Evaluation of Surface versus Total Permethrin Content in Permethrin-Treated Clothing: Considerations for Mosquito Repellency

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Abstract

Permethrin is a synthetic pyrethroid insecticide that has been used to treat military uniforms to protect personnel from pests such as mosquitoes, ticks, and lice. Permethrin-treated clothing (PTC) can also be used by the public and outdoor workers such as foresters. Pests contacting the surface of PTC may be repelled or killed, depending on exposure dose and duration of exposure. Hence, it is important to assess surface permethrin concentration of clothing for repellency and/or control. Fabric swatches prepared using two commercially available permethrin treatments (Insect Shield® & Sawyer Repellant) and one laboratory permethrin-treated fabric (4 g/L) were tested to respectively compare surface and total permethrin content using a Martindale Abrasion and Pilling Tester and an Agilent Technologies 6850 Gas Chromatograph. Findings indicate that surface permethrin content (after 1000 rubs) for Insect Shield®, Sawyer, and 4g/L permethrin groups was significantly lower than total permethrin content (P=0.011, P< 0.001, and P=0.001, respectively). The relationship between surface permethrin content (SPC) and total permethrin content (TPC) varies widely between the different treatment methods evaluated here and practical implications for this are discussed. Mosquito repellency tests indicate that Insect Shield®, Sawyer, and laboratory-treated (4g/L) permethrin fabrics showed a higher repellency rate than control (untreated) fabrics (P-values: P=0.001, P<0.0001, P<0.0001, respectively). Assessing the SPC can be correlated with the amount of permethrin that comes into contact with human skin when wearing PTC. Exposure to high concentrations of permethrin may cause rashes and increase the absorption of the pesticide into the body.

Evaluation of Surface versus Total Permethrin Content in Permethrin-Treated Clothing: Considerations for Mosquito Repellency

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Considerations for Mosquito Repellency

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CHAPTER 1: INTRODUCTION AND PURPOSE OF THE STUDY

Permethrin-Treated Clothing and Mosquitoes

Permethrin is a synthetic pyrethroid active ingredient (AI) that has been registered with the Environmental Protection Agency (EPA) since 1979 (EPA, 2006). Pyrethroids can impact the central nervous system of arthropods via ingestion and/or direct contact (Fujino et al., 2019) and are used as insecticides or repellents (Bowman et al., 2018). Permethrin is a commonly used pyrethroid that has a low risk of causing cancer in humans, but is highly toxic for fish and aquatic invertebrates (EPA, 2006). This AI is used in lotions and other formulated products that can be used to treat lice infestations (Meinking et al., 2007). Permethrin can also be applied on livestock housing and transportation vehicles, other buildings, and clothing worn by people and/or animals (EPA, 2006). The first use of permethrin on clothing as a repellent was in 1990 by the military (EPA, 2006). It was then approved for consumers as a spray in the early 2000's (EPA, 2006). This spray could be applied on clothing and gear such as backpacks. Factory-treated permethrin-treated clothing (PTC) was not available to the consumer market until 2003 (EPA, 2006). Permethrin is currently the only insecticide approved by the EPA to be applied on clothing.

Factory-treated PTC (e.g., shirts, jackets, pants, and socks) must be marketed and labeled as an insecticide product (e.g., insecticide label) (EPA, 2006). Manufacturers of PTC are also required to provide data to the EPA showing that the product can repel arthropods (EPA, 2006). The PTC can be useful in many different outdoor occupational settings such as forestry. Being outdoors can expose forestry workers to a wide variety of blood feeding pests, such as mosquitoes and ticks (Centers for Disease Control and Prevention [CDC], 2016).

The objectives of the current study were to: 1) compare the total permethrin content and surface permethrin content of three types of PTC (Insect Shield®, Sawyer, laboratory-treated [4g/L permethrin]), 2) compare the mosquito repellency of three types of PTC using a modified cone bioassay, 3) compare the repellency rate of a permethrin-susceptible mosquito colony (*Ae. albopictus*) against a colony that has been previously exposed to permethrin.

CHAPTER 2: LITERATURE REVIEW

Vector-borne Diseases

Tick-borne diseases such as Lyme disease and Rocky Mountain spotted fever are some of the most common tick-borne diseases in the US (Sullivan et al., 2019). Nearly 75% of US vector-borne disease cases that are reported to the CDC are from ticks (Sullivan et al., 2019). PTC can be used as a barrier and repellent since ticks require direct contact to spread pathogens that cause diseases (Sullivan et al., 2019). Mosquitoes are also known to vector pathogens that cause diseases such as malaria, chikungunya, dengue, West Nile encephalitis, and Zika (CDC, 2016). Malaria alone causes more than 400,000 global deaths annually (WHO, 2020). Mosquitoes vector pathogens to humans and other animals through saliva injected via blood feeding (Tangena et al., 2018; CDC, 2016).

Insecticide Resistance in Mosquitoes

Mosquito populations are expanding their geographic range due, in part, to weather and climate changes, such as increased precipitation and temperatures (Ogden et al., 2019). One way that Integrated Mosquito Management Programs (IMMP) control mosquitoes is by applying insecticides with truck-mounted equipment which is then followed up with surveillance to evaluate efficacy (CDC, 2019). Follow up surveillance conducted by IMMPs can help determine the extent to which insecticide resistance (IR) exists in a mosquito population (CDC, 2019). Surveillance has shown a correlation between over-application of insecticides and development of IR in mosquitoes (CDC, 2019). Ideally, the application of insecticides should be justified by surveillance before and after insecticide application. The IMMPs often survey before application to ensure that there is a probable cause for insecticide application.

Truck-mounted insecticide application (TMIA) is a common application method for IMMPs. A TMIA includes using an aerosol generator placed in the bed of a truck that releases ultra-low volumes (ULV) of insecticides. ULVs are used specifically to target mosquitoes and may lessen impacts on other insect populations such as bees, although timing of applications is also important for avoiding non-targets (Pokhrel, 2018). The effectiveness of TMIAs is limited by distance from the road to the targeted mosquito population, weather, and other variables (Farooq et al., 2017; Salvani, 2020). Wind velocity above 10 mph can help carry aerosols farther but may cause droplets to miss the targeted area. Wind velocities below 10 mph are ideal to maximize the contact time between ULV aerosols and flying mosquitoes. High temperature (> 90°F) is also not desired since this promotes evaporation of aerosols and can cause vapor drifting. Vapor drifting is the unwanted movement of insecticide into off target sites (Salyani, 2020).

Aedes albopictus is one of the vectors of dengue virus, a prevalent vector-borne disease (Auteri et al., 2018). It is possible that the increased use of insecticides can also increase the IR of *Ae. albopictus* and other species of mosquitoes (Auteri et al., 2018). Knockdown resistance is when a mosquito is exposed to a lethal dose of insecticide, but the mosquito survives and maintains the ability to fly (Sodurlund et al., 2003). The resistance acquired from the insecticide exposure can then be passed onto its offspring, in some cases (Smith, 2016).

There are three types of resistance mosquitoes can experience after exposure to sublethal doses of insecticides: 1) metabolic, 2) behavioral, and 3) target-site (Auteri et al., 2018). Metabolic resistance occurs when enzymes mutate. These mutated enzymes become detoxifying enzymes used to breakdown insecticides (Marcombe et al., 2009). Behavioral resistance occurs when mosquito behavior changes to avoid areas previously sprayed with insecticides (Auteri et al., 2018). Target-site resistance occurs when a targeted area of the insect becomes mutated after contacting a non-lethal dose of insecticide (Auteri et al., 2018). A target-site mutation can prevent future binding of the same insecticide, hence canceling out the lethality of the insecticide. Once metabolic resistance and target site resistance occurs in mosquitoes, it can be passed onto offspring (Marcombe et al., 2009).

When pyrethroids (such as permethrin) enter the mosquito's body, the AIs stop voltage-gated sodium channels from closing (Auteri et al., 2018). Voltage-gated sodium channels are what promote the action excitatory cells that are involved in the insect nervous system (Auteri et al., 2018). If mosquitoes are exposed to sub-lethal doses of insecticides, voltage-gate sodium channels may mutate, thereby increasing IR (Corbel et al., 2007). With increasing IR to pyrethroids, personal protective equipment (PPE) used to deter pests such as PTC should be routinely assessed for effectiveness and repellency against mosquitoes.

Permethrin-Treated Clothing and Repellency of Mosquitoes

Permethrin-treated clothing (PTC) was initially created for US military use to combat sand flies that cause leishmaniasis (EPA, 2006). This clothing can also be used by the public to repel pests such as ticks, fleas, mosquitoes, and other flies. Since PTC can be worn, they are ideal for personal protection when entering heavily forested areas (Tangena et al., 2018).

Foresters often work in rural areas which may not be accessible to insecticide spray truck equipment. Wearing PTC in such areas can help reduce mosquito (and tick) biting (Osborne et al., 2016). However, the concentration of permethrin in PTC decreases after being washed multiple times (DeRaedt et al., 2015, Osborne et al., 2016, Richards et al., 2018).

Insecticide resistance may impact effectiveness of PTC. A study conducted by Londono et al. (2015) assessed the repellency of long-lasting PTC in outdoor workers over two years. Long-lasting PTC are treated with a proprietary impregnating method which allows them to retain efficacy through washes. The same type of long-lasting PTC was used in their previous experiments and protected wearers from tick bites up to one year without retreatment of the fabric (Londono et al., 2015). The study conducted by Londono et al. (2015) asked participants how many times they were bitten by mosquitoes each season. The results acquired from the study show that long-lasting PTC was still effective up to 20 washes and can protect against mosquito bites up to one year (Londono et al., 2015). The results also support that long-lasting PTC would be a sustainable method of preventing mosquito-borne diseases since it requires fewer treatments than regular PTC to maintain repellency (Londono et al., 2015). Another commonly studied factor of PTC is how it performs over time and after being washed multiple times. Studies have shown that washing decreases the effectiveness of PTC (DeRaedt et al., 2015, Osborne et al., 2016, Richards et al., 2018). The results from these studies also support that, as the number of washes increased, the total content of permethrin decreased.

World Health Organization (WHO) Cone Assay

A common mosquito repellency/toxicity test used in the studies conducted by DeRaedt et al. (2015) and Richards et al. (2018) was the WHO Cone Assay. This assay was developed to test the efficacy of insecticide treated bed nets (WHO, 1998). The procedure for the WHO Cone Assay involves placing three clear glass cones on top of tested fabric and transferring five female mosquitoes into each of the cones (WHO, 1998). Mosquitoes are exposed to the fabric for three minutes and then transferred to an insecticide free container and further observed for knockdown after one-hour (WHO, 1998). The mortality rate is then calculated 24 h after initial exposure and efficacy is based on the number of knockdowns and mosquito mortality (WHO, 1998).

Arm-In-Cage Repellency Test

Another test conducted by DeRaedt et al. (2015) and Osborne et al. (2016) was the arm-in-cage repellency test and this requires 30 female mosquitoes in a mosquito rearing cage. The forearms of a participant are inserted inside the cage for 30 s and the number of mosquitoes landing on the forearm are counted (WHO, 2009). The number of mosquitoes which have blood fed are then counted and removed and new unfed mosquitoes are introduced into the cage for further replicates (WHO, 2009). In one study, the arm-in-cage test used 100% cotton PTC against *Aedes aegypti* (Osborne et al., 2016). The same study showed greatest reduction in repellency of PTC against *Ae. aegypti* between 0 and 10 washes (Osborne et al. 2016). At 0 washes, a forearm fully covered with PTC gave a 58.9% protection rate against both landing and biting while PTC with 10 washes only gave a 18.5% protection rate (Osborne et al., 2016). This means that, even with full arm protection, mosquitoes are still prone to bite through the clothing and PTC. This study also compared a bare arm to a fully clothed arm, and found that PTC gave a 97% bite protection rate compared to a bare arm (Osborne et al., 2016). The arm-in-cage repellency conducted by DeRaedt et al. (2015) showed similar results with a significantly greater protection by treated fabrics compared to bare arm.

Fabric Type and PTC Efficacy

Fabric types could be a factor in the efficacy of PTC. In the study conducted by Richards et al. (2018), there were two different types of fabrics evaluated (50% cotton/50% polyester and

100% cotton) and two species of mosquitoes being tested against the PTC (*Ae. aegypti* and *Ae. albopictus*). *Aedes albopictus* showed no significant differences in knockdown or mortality rates among the fabric types for either petri dish or cone assay exposure methods (Richards et al., 2018). The petri dish exposure method involves placing a fabric swatch inside a petri dish and securing a lid on top. A hole is created on top of the lid to introduce mosquitoes. The highest mortality and knockdown rates were achieved with swatches that had 0 washes against *Ae. albopictus* mosquitoes (Richards et al., 2018). *Aedes aegypti* exposed to PTC (50% cotton/50% polyester) washed 0 and 5 times, had mortality/knockdown rates of 2 ± 0.09% and 2 ± 0.07%, respectively. The exposure of *Ae. aegypti* to the 100% cotton PTC resulted in no knockdown or mortality, indicating some level of IR in the tested population (Richards et al., 2018).

DeRaedt et al. (2015) also studied PTC as protection against *Ae. aegypti,* a common vector of dengue and Zika viruses. The studies conducted by DeRaedt et al. (2015) and Richards et al. (2018) evaluated differences in duration of efficiency of the PTC based on type of fabric treatment. The four types of PTC tested in the Thailand study were factory-dipped, home-dipped, microencapsulated, and treated school uniforms (collared shirts and skirts) (DeRaedt et al., 2015). Both factory and home-dipped clothing used in the study were made from 100% cotton. The treated school uniforms were also made from cotton, but the percentage of cotton was unknown (DeRaedt et al., 2015). Results of the WHO cone bioassay showed no significant difference in protection from *Ae. aegypti* between factory-dipped clothing, factory-dipped school uniforms, and microencapsulated treated clothing after three minutes of mosquito exposure (DeRaedt et al., 2015). Washing techniques also showed a significant difference in permethrin degradation, i.e., handwashed fabrics were half as likely to lose permethrin compared to machine-washed fabrics ((DeRaedt et al., 2015).

Another study assessed permethrin content and washing degradation of permethrin for five different types/brands of factory-treated PTC: 1) Battle Dress Uniforms (BDU), 2) ExOfficio, 3) Insect Shield®, 4) Labonal, and 5) Sol's Monarch. Sol's Monarch and Labonal had the highest initial permethrin concentration prior to washing, with 4,000 mg/m^2 and 4,300 mg/m^2, respectively (Faulde et al., 2016). After 100 machine washes, Insect Shield® and ExOfficio had the lowest concentrations of permethrin at 20 mg/m^2 and 40 mg/m^2, respectively (Faulde et al., 2016). The same study showed that Insect Shield®-treated clothing showed the greatest reduction in permethrin concentration after 100 machine washes, compared to the other brands, from 1300 mg/m^2 to 20 mg/m^2 (98.5% decrease). The same study showed that, as wash frequency increases, the knockdown time for *Ae. aegypti*, *Anopheles stephensi,* and *Culex pipiens* also increased. Results from DeRaedt et al. (2015) and Faulde et al. (2016) support that, as the number of washes increase (machine and/or handwashing), the concentration of permethrin in many brands of PTC will decrease; resulting in a decrease in repellency and knockdown potential and, hence protective effect.

Mosquito repellency effects and permethrin content between home-dipped and factory-treated fabric were similar prior to washing (Banks et al., 2015). In this study, factory PTC was obtained from Insect Shield® and mome-dipped PTC was treated using a Sawyer home dipping kit (Banks et al., 2015). The arm-in-cage method was used to assess the repellency of both types of PTC over eight washes against *Ae. aegypti* (Banks et al., 2015). The home-dipped PTC had 91.5% bite protection, higher than the factory PTC (79.9%); however, this difference was not significant (Banks et al., 2015). Home-dipped (49.9%) also showed a higher degree of landing protection against *Ae. aegypti* compared to factory (40.9%) PTC (Banks et al., 2015).

Bowman et al. (2018) assessed the repellency of PTC against resistant and susceptible colonies of *Ae. aegypti*. The same study used the arm-in-cage method and showed a low mortality rate (0-11%) for both resistant and susceptible mosquitoes exposed to the PTC. The landing rates for the resistant and susceptible mosquitoes were 44% and 63%, respectively (Bowman et al., 2018). However, the reduction in blood feeding due to PTC for both resistant and susceptible colonies was 100% (Bowman et al., 2018). Richards et al. (2018), Osborne et al. (2016), DeRaedt et al. (2015), Faulde et al. (2016), and Banks et al. (2015) indicate that, as PTC is washed and permethrin content decreases, the efficacy for repellency decreases against both susceptible and resistant mosquito populations. These studies also show that initial (relatively high) permethrin content of PTC (before washing) helped to decrease biting and landing rates; however, this effect decreased as the number of washes increased. Home-dipped PTC (Sawyer kit) showed a higher degree of mosquito bite protection than factory-treated PTC (Insect Shield®) (Bowman et al., 2015). Insect Shield®-treated clothing showed the highest reduction rate in total permethrin content after 100 washes when compared to other factory treated PTCs (Faulde et al., 2016). DeRaedt et al. (2015) showed that the washing method can affect permethrin content over extended washes. Handwashing the PTC is better and can help reduce the loss of permethrin content by half (DeRaedt et al., 2015). Bowman et al. (2018) suggests that permethrin-susceptible mosquitoes are more likely to land on PTC and resistant mosquitoes may be repelled. However, these effects may vary depending on permethrin dose and other unknown factors.

Determination of Surface Insecticide Content on PTC

A previous study conducted by Dieval et al (2017) focused on using the MAT as an improved method of assessing active ingredients of pesticides on textile surfaces.

CHAPTER THREE: STUDY OBJECTIVES

Study Objectives

The main purpose of this study was to evaluate the relationship between total permethrin content and surface content of different treatment methods and their repellency rate. We aimed to:

1) compare the total permethrin content and surface permethrin content of three types of PTC (Insect Shield®, Sawyer, laboratory-treated [4g/L permethrin]),
2) compare the mosquito repellency of three types of PTC using a modified cone bioassay; and,
3) compare the repellency rate of a permethrin-susceptible mosquito colony (*Ae. albopictus*) against a colony that has been previously exposed to permethrin.

Research Questions

To reach these aims, we answered these research questions:

1) Which type of PTC showed the highest total permethrin content?
2) Which type of PTC showed the highest surface permethrin content?
3) What PTC showed the highest repellency against susceptible mosquitoes?
4) What PTC showed the highest repellency against mosquitoes that had been previously exposed to permethrin?

Hypotheses

The following hypotheses were tested:

First Hypothesis: *Insect Shield® PTC will have the highest total permethrin content among the different types of PTC.* Insect Shield® is a permethrin treated clothing that is treated in a factory setting with a proprietary impregnation method.

Second Hypothesis: *Insect Shield® PTC will have the highest surface permethrin content among the different types of PTC.* If Insect Shield® PTC has the highest total permethrin content, it is expected to have the highest surface permethrin content.

Third Hypothesis: *Insect Shield® PTC will have the highest repellency among the different types of PTC.* If Insect Shield® PTC has the highest surface permethrin content, it is expected to have the highest repellency rate against both susceptible and previously exposed mosquitoes

CHAPTER FOUR: MATERIALS AND METHODS

Development of Permethrin-Susceptible and Permethrin-Exposed Mosquito Colonies

The two *Ae. albopictus* colonies used in the cone bioassay were derived from the same colony originating from Louisiana (LA colony) which was previously determined susceptible to permethrin (data not shown). Eggs of the LA colony were split into two separate colonies (susceptible and exposed) at generation F-38. The susceptible colony was created by placing F-38 egg strips into two separate pans (33 x 28 cm) containing 2 L of tap water housed in incubator at 28°C. As the eggs hatched, the larvae were fed 2 mg of a 1:2 ratio of yeast:liver powder. Larvae were fed every two days. As the larvae turned into pupae, they were transferred into a 177 mL plastic cup containing 88.7 mL of tap water and placed into a large holding cage (30.5 x 30.5 x 30.5 cm) until adulthood. The adult mosquitoes fed on cotton balls containing 20% sucrose solution *ad libitum*. One week old female adults were fed defibrinated bovine blood (Hemostat, Dixon, CA) warmed to 37°C using the Hemotek system (Hemostat, Dixon, CA). At 24 h before blood feeding, the sugar cotton ball was replaced with a cotton ball containing only water (to promote blood feeding). At 48 h after the blood feeding session, three (2.5 x 7.6 cm) ovistrips (seed germination paper) were placed into 60 mL black plastic cups filled with tap water and positioned in the cages containing adult mosquitoes. These strips were harvested 48 h later and another generation was propagated as done with the F-38 LA colony. The propagation process continued for nine generations until the F-47 LA colony was created which was used for the cone assay.

The permethrin-exposed colony was created by placing egg strips containing F-38 LA colony eggs into two pans (33 x 28 cm) filled with 2 L of tap water. These two pans were then incubated at 28°C with 14:10 L:D cycle. As the eggs hatched, larvae were fed in the same manner as the susceptible colony. When the larvae reached the 2nd instar developmental stage, they were collected and separated between two pans containing 2 L of a 0.8 mg/L permethrin + tap water solution. These methods were adapted from a study conducted by Vera-Maloof et al. (2020), which used a lethal concentration 50 (LC50) of permethrin between 0.4 µg and 0.6 µg for *Ae. albopictus*. Here, the permethrin solution was created from a mixture of granulated permethrin and tap water. Pupae were transferred to 177 mL clear plastic cups containing tap water and placed into a holding cage (30.5 x 30.5 x 30.5 cm). The sugar ball feeding process, blood feeding process, and egg strip harvesting process for the exposed colony was the same as the susceptible colony to ensure comparability between groups. The same culturing process for the exposed F-38 LA colony was used on the exposed F-39, F-40, F-41, and F-42. However, at generation F-40 and F-41, the concentration of the solution used for 2nd instar larvae was increased to 1.0 mg/L of permethrin. The permethrin concentration of the rearing water was further increased to 1.2 mg/L during propagation of the exposed F-42 LA colony. The mortality among generations F-38, and F-39 larvae ranged from 10-15% after being exposed to 0.8 mg/L of permethrin. The increase in dose of permethrin from 1.0 mg/L and 1.2 mg/L increased the mortality rate of the larvae to approximately 25%.

The susceptible (*Ae. albopictus* F-47) and exposed (*Ae. albopictus* F-42) colony were tested for resistance using a modified CDC bottle bioassay method (CDC 2020). The modified CDC bottle bioassay involves the use of three glass 250 mL Wheaton bottles. The three 250 mL bottles are coated with a 2mL solution of 4µg/L of permethrin. The 4 µg/L permethrin solution was created with acetone and granulated permethrin (ChemService, Westchester, PA). After coating, the bottles were stored in a dark and dry area for up to 12 h (CDC 2020). Another three 250 mL bottles were designated as a control and were coated with 2 mL of acetone and stored in a dark and dry area for 12 hours. One set of bottles for testing contained three control bottles and three 4 µg/L permethrin bottles. A total of two sets were created (one set for each mosquito group).

The susceptible and exposed group bioassays were conducted separately and in two sessions. Both sessions were conducted in a laboratory at room temperature (*ca.* 27°C). For the susceptible mosquito bioassay, approximately 15 mosquitoes were aspirated into each of the three control and three 4 µg/L permethrin bioassay bottles. Following standard protocols, mosquitoes were observed for mortality at five-minute intervals until 15 minutes. The intervals are then increased to 15 min until 120 min was reached (CDC 2020).

The same modified CDC bottle bioassay used for the susceptible group was used to assess resistance in the exposed group during generation F-42. Approximately 15 mosquitoes from the exposed group were aspirated into each of the three control bioassay bottles. Approximately 50 of the exposed group mosquitoes were aspirated into the 4 µg/L permethrin bioassay bottles. The susceptible mosquito group has been previously tested and are classified as susceptible. Since the exposed mosquito group has not been previously tested, a higher number of mosquitoes were used in the 4 µg/L permethrin bioassay bottles to better determine their resistance.

The concentration of permethrin used in this bottle bioassays was 4 µg/L for both susceptible and exposed colonies. We determined the diagnostic time (DT) for 100% mortality at this diagnostic dose was 15 min. The WHO recommends that mosquitoes experiencing a mortality rate > 96% at the DT are considered susceptible. A mortality rate < 90% at the DT is considered resistant, while a 90-96% mortality rate at the DT is considered as potential development of resistance (CDC 2020). Since the control bottles are coated with acetone that has been allowed to evaporate, the expected mortality rate would be 0% (CDC 2020).

Treatment Methods for PTC

A diagram showing the treatment methods for PTC in the current study is provided (Figure 1). These methods were used to determine total permethrin content of fabric swatches treated using three different methods.

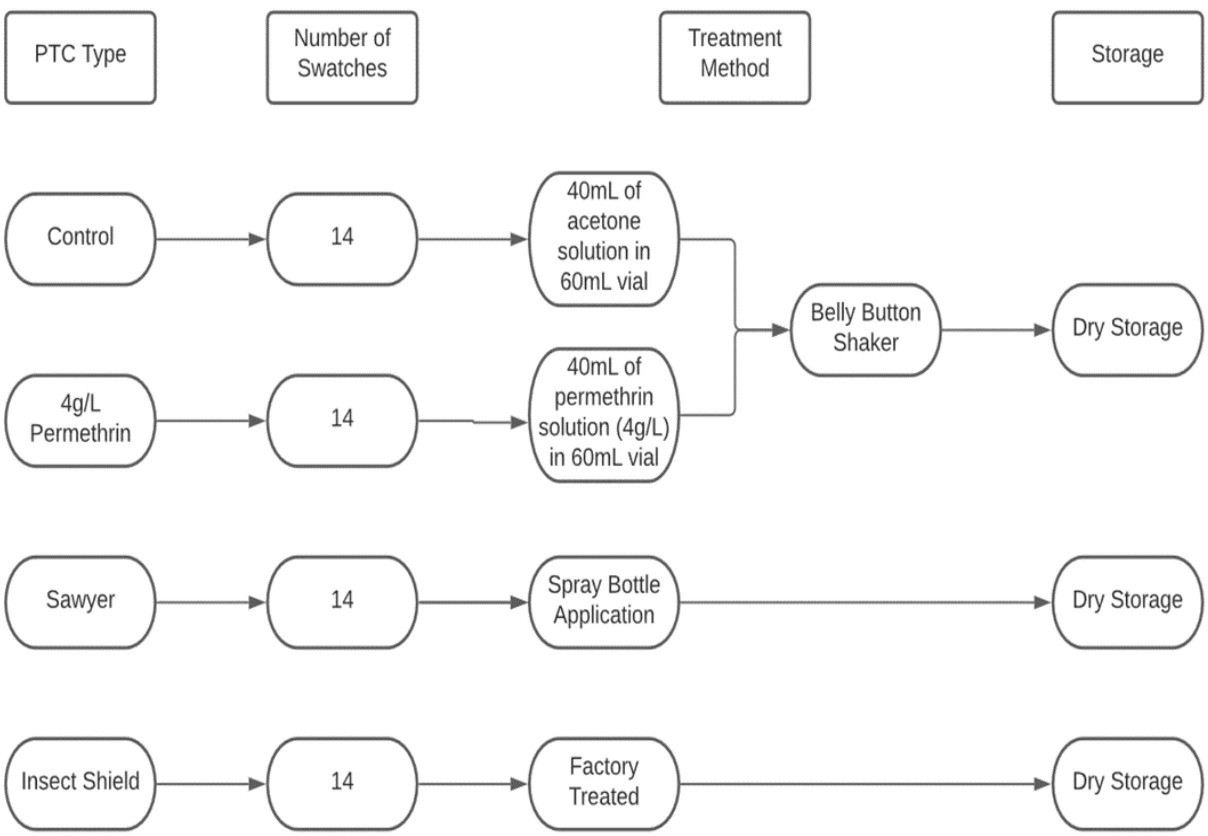

Figure 1. Flowchart of Treatment Methods of PTC

Insect Shield®

Permethrin-treated clothing (short-sleeved shirts [Hanes®], 100% cotton) was purchased from Insect Shield® (www.insectshield.com). Circular swatches (N=14 replicates for treated fabric) were cut for use in experiments. Each swatch weight was recorded before processing.

Sawyer Premium Insect Repellant (SPIR)

A 709.77 mL spray bottle of Sawyer Premium Insect Repellant (SPIR) (Sawyer Products, Inc., Safety Harbor, FL) containing 0.5% permethrin was purchased (www.amazon.com). Six additional untreated (control), short-sleeve shirts [Hanes®], 100% cotton were purchased (www.amazon.com) to provide controls for this group. Circular swatches (N=14 replicates for treated fabrics, 8.5 cm diameter) were cut for use in experiments. Swatches were placed in individual petri dishes (8.5 cm diameter) and sprayed with SPIR three times on both sides of the fabric until completely damp. These treated swatches were removed from their respective petri dishes and set on individual foil sheets and stored in a dark drawer (to avoid photodegradation) at room temperature until processed (at least 24 h to allow fabric to air dry) (Holmstead et al., 1978). Each swatch was weighed and data recorded before and after processing.

Laboratory-Created Permethrin-Treated Clothing (Permethrin Concentration: 4g/L)

There were 14 circular swatches (8.5 cm diameter) cut from untreated 100% cotton short-sleeve shirts [Hanes®] for laboratory treatment with permethrin. Six bottles containing 250 mg of granulated permethrin (Chem Service, West Chester, PA) were used to create the solutions for the treatment process of the laboratory treated fabrics. Each permethrin solution (4 g/L: 160 mg permethrin + 40 mL acetone) was created in 60 mL amber vials. All samples were hand shaken for 3 min and sonicated for 10 min using the 2.8L ultrasonic bath (Fisher Scientific, Watham, MN) to ensure complete mixture.

The 100% cotton, untreated fabrics (14 circular swatches, 8.5 cm diameter) were used for the laboratory dipping process to create the 4g/L permethrin swatches. The dipping process involved placing the fabric into an amber vial containing the designated permethrin concentration. Amber vials containing the swatches were placed on a rack connected to a Belly Button Shaker (IBI Scientific, Dubuque, IA) and shaken for 1 h at 75 rounds per minute (rpm) to maximize permethrin-impregnation onto the fabrics. Each swatch was removed from their designated vials and weighed. Swatches were allowed to air dry in a dark drawer at room temperature and reweighed after 24 h. Each swatch was weighed after being cut and after being dipped in respective permethrin solutions.

Control

An additional 14 swatches were cut from the 100% cotton, short-sleeve shirts [Hanes®]for use as untreated control swatches. These 14 swatches were placed inside 60 mL amber vials containing 40 mL of acetone (0.00 mg permethrin + 40 mL acetone). These controls followed the same procedures as the other laboratory-created PTC except swatches were dipped in acetone containing no permethrin. Vials were shaken for 1 h at 75 rpm on the Belly Button Shaker and then removed from their designated vials and allowed to air dry in a dark drawer at room temperature and reweighed after 24 h. These swatches were also weighed before and after being dipped.

Total Permethrin Content Testing

Figure 2 shows a flow chart of exposure and testing methods for PTC. Total permethrin content was calculated for each of the four PTC groups. Four of the 8.5 cm control swatches were inserted into four individual 60 mL amber vials containing 40 mL of acetone. These vials were then placed in the 2.8L ultrasonic bath (Fisher Scientific, Watham, MN) and sonicated for 60 min. The ultrasonic bath is used to further elute the permethrin from fabric into the acetone. After 60 min of sonication, 2 mL of solution was transferred into two 2 mL glass amber vials each. These vials were tested for total permethrin using the Agilent 6850 gas chromatograph (GC) (Agilent Technologies, Alpharetta, GA). This procedure was repeated for each type of PTC (control, Insect Shield®, Sawyer, and 4g/L permethrin).

The capillary column used was an Agilent Technologies DB-5MS (5% phenyl-methylpolysiloxane) with a 0.25 µm film thickness. The specific settings for the GC are adapted from Hengel et al. 1997. The injector temperature was set at 250℃ and the detector temperature was set at 260℃. The oven temperature was programmed to run at 200℃ to 250℃ at 10℃ per min and held for 7 min. The total run time for each sample was 17 min. Nitrogen was used as both carrier and make-up at 32.6 mL/min and 10 mL/min, respectively. Hydrogen was used as the detector gas at 30 mL/min. The calibration standards were created using a permethrin stock with 0.01 g permethrin dissolved in 40 mL of acetone. A blank vial containing 2.0 mL of acetone was used between sample runs to avoid false peaks.

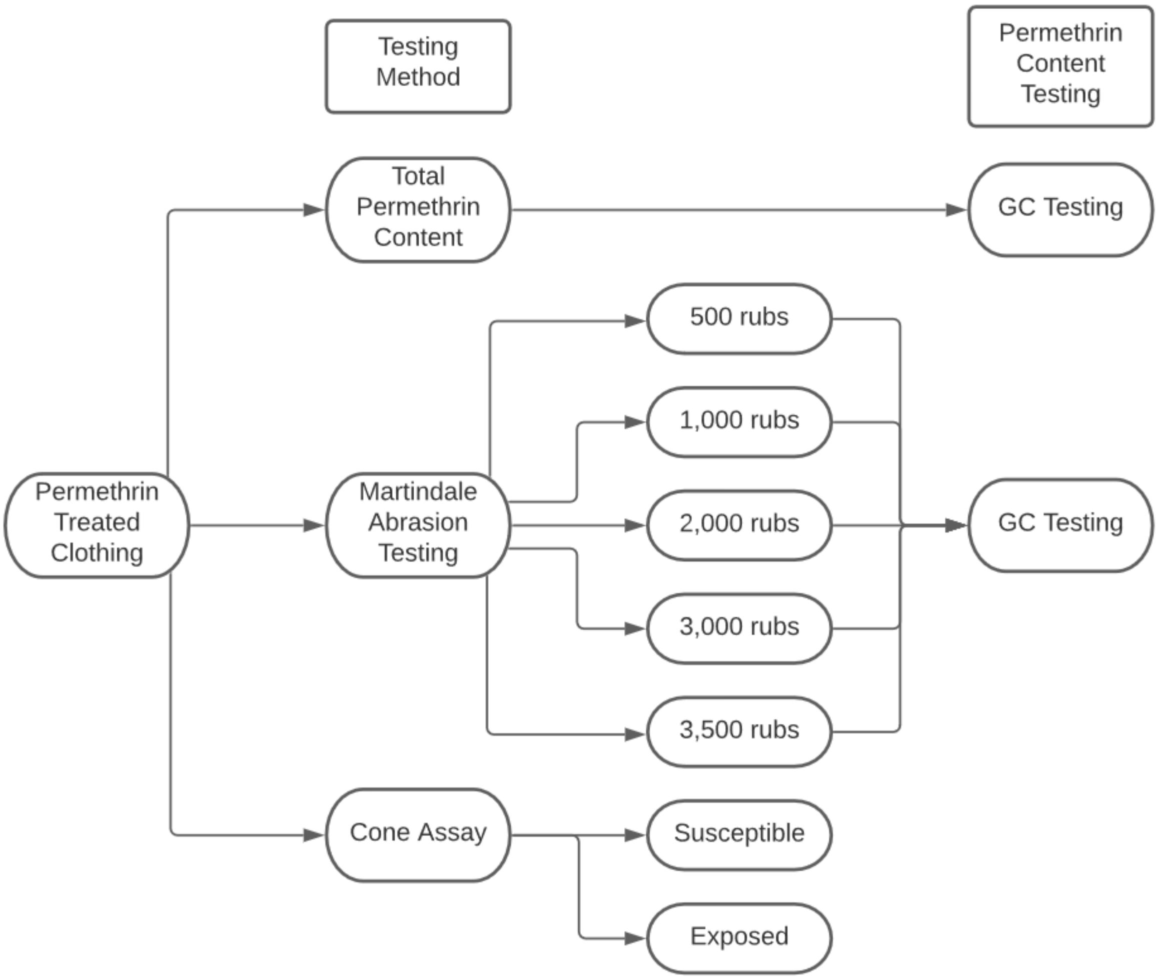

Figure 2. Flowchart of Testing Methods

Martindale Abrasion Testing for Surface Permethrin Content

To conduct the testing for surface permethrin content, the Martindale Abrasion Tester(MAT) (Testex, Guangdong, China) was used. The MAT consists of four separate parts (rubbing media, rod, treated fabric, and 9 kPa weights). The untreated rubbing media used for this MAT test were 13.5 cm circular swatches cut from untreated short-sleeve shirts [Hanes®] made of 100% cotton. The rubbing media is the part that receives the permethrin from the treated fabric through the process of rubbing. The treated fabric is a smaller circular swatch with a diameter of 3 cm cut from the different types of PTC. This was done so that the fabric could be placed within the fabric holder on the MAT. The fabric holder is then pressed and rotated against the rubbing media by the rod and weight. The weight which was set on top of the rod was 9 kPa. The rubs per minute (rpm) setting used for this study was 30 rpm. After the designated number of rubs had been reached, the rubbing media was removed and placed into a 60 mL amber vial containing 40 mL of acetone for later determination of permethrin content. The vial was then sonicated using the 2.8L Fisher Scientific Ultrasonic Bath for 60 min. Sonication was done to extract the permethrin that had transferred to the rubbing media from the treated fabric. After sonicating, 2 mL of the solution was added into 2 mL amber vials (two vials for each individual rubbing) and were tested on the GC for permethrin content rubbed from the surface of respective swatches (i.e., surface permethrin content).

The MAT test was conducted on three types of PTC (Insect Shield®, Sawyer, 4 g/L permethrin) and control. The designated number of rubs used for this group were 500, 1000, 2000, 3000 and 3500 rpm. Two swatches of each PTC were used at each designated number of rubs (10 swatches total per PTC type). In total, there were 40 swatches tested using the MAT.

Cone Bioassay with Mosquitoes

The PTC (Insect Shield®, Sawyer, and 4g/L permethrin) and control fabrics were also used for the cone bioassay. A modified cone assay method was used derived from DeRaedt et al. (2015) and Richards et al. (2018). Six swatches from Insect Shield®, Sawyer, 4 g/L permethrin, and control groups were tested against two groups of *Ae. albopictus* mosquitoes for repellency using the modified cone assay method. Both groups of *Ae. albopictus* originated from a susceptible lab colony (F-38), originating from LA. One group of mosquitoes was a permethrin-susceptible colony (F-47). The other group was a permethrin-exposed group (F-42) which also derived from the LA Colony, but had been exposed to permethrin as described previously.

The modified cone bioassay method consisted of placing six 8.5 cm glass cones/funnels on top of separate replicate 8.5 cm diameter swatches from the four designated types of fabrics. Five to eight mosquitoes were introduced into each cone and timed for one hour. Within the first three minutes of the hour, the number of mosquitoes which showed repellency to the fabric were recorded based on visual observation. Repellency for this test was defined as the mosquito flying away from the treated fabric or sporadic flying and landing onto the fabric. The definition of knockdown used here was death or inability to fly after being exposed to the fabric. Two cone assays sessions were conducted for each fabric type since a susceptible colony and exposed colony of mosquitoes were used. The same 8.5 cm swatches of fabric used in cone bioassays were tested for total permethrin content using the GC as previously described.

Table 1. Number of Swatches used for Each Test

Number of Swatches (N) for Each Test					
	Control	Insect Shield®	Sawyer	4g/L Permethrin	Total # of Swatches
Total Permethrin Content Testing	2	2	2	2	8
Martindale Abrasion Test: 500 rubs at 30rpm	2	2	2	2	8
Martindale Abrasion Test:1000 rubs at 30rpm	2	2	2	2	8
Martindale Abrasion Test: 2000 rubs at 30rpm	2	2	2	2	8
Martindale Abrasion Test: 3000 rubs at 30rpm	2	2	2	2	8
Martindale Abraision Test: 3500 rubs at 30rpm	2	2	2	2	8
Cone Assay (susceptible & exposed colonies)	6	6	6	6	24
Total # of Swatches	18	18	18	18	72

Calculation of Permethrin Concentration

A five-point calibration curve was created using the GC and permethrin stocks at different concentrations. The five points of calibration of permethrin concentration were at 12.5 µg/L, 25 µg/L, 50 µg/L, 100 µg/L, and 200 µg/L. The formula acquired from the five-point calibration curve is $Y= 0.7484(x) – 0.2208$ with an R^2 of 0.99999. The data area acquired from the GC testing was plugged into the calibration curve formula as (x). The results were then divided by the weight (in grams) of the swatch that the acquired area came from.

Statistical Analysis

Analysis of variance (ANOVA) was used to evaluate the differences in total permethrin content (TPC and surface permethrin content (SPC) between the types of PTC. *P*-values < 0.05 were considered significant. ANOVA was also used to evaluate the repellency between the susceptible and exposed mosquito colonies. The Bonferroni comparison was used to compare means between fabric treatment types and to compare means between TPC and SP

CHAPTER FIVE: RESULTS

Modified CDC Bottle Bioassay

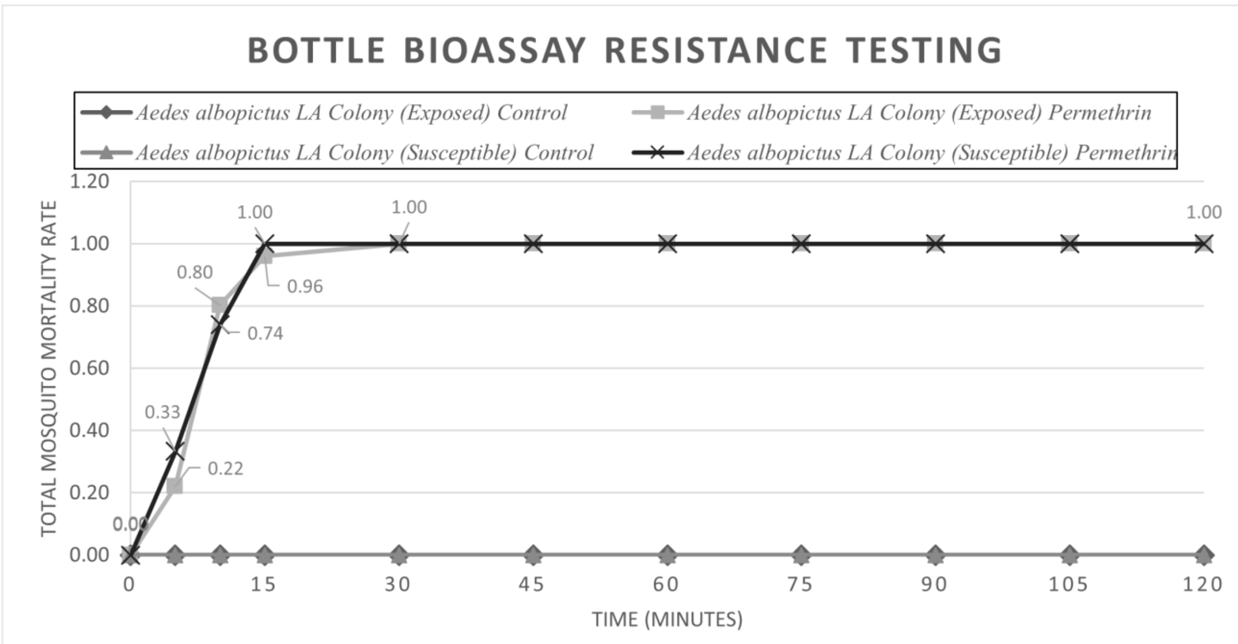

Figure 3. Bottle Bioassay Resistance Testing

The diagnostic time used for this modified CDC bottle bioassay was 15 minutes. At 15 minutes, the LA Colony F-47 (Susceptible) mosquitoes had a 100% mortality rate which classifies them as susceptible (Figure 3). The LA Colony F-42 (Exposed) mosquitoes had a 96% mortality rate at 15 minutes and are considered as developing a resistance (CDC, 2020). A 0% mortality rate was recorded for both mosquito groups in the control bottles.

Total Permethrin Content (TPC)

Table 2 shows the means ± standard error (SE) of means of TPC for the three fabric treatment types and control fabrics. Sawyer PTC had the highest TPC per swatch (289.17 ± 0.51

μg/g), followed by Insect Shield® (53.90 ± 6.79 μg/g) and the lab-created 4 g/L permethrin swatches (12.67 μg/g ± 0.35). The control swatches had the lowest mean TPC (0.2620 μg/g ± 0.00). The reported means and SEM above can be found in Table 2.

Table 2. Total Permethrin Content (μg/g) for Different Types of Fabric

Fabric Treatment	Mean	N	Std. Deviation	Std. Error of Mean
4 g/L Permethrin	12.6655	2	.50134	.35450
Control	0.2620	2	0.00000	.00000
Insect Shield®	53.9010	2	9.60817	6.79400
Sawyer	289.1655	2	7.17360	5.07250

Table 3 shows differences of means between Insect Shield®, Sawyer, 4 g/L permethrin, and Control. When comparing the differences in means of the three fabric treatments (Insect Shield®, Sawyer, 4 g/L permethrin) against Control fabric treatment, Sawyer had the highest difference in mean (288.90 μg/g), which was found to be significant ($P<0.0001$). The difference of means between Insect Shield® and Control (53.64 μg/g) was also found to be significant ($P=0.005$).

Table 3. Comparisons of Total Permethrin Content (µg/g) by Fabric Treatment

Multiple Comparisons (Total Permethrin Content)						
Dependent Variable: Permethrin Concentration						
Bonferroni						
(I) Fabric Treatment	(J) Fabric Treatment	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
4g/L Permethrin	Control	12.4035	6.00060	.646	-16.7055	41.5125
	Insect Shield®	-41.2355*	6.00060	.014	-70.3445	-12.1265
	Sawyer	-276.5000*	6.00060	$7.960E^{-6}$	-305.6090	-247.3910
Control	4g/L Permethrin	-12.4035	6.00060	.646	-41.5125	16.7055
	Insect Shield®	-53.6390*	6.00060	.005	-82.7480	-24.5300
	Sawyer	-288.9035*	6.00060	$6.681E^{-6}$	-318.0125	-259.7945
Insect Shield®	4g/L Permethrin	41.2355*	6.00060	.014	12.1265	70.3445
	Control	53.6390*	6.00060	.005	24.5300	82.7480
	Sawyer	-235.2645*	6.00060	$1.517E^{-5}$	-264.3735	-206.1555
Sawyer	4g/L Permethrin	276.5000*	6.00060	$7.960E^{-6}$	247.3910	305.6090
	Control	288.9035*	6.00060	$6.681E^{-6}$	259.7945	318.0125
	Insect Shield®	235.2645*	6.00060	$1.517E^{-5}$	206.1555	264.3735
Based on observed means. The error term is Mean Square (Error) = 36.007.						
*. The mean difference is significant at the 0.05 level.						

Surface Permethrin Content (SPC)

Table 4 shows the pooled mean surface permethrin content for Insect Shield®, Sawyer, 4 g/L permethrin, and control groups. Sawyer PTC had the highest pooled mean surface content with 32.68 ± 14.55 µg/g. Insect Shield® had the second highest pooled mean surface content with 23.35 ± 2.71 µg/g. The lab-created 4 g/L permethrin had the third highest pooled mean surface content with 8.7 ± 0.78 µg/g. The control had the lowest mean surface content with 0.26 ± 0.0005 µg/g.

Table 4. Means of Surface Permethrin Content (µg/g) for Different Fabric Treatments

Surface Permethrin Content * Fabric Treatment Type				
Fabric Treatment	Mean	N	Std. Deviation	Std. Error of Mean
4g/L Permethrin	8.6908	10	2.47041	0.78121
Control	0.2604	10	.00143	0.00045
Insect Shield®	23.3453	10	8.56055	2.70708
Sawyer	32.6814	10	46.02245	14.55358

A one-way ANOVA was carried out for the surface permethrin content of each fabric treatment (Insect Shield®, Sawyer, 4 g/L permethrin) per number of rub (Table 5). Significant (P<0.0001) differences were observed in surface permethrin content between groups. The control data set was omitted in this analysis to better assess the variance between fabric treatment and surface permethrin content due to the Control TPC and SPC being the same.

Table 5. ANOVA Analysis (Rubs vs. Fabric Treatment)

Tests of Between-Subjects Effects (Surface Permethrin Content)					
Dependent Variable: Surface Permethrin Content					
Source	Type III Sum of Squares	df	Mean Square	F	*P*-value
Corrected Model	158665.844[a]	17	9333.285	726.761	$4.860E^{-22}$
Intercept	51274.998	1	51274.998	3992.664	$1.373E^{-22}$
Rubs	53680.903	5	10736.181	836.001	$1.201E^{-20}$
Fabric Treatment	27753.338	2	13876.669	1080.544	$1.791E^{-19}$
Rubs * Fabric Treatment	77231.602	10	7723.160	601.384	$1.328E^{-20}$
Error	231.161	18	12.842		
Total	210172.003	36			
Corrected Total	158897.005	35			
a. R Squared = .999 (Adjusted R Squared = .997)					

A comparison between surface permethrin content and fabric treatment types is shown in Table 6. When comparing the surface content of Insect Shield®, Sawyer, and 4 g/L permethrin groups to the surface content of the control fabric, Sawyer had the significantly (*P*<0.0001) highest difference in mean (75.17 ± 1.27 µg/g). The second highest mean difference (*P*<0.0001) compared to the control was Insect Shield® (28.18 ± 1.27 µg/g). The lowest mean difference was found between control and 4 g/L permethrin groups (*P*<0.001; 9.09 ± 1.27 µg/g).

Table 6. Comparison of Surface Permethrin Content (µg/g) Between Fabric Treatments

Multiple Comparisons (Surface Permethrin Content)						
Dependent Variable: Surface Permethrin Content						
Bonferroni						
(I) Fabric Treatment	(J) Fabric Treatment	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval Lower Bound	Upper Bound
4g/L	Control	9.0926*	1.26700	$1.225E^{-6}$	5.4498	12.7353
	Insect Shield®	-19.0847*	1.26700	$5.947E^{-13}$	-22.7274	-15.4419
	Sawyer	-66.0755*	1.26700	$1.948E^{-25}$	-69.7182	-62.4328
Control	4 g/L Permethrin	-9.0926*	1.26700	$1.225E^{-6}$	-12.7353	-5.4498
	Insect Shield®	-28.1773*	1.26700	$9.541E^{-17}$	-31.8200	-24.5345
	Sawyer	-75.1681*	1.26700	$9.032E^{-27}$	-78.8108	-71.5253
Insect Shield®	4 g/L Permethrin	19.0847*	1.26700	$5.947E^{-13}$	15.4419	22.7274
	Control	28.1773*	1.26700	$9.541E^{-17}$	24.5345	31.8200
	Sawyer	-46.9908*	1.26700	$6.304E^{-22}$	-50.6336	-43.3481
Sawyer	4 g/L Permethrin	66.0755*	1.26700	$1.948E^{-25}$	62.4328	69.7182
	Control	75.1681*	1.26700	$9.032E^{-27}$	71.5253	78.8108
	Insect Shield®	46.9908*	1.26700	$6.304E^{-22}$	43.3481	50.6336
Based on observed means. The error term is Mean Square(Error) = 9.632.						
*. The mean difference is significant at the 0.05 level.						

Total Permethrin Content (TPC) vs Surface Permethrin Content (SPC)

A comparison was made between TPC and SPC for each type of fabric treatment (Table 7). For Insect Shield®, the difference between TPC and SPC was significant for all number of rubs except at 3000 rubs. At 3000 rubs, the Insect Shield® mean difference was 22.62 µg/g with *P*=0.054. The highest mean difference for TPC and SPC in Insect Shield® was found at 3500 rubs with a mean difference of 45.21 µg/g (*P*=0.001).

For Sawyer, comparisons of TPC and SPC at all quantities of rubs were found to be significant (*P*<0.0001). The highest mean difference between TPC and SPC was found at 2000 rubs with a mean difference of 283.11 µg/g (*P*<0.0001). The lowest mean differences were found at 500 rubs and 3500 rubs with mean differences of 169.76 µg/g (*P*<0.0001).

For the 4 g/L permethrin group, the highest mean differences were found in TPC between 500 and 1000 rubs. The highest mean difference was found at 500 rubs with a mean difference of 6.58 µg/g (*P*=0.001). The lowest mean difference (1.42 µg/g; *P*=1.00) between TPC and SPC was found at 3000 rubs. A comparison between Control TPC and Control SPC was not made since the TPC and SC were similar.

Table 7. Comparison of Total Permethrin Content (TPC) to Surface Permethrin Content (SPC) of Permethrin

Multiple Comparisons of Total Permethrin Content (TPC) to Surface Permethrin Content (SPC)

Dependent Variable: Permethrin Concentration

Bonferroni

Fabric Treatment	TPC	SC (J) Rubs	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Insect Shield®	TPC	500.00	26.3360*	4.89526	.025	3.3384	49.3336
		1000.00	30.9420*	4.89526	.011	7.9444	53.9396
		2000.00	27.6720*	4.89526	.020	4.6744	50.6696
		3000.00	22.6180	4.89526	.054	-.3796	45.6156
		3500.00	45.2105*	4.89526	.001	22.2129	68.2081
Sawyer	TPC	500.00	169.7610	3.74735	$1.162E^{-7}$	152.1562	187.3658
		1000.00	270.0375	3.74735	$7.209E^{-9}$	252.4327	287.6423
		2000.00	283.1050	3.74735	$5.431E^{-9}$	265.5002	300.7098
		3000.00	282.7785	3.74735	$5.468E^{-9}$	265.1737	300.3833
		3500.00	169.7610	3.74735	$1.162E^{-7}$	152.1562	294.3433
4g/L Permethrin	TPC	500.00	6.5785	.72159	.001	3.1885	9.9685
		1000.00	6.8620	.72159	.001	3.4720	10.2520
		2000.00	2.6925	.72159	.146	-.6975	6.0825
		3000.00	1.4180	.72159	1.000	-1.9720	4.8080
		3500.00	2.3225	.72159	.273	-1.0675	5.7125
	*. The mean difference is significant at the 0.05 level.						

Cone Bioassay Repellency Testing

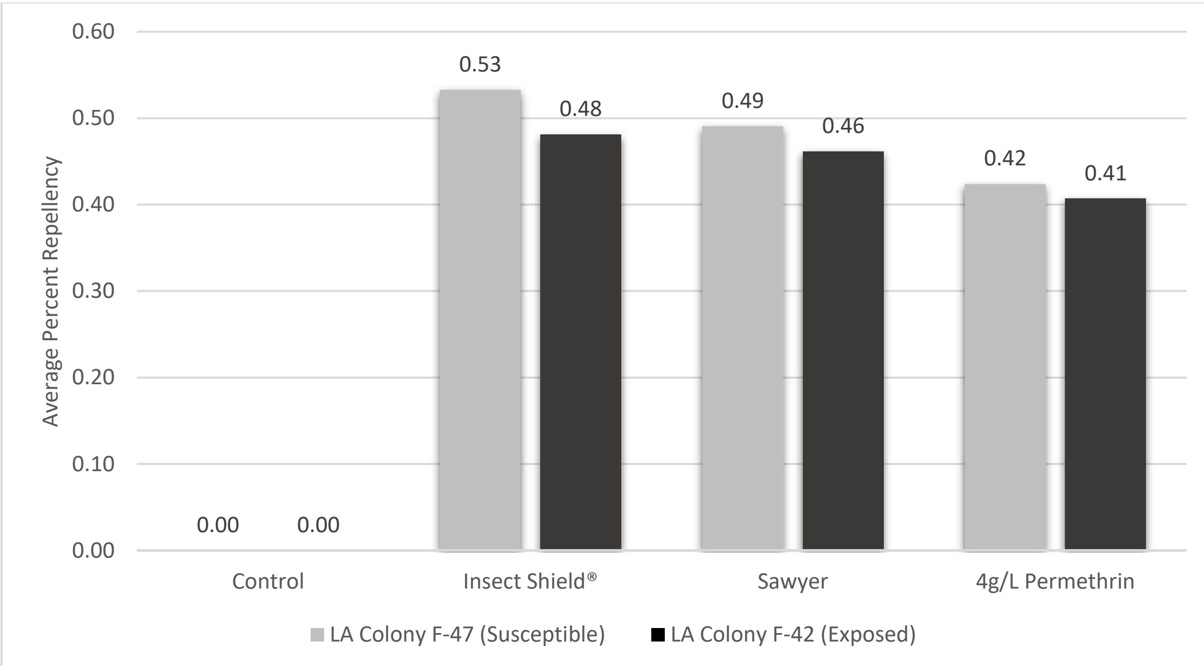

Figure 4. Cone Bioassay Repellency Testing by Fabric Treatment

A cone bioassay consisted of the six swatches from each PTC fabric being tested against mosquitoes for 3 min (Figure 4). Since there were two mosquito groups (susceptible & exposed), two cone bioassays were conducted using the same swatches for both assays. The cone bioassay results were compared between PTC (Table 8).

As expected, for the control fabric, both groups showed zero repellency. Insect Shield® performed the best against both susceptible and exposed mosquito groups with 53% and 48% repellency. The repellency rates for Insect Shield® for both mosquito groups were not significantly different when compared to Sawyer and 4g/L permethrin. The Sawyer swatches showed the second highest repellency against both mosquito groups with 49% repellency for susceptible and 46% for exposed mosquitoes. The repellency rates for Sawyer were not significant when compared to both Insect Shield® and 4 g/L permethrin. The third highest repellency was observed in the 4 g/L permethrin group (42% repellency against susceptible group and 41% against exposed group). No significant differences in repellency rates were observed between the 4 g/L group, Insect Shield® and Sawyer. Repellency rates for Insect Shield®, Sawyer, and 4 g/L permethrin were significantly higher ($P<0.05$) than the control group.

Table 8. Comparisons of Repellency among Permethrin Treated Clothing

Multiple Comparisons

Dependent Variable: Repellency

Bonferroni

Mosquito Colony	(I) PTC	(J) PTC	Mean Difference (I-J)	Std. Error	P-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Susceptible	Control	4g/L Permethrin	-.4233*	.09270	$1.00E^{-3}$	-.69	-.1520
		Insect Shield®	-.5183*	.09270	$1.08E^{-4}$	-.79	-.2470
		Sawyer	-.5067*	.09270	$1.43E^{-4}$	-.78	-.2353
Exposed	Control	4g/L Permethrin	-.4600	.04433	$1.74E^{-8}$	-.5905	-.3295
		Insect Shield®	-.5783	.03505	$6.10E^{-12}$	-.6815	-.4752
		Sawyer	-.4633	.03505	$2.98E^{-10}$	-.5665	-.3602
Susceptible	Insect Shield®	4g/L Permethrin	.0950	.09270	1.00	-.1763	.3663
		Control	.5183*	.09270	$1.08E^{-4}$	.2470	.7897
		Sawyer	.0117	.09270	1.00	-.2597	.2830
Exposed	Insect Shield®	4g/L Permethrin	.1717*	.04170	$3.00E_{-3}$	.0496	.2937
		Control	.5783*	.04170	$6.08E^{-11}$	.4563	.7004
		Sawyer	.1150	.04170	$7.30E^{-2}$	-.0071	.2371
Susceptible	Sawyer	4g/L Permethrin	.0833	.09270	1.00	-.1880	.3547
		Control	.5067*	.09270	$1.43E^{-4}$	.2353	.7780
		Insect Shield®	-.0117	.09270	1.00	-.2830	.2597
Exposed	Sawyer	4g/L Permethrin	.0567	.04170	1.00	-.0654	.1787
		Control	.4633*	.04170	$3.14E^{-9}$	.3413	.5854
		Insect Shield®	-.1150	.04170	$7.30E^{-2}$	-.2371	.0071
Susceptible	4g/L Permethrin	Control	.4233*	.09270	$1.00E^{-3}$	.1520	.6947
		Insect Shield®	-.0950	.09270	1.00	-.3663	.1763
		Sawyer	-.0833	.09270	1.00	-.3547	.1880
Exposed	4g/L Permethrin	Control	.4067*	.04170	$2.89E^{-8}$	.2846	.5287
		Insect Shield®	-.1717*	.04170	$3.00E^{-3}$	-.2937	-.0496
		Sawyer	-.0567	.04170	1.00	-.1787	.0654

*. The mean difference is significant at the 0.05 level.

Slightly higher repellency rates were observed in the susceptible group of mosquitoes compared to the exposed group among all fabric types (e.g. Insect Shield: Susceptible 53%, Exposed 48%). An ANOVA was used to assess differences in repellency rates between susceptible and exposed mosquitoes (Table 9). The difference in repellency among PTC against susceptible and exposed mosquitoes was not significant (*P*=0.774).

Table 9. ANOVA Analysis Effects (Cone Bioassay Repellency)

Tests of Between-Subjects Effects (Cone Bioassay Repellency)					
Dependent Variable: Repellency					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.222[a]	7	.317	20.480	2.208E-11
Intercept	6.293	1	6.293	406.066	1.482E-22
PTC	2.204	3	.735	47.415	3.077E-13
Mosquito Susceptibility	.000	1	.000	.000	1.000
PTC * Mosquito Susceptibility	.017	3	.006	.371	.774
Error	.620	40	.015		
Total	9.135	48			
Corrected Total	2.842	47			
a. R Squared = .782 (Adjusted R Squared = .744)					

CHAPTER SIX: DISCUSSION

TPC vs. SPC

The TPC and SPC were tested and compared for four PTC (Insect Shield®, Sawyer, 4 g/L Permethrin, Control). We observed a significant difference between TPC and SPC among the four PTC types. Sawyer-treated fabrics showed the highest TPC (289.17 µg/g) and this amount was significantly ($P<0.0001$) higher than Insect Shield®, 4 g/L permethrin, and control groups. The Sawyer-treated fabrics also had the highest pooled mean of SPC (32.68 µg/g) and this was significantly ($P<0.0001$) higher than Insect Shield®, 4 g/L permethrin, and control groups. These findings do not support the first and second hypotheses of Insect Shield® having the highest TPC and SPC. These findings also indicate that PTC with higher TPC typically have a higher SPC.

Sawyer may have the highest TPC and SPC due to its application method. For Sawyer, the permethrin was not impregnated into the cotton fabric, but sprayed onto the surface. The directions on the bottle which stated to spray until damp were also followed in this experiment. Applying Sawyer onto the cloth without a direct measure of the pesticide could also be an explanation for why it has the highest TPC.

When comparing TPC and SPC, a significant mean difference indicates a lower concentration of permethrin found on the fabric surface. If the mean difference between TPC and SPC is insignificant, it indicates that a higher concentration of permethrin is found on the fabric surface. The number of rubs in which these insignificant mean differences are found may indicate an optimal number of rubs that can be used to assess the SPC of each PTC.

For Insect Shield®, the insignificance in mean difference was found at 3,000 rubs (*P*=0.054). At 3,500 rubs, the mean difference was found to be significant. These results indicate that the transfer of permethrin is lost or destroyed for Insect Shield® after 3,000 rubs.

Sawyer did not have any insignificant mean differences between TPC and SPC. The lowest mean differences were found between 500 rubs and 3,500 rubs (mean difference: 169.76 µg/g). The disparity in the number of optimal rubs may be due to the treatment method used for the swatches. Since a spray bottle application method was used, a uniform application of permethrin may not have been achieved and the concentrations could vary between each swatch. Future studies could use a uniform application method for Sawyer to further assess the number of optimal rubs.

4g/L Permethrin had three mean differences between TPC and SPC that were insignificant (2000, 3000, 3500 rubs). The insignificant mean differences between TPC and SPC for 4g/L Permethrin at 2000, 3000, and 3500 rubs were 2.6925 µg/g, 1.4180 µg/g, and 2.3225 µg/g respectively. These results indicate that the optimal number of rubs for 4g/L Permethrin is 3000 rubs since the mean difference from 2000 to 3000 decreases from 2.6925 µg/g to 1.4180 µg/g. The mean difference then increases between 3000 and 3500 rubs from 1.4180 µg/g to 2.3225 µg/g. The increase of mean difference after 3000 rubs may indicate the loss or destruction of permethrin which could be due to factors such as friction and fabric type.

Cone Bioassay

A modified WHO cone bioassay was conducted on types of PTC against a susceptible group and a permethrin exposed group of mosquitoes. Among the PTC, Insect Shield® had the highest repellency rate against both susceptible (53%) and exposed (48%) groups of mosquitoes.

Insect Shield® repellency rates for both susceptible and exposed groups were significantly (P<0.0001) better than the control fabrics. However, no significant differences were observed in repellency rates between Insect Shield®, Sawyer, and 4 g/L permethrin groups. These results do not support our third hypothesis of Sawyer having the highest repellency rates for both susceptible and exposed mosquito groups.

The mosquito repellency rate among PTC found in this study indicate that other factors besides SPC may affect repellency rate. A possibility may be the difference in treatment methods. An impregnation method helps an active ingredient (permethrin) bind to a fabric and allows the pesticide to be resilient to corrosion through normal washing and wearing. It is also possible that other ingredients such as binding agents may be present and can increase the repellency rate of a PTC. Insect Shield® PTC having the highest repellency rate can be correlated with the use of a proprietary impregnation method by Insect Shield®.

Sawyer PTC was treated in accordance with the bottle directions. The solution of Sawyer only contains 0.5% permethrin while 99.5% of the solution is listed as other ingredients. These other ingredients may be binding agents or ingredients that increase the potency of permethrin and may explain the higher repellency rate in Sawyer than 4g/L Permethrin. 4 g/L permethrin was treated using a lab created method with a solution only containing acetone and permethrin.

Susceptible vs. Exposed Mosquito Groups

This study used two *Ae. albopictus* mosquito groups; one previously exposed to permethrin and one susceptible. Permethrin was introduced five times at low concentrations of permethrin between generation F-38 to F-42 for the exposed mosquito group. A CDC bottle bioassay was conducted using permethrin for both mosquito groups. The exposed mosquito group had a 96% mortality rate at the diagnostic time of 15 minutes and was determined to be developing resistance to permethrin but was not yet classified as resistant.

The same two mosquito groups were also tested using a modified WHO cone assay against different types of PTC. A higher repellency rate was found in susceptible mosquitoes when compared to exposed mosquitoes. This finding is consistent with those found in Bowman et al. (2018) where a higher repellency rate was observed in mosquitoes previously exposed to permethrin in comparison to susceptible mosquitoes. In the study conducted by Bowman et al. (2018), an arm-in-cage test was used with PTC instead of a cone assay.

The findings of the cone assay when comparing the difference in repellency rate between the two mosquito groups was not significant. However, these results do support that the *Ae. albopictus* F-42 exposed mosquitoes were building a slight resistance to the PTC used in this study. It is possible that continued permethrin exposure through additional mosquito generations could lead to a permethrin-resistant colony and further studies are warranted to evaluate this.

Recommendations for Future Studies

A possible future study could focus on testing the effectiveness of the MAT to transfer the surface content of pesticide treated clothing to other media. Doing so will allow for more accurate surface content readings that can be used to assess the effectiveness of PTC against pests. Future studies could also assess the transfer of pesticides among different fabric types (i.e., polyester, cotton, or wool) using the MAT. A future study could also be conducted using landing counts of mosquitoes in the field to assess and compare the PTC used in this study.

Limitations of this Study

Few studies have used a cone assay to assess and compare the repellency rates for an exposed mosquito group to a susceptible mosquito group. Few studies have also used the MAT to assess surface content of pesticides on fabric. The current study was conducted using a mosquito colony reared in a laboratory; and results may not be applicable to evaluate field mosquito populations

CHAPTER SEVEN: CONCLUSION

The first use of permethrin on clothing as a repellant occurred in 1990 by the military (EPA, 2006). Since then, many studies have been conducted to demonstrate the effectiveness of permethrin in repelling and killing mosquitoes. However, few studies have focused on the surface of clothing that has been treated with permethrin. In this study, we evaluated concentrations of TPC and SPC with the repellency rate of mosquitoes. Our findings show that Sawyer-treated fabrics had significantly higher TPC and SPC compared to the other tested fabrics but no significant differences were observed in mosquito repellency rates between any treated fabrics. More studies are needed to evaluate the relationship between SPC and repellency. This study also assessed the difference between TPC and SPC. As expected, we show that SPC was lower than TPC. This has important public health implications as the surface of fabrics are what the potential pests (ticks, mosquitoes, etc.) contact when attempting to blood feed. Additional studies should assess the MAT's ability to transfer insecticides and further refine this valuable assessment method. Studies could also further assess the relationship between washing of fabrics and SPC/TPC.

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