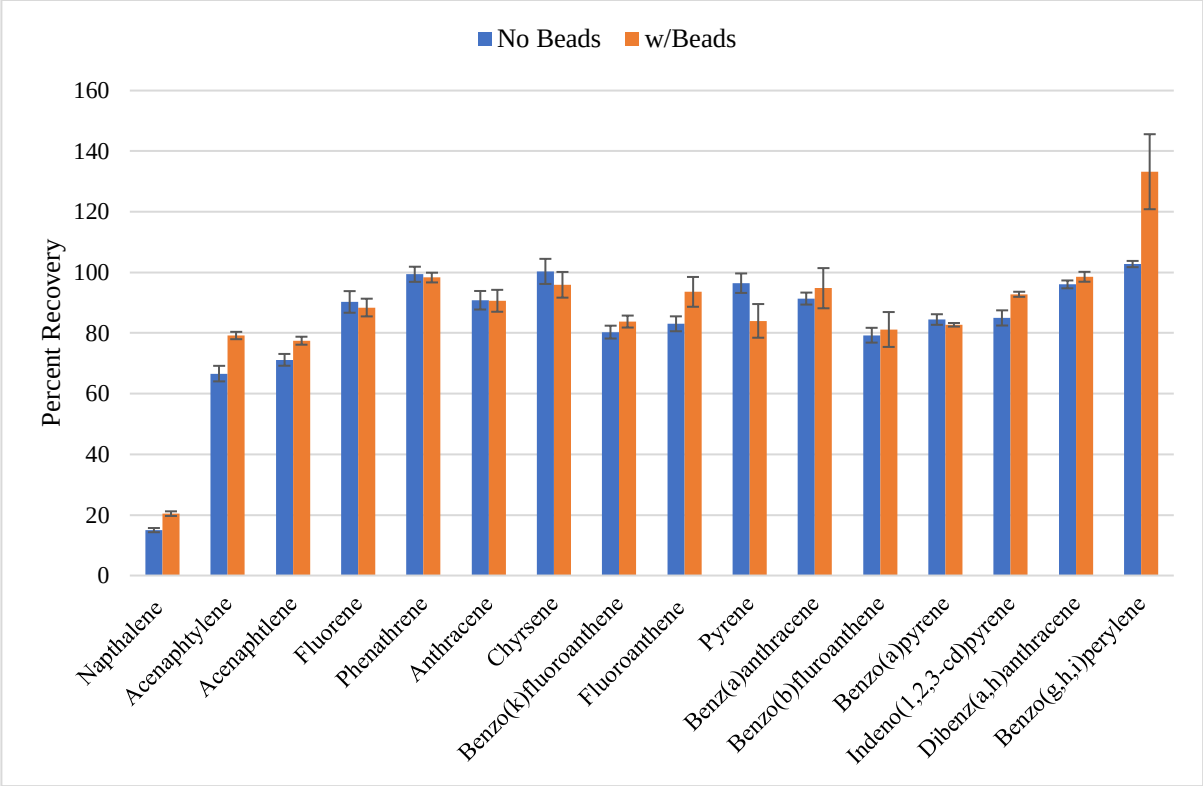


Figure 22: Pressurized solvent extraction method optimization effect of glass beads on recovery of PAHs using three extraction cycles (n=3).

Effect of extraction solvent on recovery of PAHs from outer shell material

Several solvents are compatible with the Buchi speed extractor and have been used to extract PAH compounds from various media. However, the goal of this work was to develop a simple and quick extraction method that used a single solvent and minimize the number of steps. Hexane and cyclohexane are two popular solvents for PAH extraction but were not used to preserve the HPLC system. Acetonitrile and methanol were chosen and evaluated because of their compatibility with the PAHs and HPLC column. For all PAH compounds, with the exception of naphthalene and acenaphthylene using methanol, both extraction solvents were able to recover at least 80% of each PAH, which falls within the Association of Official Analytical Chemists (AOAC) recovery limit of 80-115% for liquid extraction, seen in Figure 23.

The effect of extraction solvent did produce some significant differences for certain PAHs. At p-value threshold of $p<0.05$, a significant difference was found for acenaphthylene, fluoranthene, benzo(k)fluoranthene, and benzo(g,h,i)perylene. Despite a significant difference being found for four PAHs, this difference does not affect the recovery of the majority of PAHs. Overall, either acetonitrile or methanol are acceptable extraction solvents for the recovery of PAHs from outer shell materials.

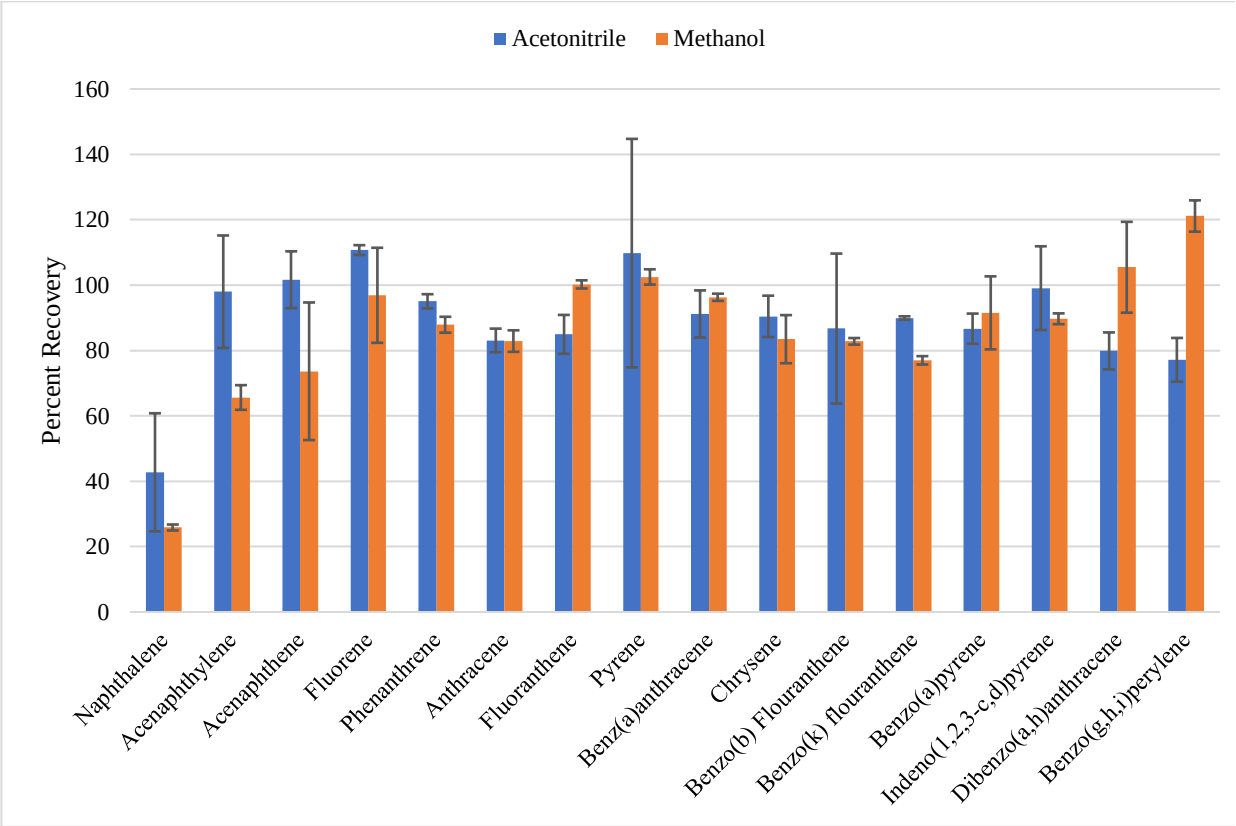


Figure 23: Pressurized solvent extraction method optimization effect of extraction solvent on recovery of PAHs using three extraction cycles and glass beads (n=3).

Recovery of PAHs from SynDaver™ skin

After outer shell extraction experiments were concluded, SynDaver™ skin samples were extracted to identify the best extraction parameters. Based on the results from outer shell experiments, the number of extraction cycles was set to three cycles with glass beads. Since

some differences were found between extraction solvents during outer shell extractions, both acetonitrile and methanol were used to extract PAHs from SynDaver™ skin samples.

Acetonitrile and methanol were found to be significantly different ($p<0.05$) for the SynDaver™ skin extractions, except for chrysene, benzo(b)flouranthene, and benzo(a)pyrene, seen in Figure 24. Acetonitrile tended to have a higher recovery for each PAH compound, recovering 74 – 133%, excluding naphthalene. Meanwhile, methanol recovered at most 80% of benzo(k)flouranthene, and generally recovered 60-80% of each PAH, excluding naphthalene. The recoveries of both of extraction solvents used were not within AOAC guidelines for recovery analytes using liquid extraction.

The differences in recovery of methanol and acetonitrile in the outer shell and SynDaver™ skin extractions indicate that there is a relationship between the material being extracted and the extraction solvent. Simply put, the best extraction solvent for one material might not be the best for another material even though the same compounds are being extracted. In the outer shell extractions, acetonitrile and methanol recovered similar amounts of each PAH compound; however, the SynDaver™ skin extractions showed that acetonitrile was significantly better for recovering most of the PAH compounds.

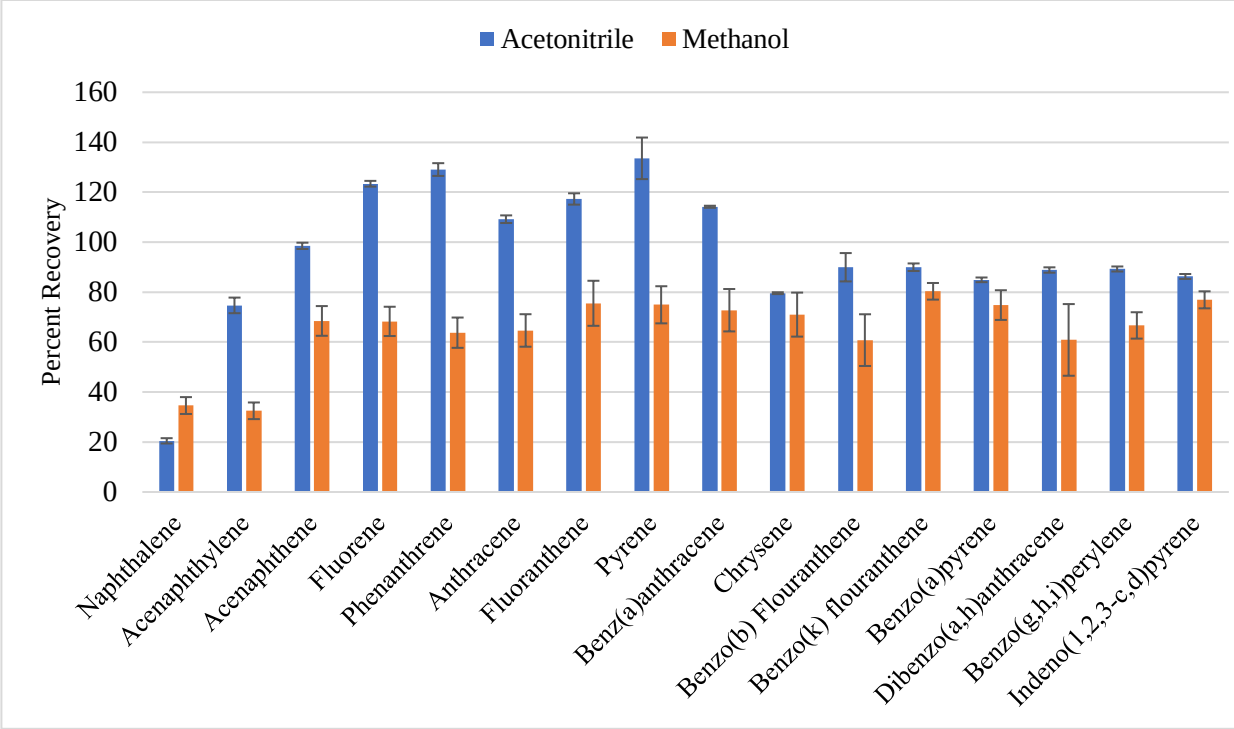


Figure 24: Pressurized solvent extraction method optimization effect of extraction solvent on recovery of PAHs from SynDaver™ using three extraction cycles and glass beads (n=3).

3.4. Conclusions

Pressurized solvent extraction was found to be a suitable method for the recovery and extraction of PAH compounds from firefighter turnout material. No significant differences were found between results using five or three extraction cycles. In future extractions, three extraction cycles should be used to save time and solvent. Likewise, the addition of glass beads did not significantly affect the recovery of most PAHs; however, four PAHs were found to have increased recovery when glass beads were used. Additionally, the glass beads were found to reduce the amount of solvent used per extraction sample.

In the case of the best extraction solvent for outer shell samples, significant differences were found between the extraction solvents for four PAHs. Acetonitrile had higher recovery of acenaphthylene and benzo(k)fluoranthene, while methanol had higher recovery of fluoranthene and benzo(g,h,i)perylene. Either solvent would be acceptable to use for recovering PAHs from

outer shell material because both solvents were within the AOAC recovery limits for liquid extraction. Alternatively, when extracting SynDaver™, skin acetonitrile had a higher recovery for nearly all PAHs and was significantly different from methanol, but both solvents had recoveries that were outside the AOAC recovery limits. When selecting the best extraction solvent, multiple solvents should be tested because the interaction with the sample matrix will influence the extraction of the analytes.

Based on the extraction experiments done to recover PAHs from the outer shell and SynDaver™ skin future extractions will use the following parameters: three extraction cycles, add glass beads to the extraction cells, use either acetonitrile or methanol for outer shell samples, and use acetonitrile for SynDaver™ skin samples.

Chapter 4: Skin Surrogate Comparison using Flow-Through Diffusion Cell Experiment

Abstract

Human skin is a highly organized complex structure consisting of several layers of tissue that acts as a barrier to penetrating chemicals. Several synthetic membranes have been developed to replace human skin models for *in vitro* permeation studies. However, most synthetic skin models struggle to replicate the skin's highly ordered structure and metabolic activity. Regardless, the main goal of the synthetic skin models is to simulate the absorption and permeability of human skin for chemical permeation studies.

This study used naphthalene (NAP) and orthophenylphenol (OPP) to compare the absorption (μg cm^{-2}) and permeability (K_p cm h^{-1}) of two synthetic skin models, SynDaver™ skin and Strat-M® membrane, to porcine skin. SynDaver™ skin was found to be three times more permeable for orthophenylphenol and 50% more permeable for naphthalene compared to porcine skin. Conversely, the Strat-M® membrane was relatively less permeable for naphthalene) and nearly impermeable for orthophenylphenol compared to porcine skin. Overall, the SynDaver™ skin overestimated absorption while the Strat-M® membrane underestimated absorption of the test compounds. However, SynDaver™ skin absorption correlated to porcine skin absorption of both chemicals. Meanwhile, Strat-M® correlated well to porcine skin only for the absorption of naphthalene. For use as a skin surrogate for human skin, SynDaver™ skin should be selected over the Strat-M® membrane. The overestimation tendency for SynDaver™ skin can be corrected using a correlation model. Although this method may not be accurate, these preliminary data suggest that SynDaver™ skin would serve as an estimation of the penetration capability of PAHs through human skin.

4.1. Introduction and Background

Human skin is a complex organ consisting of several layers of tissue, as seen in Figure 25. The stratum corneum (SC) is the outermost layer of the epidermis, consisting of corneocytes (dead cells) stacked 10 – 25 layers, about 10 – 40 μm, embedded in an intercellular lipid matrix, in a brick and mortar arrangement [87]. The brick and mortar arrangement of the cells create tortuous pathways that chemicals must travel to penetrate the skin. Furthermore, the tight molecular packing of the lipid matrix limits molecules that can pass through the skin barrier based on their physical size. Few molecules with a molecular weight of 500 Dalton are capable of passive diffusion through the skin; this is referred to as the "rule of 500" [92]. The lipid matrix contains various lipids, such as phospholipids and ceramides, which impart hydrophobic characteristics on skin [105]. The corneocytes of the stratum corneum are sometimes considered hydrophilic domains, and being surrounded by the lipid matrix creates amphiphilicity within the stratum corneum, protecting against lipophilic and hydrophilic chemicals. All of these factors provide a barrier to chemicals that may penetrate the skin. If a chemical is capable of penetrating the stratum corneum it is subject to systemic absorption at the capillary loops where the epidermis and dermis layers meet [91]. In short, the stratum corneum acts as the rate-limiting step in chemical absorption [82].

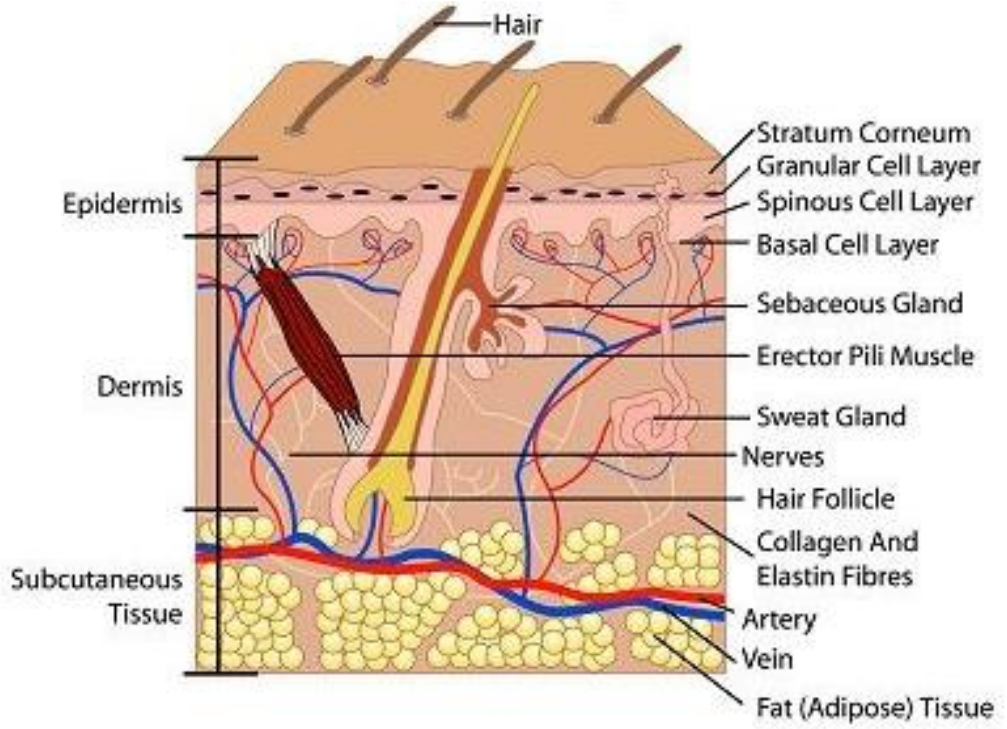


Figure 25: Structure of human skin detailing the epidermis, dermis, and hypodermis layers [86]

Human skin models are the gold standard for permeation studies; however, using human skin is difficult to obtain due to ethical reasons and limited supply. Animal skin models are acceptable alternatives to use for chemical permeation studies but come with some disadvantages. Rodent skin is known to overestimate the absorption of chemicals in human skin, due to histological differences of their skin [82]. Porcine skin, which is widely used in *in vitro* tests, is the best animal model to represent human skin because of the similar cell structure [97]. Dalton and coworkers (2006) found no significant difference in the *in vitro* absorption of VX nerve agent through human and pig skin under multiple test conditions [122]. If human skin is unavailable, then porcine skin would be the best animal skin model alternative.

Synthetic membranes were initially developed to replace human skin models for *in vitro* permeation studies [123]. Artificial skin models range from homogeneous polymeric materials to lipid-based parallel artificial membrane-permeability assays [85]. However, most synthetic

models lack the highly ordered structures, metabolic processes, and interactions of proteins that occur in the human epidermis [105]. Even though most synthetic membranes lack the higher order and functionality of human skin they are used in *in vitro* permeation studies because of their low cost, ease of customizing, and reduced variability relative to animal or human skin models [82].

Strat-M® membrane is a synthetic, non-animal-based model designed for transdermal diffusion testing to predict the diffusion of chemicals into human skin without lot-to-lot variability or safety and storage limitations [124]. Strat-M® membranes are designed to mimic the layered structure and lipid chemistry found in the human epidermis, seen in Figure 26. The thickness of each Strat-M® is approximately 300 µm and comprises a top layer supported by two layers of porous polyether sulfone (PSE) on top of one single layer of polyolefin non-woven fabric support. Membrane layers are increasingly more porous and open, and also increasingly larger in thickness to mimic different layers of human skin. The porous layers of Strat-M® are treated with a proprietary blend of synthetic lipids containing a combination of ceramides, cholesterol, free fatty acids and others in a ratio similar to what is found in the human stratum corneum [105].

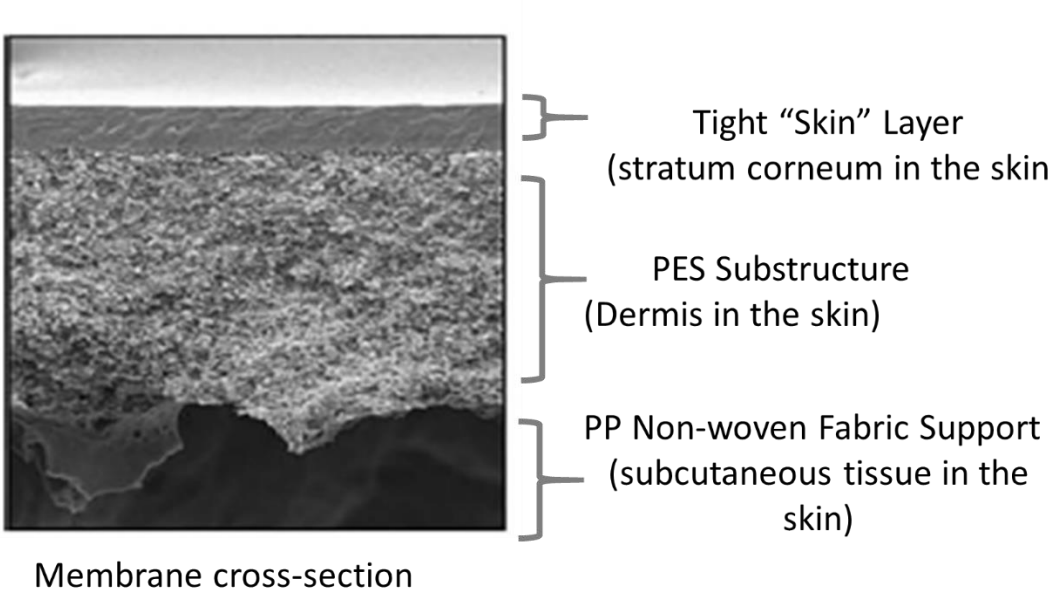


Figure 26: Illustration of the multilayered structure of Strat-M® membrane [107]

SynDaver™ designs synthetic models of canine and human organs, biological systems, and individual parts of the anatomy. SynDaver’s products are designed for medical device design verification, validation testing, practicing intradermal injections, and surgical procedures. SynDaver™ offers several skin models, including muscular tissue plate, abdominal tissue plate, basic tissue plate, skeletal muscle tissue plate, and adult skin, along with several others. An example of the muscular tissue plate is shown in Figure 27. Each model has a highly specific purpose and employs materials designed to match results obtained from testing on living tissue. This gives the synthetic tissue models realistic appearances and physical properties. The basic tissue plate consists of an upper layer of “adult skin” and a layer of “subcutaneous fat.” The standard thicknesses for the skin and fat are one millimeter and five millimeters, respectively [125]. According to the manufacturer’s website, the synthetic tissues are made from salt, water, and fiber. The basic tissue plates were selected to be used in this study because of their realistic appearance and feel. Furthermore, no previous research testing the permeability of SynDaver™

tissue plates to serve as a synthetic skin model for human skin for *in vitro* permeation studies was identified. Having gone untested thus far, the opportunity exists to investigate SynDaver™ tissue plates as a surrogate for human skin models, specifically in laboratory-based contact transfer evaluations.

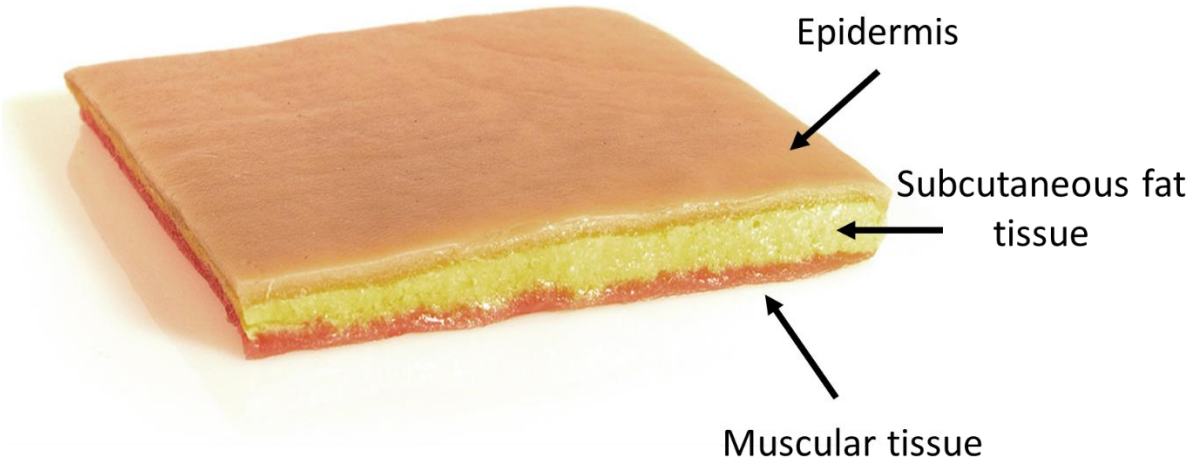


Figure 27: Image of SynDaver™ tissue plate with epidermis layer, subcutaneous fat tissue, and muscular tissue [125]

Flow-through diffusion cell experiments are currently used to determine the rate and extent of absorption of chemicals through skin *in vitro*, seen in Figure 28 [109]. *In vitro* methods used to determine percutaneous absorption typically utilize excised human or animal skin to simulate the living skin *in vivo* [126]. At the time of this study human skin was unavailable, resulting in the use of porcine skin. Percutaneous absorption rates measured by *in vitro* techniques are determined by passive diffusion through the non-living stratum corneum [126]. The main advantages of *in vitro* experiments over *in vivo* experiments that they are: easier to perform, less expensive, provide greater control of environmental factors, and sampling can be done frequently and directly beneath the skin [126]. Conversely, there are several disadvantages

of *in vitro* experiments: they do not take into account the cutaneous metabolism of the dermis or the inevitable changes to the stratum corneum after it is excised from the rest of the epidermis and no longer receives blood [126]. Although there may be several disadvantages, *in vitro* flow-through diffusion testing is the best alternative to *in vivo* animal testing.

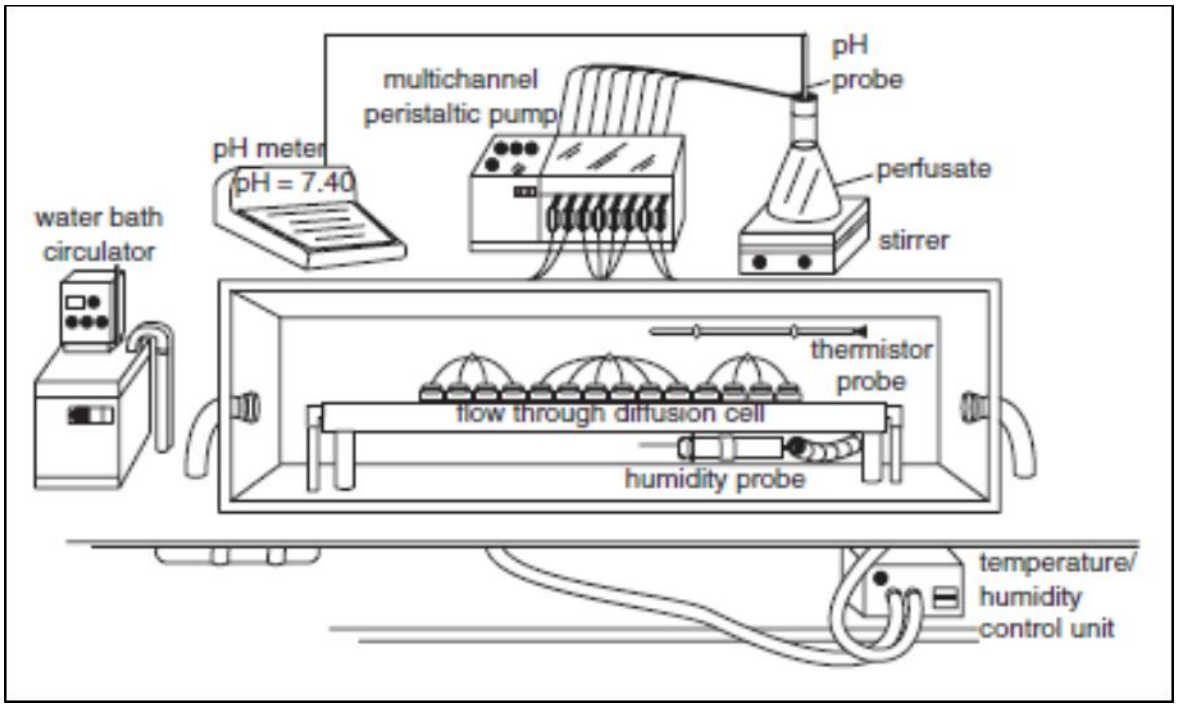


Figure 28: Illustration of the flow-through diffusion cell system in an environmentally controlled chamber

Ultimately, artificial skin models remain to be a potential replacement for animal and human skin models but require comparative testing to obtain absorption rates for chemicals of interest in each skin model. The following study used an *in vitro* flow-through diffusion cell system to compare the percutaneous absorption of naphthalene (NAP) and orthophenylphenol (OPP) through porcine skin, Strat-M® membrane, and SynDaver™ skin for their potential use as a human skin surrogate. It was crucial to identify a skin surrogate so that it could be used to simulate human skin absorption in the contact transfer test method, as human skin and porcine

skin would not be ideal for use in developing the contact transfer test method. Naphthalene was chosen out of the sixteen-priority polycyclic aromatic hydrocarbons (PAHs) because of cost, availability, and favorable physicochemical properties. Orthophenylphenol (OPP) was utilized as a control for its comparability to an abundance of literature, which allowed for an assessment of the experiment's validity [94, 96]. The two test chemicals were administered to the skin models in an artificial sweat vehicle to simulate the sweating firefighters experience during fire response.

4.2. Materials and Methods

Flow-through set up

Two 12-hour flow-through experiments were run simultaneously, each containing fourteen cells. The skin samples were distributed among the two flow-through systems prior to the beginning, seen in Table 11. Fresh porcine skin was obtained from a Yorkshire/Landrace pig (30 – 40 kg). The pig was shaved and dermatomed to a thickness of 450-550 micrometers with an electric dermatome. Afterward, each piece of skin was cut into a circular disk using a biopsy tool, placed into the diffusion cell, and secured in place by a screw cap, providing a dosing surface area of 0.64 cm^2. SynDaver™ and Strat-M® membranes were prepared and placed into the diffusion cells in an identical manner as the porcine skin. The skin membranes were dosed within 60 minutes of death of the porcine skin donor, so skin integrity testing was not necessary [127].

The underside of the skin disks was perfused with Bioscint and bicarbonate buffer spiked with dextrose and bovine serum albumin (BSA) to maintain pH between 7.3 and 7.6 and mimic the oncotic pressure found in the blood. The temperature of the perfusate and diffusion cells were maintained at 37°C. The flow rate was maintained at 4 mL h^{-1} using a peristaltic pump. The room temperature and relative humidity were recorded throughout the experiment for record-

keeping. Perfusate samples were collected in glass scintillation vials (Fisher Scientific) at times: 0, 15, 30, 45, 60, 75, 90, 120, 180, 240, 300, 360, 420, 480, 540, 600, and 720 minutes. After the flow-through diffusion cell systems were set up the chemical doses were added to each cell.

Dosing procedure

To eliminate the need for sample clean-up and maintain accuracy radiolabeled chemicals were used. Radiolabeled test chemicals ^{14}C-ortho-phenylphenol (concentration 0.1 mCi/mL, specific activity: 150 mCi/mmol, 250 µCi/vial) and ^{14}C-naphthalene (concentration: 0.1 mCi/mL, specific activity: 57 mCi/mmol, 50 µCi/vial) were obtained from American Radiolabeled Chemicals (Saint Louis, MO). Artificial eccrine perspiration (stabilized 200mL bottle) was used as an artificial sweat and was obtained from Pickering Laboratories. Acetone (purity 99%) was obtained from Millipore Sigma. Two separate dose mixtures, one for each test chemical, were prepared by dissolving 500µL of test chemical in 50µL of acetone and then vortexed. Afterwards, 4500µL of eccrine artificial sweat was then added to the stock mixture and vortexed again, producing 100µg/mL concentration. Skin disks were dose with 250 µL of the respective stock administered through a delivery channel to the top of the skin disk, according to Table 11Table 11. After dosing, diffusion cells were covered with Parafilm® pieces (Pechiney Plastic Packaging, Chicago, IL, USA) to minimize the loss of naphthalene.

Sample Analysis

After the experiment, 500 µL of perfusate for samples at times 15 – 90 minutes, and one milliliter of perfusate for samples at times 0 and 120 – 720 minutes were transferred to new vials. Different volumes were transferred to new vials based on the amount of perfusate available, as the amount of perfusate is dependent on the time between sample collection. At the end of the experiment the remaining dose was removed from the surface of the skin membrane with a

cotton swab, and the skin disk was transferred to wax paper. The surface of each skin disk was swabbed with a 1% soap solution using a cotton swab. The skin disks were then tape-stripped (Scotch Tape; 3M, St. Paul, MN, USA) six times, placing three strips into a single scintillation vials, and 10 mL of ethyl acetate were added. After tape-stripping, the center of the skin disks was punched with an 8 mm biopsy tool. The center and peripheral skin were separated and placed into individual scintillation vials along with 2 mL of BioSol. The fingertips of the gloves used during swabbing and tape stripping were extracted with ethanol. Samples were incubated at 50°C for 8 – 12 hours and analyzed using a liquid scintillation counter for ^{14}C determination.

Table 11: Diffusion Cell set up across the two diffusion cell systems

System 1 Set Up			**System 2 Set Up**	
1. Pig Skin + NAP	2. Pig Skin + NAP		15. Pig Skin + NAP	16. Pig Skin + NAP
3. Pig Skin + NAP	4. SynDaver + NAP		17. SynDaver + NAP	18. SynDaver + NAP
5. SynDaver + NAP	6. Strat-M + NAP		19. SynDaver + NAP	20. Strat-M + NAP
7. Strat-M + NAP	8. Strat-M + NAP		21. Strat-M + NAP	22. Pig Skin + OPP
9. Pig Skin + OPP	10. Pig Skin + OPP		23. Pig Skin + OPP	24. Pig Skin + OPP
11. SynDaver + OPP	12. SynDaver + OPP		25. SynDaver + OPP	26. SynDaver + OPP
13. Strat-M + OPP	14. Strat-M + OPP		27. Strat-M + OPP	28. Strat-M + OPP

Absorption Parameters Calculations

Absorption was defined as the total percentage of initial dose detected in the perfusate. Cumulative absorption (µg cm^{-2}) was calculated by summing the total dose that was detected in the perfusate at each sampling time. Flux (µg cm^{-2} hr^{-1}) was obtained from the steady-state slope of the cumulative absorption amounts versus time curves. The permeability coefficient (K_p) (cm hr^{-1}) was calculated from the ratio of the flux (µg cm^{-2} hr^{-1}) to the concentration (C_s) (µg/cm^{-3}) of the dose. The concentration (C_s) of the dose was 13.47 µg/mL and 3.29 µg/mL for naphthalene and orthophenylphenol respectively. The dose concentration was obtained from 2-µL dose checks ran on the scintillation counter. Calculations used to find the absorption parameters can be found in Table 12.

Table 12: Equations used to calculate absorption parameter values

Equation 5: Absorption (%Dose)	$= \frac{\text{Total µg in Perfusate}}{\text{µg in Initial Dose}}$
Equation 6: Cumulative Absorption	$= \frac{\text{Total µg in Perfusate}}{0.64\text{cm}^2}$
Equation 7: Flux	$= \frac{\text{µg}}{0.64\text{cm}^2 * \text{hr}}$
Equation 8:Permeability Coefficient (K_p)	$= \frac{\text{µg}}{0.64\text{cm}^2 * \text{hr}} / \frac{\text{Dose µg}}{\text{cm}^3}$

4.3. Results and Discussion

Absorption Profiles

Due to leaks during the experiment, cells 9, 10, 11 and 20 (shown in Table 11) could not be included in the results. Looking at the absorption profiles, seen in Figure 29, the cumulative absorption of both compounds in porcine skin fell in between the synthetic skin models. The absorption profiles for NAP were comparable in each skin model (Figure 29A). However, the

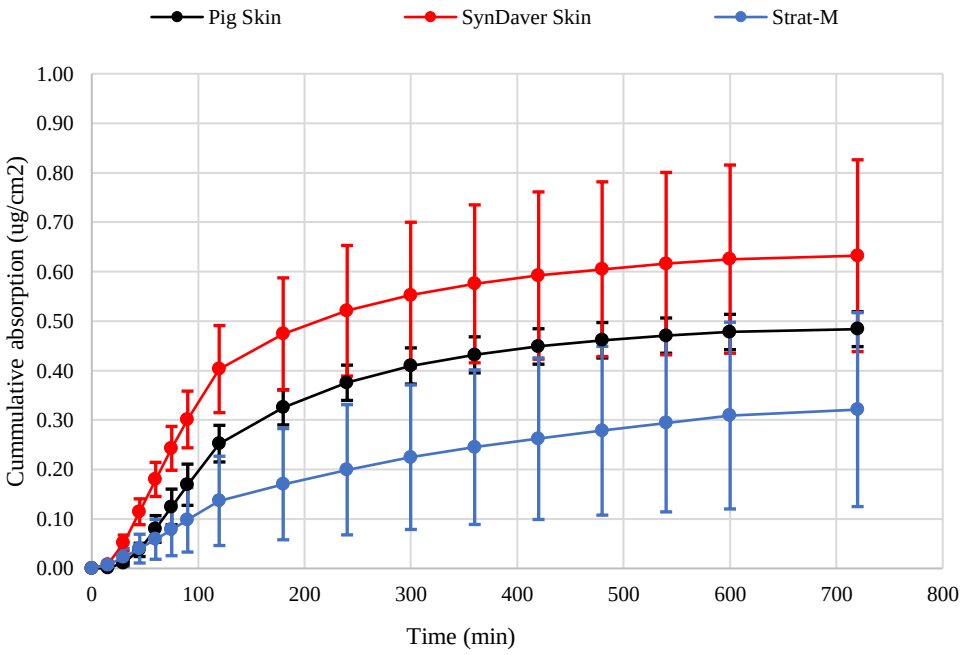
absorption profiles for OPP were different in each skin model. SynDaver™ skin was seen to begin absorbing the OPP dose nearly as soon as the experiment began and its rate of absorption changed after 2 hours, whereas the absorption of OPP in porcine skin had a greater lag time and absorbed at a consistent rate for the majority of the experiment. The absorption of OPP in the Strat-M® membrane was minimal, absorbing only two percent of the dose, and remained consistent throughout the experiment, indicating that OPP struggled to fully penetrate the membrane. Finally, the variability of the synthetic skin models was greater than porcine skin for NAP and less than porcine skin for OPP. The reduced variability in absorption would be advantageous for the synthetic skin models; unfortunately, the reduced variability is observed for both test compounds. Overall, SynDaver™ skin had higher cumulative absorption at all sampling times, thus overestimating the absorption of NAP and OPP when compared to porcine skin. The Strat-M® membrane tended to underestimate the absorption of NAP as it had lower cumulative absorption values for all sampling times. The Strat-M® membrane absorbed less than two percent of the OPP dose indicating it is not a good skin model to predict the absorption of OPP in the skin. The absorption of naphthalene and orthophenylphenol in all skin models were compared using a one-way ANOVA (p-value < 0.05).

Despite the differences in cumulative absorption of NAP between porcine skin and the synthetic skin models no statistically significant difference was found between porcine skin and SynDaver™ skin ($p = 0.065$), and porcine skin and Strat-M® membrane skin ($p = 0.053$). However, there was a statistically significant difference between SynDaver™ skin and Strat-M® membrane ($p = 0.024$). This difference leads to a decision having to be made between the two synthetic skin models, whether it is better to use a model that overestimates absorption or underestimates absorption.

The statistical analysis done on OPP cumulative absorption revealed that Strat-M® membrane was significantly different from porcine skin ($p = 0.003$) and SynDaver™ skin ($p = 0.00002$). Looking at Table 13, a majority of the dose was found remaining in the skin disk, thus indicating that OPP could not fully penetrate the Strat-M® membrane. Although the Strat-M® membrane was not suitable to measure the absorption of OPP it was a better predictor for NAP. SynDaver™ skin again overestimates the absorption of the test compound, but no statistically significant difference was found ($p = 0.13$).

The large disparity between the cumulative absorption of NAP and OPP in the Strat-M® membrane highlights that chemicals with similar physicochemical properties will penetrate a membrane to different magnitudes. There are several physicochemical factors that greatly influence the absorption through human skin, and one important factor relative to the differences seen in this study is the solubility of the chemical in the dosing vehicle. In dermal permeation testing the solubility of a chemical predicts how a chemical partitions from the dosing vehicle to the skin. This phenomenon was highlighted well in a study by Roux and coworkers (2014). They found differences in skin absorption of several amines based on the chemical's partition coefficient and solubility in the dosing vehicle [96]. In the case of this study, artificial sweat was the dosing vehicle, which is primarily water. OPP is greater than 20 times more soluble in water than NAP at 25°C [128, 129]. The difference in solubility in the dosing vehicle is most likely why the two compounds had different absorption in porcine skin.

(A)



(B)

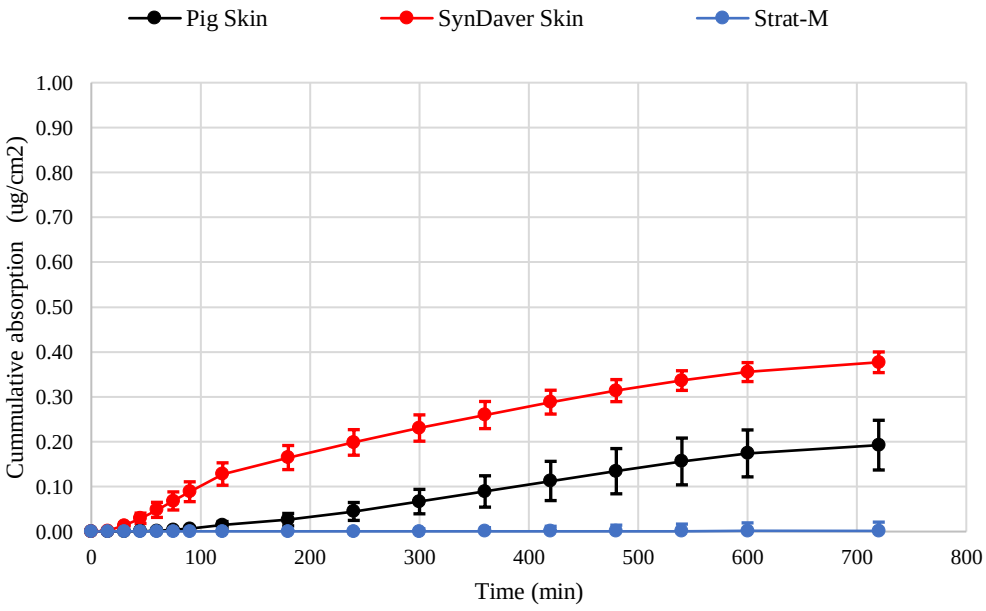


Figure 29: Absorption profiles of (A) naphthalene in each test membrane and (B) orthophenylphenol in each test membrane the dose for naphthalene was 3.37 µg and the dose for orthophenylphenol was 0.82 µg.

Time point linear correlations between the synthetic skin models and porcine skin, shown in Figure 30, were created by plotting the cumulative absorption of porcine skin against SynDaver™ skin and Strat-M® membrane for NAP and OPP at each time point. SynDaver™ skin was found to have a greater correlation (R^2 value = 0.9968) relative to porcine skin than the Strat-M® membrane (R^2 value = 0.9789) for the absorption of NAP. The high correlation between the two synthetic membranes and porcine skin and similar percent dose absorbed indicate that either SynDaver™ skin or Strat-M® membrane would be suitable skin surrogates for the absorption of naphthalene.

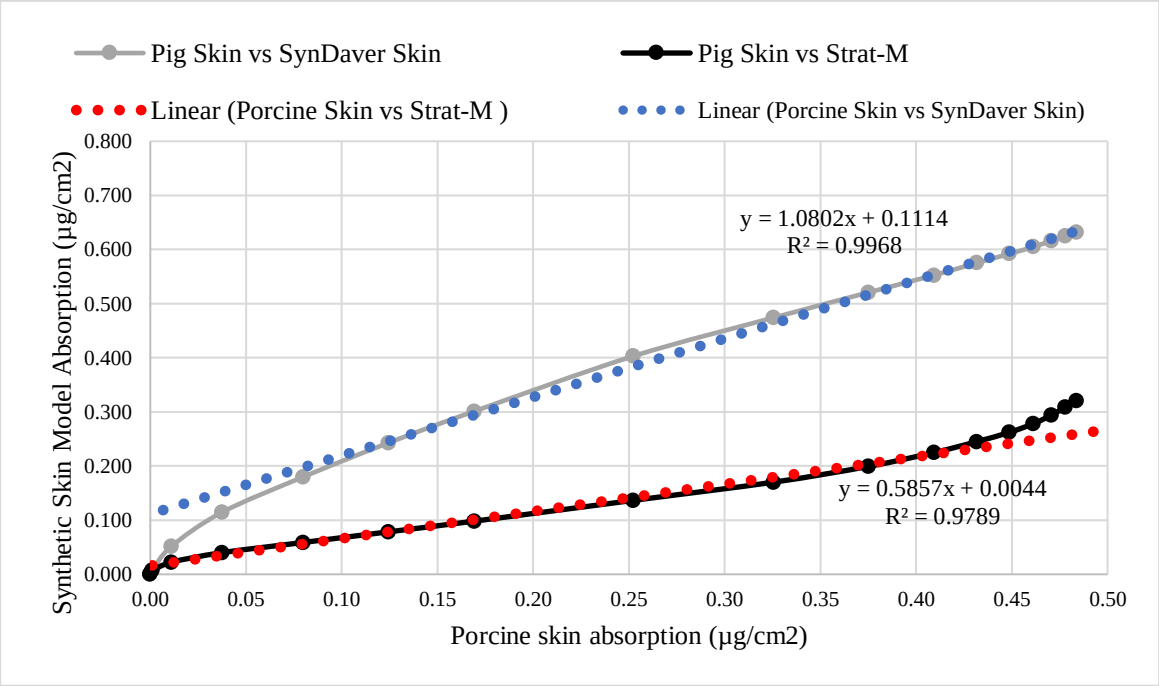
Similarly, both synthetic models had positive correlations relative to porcine skin for the absorption of OPP; SynDaver™ skin (R^2 value = 0.9938) and Strat-M® membrane (R^2 value = 0.9562). However, the high correlation between Strat-M® membrane and porcine skin is misleading. Without looking at the absorption profile or absorption values the fact that the Strat-M® membrane absorbed less than two percent of the OPP dose may go overlooked. The low absorption of OPP in Strat-M® could result in failed lab experiments if analysis equipment is not sensitive enough. The results of this study indicate that Strat-M® membrane should not be used as a model for the absorption of OPP.

Some differences in absorption and permeability between the synthetic skin models and porcine skin are to be expected, as they lack the high order structure and functionality of living skin. Even though SynDaver™ skin overestimates absorption and Strat-M® membrane underestimates absorption they can still be related by a correction factor. A study by Haq and coworkers demonstrated a high correlation (R^2 value > 0.90) between the cumulative absorption of several transdermal formulations in human skin and Strat-M® membrane [105]. Haq's study also demonstrated that Strat-M® could not accurately predict the absorption of nicotine;

however, it would be beneficial to use the Strat-M® membrane as a screening tool for optimizing drug formulations.

The low absorption of OPP in the Strat-M® membrane shows that the synthetic skin model may struggle to simulate the absorption of chemicals in human skin, even though its structure is intended to mimic the layers of human skin. Lab experiments that use non-radiolabeled chemicals and Strat-M® membrane for permeability tests would require the concentration of the chemical in the perfusate to be above the limit of detection. The low permeability of the membrane could prevent a detectable amount of analyte from being collected in the perfusate, therefore eliminating its potential for use as a qualitative or quantitative model. Ideally, a synthetic skin model that matches the absorptive properties or permeability of human skin would be best, but it has been demonstrated that this is hard create within a single synthetic model. A synthetic skin model that overestimates the absorption of a chemical, like SynDaver™ , may not be accurate but would allow lab experiments to obtain detectable amounts of analytes and use a correction factor to compare results back to human skin.

(A)



(B)

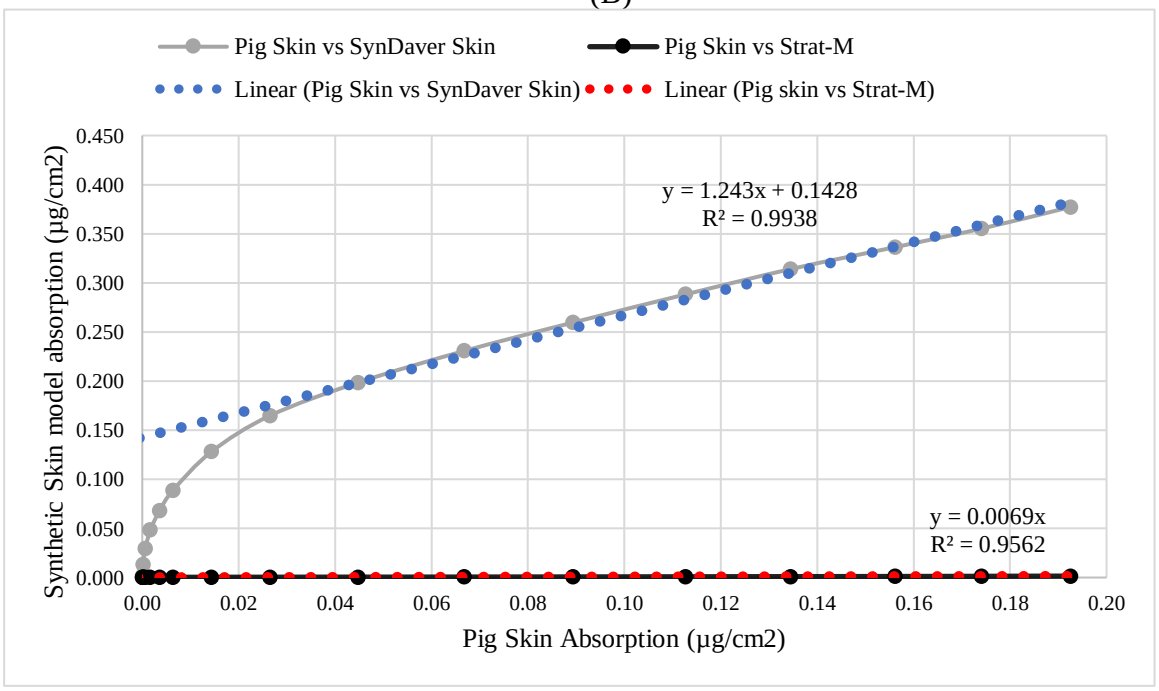


Figure 30: Absorption profiles of time point correlations between porcine skin and synthetic skin models: SynDaver™ skin and Strat-M® membrane. A) Naphthalene time point correlations between porcine skin and synthetic skin models for naphthalene. B) Orthophenylphenol time point correlations between porcine skin and synthetic skin models.

Permeability

The cumulative concentration ($\mu g\ cm^{-2}$) of both test compounds collected in the perfusate were used to calculate the permeability. The permeability and other absorption parameters are shown in Table 13. The permeability ($cm\ h^{-1}$) of OPP and NAP in porcine skin using an artificial sweat vehicle was 0.0091 ± 0.0034 and 0.013 ± 0.0008, respectively. Comparing the permeability values found in this study to values in previous studies that used porcine skin and aqueous dosing vehicles, the permeability of OPP was outside the range of previous studies K_p values (0.016 – 0.032) [130] [95]. The permeability value found in this study for NAP (0.013 ± 0.0008) was consistent with permeability values using animal skin models and aqueous dose vehicles (0.006 - 0.039) [131] [94].

The synthetic skin models, SynDaver™ skin and Strat-M® membrane, were roughly 50% more permeable and roughly 50% less permeable, respectively, for NAP compared to the porcine standard. These differences explain the increased and decreased absorption shown in the absorption profiles in Figure 29. The differences in permeability were even greater for OPP. The permeability for SynDaver™ skin was nearly three times more permeable, and the Strat-M® membrane was over an order of magnitude less permeable relative to porcine skin. The increased permeability of the SynDaver™ skin could be due to its structure. However, no cross-sectional image analysis was found on SynDaver™ skin. If the material has increased pore sizes compared to the Strat-M® membrane it would explain the increased permeability.

Table 13: Mean experimental (±SEM) absorptions (% Dose), permeability coefficients (cmh^{-1}), and amounts of test compounds in the remaining dose (% Dose), tape strips (% Dose), skin (µg) and the absorption plus the skin amount (% Dose) of naphthalene and orthophenylphenol. The dose for naphthalene was 3.30 µg and the dose for orthophenylphenol was 0.82 µg.

Compound Name	Skin Model	Absorption (% Dose)		Permeability ($cm\ h^{-1}$)		Remaining Dose + Swabs (% Dose)		Tape Strips (% Dose)		Skin (% Dose)		Skin+ Absorption (% Dose)	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
NAP	Porcine	14.36	0.47	0.013	0.0008	0.49	0.12	0.04	0.27	1.11	0.27	15.47	0.40
NAP	SynDaver	18.77	2.58	0.019	0.0013	0.85	0.51	0.003	0.002	0.38	0.14	19.15	2.45
NAP	Strat-M	9.53	3.36	0.006	0.0020	0.83	0.25	0.19	0.002	24.23	2.95	27.71	4.29
OPP	Porcine	31.65	10.03	0.009	0.0034	18.14	9.48	0.22	0.11	23.42	12.1	55.07	7.88
OPP	SynDaver	62.94	17.13	0.026	0.0036	13.61	6.54	0.012	0.007	5.50	1.99	68.43	15.37
OPP	Strat-M	1.37	1.19	0.0004	0.0004	32.21	20.96	0.014	0.003	120.18	36.32	121.5	36.56

4.4. Conclusions

The primary focus of this study was to compare the absorption properties of two synthetic skin models (SynDaver™ skin and Strat-M® membrane) to porcine skin using naphthalene and orthophenylphenol. SynDaver™ skin was more permeable than porcine skin, while the Strat-M® membrane was less permeable for both NAP and OPP, accounting for the differences in absorption for each material. The absorption profiles demonstrate that the synthetic skin models cannot exactly mimic the absorption of NAP or OPP in porcine skin. However, the only statistically significant differences in cumulative absorption regarded the Strat-M® membrane. For the absorption of NAP, SynDaver™ skin and Strat-M® membrane were significantly different ($p = 0.024$), whereas Strat-M® membrane was significantly different compared to porcine skin ($p = 0.0028$) and SynDaver™ skin ($p = 0.00002$).

Even though the synthetic skin models could not simulate the absorption of NAP or OPP in porcine skin, they could be used to predict the penetration capability of chemical compounds. Time point correlation plots showed that both SynDaver™ skin and Strat-M® membrane, R^2 value = 0.9861 and 0.9784, respectively, could be used to estimate the absorption of NAP in porcine skin. However, out of the two synthetic skin models, SynDaver™ skin may be the better surrogate for human skin. Although it overestimates the absorption of NAP and OPP, it had positive correlation values (R^2 value > 0.90) for both test compounds. With additional experimentation, a model could be developed to predict the absorption of PAHs in human skin using the SynDaver™ skin surrogate. Furthermore, the increased permeability of the SynDaver™ skin material will aid in allowing detectable amounts of test compounds into the perfusate during lab experiments.

Chapter 5: Development of Contact Transfer Test Method for Fireground Contaminants

Abstract

Firefighters are up to two times more susceptible to several cancers, and in 2010 the International Agency for Research on Cancer classified firefighting as possibly carcinogenic. Firefighters are exposed to carcinogenic combustion products via inhalation and dermal exposure, and exposure studies clearly demonstrate that polycyclic aromatic hydrocarbons (PAHs) are repeatedly found on the turnout gear and skin of firefighters. Contact with contaminated turnout gear can spread the PAH contaminants from the gear to firefighter skin, increasing firefighter's exposure to these carcinogenic contaminants.

This study developed a contact transfer test method to assess the transfer of polycyclic aromatic hydrocarbon contaminants from firefighter turnout gear to human skin. Turnout outer shell material was contaminated with liquid contaminants and put in contact with a human skin surrogate. After fifteen minutes, both materials were extracted. Out of the sixteen PAHs used in this study, only three were found to transfer from firefighter outer shell material to the skin surrogate. Minimal amounts of naphthalene (< 3%), phenanthrene (< 1%), and anthracene (<1%) were extracted from the skin surrogate material after contact. The remaining thirteen PAHs had no amounts found in the skin surrogate material. The lack of transfer found indicates that liquid PAHs are unlikely to transfer from firefighter outer shell material to human skin. However, alternate methods of contamination, such as particulate matter, would provide a more realistic representation of the possible transfer of fireground contaminants. Further testing is required with particulate contaminants to confirm that the threat of dermal exposure to PAHs by means of contact transfer is minimal.

5.1. Introduction and Background

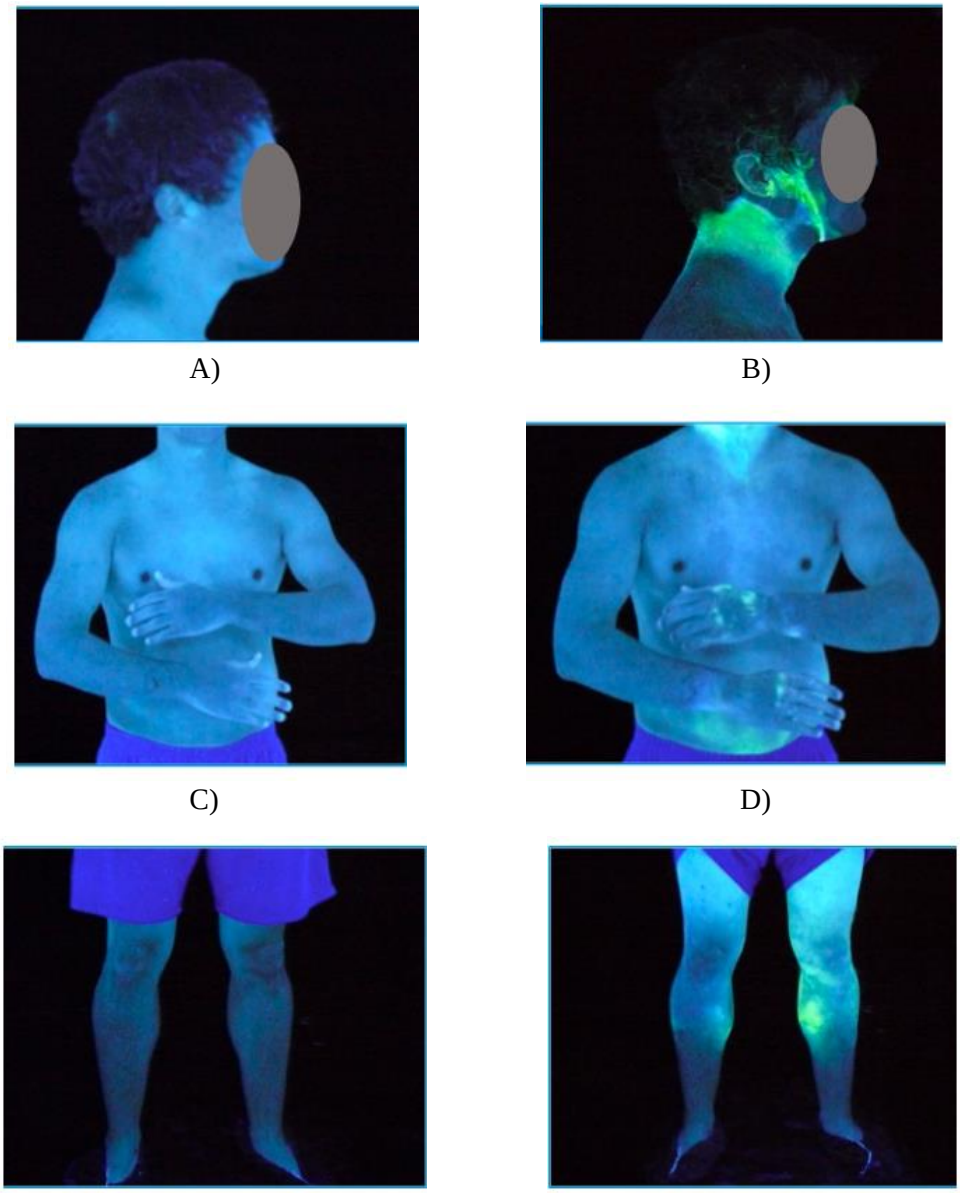
After the events at the World Trade Center in 2001, several American studies have investigated cancer incidence in firefighters in states such as Washington [7], Massachusetts [6], Florida [8], and California [5]. Furthermore, meta-analysis and cohort studies reviewed previous research on firefighter cancer incidence and confirmed that firefighters have elevated risk for several cancers, seen in Table 14 [3] [4] [5] [6] [7] [8] [21]. All of these studies found an increased incidence of at least one cancer or disease in firefighters. With the evidence building that showed firefighters have increased risk of various cancers, the International Agency for Research on Cancer (IARC) investigated firefighter's risk of developing cancer due to their occupational expousres. In 2010, the IARC classified the occupational exposures of a firefighter as possibly carcinogenic to humans (Group 2B) [21]. Follow up studies such as the Pooled Cohort study which included nearly 30,000 firefighters continue to connect firefighters with an increased risk of several cancers such as skin, prostate, testicular cancer as well as other diseases like malignant mesothelioma [3].

Table 14: Previous Cancer Incidence in Firefighter Studies

Study	Location	Length of Study	Study Population	Results of the Study
Cancer incidence among firefighters in Seattle and Tacoma, Washington (United States) [7]	Seattle and Tacoma, Washington	1974 – 1989	2500 male firefighters	Elevated risk of prostate cancer and slightly elevated risk of colon cancer. Risk of colon cancer increased with duration of employment.
Cancer Incidence Among Male Massachusetts Firefighters, 1987 – 2003 [6]	Massachusetts	1986 – 2003	2200 white male firefighters	Moderately elevated risk of colon cancer and brain cancer, meanwhile there was weaker evidence of increased risk for bladder cancer, kidney cancer and Hodgkin's lymphoma
Cancer Incidence in Florida Professional Firefighters, 1981 to 1999 [8]	Florida	1981 – 1999	35000 male and 2000 female firefighters	Male firefighters had significantly increased risk of bladder, testicular, and thyroid cancers. Female firefighters had significantly increased risk of overall cancers, cervical cancer, thyroid cancer, and Hodgkin disease.
Risk of Cancer Among Firefighters in California 1988 – 2007 [5]	California	1988-2007	4000 male firefighters	Significantly elevated risk of melanoma, prostate and brain cancer for all firefighters. Significantly elevated risk of esophagus, lung cancers, and acute myeloid leukemia in white firefighters. Significantly elevated risk of kidney cancer, multiple myeloma, and overall leukemia in non-white firefighters
Mortality and cancer incidence in a pooled cohort of firefighters from San Francisco, Chicago and Philadelphia (1950 – 2009) [3]	Philadelphia, Chicago, and California	1950 – 2009	30000 male firefighters	Excess cancer mortality and incidence for digestive, respiratory cancers, malignant mesothelioma. However, evidence of excess lymphatic or haemopoietic cancers were lacking.

Table 14: (continued)				
Cancer Risk Among Firefighters: A Review and Meta-Analysis of 32 Studies [4]	N/A	N/A	32 previous studies on firefighters	Probable cancer risk for multiple myeloma, non-Hodgkin lymphoma, prostate cancer, and testicular cancer. Possible risk of skin cancer, Malignant Melanoma, brain cancer, rectum cancer, buccal cavity and pharynx cancers, stomach cancer, colon cancer, and leukemia
IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 98 Painting, Firefighting, and Shiftwork [21]	N/A	N/A	Previous studies on firefighters	There is limited evidence in humans for the carcinogenicity of occupational exposure as a firefighter. Occupational exposure as a firefighter is possibly carcinogenic to humans (group 2B)

Firefighters have three primary exposure pathways to carcinogenic combustion products; dermal absorption, inhalation, and ingestion. Ingestion is the least worrisome exposure pathway, and the inhalation pathway is protected when firefighters wear their self-contained breathing apparatuses (SCBA). Unlike the SCBAs, turnout coats and pants do not provide an adequate amount of protection against the vapors and particulates produced during a fire. The interface areas of the turnout ensemble are highly susceptible to particulate penetration. A full ensemble penetration test conducted at RTI International produced images showing heavy fluorescent particle contamination around the neck, wrist and hands, thighs, and calf areas of the body; areas that all correspond to the interface areas of turnout ensembles, see Figure 31Figure 2. The National Institute for Occupational Safety and Health (NIOSH) conducted a study to investigate firefighter's exposure to polycyclic aromatic hydrocarbons (PAHs), a common fireground contaminant. They found PAH concentrations on the necks of the firefighter were higher after fire response and observed in urine samples collected three hours after fire exposure [117]. Furthermore, several studies have observed PAH contamination on the outside and inside of turnout ensembles, and on the skin of firefighters after fire exposure [117, 10, 132, 36, 12, 38].



A) B)

C) D)

E) F)

Figure 31: Fluorescent particle deposition on the skin after fluorescent particle exposure while wearing full turnout gear [26]. Part A and B show the before and after of the head area. Part C and D show the before and after of the torso area. Part E and F show the before and after of the lower body area.

Substantial evidence has shown firefighter turnout gear, and their skin is contaminated with PAHs after fire response. The interfaces of turnout gear leave the neck, hands, and calves most susceptible to particulate deposition during the fire. However, firefighters may be increasing their exposure to fireground contaminants, such as PAHs, through cross-contamination. Wearing dirty or contaminated turnout gear has often been regarded as a "badge of honor" in the fire service industry. Even though the mindset of the fire industry is slowly changing and begin to implement more on-scene decontamination procedures, firefighters are not confident in their ability to properly clean their turnout gear. A 2017 survey study showed that less than 20% of firefighters frequently or always cleaned their gear before leaving the fire-scene or used decontamination wipes [18]. Another survey study found that 61 out of 250 firefighters had not cleaned their gear after responding to a fire within the last year. Furthermore, this study found that firefighters are not always confident in their cleaning abilities when they do decide to clean their gear [42]. Repeated exposure to fireground contaminants and infrequent cleaning of turnout gear creates a likely scenario that firefighters may be increasing their exposure to combustion products when they are handling their turnout gear.

This study aims to develop a contact transfer method to evaluate the threat of PAHs, a common type of carcinogenic fireground contaminant, transferring from the turnout coats of firefighter protective gear to the skin. By using a realistic skin surrogate, this study aims to provide a more realistic test method compared to methods that have used "textured boards" as skin surrogates. Ultimately, the goal of this contact transfer test method is to assess the transfer of PAH contaminants from firefighter turnout gear to the skin and predict firefighter's dermal exposure when handling contaminated turnout gear.

5.2. Materials and Methods

Turnout Gear Outer Shell Material

Firefighter outer shell samples were prepared using PBI Max 7.0 obtained from Safety Components Fabric Technologies Inc. The PBI Max 7.0 material is comprised of 70% PBI/para-aramid spun yarns and 30% 600 denier para-aramid filament woven together [120].

Skin Surrogate

The skin surrogate SynDaver™ basic tissue plate was purchased from SynDaver™ (Tampa, FL, USA) [121]. Little information was provided on the composition of the basic tissue plate. According to manufacturer specifications, the tissue plate consisted of water, salt, and fiber, most likely polyurethane and has a similar tensile modulus, abrasion resistance, penetration force, and coefficient of friction as human skin.

Standards and Solvents

A standard PAH mixture Certified Reference Material 31011/ SV Calibration Mix #5 was supplied by RESTEK (Bellefonte, PA, USA). The contents of the PAH mixture can be seen in Table 15. A separate PAH stock solution used to dose the outer shell samples (300 ng/µL) was created by diluting the SV calibration Mix #5 in methanol. Solvents used for extraction included (99% purity) acetonitrile and (99% purity) methanol were obtained from Fisher Chemical, (Ottawa, ON, Canada). Solvents used for HPLC analysis included: UHPLC grade acetonitrile and UHPLC grade water were supplied by Fisher Chemical, (Ottawa, ON, Canada). PTFE Syringe Filters, pore size 0.2 µm were supplied by Advanced Microdevices PVT. LTD. (Ambala Cantt, India).

Table 15: Concentration and carcinogenicity of PAH compounds in SV calibration mixture

PAH Compound	Concentration (µg/mL)	IARC Classification
Naphthalene	2018	2B
Acenaphthylene	2002	3
Acenaphthene	2009	3
Fluorene	2009	3
Phenanthrene	2016	3
Anthracene	2019	3
Fluoranthene	2011	3
Pyrene	2002	3
Benzo(a)anthracene	2014	2B
Chrysene	2017	2B
Benzo(b)fluoranthene	2006	2B
Benzo(k)fluoranthene	2010	2B
Benzo(a)pyrene	2009	1
Indeno(1,2,3,-c,d)pyrene	2002	2B
Dibenz(a,h)anthracene	2019	2A
Benzo(g,h,i)perylene	2008	3
*IARC Classifications: 1 = known carcinogen , 2A = probably carcinogenic to humans, 2B = possibly carcinogenic to humans, 3 = carcinogenicity not classifiable, 4 = probably not carcinogenic to humans		

Contact Transfer Method

Skin surrogate samples were cut into 7.6 cm x 7.6 cm samples from the SynDaver™ basic tissue plate. Outer shell material (PBI MAX™) was cut into the same size and contaminated by pipetting 100 µL of a 300 ng/µL PAH stock solution, applying roughly 30,000 ng of each PAH onto the surface of the outer shell sample. The contamination droplets were given 20 minutes for the solvent to evaporate. The contaminated outer shell material was then placed on top of the SynDaver™ skin surrogate. The two materials were held in contact with a weighted plate, applying roughly 2.5 psi. A piece of aluminum foil was placed between the contaminated outer shell material and the weighted plate to ensure that the PAHs could only transfer to the skin surrogate. Figure 32 illustrates the arrangement of materials used in the contact transfer test method. The outer shell and skin surrogate were held in static contact for 15

minutes. Afterward, the materials were separated and then extracted using the pressurized solvent extraction method previously developed.

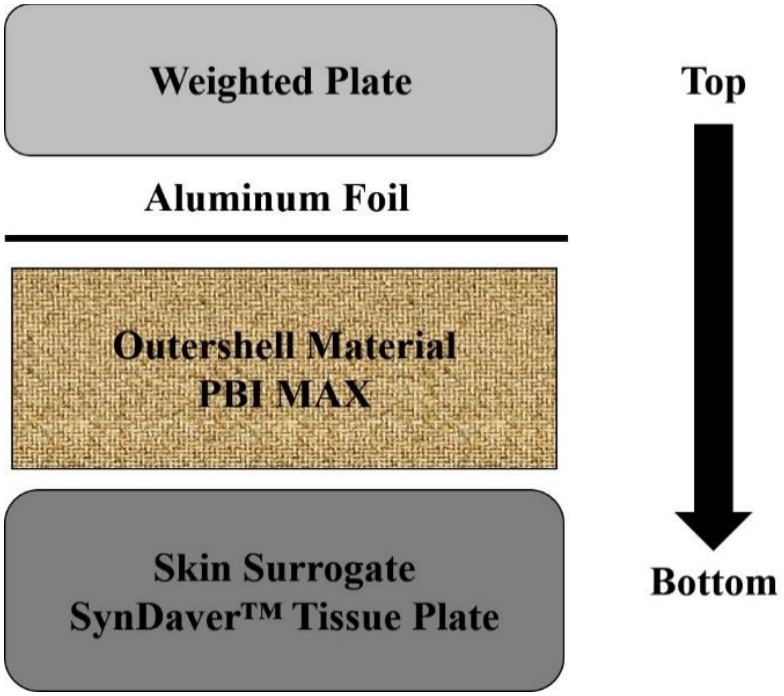


Figure 32: Illustration of the arrangement of materials used in contact transfer test method

Material Extraction

After the contact transfer test, the outer shell and skin surrogate materials were separated and extracted using a Buchi Speed Extractor E-916 pressurized solvent extractor. The Buchi Speed Extractor E-196 was equipped with 10-mL stainless steel extraction cells. The extraction cells were prepared by placing a small cellulose filter at the bottom of the extraction cell prior to the addition of either the outer shell or skin surrogate. Samples were placed into the extraction cells along with 5 grams of 4-mm glass beads. A cellulose filter is placed at the top and bottom of the extraction cell to prevent clogging of the condensing coils. Two trials of the contact test were run along with positive and negative controls. The positive control had an equal amount of

PAH stock spiked directly into the extraction cell. Negative controls of the outer shell and skin surrogate materials were extracted, having no added chemicals. Samples occupied all six extraction cells in the extractor and were ran simultaneously.

Extractions were performed with the parameters shown in Table 16Table 9, and the steps of the extraction method are illustrated in Figure 33. Before starting the extraction, the extractor conducts a tightness check to ensure there are no leaks in the system. Subsequently, the extraction begins and increasing pressure and temperature. Following the static and dynamic steps of the extraction method, the solvent is discharged into glass scintillation vials.

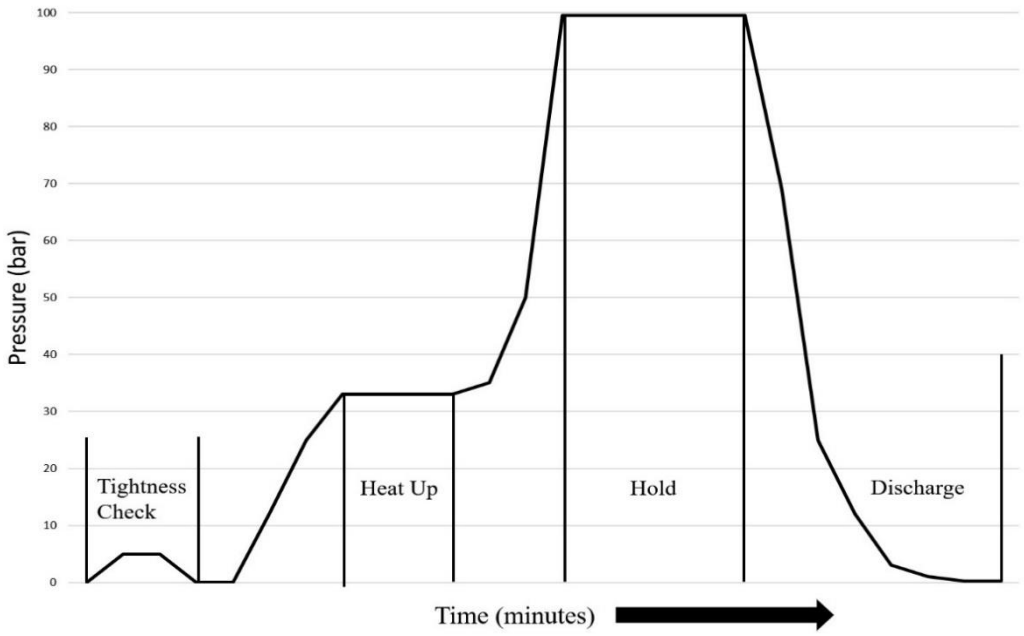


Figure 33: Illustration of pressurized solvent extraction method steps

Table 16: Pressurized solvent extraction parameters

Pressure	100 bar
Temperature	100°C
Gas	N_2
Cell Size	10mL
Vial Size	60mL
Solvent Flush Time	1 minute
Gas Flush Time	3 minute
Glass Beads	5 grams
Number of Extraction Cycles	3 cycles
Solvent	methanol

Each extraction cycle was collected independently, with each cycle producing six to nine milliliters of solvent. Upon completion of each extraction cycle the samples were diluted to 10 mL with methanol to ensure equitable dilutions for each sample before analysis via high-performance liquid chromatography (HPLC). Subsequently, 2 mL of extract were transferred into HPLC vials through 0.20 µm PTFE filters to prevent any loose fibers, particulates, or potential obstructions from damaging the HPLC column or system.

High Performance Liquid Chromatography Analysis

Liquid chromatography analyses were performed on an Agilent 1260 Infinity II LC system coupled with Infinity Lab LC/MSD along with the following modules: Agilent 1260 Infinity Binary Pump (G1312B), Agilent 1260 Infinity Autosampler (G1392B), Agilent 1260 Infinity Diode Array Detector (G4212B) with 10 mm Max-Light flow cell (Agilent Technologies). Chromatographic separation was done using a PAH Zorbax Eclipse column (1.5 x 150mm 3µm pore size; Agilent Technologies). The HPLC operated under conditions: column temperature: 35°C; mobile phase: gradient method shown in Table 17Table 10; 5 µL injection volume; Diode Array Detector was set to wavelengths 220nm, 254nm, 270nm, 285nm;

Fluorescence detector was set to emission λ: 425nm; excitation λ 340nm. Data were analysed with Open Lab CDS Chemstation (Agilent Technologies).

Table 17: HPLC gradient method for PAH analysis

Time min	Flow mL/min	% A (Water)	% B (Acetonitrile)
0	0.5	60	40
0.66	0.5	60	40
20	0.5	0	100
25	0.5	0	100
27	0.5	60	40
30	0.5	60	40

5.3. Results and Discussion

Extraction of Materials

To ensure there was not a significant loss of PAH compounds the recovery of each compound was calculated. Figure 34 shows the percent recoveries of each PAH compound for trials 1 and 2. The recovery of naphthalene was 183% and 230% for trials 1 and 2, respectively. The high recovery of naphthalene was likely due to cross-contamination between samples. Naphthalene has the lowest boiling point and known to volatilize at room temperature, which explains why no other compound had elevated recovery. A study by Lundstedt and coworkers (2000), used a pressurized solvent extraction technique to analyze PAH contamination in soil samples and obtained recoveries ranging from 83% to 120% [51]. The recovery of acenaphthylene was slightly elevated compared to recoveries reported by Lundstedt and coworkers (2000). The remaining PAH compounds had recoveries ranging from 61 - 108% for and 85 – 110% for trial 1 and 2, respectively. The recoveries of trial 2 are comparable to those in the Lundstedt and coworkers (2000) study.

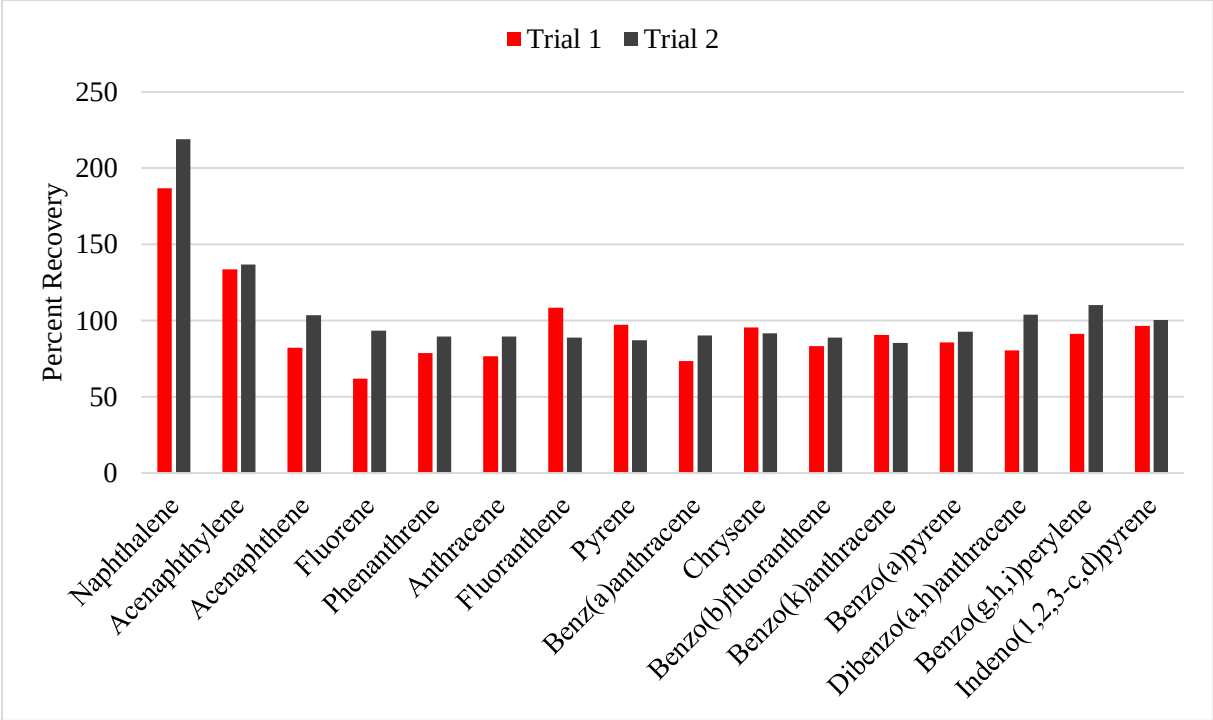


Figure 34: Percent recovery of polycyclic aromatic hydrocarbons during contact transfer trials

Transfer of polycyclic aromatic hydrocarbons

After the contact transfer test, the outer shell and skin surrogate materials were extracted. The percent dose on the outer shell and SynDaver™ skin surrogate was used to determine if any PAH compounds transferred from the outer shell material to the skin surrogate, seen in Table 18. Out of the 16 PAH compounds, only naphthalene transferred from the outer shell material to the skin surrogate in both trials; however, minimal amounts, less than three percent, of the spiked amount transferred to skin surrogate. This observation could be explained by naphthalene's ability to volatilize at room temperature, thus giving the appearance of naphthalene transferring to the skin surrogate material. Only two other PAH compounds (phenanthrene and anthracene) were found to transfer to the skin surrogate in at least one trial. Similar to naphthalene, the amount that transferred was minimal, less than one percent of the spiked amount transferred. The

remaining 13 PAH compounds were not found to transfer from the outer shell to skin surrogate in any capacity.

The lack of transfer demonstrates that liquid contaminants are unlikely to transfer from firefighter outer shell material to skin during static contact. However, there are some limitations to this contact transfer test method. The use of liquid chemicals does not accurately represent the contaminants found at fire scenes; most larger PAHs are frequently found in the particulate phase (smoke and soot) of fire contaminants. Using a particulate contaminant would allow for more realistic behavior of PAH transfer. Furthermore, the contact transfer method in this study could be adapted to a dynamic contact method. The dynamic motion of the contaminated outer shell moving across the skin would increase the surface area of contact between the outer shell and skin surrogate and increase the number of removal mechanisms for the particles to transfer from the outer shell surface, thus increasing the opportunity for the contaminants to transfer. Finally, the dynamic motion would also create frictional forces that would simulate firefighters handling and removing their turnout gear after fire response.

Table 18: Percent Dose on Outer shell and Skin Surrogate after Contract Transfer Test

Trial 1 / Trial 2	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene
% Dose on Fabric	97.49 / 98.71	100.00 / 100.00	100.00 / 100.00	100.00 / 100.00
% Dose on Skin Surrogate	2.51 / 1.29	0.00 / 0.00	0.00 / 0.00	0.00 / 0.00
	Phenanthrene	Anthracene	Fluoranthene	Pyrene
% Dose on Fabric	99.37 / 100.00	99.61 / 100.00	100.00 / 100.00	100.00 / 100.00
% Dose on Skin Surrogate	0.63 / 0.00	0.39 / 0.00	0.00 / 0.00	0.00 / 0.00
	Benz(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(k)anthracene
% Dose on Fabric	100.00 / 100.00	100.00 / 100.00	100.00 / 100.00	100.00 / 100.00
% Dose on Skin Surrogate	0.00 / 0.00	0.00 / 0.00	0.00 / 0.00	0.00 / 0.00
	Benzo(a)pyrene	Dibenzo(a,h)anthracene	Benzo(g,h,i)perylene	Indeno(1,2,3-c,d)pyrene
% Dose on Fabric	100.00 / 100.00	100.00 / 100.00	100.00 / 100.00	100.00 / 100.00
% Dose on Skin Surrogate	0.00 / 0.00	0.00 / 0.00	0.00 / 0.00	0.00 / 0.00

5.4. Conclusions

Pressurized solvent extraction was able to recover on average 74 – 109% of the PAH compounds from the outer shell and SynDaver™ skin materials, except for naphthalene. The recovery of PAH compounds was similar to a study by Lundstedt and coworkers (2000) who used a pressurized solvent extraction technique to assess PAH contamination in soil samples. Pressurized solvent extraction is an appropriate method of extraction for the materials used in the contact transfer test method.

Only three PAH compounds, naphthalene, phenanthrene, and anthracene, were found to transfer from the outer shell material to the SynDaver™ skin. However, only minimal amounts (less than three percent) transferred. The remaining 13 PAH compounds were not seen to transfer. Thus, demonstrating that liquid PAHs are not likely to transfer from outer shell material to the skin.

The initial contact transfer test method remains a possible method to assess the potential transfer of fireground contaminants from firefighter turnout gear to the skin of a firefighter with some modifications. It was found that nearly all liquid PAH compounds were unable to transfer from the outer shell material to the skin surrogate when held in static contact. A particulate contamination method could provide better means of transfer and be a more realistic method of simulating the transfer of PAHs adsorbed onto particulates found at fire scenes. Additionally, the contact method could be changed to simulate frictional forces created when firefighters remove their turnout gear by sliding the materials across one another.

Chapter 6: Conclusions and Future Works

As more evidence mounts a clearer relationship between firefighting and an increased risk for cancer begins to form, and the need for a better understanding of firefighter exposure becomes ever more pressing. Current research estimates that firefighters have 1.3 – 2.0 times increased risk of developing cancer than the general public. Several exposure studies have confirmed the presence of carcinogenic contaminants in the smoke and soot created during a fire. These contaminants are then deposited both inside and outside the turnout gear as well a on the skin of firefighters. Among researchers, the popular belief is that dermal exposure to carcinogenic combustion products is the primary pathway for chemical exposure for firefighters when standard turnout gear and SCBAs are worn. However, assessing dermal exposure can be quite complex for firefighters. After fire response or training exercises, the contaminants that have deposited onto their gear may be transferred to their skin, thus increasing their exposure. Furthermore, there is data on the penetration capabilities of only a few chemicals present at most fire scenes.

While developing the contract transfer test method, extraction methods were evaluated to ensure the test chemicals could be recovered from the materials used in the test method. Also, permeation studies were done to compare the absorption rates of synthetic skin models to porcine skin to identify a suitable skin surrogate for human skin. The purpose of this research was to start the development of a contact transfer test method using a skin surrogate and firefighter turnout gear material to simulate the contact made when firefighters handle their contaminated gear after fire response. The information obtained from this transfer test aimed to provide an understanding of how fireground contaminants, specifically PAHs, can transfer from the firefighter turnout gear to firefighter skin after deposition during the fire. Furthermore, the contact transfer test method

would serve as a blueprint for the development of a standardized test method to evaluate the efficacy of decontamination wipes that firefighters have started using to reduce their exposure to fireground contaminants.

6.1. Extraction and Analysis Methods

The results produced from the extraction and analysis trials (Chapter 3) demonstrated that pressurized solvent extraction serves as a reliable and effective method for the recovery of PAHs from firefighter turnout gear and synthetic materials. Out of the 16 PAHs, 15 were recovered within AOAC guidelines, the only chemical found to have repeatedly low or extraneous recoveries was naphthalene. Special care must be taken when handling volatile chemicals, such as naphthalene, to prevent evaporation of the chemical resulting in low recoveries or cross-contamination.

The promising results from the extraction tests warrant future testing to investigate the possibility of using the pressurized solvent extraction method for other classes of fireground chemicals. Continuous improvements in the extraction method indicate that it could be further improved. Additional optimization studies should focus on other extraction solvents, increased temperature and pressures, and using fewer extraction cycles to maximize extraction efficiency while minimizing time and solvent used for each extraction. Further, the work done in the extraction and analysis section (Chapter 3) only used outer shell material, additional layers of the turnout jacket could be tested. However, it is recommended to use a lower temperature when extracting the moisture barrier to prevent melting of the material. Other parts of the turnout ensemble, like the particulate-blocking hoods and gloves could also be extracted to determine amounts of contamination after firefighting activities.

6.2. Skin Surrogate Comparison

To identify an appropriate human skin surrogate with similar rates of absorption, diffusion cell experiments with two synthetic skin models were compared to porcine skin (Chapter 4). It was important that the skin surrogate have similar absorption and permeability properties to human skin because it would be used in the contact transfer test method and indicate that if any chemicals were found to transfer, they would be absorbed into human skin. The two synthetic skin models, SynDaver™ skin and Strat-M® membrane, had comparable rates of absorption for naphthalene relative to porcine skin; however, the Strat-M® membrane failed to produce comparable results for orthophenylphenol. The SynDaver™ skin had increased permeability and absorption for both naphthalene and orthophenylphenol, which eased the decision in choosing which surrogate material should be used for the contact transfer test method.

Comparable absorption rates of naphthalene seen in the two synthetic skin models show the potential for synthetic skin models to replace animal skin models in permeation studies. Although the results from the absorption experiments are promising, the experiment should be repeated to increase replicates and the data set since it is the first study to test this combination of chemicals and materials. Future tests should also evaluate the penetration capability of other PAHs, such as benzo[a]pyrene, in the synthetic skin models. If the other PAHs are not found to penetrate the synthetic skin material, another surrogate will have to be used. Additionally, flow-through diffusion cell permeation tests can be used to test the penetration capability of PAHs in different dosing vehicles. It would be more indicative of firefighters' exposure if a particulate vehicle were used to simulate absorption if firefighters did not do any post-fire decontamination.

Other vehicles, like the solutions used in decontamination wipes, should be tested to address the concerns that the alcohols and additives in the wipes are possibly increasing dermal absorption.

6.3. Development of Contact Transfer Test Method

After the preliminary work to ensure adequate recovery of the test chemicals from the materials that would be used in the contact transfer and identifying a suitable skin surrogate was completed, the development of the contact transfer test method followed. Early testing of the contact transfer test method indicates that liquid contaminates are unlikely to transfer from turnout gear to human skin. No further testing was done due to the response to the 2020 COVID-19 pandemic. Although the results of the initial testing were discouraging, the application of the contact transfer test method remains a viable method to evaluate the potential transfer to fireground contaminants to human skin. However, several modifications to the current contact transfer test method can be made to better represent transfer scenarios at fire scenes. The use of particulates would provide a more realistic representation of the contamination and prevent the outer shell from absorbing the liquid contaminants. Particulate-bound PAHs are more likely to transfer from the outer shell to skin if the particulates, themselves, transfer, which is exemplified when firefighters smear the soot on their hands to their arms or face. Additionally, employing a method to drag the outer shell across the skin surrogate would simulate the frictional forces when firefighters doff their gear.

Future work is needed in order to adapt the contact transfer test method into a standardized test method to evaluate the efficacy of decontamination wipes at removing fireground contaminants from the skin of firefighters. Efficacy testing is needed to evaluate the decontamination wipes that firefighters have started using to clean their bodies. Some firefighters worry that the alcohols and other chemicals used in the decontamination wipes may be

increasing their dermal absorption, which is a strong possibility as ethylene glycol and ethanol are known dermal absorption enhancers and are present in the decontamination wipes.

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