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Permethrin: An Assessment of Exposure During a Military Uniform Mass Spray Application

by

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DEDICATION

"As you go through life, you will see that there is so much that we don't understand. And the only thing we know is things don't always go the way we plan."

- Simba, *The Lion King*, 1994

Dedication is something that motivates and inspires you. Two forces drove me throughout my graduate studies journey, but both begin and end with family. The eternal love and power of family is the foundation of who I am. We take for granted that those closest to us will always be there to share in the happy and sad moments. It takes one minute to change the future for so many lives.

Mental illness is the silent killer, not only to the one suffering but for everyone who loves you. Ben, I bring you to light in this dedication. Not that you were particularly involved in science or public health, but your passion for life is something that inspired. Ben, your acceptance of everyone, your smile and energy, ever since you were a kid, brought joy to everyone around you. I loved your goofy-scared smile when we would watch *Are You Afraid of the Dark?* and your mischievous side when you would assure Eric and me that you were "allowed" to do something that you most certainly were not. You are in my mind and my heart, always. You left this world far too soon but left an everlasting mark. The way to honor you, and to help others who silently suffer is to promote mental health awareness.

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Lynnea K. Jensen

June 19, 2020

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ABSTRACT

Title of Thesis: Permethrin: An Assessment of Exposure During a Military Uniform Mass Spray Application

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2020

Thesis directed by: Alex H. Stubner, PhD, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences

For more than 30 years, the US military has relied on permethrin for protection against biting arthropods and exposure to arthropod-borne diseases. Most personnel have worn uniforms treated with and sprayed products containing permethrin. Permethrin can be applied to uniforms by trained personnel in a mass spray event using permethrin. During a uniform spray event, exposure is possible through the dermal, inhalation, and ingestion routes. All the potential routes of exposure are heavily influenced by personal behaviors of the users of permethrin.

The potentially exposed population during a spray event includes the Applicator and Working Party, or personnel who handle uniforms, including positioning on the ground, flipping, and removing uniforms. Though the expected risk to this population is low because they should wear protective gloves, a risk assessment has not been conducted previously. This study is a multi-route exposure assessment for personnel participating in the spray treatment of uniforms and also aims to identify permethrin in

the vicinity of a uniform spray event to identify potential exposure routes. Sampling occurred during a routine spray performed by 13 Navy personnel (3 Applicators; 10 Working Party) over two days.

Human exposures were measured through biological monitoring and dermal sampling. Baseline urine samples were collected before spraying to measure metabolites *cis*-DCCA and *trans*- DCCA. Additional urine samples were collected at least 4-hours and at least 12-hours after spraying started. The urine sample analysis is pending due to the current COVID-19 limitations. Two, two-way repeated-measures Analysis of Variance will be used to compare data from urine samples. The first will compare the mean difference and interaction between the role (Applicator or Working Party) to the DCCA measured in urine. The second repeated-measures ANOVA will compare isomer (*cis*- and *trans*-) and time (baseline, 4-hr, 12-24-hr) to understand if there was an interaction on the dependent variable.

Dermal exposures were measured using dosimeter gloves (n=26) and dermal patch (n=26) worn on the right forearm of study participants. A repeated-measures ANOVA showed the average value of exposure is similar between the Applicators versus Working Party in patches as in gloves, and not statistically significant (p-value= 0.8340). Where data about dermal exposures comes from a routine uniform spray event, care is needed to estimate the mean permethrin measured in patches because there was 65% censored data. However, similar dermal exposures may be a reason to reconsider training and PPE requirements during a mass spray uniform treatment. This study suggests the Working Party should be considered along with the Applicators in risk assessments and published guidance, and PPE requirements should be for all personnel.

Collection of permethrin in the air around a spray event used OVS-2 tubes and rotating spinners that were co-located and placed using a stratified random sampling approach. The collection of aerosol and vapor phases of permethrin on the OVS-2 tube (n=36) followed OSHA Method 70. All OVS-2 tube samples were reported below LOQ.

Rotating impactors were used to collect and measure droplet density, size, and distribution as an indication of dermal and indirect ingestion risks. DropVision® system analyzed the number of drops per slide and provided volume distributions of slides (n=72). Four one-way analysis of variance (ANOVA) tests compared sampling locations to average droplet span and density and showed no significant difference (p=0.9262) in mean droplet densities between zones. However, there was a difference in droplet size distribution, using the span between zones (p=0.0206). Differences in the mean span (distribution width) between the zones, despite no difference in the number of droplets, indicated the sizes of the droplets vary by zone, and there might be an Applicator behavior or aspect of the equipment producing this difference. Neither mean droplet density (p=0.2056) or mean span (p=0.1958) had statistical difference when collected at 0, 1, 2, and 3 feet off the spray line. Multiple variables influence a mass spray treatment of uniforms with permethrin, such as human behaviors, and performing a spray during high humidity may increase the dispersion of permethrin droplets. The distances from the spray line used in this study showed permethrin persists in the air similarly up to 3 feet, and further knowledge is needed beyond that distance.

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CHAPTER 1: Introduction and Background

1.1 INTRODUCTION

Most people are aware they can purchase pesticides from a local store. However, it may not be common knowledge that pesticides are not limited to insecticides and herbicides. Pesticides include any substance or mixture intended for preventing, destroying, or repelling any pest and includes insecticides to repel or kill insects and some other arthropods, herbicides to control weeds and unwanted vegetation, fungicides to inhibit or kill mold and mildew, and disinfectants to stop the spread of bacteria.

The Environmental Protection Agency (EPA) regulates pesticides in the United States. All pesticides must also be registered with the EPA, which review the ingredients, assesses the quantity required , determine personal protective equipment to mitigate exposure risks, approve labeling, and restrict where and how the product will be used, stored and disposed (36). The EPA also appoints a timeline for the reassessment of the product to ensure it continuously meets evolving standards as there are advances in scientific knowledge. Permethrin is a type of insecticide that can be applied as a lotion to treat scabies and lice and sprayed on clothing, including US military uniforms.

In the fall of 2018, a Freedom of Information Act (FOIA) question was submitted to the Navy and Marine Corps Public Health Center (NMCPHC) concerning permethrin exposure to individuals performing large-scale, manual field uniform spray treatments. The question was brought to the attention of Preventive Medicine and Biostatistics (PMB) faculty at Uniformed Services University of the Health Sciences (USUHS). Upon further investigation and review of the literature, a determination was made that a

comprehensive exposure assessment to permethrin among the Applicators and Working Party personnel during the field treatment of US Navy field uniforms had not been conducted.

1.2 BACKGROUND

The United States military has relied on permethrin for protection against biting arthropods and exposure to arthropod-borne diseases since the 1990s. For almost 30 years, most personnel have worn uniforms treated with permethrin. Without an assessment of potential permethrin exposures during a uniform spray, we cannot confidently assess exposure risk. Some people potentially have increased exposure if they participated in treatment of uniforms by spraying them with a concentrated permethrin solution.

Uniform treatment is a technical service offered by Department of Defense (DoD) preventive medicine units, such as the Navy Environmental Preventive Medicine Units (NEPMU) (located both within and outside the U.S.) and the Navy Entomology Center of Excellence (NECE) in Jacksonville, FL for units preparing for contingency operations. Large scale field treatment of uniforms requires a Working Party to layout, flip, and collect uniforms that have been saturated with the permethrin solution. It may be assumed that since the Working Party is just laying out uniforms and flipping them over, they do not have any significant exposure during these tasks. However, an assessment of exposure to Working Party has not been conducted. This study seeks to investigate if the Working Party potentially face dermal, inhalation, or ingestion exposures from handling and working near the spray efforts and handling wet uniforms.

The potentially exposed population during a field uniform spray event typically includes the Applicator and Working Party, which is defined as the personnel who handle uniforms, to include positioning on the ground, flipping for drying, and removal of uniforms after the treatment evolution. The Working Party are personnel assigned to assist by the unit requesting the uniform treatment and are typically enlisted personnel. Though the expected risk to this population is low because they should wear protective gloves, a risk assessment has not been conducted previously. It is essential to understand potential permethrin exposure risks to personnel operating within the DoD, so adequate precautions are taken to protect their health and well-being.

1.3 Description and Use of Permethrin

Permethrin ($C_{21}H_{20}Cl_2O_3$) is a synthetic pyrethroid compound derived from pyrethrins which are naturally occurring substances extracted from chrysanthemum flowers (2). Pyrethroid compounds such as permethrin are derived from pyrethrins to provide a more stable biting arthropod and pest repellent (114). Pyrethroids are effective for use in agriculture, forestry, horticulture, for the protection of textiles, public health, and in homes (62). Permethrin is odorless and ranges from colorless to yellow solid to an oily liquid.

1.4 Potential Permethrin Exposures

Permethrin is a broad-spectrum pesticide that targets pests, including those of interest to public health, such as mosquitoes and fleas. Exposure to the general public can occur through residue left on food surfaces, mosquito abatement, topical application for the treatment of scabies or lice, and pest control around homes. Occupational exposures are of interest for people who work in agriculture and pest control, pesticide

manufacturing, and textile impregnation industries. Family members of these workers may also face higher exposure than the general public because of take-home residual pesticides (121). A study investigating the exposure pathways to *cis-* and *trans-* permethrin of 127 preschool children (87) found dietary and indirect ingestion are the primary and secondary routes of exposure, which renders take-home residual pesticides relevant.

Exposure to permethrin is possible through ingestion, inhalation, and dermal routes of exposure (68). There is no consensus on which exposure route is most significant (57). Ingestion of permethrin by the general population may occur through direct or indirect ingestion and is thought to be the primary exposure route (29; 62). Toxicokinetic studies have shown that dermal exposures to pesticides contribute minimally, and inhalation or oral exposures contribute the highest exposure risks (28; 72; 125). However, studies by Atabila, Phung, Sadler, Connell, and Chu (2019), MacFarlane, Carey, El-Zaemay, and Fritschi (2013), Vitali, Protano, Del Monte, Ensabella, and Guidotti (2009), Van Hemmen and Brouwer (1995), and Bradberry, Cage, Proudfoot, and Vale (2005) suggest dermal exposure may be a major route among agricultural pesticide applicators (11; 17; 81; 122; 125).

1.5 Mode of Action

According to Chrustek et al. (2018), permethrin acts by interfering with brain functions and sodium channels of the sensory nerves of mammals and insects (23). In insects, the voltage-gated sodium channels are disrupted, delaying their inactivation, which leads to premature opening and extended opening, which therefore increases impulses leading to paralysis and death. In humans, permethrin disrupts sodium channels

but does not have the same effect as on insects because of human's larger size, higher body temperature, and less sensitive channels.

1.6 Potential Health Impacts

Health effects to permethrin can be local or systemic, and health effects are dependent on the route of exposure, dose, and duration, as well as individual metabolic and excretion responses (112). Symptoms of acute exposures may include reversible effects that subside after the exposure stops, such as running nose or cough. Chronic exposures to permethrin may induce hyperexcitability and whole-body tremors. The impact of long-term acute exposures needs further investigation. Between 2010 and 2020 there have been an increasing number of studies that investigate the concern for possible male reproductive effects (57; 84).

1.7 Sampling for Exposures

Exposure to permethrin can occur through the dermal, inhalation, and ingestion routes (68). Each route of exposure presents unique absorption tendencies that contribute to the overall dose. Biological monitoring is a way to measure the absorption of a chemical of interest where biomarkers can be indicators of multiple routes of exposure to a chemical. Compared to measuring personal exposures to environmental concentrations of a chemical, biomonitoring is more expressive of the actual risk of adverse health effects, as biological monitoring measures the actual absorbed dose experienced by a worker. Biomonitoring also accounts for interpersonal differences between individual absorption and metabolic rates.

Dermal exposure can occur through direct contact with a chemical, indirect contact with surfaces, clothing, or residues, and through the air. Skin exposure to a

chemical will contribute to the overall body burden and can lead to localized responses. There is agreement among studies that dermal exposures depend on processes and individual behaviors and can have high levels of variability among individuals (11; 81; 107; 108; 110; 122).

Permethrin has a low vapor pressure, 2.15×10^{-8} mmHg (120), which makes aerosols, spray droplets, and dust more of an inhalation risk than vapors. Air sampling methods for pyrethroid exposures are much more developed than dermal sampling methods. Both the National Institute for Occupational Safety and Health (NIOSH) (9) and Occupational Safety and Hazard Association (97) have validated methods for air sampling for permethrin.

Respiratory exposures to permethrin are challenging to accurately measure, due mainly to the lack of homogeneity in atmospheric concentration levels and individual differences. Variables, such as wind, sunlight, humidity, as well as droplet size and pressure of the spray affect the behavior of pesticides. Each individual has different inhalation and exhalation performance that affects the amount of permethrin delivered into the body as an exposure.

1.8 U.S. Military Uniforms and Permethrin Treatment

There are many uniform types worn across each of the military branches, but not all uniforms are approved for permethrin treatment, with some exceptions because some fire-retardant textiles do not allow the binding of permethrin. In general, uniforms must contain approximately 50% cotton in order for the permethrin formulation to bind to the textile. Some are treated during the uniform fabrication process, while others are treated in the field using individual dynamic absorption kit (IDA), or spray application methods.

Uniforms that are factory-treated are currently not approved for retreatment with permethrin. The Armed Forces Pest Management Board (AFPMB) has published a uniform treatment matrix to assist with the management of uniform treatment options (8).

In 2009, the EPA completed a human health and environmental risk assessment for permethrin (33). This report reassessed the potential adverse health effects resulting from wearing permethrin-treated uniforms and revalidated that wearing these uniforms is unlikely to cause adverse health effects. Permethrin in treated uniforms is chemically bound to the material once it is dry and therefore is not likely to be released and absorbed or ingested by the wearer. The EPA concluded that treated uniforms are safe to wear, even when sweating, in the rain, or in water (33).

1.9 Environmental Conditions and Effects During Uniform Treatment

Environmental conditions can play an essential role during the treatment of military uniforms and can have an effect on small droplet deposition on the target. Several factors influence droplet size and potential drift, including wind speed and direction, humidity, temperature, and sun intensity. These conditions are relevant when investigating potential exposures to personnel and will be investigated further as part of this research.

1.10 Permethrin and Public Health

Permethrin is a substance that provides benefits, but it may not be free of public health risks. The EPA balances the risk of exposure against the benefit of permethrin through regulating its use, and through the re-registration process.

1.10.1 Reduction of Disease

Permethrin provides many benefits. It provides a means of control of scabies and lice, which are significant public health concerns. Permethrin is used in mosquito control programs, used to protect food and feed crops, on livestock and pets, and on structures. In clothing, permethrin has proven useful in the repellency and control of mosquitoes and, therefore, the diseases mosquitoes transmit. The use of various insecticidal compounds has evolved throughout history. Permethrin has the desired properties of a short half-life, low mammalian toxicity, and useful general insecticidal properties.

1.10.2 Exposure to Permethrin

Exposure to permethrin may occur through multiple routes, including ingestion, inhalation, and dermal. The daily exposure to permethrin for an average person is estimated at 1,000 times less (1) than the average daily intake (ADI) established by the FAO and World Health Organization (WHO) (46). Further discussion on exposures and cancer classifications are in chapter 2.

1.11 Military Health Relevance

Service members have many occupational and environmental exposures, and characterization is difficult because of the volatile nature of a military career. For example, accurately establishing similar exposure groups (SEGs) and understanding exposure profiles proves difficult when personnel move to new duty stations every 2-4 years, deploy to combat zones, are tasked with jobs outside of their career field, and have duties that range across various environments.

However, short term exposure assessments can fill in some of the knowledge gaps to better understand potential exposures in specific scenarios. The field treatment of

uniforms is one such scenario that has taken place in the military since 1990 and permethrin application techniques and uniform material has been evolving since that time. Gaining an understanding of potential exposures that may occur during uniform treatment with permethrin in the field is highly relevant to preventive medicine units currently conducting uniform sprays. Also, there is future relevance as new products become available for use on uniforms and as the safety label on uniforms is reconsidered for re-treatment of factory pre-treated uniforms, increasing the need for field uniform treatment evolutions.

1.12 RESEARCH QUESTIONS

The objective of this study is to conduct a multi-route exposure assessment for permethrin during the field treatment of uniforms with a 40%-strength permethrin concentrate for personnel serving as Applicators and Working Party.

Research Question #1: What is the measure of absorbed pesticides in urine resulting from exposure via all routes during field uniform spray treatment using 40% permethrin?

Research Question #2: Using dosimeter gloves (pairs) and dermal patch sampling, what is the dermal exposure to permethrin?

Research Question #3: What is the dispersion of permethrin in the vicinity of spray applications when measured with the FLB impactor and the area air sampler?

CHAPTER 2: Literature Review

2.1 INTRODUCTION

There have been many studies on the safety and effectiveness of permethrin. One review by the World Health Organization (2015) found pyrethroids characterized by moderate acute toxicity and does not show any evidence of long-term toxicity in humans (133). As such, service members have spent a majority of their careers wearing uniforms that have been treated with permethrin during manufacture and/or applied to uniforms in the field. Until recently, it has been assumed that since the Working Party is expected to have minimal contact with freshly treated uniforms while laying out uniforms and flipping them over, they do not have any significant exposure during these tasks. Understanding the potential permethrin exposures to personnel performing a field spray treatment of military uniforms using 40% permethrin is an essential step in ensuring the safety of our service members as research into new products continues, and consideration into the authorized use of permethrin transforms.

2.2 PYRETHRUM

Using plants as an insecticide was first recognized as early as 1300 in Europe (73) and 1800 in Asia (1). **Pyrethrum** is a naturally occurring chemical found in the dried flower head of some chrysanthemum flowers that contains six active insecticidal chemicals called **pyrethrins** (1). Pyrethrins were first recognized in Europe during the 1820s but not introduced for insecticidal use in the US until 1876. The US military played an early part in employing pyrethrum by percolating the flower heads with kerosene to produce an extract that was effective for preventing insect bites. Though

useful for the time, pyrethrins proved not to be persistent in the environment, breaking down easily when exposed to sunlight and the atmosphere (1). The demand for pyrethrins during World War II was very high, and supply was insufficient, motivating a need to find a synthetic insecticide that could be manufactured (114). DDT was a common pesticide used during WWII because of its persistence in the environment, rendering its effects to be enduring. It was widely used on crops in the 1950s and 1960s until the US EPA no longer approved DDT for use in 1972 due to its environmental toxicity. Organophosphates followed as the most widely used insecticides. They are less persistent than DDT but highly toxic to non-target species. They are also readily stored in fat tissues, and accumulation can lead to neurotoxicity. Research and development of an effective pyrethroid continued despite the introduction of organochlorines, organophosphates, and carbamates, which today are known to be more environmentally toxic due to their biomagnification and high non-target organism toxicity.

2.3 PYRETHROIDS

Structural modifications to the chemical structures of pyrethroids gave birth to four generations of insecticides with progressively increased potency and stability to air and sunlight. The increased insecticidal activity is a result of the fit of an entire molecule at the site of action. Modifications were made through sequential replacement of the elements that make up pyrethroids. The replacement of structural elements within the compound needs to maintain the molecular shape of the compound to conserve the physical properties of the template structure. In 1949, a first-generation pyrethroid, allethrin, was the first commercially successful pyrethroid to be introduced. Resmethrin is a second-generation pyrethroid developed in 1967 that has increased photostability,

potent insecticidal properties, and lower acute mammalian toxicity (15). Permethrin is a third generation pyrethroid. It was discovered in 1973 as the first pyrethroid with adequate photostability for use in agriculture but was not registered for agricultural use until 1979 by the EPA (73). Third generation pyrethroids include products such as deltamethrin and permethrin and are the largest group of pyrethroids in current use. Despite the widespread use of permethrin, efforts to find a pesticide with even more effectiveness and reduced toxicity to non-target organisms gave way to a fourth generation (Table 2.1).

Etofenprox is fourth generation but considered a pseudo pyrethroid. Pseudo pyrethroids are non-ester pyrethroids with active insecticidal properties and low mammalian toxicity, but lower toxicity to aquatic organisms than permethrin. Traditional pyrethroids are broken down by hydrolysis. Pseudo pyrethroids are broken down by oxidation and are approximately 98% less toxic to aquatic organisms (114). Though mainly developed in the 1980s, pseudo pyrethroids are not widely used in the US. Etofenprox was registered for use on military uniforms in 2016 (34), and is currently being researched for use in the DoD.

PARENT CHEMICAL	INSECTICIDE	TYPE (I or II)	GENERATION	DISCOVERED	DESCRIPTION
Pyrethrum	Naturally occurring	---	---	1300	Found in dry flower heads Contain 6 active insecticide chemicals, pyrethrins
Pyrethrins	Naturally occurring	---	---	1820s	6 types: pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I, and jasmolin II
Pyrethroids	Synthetically derived	---	---	1940s	Synthetic insecticides with increased potency and stability in air and sunlight comparted to natural chemicals There are four generations
	Allethrin	I	First	1949	First commercially successful pyrethroid to be introduced
	Bifenthrin	I	Third	1985	Pyrethroid with the longest known residual time in soil

	Cypermethrin	II	Fourth	1974	Large scale commercial agriculture applications; easily degraded on soil, especially with sunlight, water, and oxygen exposure) Resistance is growing
	Deltamethrin	II	Fourth	Described-1974 Marketplace-1978	Used worldwide for malaria control efforts. Resistance is widespread
	Etofenprox	Non-ester pyrethroid	Fourth	1980	Pseudo-pyrethroid Broken down by oxidation Reduced toxic to aquatic organisms
	Fenvalarate	II	Third	1972-73	photostable
	Permethrin	I	Third	1973 (agric. Use-1979)	Photostable Reduced non-target species toxicity
	Resmethrin	I	Second	1967	Increased photostability with potent insecticidal properties; lower acute mammalian toxicity. 20 times as effective as pyrethrum
	Tetramethrin	I	Second	1965	

Table 2.1. Evolution of Pyrethroids

Synthetic pyrethroids are among the most effective insecticides available. There are 18 pyrethroids registered for use in the US (29), including (but not limited to) permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, cyfluthrin, and fenvalerate (12). Approximately 100,000 to 200,000 pounds of pyrethroids are used annually in the US for non-crop applications such as clothing treatment and household insecticides, with an additional 5,000 to 10,000 pounds used in agriculture (31).

Regardless of the generation, pyrethroids are divided into type I and type II categories based on the presence of an alpha-cyano group, their actions on the body, and the production of undesirable symptoms (23). Actions in the body are different between type I and II pyrethroids, but the mechanisms of toxicity between them are not of clinical value (17).

2.3.1 Type I Pyrethroids

Type I pyrethroids lack the alpha-cyano group in their structure. Sometimes referred to as "T" or T-syndrome, type I pyrethroids may cause hyperexcitability and whole-body tremors, especially in insects (1). Permethrin is a type I pyrethroid; it acts by interfering with brain functions and with sodium channels of the sensory nerves of mammals and insects. In insects, the voltage-gated sodium channels are disrupted, delaying their inactivation, leading to premature opening and increased impulses, causing tremors, paralysis, and death. As a generalization, type I pyrethroids modify sodium channels in the closed state. However, *cis*- permethrin interacts with sodium channels in both open and closed phases and binds with a stronger affinity to the open state. These interactions may account for the higher toxicity of the *cis*- isomer over the *trans*- isomer (discussed in section 2.3.2). In humans, permethrin disrupts sodium channels but does not have the same effect as on insects because of human's larger size, higher body temperature, and less sensitive channels (23).

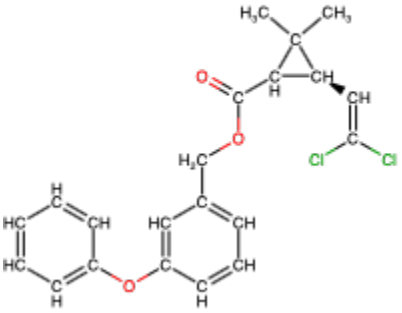


Figure 2.1. Permethrin (22), a Type I pyrethroid, lacking the alpha-cyano group

2.3.2 Type II Pyrethroids

Type II pyrethroids are synthetic derivatives of naturally occurring pyrethrins with further enhancement of type I pyrethroids, by the addition of a cyano group. The

properties of type II pyrethroids cause the prolonged opening of sodium channels. They are referred to as CS-syndrome because they tend to elicit choreoathetosis (twisting and writhing) and salivation (1). In addition to disrupting the sodium channels, type II pyrethroids can also disrupt chloride channels (47). The disruption in these channels explains symptoms of type II poisoning, such as salivation and seizures.

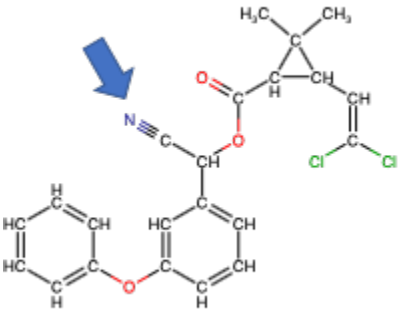


Figure 2.2. Cypermethrin (22), a Type II pyrethroid, containing the alpha-cyano group

2.4 TOXICOKINETICS

In animal studies, permethrin is rapidly absorbed, distributed, and excreted within 12 days (33). When sampling for permethrin exposures, the toxicokinetics must be understood to effectively capture the excretion of metabolites, based on multiple routes of exposure and individual variability. This study examines dermal exposures, the dispersion of permethrin in the air, and permethrin metabolites in urine. Collection of biomonitoring samples before spraying starts, at 4-hours, and 12-24-hours after spraying ends to determine the overall absorbed and metabolized permethrin by each individual.

The toxicity of permethrin is complicated because of inter- and intra-individual variances and the function of dose, duration, and site of exposure. Toxicity to mammals

is thought to be minimal, perhaps due to poor dermal absorption and rapid metabolism of permethrin into non-toxic metabolites that are quickly eliminated from the body (17). The US EPA designated permethrin to have low toxicity for acute exposures for all three exposure routes (33).

2.4.1 Absorption

Inhalation exposure to permethrin results in the absorption of the pesticide through the respiratory tract and passively diffused into the bloodstream. There is insufficient data to indicate the amount of the dose absorbed following brief or prolonged inhalation exposures. Oral exposure results in immediate absorption of permethrin in the gastrointestinal tract as the route of entry and metabolic site (68). In a study of oral exposure to permethrin in the rat, 60% was absorbed with a demonstrated half-life of less than one-hour (92). When applied to the skin for the treatment of lice or scabies, it is estimated that only a small fraction (about 0.5%) of the applied dose is absorbed into the body (41; 119). Brand *et al*. suggests that dermal exposure is a primary site for xenobiotic absorption, particularly agricultural chemicals, such as permethrin (18). However, results from Kuhn *et al*. suggest that oral and inhalation are the major sources of absorption following exposure (75). There is no consensus on which route of exposure presents the most significant absorbance.

2.4.2 Distribution and Metabolism

Regardless of the exposure route, once absorbed into the body, permethrin is rapidly distributed with minimal accumulation in the brain and fat tissue. Permethrin is thought to have low toxicity to mammals, which is mainly attributed to the rapid

metabolism from the parent compound to metabolites (78). Permethrin is metabolized rapidly in the body through hydrolysis, esterification, oxidation, and conjugation. There are several products formed during these processes, including three metabolites with relative importance for this study: *cis*-DCCA, *trans*-DCCA, and 3- PBA (23). In many pyrethroid studies, the urinary metabolites *cis*-DCCA, *trans*- DCCA, and 3-PBA are measured in urine to determine the absorption of pesticides into the body following. Permethrin is enzymatically transformed into a very general metabolite, 3-PBA, and two less general metabolites *cis*- and *trans*- DCCA (117). Metabolites of permethrin are also metabolites of many other pyrethroids. 3-PBA is the most prevalent metabolite among pyrethroids. It is a metabolite of ten of the 18 pyrethroids registered in the US (29), including cyhalothrin, cypermethrin, deltamethrin, and fenvalerate. *Cis*-DCCA and *trans*-DCCA are somewhat more specific, but are metabolites of cyfluthrin and cypermethrin (117). C*is*- and *trans*-DCCA are the metabolites used for biomonitoring in this current study. The metabolites of permethrin do not have higher acute toxicity than the parent compound (33), and the rapid metabolism of permethrin into metabolites promotes low mammalian toxicity.

2.4.3 Excretion

After metabolism into *cis*-DCCA, *trans*-DCCA, and 3- PBA, all are excreted primarily in urine, with trace amounts in feces (46). Despite the lipophilicity of permethrin, it is quickly eliminated from the body because of colloidal water dispersion, which allows rapid metabolism and elimination mainly through urine.

First-order elimination of 97-99% of a synthetic compound is considered complete elimination and occurs during five to seven half-lives (75). The half-life of

permethrin in the body is 12.3 hours (92). When temporarily stored in the brain and fat tissues due to its lipophilic nature, the half-life of permethrin is between 9 to 23 hours. Using the studies mentioned previously, permethrin is expected to be eliminated from the body within seven days.

Accumulation in fatty tissues after repeated or prolonged exposure is rapidly eliminated after exposure ceases (46). Following dermal exposures, metabolites are almost entirely excreted from the body within 72 hours after exposure ceases (118). In a case study on the ingestion of permethrin in a suicide attempt, the serum output in urine peaked at 3-4 hours after consumption (54).

2.4.4 Interferences

Based on the characteristics of agricultural chemicals and pyrethroids, other factors may increase the transdermal penetration of permethrin. Such penetration enhancers include the consumption of alcohol and the topical application of sunscreen (18). An association was made between smoking and higher urinary metabolite levels (71) which may be attributed to hand-mouth ingestion.

Permethrin toxicity may become more complicated when amalgamated with PBOs or when potential exposures to organophosphates are added to the exposure scenario (13; 17). Ingestion of just a single dose of ibuprofen may interfere, and even mask, detection of the metabolite 3-PBA for up to 48 hours because the metabolites of ibuprofen have identical reference spectra as 3-PBA on a GC-MS (gas chromatography-mass spectrometry) (59). Since the collection of urine for this study will occur at 4 hours and 12-24 hours post permethrin application, ibuprofen has the potential to mask 3-PBA for the entire sample collection period.

2.5 PERMETHRIN

Approved for a variety of uses, including over the counter lotions for topical treatment of scabies and lice, clothing treatment to repel and kill mosquitoes and ticks, and environmental spraying for mosquito abatement and crop protection, permethrin is a widely used pesticide worldwide. It is becoming more widely used because of its low toxicity to mammals. Permethrin is lipophilic and has low water solubility, low volatility, and a high octanol-water coefficient. The molecular formula, $C_{21}H_{20}C_{l2}O_3$, is a racemic mixture of the *cis-* and *trans-* isomers with a molecular weight of 391.3 grams. Permethrin, 3-phenoxybenzyl-(+ or -)-cis, trans-3-(2,2 dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (CAS Number: 52645-53-1) is a synthetic pyrethroid and is the most frequently used pyrethroid in the US (1).

2.5.1 Isomers of Permethrin

Pyrethroids have a complex chemical structure that often includes two, four, or eight isomers, which can attribute to differences in toxicities between products of the same compound (1). Permethrin is composed of four stereoisomers that consist of a blend of two *cis-* and two *trans*-isomers. The *cis: trans* ratio within a compound is a determinant of how the compound acts in the body. Isomers are identical molecules with the same atoms arranged in the same order, with a slightly different structural orientation at the cyclopropane moiety. Technical grade permethrin available in the DoD supply ordering system (NSN: 6840-01-334-2666) consists of 40% permethrin with a *cis: trans* ratio of *25:75*.

The *25:75* (*cis: trans*) permethrin isomer ratio is most commonly used to protect against military insect pests and for population pest control applications (46). The *trans-*

isomer of permethrin is more readily metabolized and less toxic to mammals, so *25:75* is typical in products used around humans. Agricultural products have higher *cis-* to *trans-* ratios, which resists biological and chemical degradation and is more toxic to target pests and mammalian hosts and metabolized more slowly than *trans-* isomer (115).

2.5.2 Formulation of Permethrin

Permethrin is a nonpolar substance that has low solubility in water (0.006 mg/liter at 20°C) (91). Manufactured with many *cis: trans* ratios, technical grade permethrin is generally *25: 75* or *40: 60* and mixed with inert ingredients. Identification of inert ingredients is not required according to law (1). The inactive ingredients may contain a surfactant to allow proper dilution of the concentrate.

The formulation discussed throughout this paper and used during data collection is a commercially available product commonly found in an emulsifiable concentrate with a surfactant (134). The surfactant helps to stabilize the dispersion of the water-insoluble permethrin, and the formulation allows for spontaneous emulsion when mixed with water in a spray tank.

2.6 Health Effects

Pyrethroids encompass a long list of synthetic chemicals that have insecticidal ingredients developed for their enhanced capabilities. Toxic effects of pyrethroids can range in a full spectrum of signs and symptoms from no effect to major effects, defined by the American Association of Poison Control Center (88). Mild pyrethroid toxicity, which is marked by some minimally bothersome and quickly resolved signs or symptoms, including nausea, headaches, vomiting, dizziness, fatigue, and paresthesia. Moderate toxicity is defined by more prolonged signs or symptoms that usually result in

some form of treatment, may include CNS depression, fever, increased salivation, and blurred vision. Pyrethroid exposure may lead to major effects, marked by life-threatening signs or symptoms as a result of exposure, which lead to disability or disfigurement, and even death. The American Association of Poison Control Centers reported 69,072 pyrethroid exposures from 2015-17, but only three deaths (55; 56; 89). Of the cases, 26,327 experienced no or minor effects, whereas 2,409 experienced moderate to major effects following exposure to pyrethroids. As a broad group, health effects of pyrethroids include paresthesia and local skin irritation (dermal exposures), nasal and respiratory irritation (inhalation exposure), sore throat, nausea, vomiting, and abdominal pain (following ingestion).

In animal studies, there is a positive association between pyrethroids and a gamut of system toxicities, mainly when exposure is in very high doses or to young children. A study of children under six years old suggests that environmental doses of permethrin, at levels that could be encountered by the general public, negatively affected neurobehavioral development (123). In other studies, there were associations with attention deficit hyperactivity disorder (ADHD) and autism to pyrethroid exposures (126). Viel *et al*. show the detection of pyrethroids in human cord plasma, indicating compounds can cross the placental barrier (123). Potential maternal-fetal transmission of such exposures when pregnant or when trying to conceive may have public health implications for the general public.

Military service members are at least 18 years old, so childhood and adolescent-specific exposures are not a significant concern in a military population. However, military members are in reproductive ages for most of their military careers, making

potential effects of prenatal and reproductive exposure to permethrin relevant. There are links between pyrethroid exposures and adverse male reproductive effects, such as decreased semen volume, sperm concentration, and motility through measuring for the metabolite 3-PBA (70; 136). Further discussion on reproductive effects are in section 2.4.1.

Epidemiological studies suggest a negligible risk to humans and non-target organisms when used according to the label and as outlined in the reregistration eligibility decision (RED) (33; 86). However, the route of exposure, dose, and duration of the exposure influences the potential health effects. Most documented cases of health effects caused by exposure to permethrin were reversible (112), and most patients recovered entirely within six days after exposure ceased (17).

Though not well documented, there are a handful of studies on occupational exposures to permethrin. Some suggest adverse respiratory health effects following occupational exposure (64; 135). A study that investigated the impact of permethrin via factory treated uniforms to the U.S. Army indicates a higher prevalence of urinary metabolites in workers when compared with that of the general US population. This study had a relatively small sample size signifying a need for further studies, but it does suggest that dermal exposure to permethrin via clothing worn for extended periods or for consecutive days results in higher exposures (104).

Permethrin is very lipophilic yet rapidly metabolized and excreted from mammals. Because it does not readily accumulate in the body, permethrin is an effective insecticide that is thought to pose relatively little health risk to mammals. Even with rapid mammalian detoxification, permethrin readily distributes to the nervous system.

Neurologic symptoms are most common due to acute exposures but are uncommon in mammals because of the rapid metabolism and elimination. Symptoms include skin irritation, rash, itchiness, redness, and numbness following dermal exposure; nose, throat, and lung irritation following inhalation exposure; and headaches, dizziness, fatigue, excess salivation, muscle weakness, nausea, and vomiting following oral exposure (93).

2.6.1 Reproductive Effects

Reproductive age for the general population is typically between 15-44 years. In the military, ages from 18 to 41 are an accurate snapshot of the window that includes both service and reproductive years. Military service may result in exposure to permethrin via similar routes as experienced by the general public (direct and indirect ingestion, inhalation, dermal), but military service adds additional potential for exposure. Service members may wear uniforms treated with permethrin or may be asked to participate in the spraying of uniforms. Also, indoor and outdoor pest control may take place where the military member works. These potential exposures, as well as the effects on reproduction hormones, are essential for understanding the overall potential risk faced by military members.

Several epidemiological studies have investigated the effects of pyrethroid exposure on reproductive hormones in humans (112) (57) (102) (84). There was a weak association demonstrated between 3-PBA levels in urine and reproductive toxic effects on a group of non-occupationally exposed men in China (57). The pilot study conducted by Perry suggests an association between pyrethroid exposures and reduced semen concentration but warrants further investigation due to the small sample size of the pilot study.

Studies investigating pyrethroid exposures during pregnancy considered permethrin in the air from general use pesticide application, topical application for treatment of head lice, and occupational exposure by women with farming jobs (53). Respiratory and immune system health effects were mostly found not to have a significant association with pyrethroid exposure. A retrospective assessment of occupational pesticide use by pregnant women found a significant association between pyrethroid exposure and decreased birth weights (58). Birth weights were the only outcome analyzed, so further studies are needed to confirm the absence of other confounders or trends. In one study, there was no observation of consistent associations between exposure and maternal prenatal urinary pyrethroid metabolite concentrations (124). Lack of association can be because of the rapid metabolism and excretion of permethrin from the body. However, there is an indication of bioaccumulation of pyrethroids in the human body, based on the presence in breastmilk (124). The potential daily intake of permethrin by a breastfed infant remains below the ADI, indicating no appreciable risk. Also found was an association between childhood exposures to pyrethroids and reduced neurocognitive abilities (124).

2.6.2 Carcinogenicity

Scrutiny over pyrethroid use and safety is ongoing. The 2009 US EPA Reregistration of permethrin remains conditional due to the need for additional toxicological data to fully evaluate the carcinogenicity. Permethrin is thought to be a weak tumor-former or oncogene in mice and rats (33), but it is difficult to identify a specific dose where carcinogenic effects may occur in humans because of limited human data.

There have been recent studies that suggest associations between permethrin exposures with some cancers (3; 111). In 1991, the International Agency for Research on Cancer (IARC) classified permethrin as a Group 3, not classifiable as to its carcinogenicity to humans (67). Based on benign lung and liver tumors in mice studies, in 1994, the EPA classified the ingestion of permethrin as "likely to be carcinogenic to humans," though human data were not found (92).

2.7 Routes of Exposure

Exposure to permethrin can occur from the oral, inhalation, and dermal routes of entry. It is important to not only consider multiple routes of entry, rather than single routes, for potential interactions, such as synergistic effects. According to INRS (Institut National de la Researche Scientifique), 2010 (105), the general population is exposed to permethrin mainly via ingestion, but populations with occupational exposure allow for inhalation and dermal contact routes of entry.

2.7.1 Inhalation

The EPA considers inhalation toxicity a data gap, as there is insufficient data for acute and chronic exposures (33). Some studies show inhalation as a primary exposure route for permethrin (80), where it passively diffuses in the alveolar region of the respiratory tract and is absorbed into the bloodstream. Health effects may include headaches, nasal and respiratory irritation, difficulty breathing, dizziness, nausea, and vomiting (17).

Permethrin has a low vapor pressure, 2.15 x 10-8 (120), which makes aerosol spray droplets, and dust more of an inhalation risk than vapors. The hazards produced by

aerosolized pesticides vary with time and distance from the spray source. The highest levels are expected to be near the uniform spraying, with most of the permethrin settling or dissipating within five minutes after spray ceases. Though it is expected that potential inhalation exposure to the Working Party is less than that of the Applicator, it is important to assess several group's exposures. Specifically, the Working Party's personal protective equipment requirements may differ from Applicators which would change the exposure.

There is limited data on human permethrin inhalation exposures, so information from animal studies provide some indications of exposure risks. In a study measuring metabolites in urine of rats subject to inhalation of four concentrations of aerosolized permethrin 6 hours per day, 5 days a week, for 13 weeks, metabolism excretion peaked between 3 and 4 days (51). The excretion timeline demonstrates that permethrin is quickly metabolized and excreted. Following an acute 4-hour inhalation exposure of rats to permethrin, the lethal concentration to 50% of the rats (LC_{50}) was approximately 23.5 mg/liter of air (37). This concentration is extremely low, considering the lowest relevant NOAEL for inhalation of permethrin is 0.25 mg/liter based on tremors and convulsions in rats (46).

Discussion of biological monitoring with urine following exposure to permethrin is in section 2.17, but it is relevant to mention that analysis of permethrin metabolites in urine show inhalation and oral intake have similar isomeric ratio outputs. Therefore, it is often difficult to discern a sole or individual route of exposure.

2.7.2 Ingestion

The low volatility of permethrin results in it settling on surfaces and being absorbed in dust and other airborne particles. Ingestion can occur by intentional or accidental direct ingestion, or through indirect ingestion through food or hand-to-mouth movements. Ingestion of permethrin from residue on food or contaminated house dust is thought to be the most common route of exposure to the general population (84). Following oral exposure to permethrin, it is slowly and only partially absorbed into the body. Based on the limited human studies, ingestion of permethrin may cause sore throat, nausea, vomiting, and abdominal pain, with most symptoms occurring several hours after exposure (92).

Chronic oral exposures to permethrin show increased liver weights and neurologic effects in animal studies (92). An acute and chronic reference dose (RfD) of 0.25 mg/kg per day for ingestion of permethrin was established based on animal studies (33). At this dose, a 70 kg adult can ingest 17.5 mg of permethrin every day for the rest of their life without expecting adverse health effects. An estimated oral lethal dose of permethrin is 1-2 g/kg (119). Acute ingestion lethal dose (LD_{50}) of technical grade permethrin is dependent on the age, sex, and isomeric ratio. The US EPA established the LD_{50} in rats at 2,280-3,580 mg/kg and considers permethrin to present a low risk of toxicity (92).

Although rare, there are a few cases of human ingestion of permethrin. In one case study, a 59-year old male ingested 20% permethrin with 43.5:56.5 (*cis: trans*) in a suicide attempt (54). In this case, medical care and observations commenced approximately 40 minutes after ingestion. Unconsciousness (though other central nervous

system (CNS) neurotoxicity was not seen), vomiting, diarrhea, cutaneous paresthesia (in the mouth for three months following the incident), and increased creatine and blood urea nitrogen levels were observed. Diarrhea ceased after 9 hours, and full cognizance regained after seventeen hours. The emulsion also had xylene and surfactant, which may have caused the loss of consciousness and paresthesia. At the time of the study, the standards for the metabolites of permethrin were not available, so only the parent compound was used for determining the blood level kinetics of permethrin after ingestion. With assumptions about specific gravities of the emulsion constituents, the patient in this study ingested over 2,000 mg/kg body weight (approximately 143g) of permethrin, which was near the LD_{50} of 3,080 mg/kg for male rats identified by the manufacturer of 20% permethrin (54). Blood measurements of permethrin peaked at 3-4 hours post exposure, and trans- isomer was metabolized more quickly, supporting data found in animal studies (105).

In a retrospective review of clinical records from 48 permethrin emulsion poisoning cases in Taiwan, characterization of toxic manifestations from permethrin versus other components in the emulsion (xylene and surfactant) proved difficult. However, signs and symptoms were primarily associated with the gastrointestinal tract and secondarily the CNS and lungs (134). As with the first case, there was a fair amount of uncertainty in dose because the quantity lost due to vomiting is unknown. However, in the known human cases of permethrin poisoning, CNS was not a primary symptom that manifested, rather; gastrointestinal (GI) symptoms were more common (134).

2.7.3 Dermal Absorption

The rate of dermal absorption remains in debate with studies referencing dermal absorption as low as 1% (108) to 3% (44) and as high as 15% (33). The EPA uses 5.7% for a dermal absorption factor in the cancer equation based on *in vitro* and *in vivo* data reviewed during the 2009 RED (33). The ATSDR recognizes that less than 2% of the applied dose is absorbed into the body (2). Though acute toxicity from dermal exposure is thought to be lower than from other routes (83), direct contact with permethrin may cause localized symptoms, such as tingling, burning, stinging, numbness and itching, and may lead to sensitization. Symptom onset is typically directly after contact, up to two hours (69), with symptoms seldom lasting more than 24 hours (92).

Chemical absorption through the skin may depend on variables such as species, age, gender, race, anatomical site, skin condition, temperature, and hydration levels (32). These variables affect the absorbance of permethrin through the skin and therefore make it difficult to predict how much of a contaminant penetrates the skin barrier. Dermal exposure is influenced by the amount of chemical present on the skin, or available to be absorbed. The contaminant concentration, duration and frequency of exposure, and the ability of the contaminant to be absorbed through the skin, contribute to the likelihood of a person experiencing these symptoms. Dermal exposures in humans may lead to a burning sensation, itching, or tingling of the skin (61). However, absorption of permethrin through human skin is thought to be lower than animals and is thought to have low toxicity (119).

In an *in vitro* study investigating removal of *cis-* permethrin in rat and human cadaver skin, dermal absorption of permethrin was demonstrated to be weak, with at least

50% of the dose removed through skin washing and 50% of the remaining dose removed by tape-stripping of the epidermal surface (65). While the Hughes, 2010 investigation only studied the *cis-* isomer, a study by Franz (1996) and Baynes (2002) did not find a difference between dermal absorption characteristics of *cis-* and *trans-* isomers (13; 48).

Two studies examined potential human dermal exposure using passive sampling, one after truck-mounted ULV technical-grade permethrin application (103), and the second using ULV application of malathion (86). These studies showed dermal exposures to bystanders of ULV spray were well below the dermal NOAEL of 500mg/kg/day. Though ULV application utilizes smaller droplet sizes, permethrin spray during military uniform application is expected to resemble a similar model in the potential for dermal exposures.

Wearing clothing impregnated with permethrin, especially when worn for many hours or for consecutive days, has the potential for dermal exposure. In a controlled trial of 187 volunteers and permethrin-impregnated battle dress uniforms, the maximum permethrin uptake was well below the ADI (109). Though when compared to the urine metabolites of the general population, two US military groups had noticeably higher concentrations (82).

2.8 Toxicity Classification

Permethrin is considered by the EPA to be low in toxicity for acute oral and dermal exposures, and they recognize a data gap in inhalation toxicity. The oral toxicity classification is based on an LD_{50} range of 2,280-3580 mg/kg in rats (46) and a dermal LD_{50} for rabbits of over 2,000 mg/kg (33). For use in inhalation toxicity for the re-

registration decision in 2009, one study found an LC_{50} in rats greater than 23.5 mg/L following a 4-hour exposure (33).

The ADI for permethrin, established in 1987 by the Joint Meeting on Pesticide Residues (JMPR) administered by the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), is 0.05 mg/kg. The ADI for permethrin is relatively high when compared with other pyrethroids (cypermethrin: 0.02 mg/kg and deltamethrin: 0.01 mg/kg). The Agency for Toxic Substances and Disease Registry (ATSDR) estimates the average adult's daily intake of permethrin is approximately 1,000 times lower than the ADI established by the WHO (1).

Undiluted technical-grade permethrin has low acute toxicity. The WHO identified it as class II, moderately hazardous, according to the proposed hazard classification by the twenty-eighth World Health Assembly (131). A RfD for the general population for acute and chronic exposures is established at 0.25 mg/kg per day (33). The ATSDR established an acute oral exposure minimum risk level (MRL) to technical grade permethrin of 0.3 mg/kg per day and 0.2 mg/kg per day for exposure durations up to 365 days (92).

2.8.1 Occupational Exposure Limits (OEL)

There is no specific occupational exposure limit (OEL) established for permethrin; however, the Occupational Safety and Health Administration's (OSHA's) permissible exposure limit (PEL) for pyrethrum is 5 mg per cubic meter (mg/m^3) as a time weighted average for an 8-hour workday and 40-hour workweek (20). The 5 mg/m^3limit is recommended by National Institute for Occupational Safety and Health

(NIOSH) as their recommended exposure limit (REL) and by American Conference of Governmental Industrial Hygienists (ACGIH) 2019 threshold limit value (TLV) (4). The 5 mg/m^3 TLV-TWA of pyrethrum was established in 1962 and remains relevant today. The regulatory limit was based on studies that showed dermatitis and sensitization of the skin and respiratory tract following ingestion, inhalation and contact with pyrethrum. There are limited human studies, so extrapolation from animal studies and reliance on comparison of other pesticides is beneficial to understand what is considered acceptable versus unacceptable, and at what levels we may expect individuals to exhibit signs of exposure.

Pyrethrum has been used for its insecticidal properties far longer than any pyrethroid but is far more susceptible to sunlight degradation and is oxidized more readily in the air. Though it is helpful to know the established pyrethrum limits, it is vital to remember that permethrin was synthetically derived from pyrethrum and designed to have higher toxicity and longer toxic persistence.

2.9 Environmental Fate of Permethrin

Permethrin has low water solubility and strong adsorption to soil, making it low risk to environmental transport and widespread contamination. When using permethrin to treat uniforms, the ground surface does not need to be an extensive consideration. However, knowing if there is surface water in the surrounding area where runoff could go is essential. Permethrin is highly toxic to fish and aquatic life and should not be used near water. Though, it does find its way to surface water sources through runoff from

agricultural fields and rainfall. When it finds its way to water systems, most binds to sediment and suspended solids.

Permethrin forms strong bonds with soil. Once in the soil, some permethrin will degrade by sunlight, but the majority can persist in soil for up to a year (92). Permethrin is not efficiently uptaken by roots and is not mobile in the ground, which is a useful mechanism of protection from groundwater contamination (1). Compared to its predecessors, such as organophosphates, pyrethroids degrade faster in soil with a minimum and maximum half-life between 11.6 and 113 days , but, in most cases, a half-life of 4 weeks or less (115). Pyrethroids are readily broken down in soil by microorganisms and sunlight and has not been shown to be poisonous to plants.

During the spray application of permethrin, many aerosol and spray droplets do not reach the target. The vapor pressure of permethrin is low enough at 25^0 C that evaporation is not a key concern. Though potential drift of pesticide in the form of aerosols, droplets, or with air particulates around the spray area is possible.

Low vapor pressure keeps permethrin out of the gaseous phase and therefore reduces transport in the air. However, some drift will still be present. The extent of drift is dependent on the sprayer type and application techniques employed by the Applicator, weather conditions such as wind speed, temperature, atmospheric pressure, and humidity, as well as the skill of the Applicator.

Review of the sampling collection height in several studies that measured spray drift of pesticides revealed a range between 1.5 to almost 5 feet from the ground (40) (16) (45). These studies measured the presence of pesticides in the air following a variety of

application methods, including ultra-low volume (ULV) spraying. Due to the low vapor pressure and the downward pointed spray wand during uniform treatment, permethrin is not expected to be readily lost to the atmosphere through vaporization. Therefore, the collection height for aerosol and spray droplets during this study should be at three feet from the ground or lower, to capture the exposure zone where workers bend over and breath.

2.10 Effects on Non-target Species

Though permethrin is largely considered safe for mammals, it is toxic to cats, honeybees, and aquatic organisms. Permethrin has a high octanol/water partition coefficient (log K_{ow} = 2.88), suggesting biomagnification in aquatic organisms (32). Because of this toxicity, it is vital to be aware of the surrounding area when spray treating uniforms with permethrin. Surface water should not be nearby where permethrin has the potential to contaminate the water through runoff.

2.11 Permethrin in the Military

Arthropod-borne diseases cost millions of people-hours during armed conflict, due-to illness and death, and have debilitating effects. Diseases such as malaria, leishmaniasis, and dengue fever can adversely affect unit performance and cause mission failure. U.S. Troops are an immunologically naïve population when they move into a country for contingency operations where diseases and vectors are present. Protection against the chain of infection for arthropod-borne diseases is critical, and pest management practices are essential to mission accomplishment. Coinciding with the EPA registering permethrin for agricultural use in 1979, in collaboration with the U.S.

Department of Agriculture (USDA), the DoD began evaluating the use of permethrin for clothing treatments. Application to field uniforms was initially evaluated in 1984 and approved for factory treatment of uniforms in 1990. Permethrin treatment is now common for many uniforms across the DoD (Figure 2.1).

2.11.1 FIFRA

In compliance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), registration of a pesticide is required by law for use in the US. The Food Quality Protection Act (FQPA) was enacted in 1996 and specified additional tolerances for permethrin on food and feed commodities, requiring review once every 15 years (35).

Permethrin was first registered in 1979 and underwent a review of ecological effects in 1985. It underwent a review of residue chemistry, environmental fate, and toxicological data in 1988, a series of agriculture, residential, and mosquito ULV product assessments between 1994 to 2000. The most recent reregistration process occurred in 2006, with 2007 revisions based on public comments and activities that occurred after publishing. A revised occupational and residential exposure risk assessment was published in 2009 due to reconsiderations of the dermal absorption factor risk mitigation strategies.

The registration process includes evaluation of the pesticide for human health risks to the population through all sources (considering cumulative risks that may result from the use of many products), including food, water, residential uses, and occupational risks (33). Evaluation of environmental risks captures a dynamic awareness of all

exposure routes, incorporating groundwater contamination, risks to endangered or threatened species, and effects to other possible endocrine disruptors.

2.11.2 Overseas Environmental Baseline Guidance (OEBGD)

The EPA is not applicable in all overseas US military environments. The Overseas Environmental Baseline Guidance Document (OEBGD) serves as a guideline to environmental compliance for military populations (26). Based on the OEBGD, the use of pesticides for clothing applications falls into an exempt category that does not need to be recorded and maintained as part of the pest management maintenance report. However, Applicators are required to be certified pesticide applicators.

2.11.3 DoD Repellent System

The DoD Insect Repellent System is designed for maximum protection against disease-carrying insects to enhance force health protection and readiness. There are three personal components of the system: (a) proper wear of permethrin-treated uniforms, which means that the uniform is not worn too tightly where vectors may bite through the material and that the t-shirt is tucked into the pants, and the pants are tucked into the boots, and the sleeves should be buttoned to form a close fit around the wrist; (b) application of skin repellents with 20-30% DEET (or Picardin or IR3535) to exposed skin; and, (c) use of permethrin-treated bed nets. For maximum protection, personnel should use all elements of the system (7). Several uniform treatment methods are approved by the DoD and include factory treatment, spray application methods, and individual application methods.

2.12 PERMETHRIN IN UNIFORMS

Permethrin in uniforms serves as a contact toxicant against crawling arthropods and a contact repellent against flying insects. Ticks and mites crawling across the uniform will drop off before they can attach and feed, and flying insects that land on the uniform will quickly fall from the fabric and often die from exposure to the pesticide. However, wear of the uniform does not protect exposed skin, so skin repellents with 20-30% DEET (or products with more recently approved active ingredients: Picardin and IR3535) is necessary as additional protection. Permethrin in uniforms does not deter homogeneously across all arthropods, and there is reduced insecticidal capacity after washing. However, the remaining permethrin inhibits arthropod biting sufficiently to protect service members. Permethrin is used to treat uniforms from each branch of service within the DoD via a spray method or factory treatment.

2.12.1 Types of Uniforms

There are many U.S. military uniforms within the DoD. Uniforms typically fall into one of three categories: combat uniforms, service uniforms, and dress uniforms. Combat uniforms are worn while deployed, in the field, or as the working uniform. Even within combat uniforms, there are many variations and changes are frequent. The scope of this study is not to identify every variety of uniform worn across the ranks. However, it is essential to recognize that within each service, uniforms are made of different materials, and may have different treatments applied to them that offer different magnitudes of protection. Some uniforms may have flame retardant properties, while others may allow for protection against arthropod vectors. It is important to understand

the benefits and limitations of each uniform. The AFPMB developed a matrix that shows uniforms for the different services and their compatibility with permethrin (Figure 2.3).

Uniform Permethrin Treatment Matrix 12/4/2019

Uniform	Field Method			Factory	
	Aerosol	IDA	2-Gallon	InsectShield	Warmkraft
Marines					
Marine Corps Combat Utility Uniform (MCCUU)					
Marine Corps Tropical Combat Uniform (MCTCU)					
Flame Retardant Combat Ensemble (FRCE)					
Enhanced FRCE					
Nomex					
Navy					
Navy Working Uniform (NWU II) (desert)					
Navy Working Uniform (NWU III) (green)					
Nomex					
Blue Coveralls					
Improved Flame Resistant Variant Coverall (IFRV)					
Air Force					
Air Force Operational Camoflage Pattern (OCP)					
ABU (Airman Battle Uniform)					
RABU (Ripstop ABU)					
Nomex Flight Suits					
FR ABE (Airman Battle Ensemble)					
Army					
Operational Camoflage Pattern Army Combat Uniform (OCP-ACU)					
Improved Hot Weather Combat Uniform (IHWCU)					
Fire Retardant ACU (FR ACU)					
Army Combat Pant (ACP)					
Army Combat Shirt (ACS)					
Army Aircrew Combat Uniform (A2CU)					
Nomex					
Joint Special Operations Capable					

Grey - Not Evaluated
Amber - Under Evaluation
Red- NOT Approved
Green - Approved for Treatment

Approved for Treatment means that the garment treated by the company has been tested to ensure that the garment is in compliance with the EPA labeled initial treatment concentration of 0.52% w/w. Second, the factory treated garments are then laundered IAW AATCC Method #135-204 and subsequent samples are taken at established time points based on the Service and garment. These samples are then tested for permethrin residual in the fabric and also sent to the USDA CMAVE laboratories for bite protection testing. In order to ensure that the garment is in compliance with the EPA labeled initial treatment concentration EPA label-required permethrin concentration in the garment and also provide the required % of bite protection at established time points.
For example, in the case of a factory treated ACU, a garment must have 0.52% w/w +/- 10% permethrin in the fabric after initial treatment prior to laundering. The garment must also provide 90% bite protection after 50 launderings. Failure to meet either or both of these criteria means the garment is NOT APPROVED for treatment by that method or company.

The 6 oz aerosol spray may be applied to uniforms not previously treated by the factory, IDA or 2 Gallon Sprayer.
May not be applied to uniforms containing Flame Retardant.
Impact on infrared (IR) not known, so may not be suitable for combat or hostile environments.

Figure 2.3. Uniform Treatment Matrix (8)

As of February 2020, Soldiers wear the Operational Camouflage Pattern (OCP) uniform, which is standard with factory treatment of permethrin. Though permethrin is standard for the OCP, there are special considerations for uniforms without permethrin, such as the maternity uniforms which are not factory treated with permethrin. Sailors wear the Navy Working Uniform (NWU) that has two variants. The type II desert variant is authorized for Naval Special Warfare units in desert environments, and the type III woodland variant is the standard ashore working uniform. The Navy also has unique uniforms designed for specific situations. Some of these uniforms include Naval aviation uniform and the shipboard working uniform. Marines wear the Marine Corps Combat Utility Uniform (MCCUU) and a Flame-Retardant Combat Ensemble (FRCE). They have additional uniforms that are directed by the job or task. The Air Force wears the Airman Battle Uniform (ABU), but they also have specialty uniforms for specific situations, such as Nomex flight suits.

2.12.2 Treatment with Permethrin

There are several methods of permethrin treatment of fabric or material, each having different training and personnel requirements. Available methods include aerosol spray, individual dynamic absorption kit (IDA), and a concentrated spray. Fabrics can be sprayed with permethrin using a variety of different formulations. Spray application relies on the insecticide absorbing onto the surface of the material. There is potential for uneven distribution of spray to uniforms, as well as limited wash resistance due to the

lack of bonding of permethrin to fabric (71), which may also allow higher dermal uptake by the wearer. The amount of permethrin is unvarying per uniform, without consideration to the size of the uniform. For example, one 5.1-ounce bottle of concentrated permethrin should be used to treat eight complete uniforms, front and back, and one IDA kit is used per uniform.

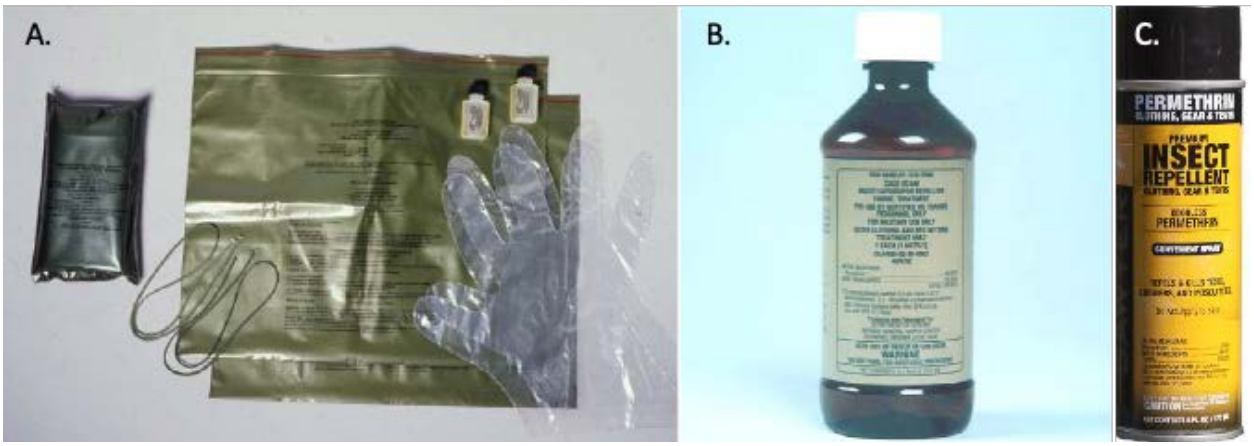


Figure 2.4. A. IDA kit; B. 5.1-oz permethrin concentrate; C. Aerosol spray (5)

The DoD authorizes the use of an aerosol spray of 0.5% permethrin as an effective countermeasure against disease vectors and nuisance pests. This method is for use on uniforms, bed nets, and socks. Because of the low concentration of permethrin, the 6-ounce aerosol can be applied by any individual, without training. This method requires uniform re-treatment after approximately six weeks. Dry-cleaning will remove permethrin from the material (5).

The IDA kit, also known as the "shake and bake" method in the field and is another useful option for an individual to treat uniforms with permethrin. This method is considered the safest and most environmentally friendly way of treatment. It consists of a

kit that uses a 40% permethrin solution but is designed to treat only one uniform at a time, so the volume of the high concentration is precise. Once treated using the IDA method, uniforms do not need to be re-treated with permethrin. Uniforms need adequate time to dry before touching or wear.

Factory treatment is accomplished by applying permethrin to the fibers of fabric after the fabric is dyed but before tailoring. This treatment method provides personnel protection against disease vectors and nuisance pests, and the permethrin lasts the lifetime of the uniform, which is estimated to be 50 launderings. After a uniform is laundered 50 times, it should be replaced. Factory treatment is prescribed to last the life of the uniform, therefore, it should eliminate the need for individuals, or units, to treat their uniforms. It also reduces environmental concerns associated with the use and disposal of permethrin containing containers when applied in the field. Factory treatment reduces exposure risks to service members related to applying the liquid and aerosol permethrin products. Because factory treatment binds permethrin tightly to the fabric of the uniform, almost no permethrin transfers to the skin. Uniforms that are factory treated should not be treated with any other treatment (7). Like with other treatments with permethrin, dry-cleaning of treated uniforms will remove permethrin from the material.

In the present study, 40% permethrin concentrate will be used to treat uniforms by certified applicators. Application of permethrin using a concentrated spray requires the EPA approved pesticide applicator certification. Special considerations for this method are necessary, as certified Applicators have current training within the last three years and

must also be properly fit with a respirator. Runoff of permethrin when using this method is a critical concern because of its toxicity to aquatic organisms, and appropriate steps must be taken to minimize contamination. There are several benefits of spray treatment with permethrin concentrate, such as the ability to treat large numbers of uniforms at one time at a relatively fast rate, with a treatment that is designed to last the life of the uniforms.

2.12.3 Retreatment of Uniforms

Currently, the EPA prohibits retreating uniforms that were previously factory treated or sprayed with permethrin concentrate. However, research for increased effectiveness of current products and a search for new products is persistent. Such research may lead to broader use of permethrin outside what is currently allowed, such as retreatment of uniforms or the adoption of new products. In practice, new uniforms are used in garrison and then transitioned to a "field uniform" once they become old, which may be after the "50 launderings" that the label states to be the extent of permethrin effectivity. When the original research regarding permethrin in uniforms was conducted, focus was on finding a formulation that caused the permethrin to stick or cling to the textiles, and the testing factor assessed was "laundering cycles" (times they were washed and dried). Since then, it has become apparent that other environmental factors are also important in reducing the effectiveness of permethrin. Even though permethrin protocols and formulations are designed to remain effective through laundering, reports from the field are clear that the permethrin does not remain effective through the rigors of wearing

the uniform in field conditions. Untested factors such as sweat, UV light exposure and abrasion may be reducing the effective life of permethrin in field-worn uniforms. More research in this area is needed to determine why field-worn uniforms are not remaining effective at providing bite-protection for the intended duration. The allowance for retreatment of uniforms could have value. Still, it would increase the use and application of permethrin across the services, as Army units would require reapplication to their factory treated uniforms, and Navy units would be authorized to retreat uniforms previously treated with permethrin.

2.13 RESPONSIBILITIES

The manufacturer's label on a bottle of permethrin concentrate provides fundamental requirements for its use, with the Applicator in mind; however, the procedures for large scale uniform spray treatment do not consider the Working Party personnel. The DoD, nor specific services, have protocols for pesticide use, which creates the need for the establishment of local standard operating procedures (SOPs). These SOPs should cover the specific formulation of permethrin and its proper use, personal protective equipment (PPE) required, treatment site requirements, and uniform saturation levels, or how many uniforms can be treated according to formulation criteria. A DoD comprehensive SOP that is consistent with the manufacturer's label, DoDI 4150.07, DoD Pest Management Program and the AFPMB Technical Information Memorandum No. 36 could normalize the application process, regardless of what service and what unit a member belongs. A comprehensive SOP could also ensure the spraying process includes adherence to regulation, human health considerations, and

environmental health precautions. Development and use of an SOP provides a framework for operations to guide personnel with varying experience to reduce exposure variability during spray operations.

2.13.1 Unit Responsibilities

Uniform treatment is a technical service offered by DoD preventive medicine units such as the Navy Environmental Preventive Medicine Units located both within and outside the U.S. and NECE in Jacksonville, FL for units preparing for contingency operations. Certified Applicators treat field uniforms, canvas general purpose (G.P.) and command post (C.P.) tents, and bed nets upon request from units. The unit requesting uniform treatment is typically responsible for providing personnel to handle, position on the ground, flip position for drying, and remove uniforms during the treatment evolution. These support personnel, who are not engaged in the actual application of permethrin but are assisting the pesticide applicators with handling the uniforms, make up the Working Party (WP).

2.13.2 Personnel

According to the manufacturer's label, uniforms treated with 40% permethrin must be allowed to dry thoroughly before being flipped, handled, moved or combined and returned to the unit requesting the service. Complete drying varies significantly depending on temperature, humidity, and wind, but often takes over three hours, and in practice, handling may occur before uniforms are completely dry (especially if an unexpected rain is looming, if schedules change or if schedules and timing aren't clearly

communicated beforehand. WP personnel handling uniforms (collecting the uniforms after the spray but before the uniforms have completely dried) could be exposed to permethrin via the dermal route during this time. Also, the proximity of the Working Party to the spray application of permethrin has potential to present inhalation and ingestion exposures during the treatment evolution. Ingestion exposures may occur through contact with permethrin on surfaces, followed by eating, smoking, and other mechanisms that may allow for indirect transfer of the contaminant into a person's mouth. Depending on the size of a spray operation (and therefore the time it takes to spray one side and dry before WP flip the uniforms), WP may take a lunch break or smoke break while waiting, which provides an opportunity for permethrin exposures.

Personnel who use pesticides on federal property, to include uniform treatment, must complete the two-week EPA approved pesticide applicator training course, which consists of in-class and hands-on training. Initial certification is good for three years, and recertification is required every three years to maintain the certification. The training ensures that pesticide applicators understand the proper way to handle, dispose of, and store pesticides used. Certified personnel, or Applicators, are responsible for correctly mixing permethrin with the appropriate amount of water, applying the correct amount of mixture onto uniforms, reducing the amount of remaining formulation for disposal at the end of the uniform treatment, appropriately disposing of any excess, and cleaning spray equipment. The Applicator is also responsible for ensuring proper calibration of the equipment.

The skill of the Applicator can influence the spray, and therefore can alter the potential exposures of personnel. The Applicator is trained to know which spray nozzle to use for a variety of purposes, from a fine point spray to a fan spray, that can create smaller spray droplets with potential for further transport. The Applicator needs to be aware of the wind speed and direction, as well as the wand distance from the target, as there is potential for impact splashback, ricochet and/or drift onto an unintentional target. The saturation level is another way that the Applicator can influence spray and its drift potential. It is possible to under-treat a uniform and possible to over-treat a uniform. If the uniforms are appropriately saturated, then most permethrin will be absorbed by the material. However, if the uniform is over-saturated, some spray will run off the material onto the ground or substrate rather than being used, creating a potential to lose spray to the environment if there is excess exposed ground, or even runoff into drainage or waterways. Too little spray leads to a false sense of security providing by the permethrin treatment, too much spray leads to waste, pollution, environmental damage or, potentially, greater permethrin exposure by the wearer.

2.14 Spray Application Methods

The DoD has several approved methods for spray treatment of permethrin using the 5.1-ounce, 40% permethrin, which include hand-carried compression sprayers (hand-can sprayers), manual compression backpack sprayers, truck or trailer carried electric, battery, or gas-powered compression sprayers (AG), and air compressed backpack carried sprayers (JQSX). However, they commonly rely on three of those methods: (i) 2- gallon compression sprayer (hand-can sprayers), manufactured by either Hudson or B&G; (ii)

Agricultural (AG) mist sprayer, manufactured by AG Spray Equipment; (iii) manual compression backpack sprayers (e.g. Solo backpack sprayers).



Figure 2.5. The AG sprayer

The AG sprayer pump is conventional in the DoD. This system is battery or electric powered with a tank that ranges from 20 to 50 gallons and mounted on a vehicle. AG sprayers require little effort by the Applicator since the pressure is mechanically maintained, and the tank is vehicle mounted. It is especially vital when using mechanical sprayers to calibrate the flow rate and ensure the Applicator delivers 32 fluid ounces of the mixture to each uniform (front and back) while preventing oversaturation of uniforms. The appropriate permethrin to water ratio must be maintained to ensure neither low concentration (reduced effectiveness) nor high concentration (increased runoff to the environment) of the solution.

2.15 Spray Applications

Regardless of the spray method, some choices can have a significant influence on droplet distribution. Nozzle type, droplet size, the pressure of the spray, and the

application height may have a considerable effect on the spray deposition. A small decrease in spray height from 0.5 m to 0.35 m was found to reduce drift by over 50% (94). Therefore, selection of the appropriate spray application method is essential before spraying uniforms with permethrin.

2.16 Exposures and Controls

A majority (70%) of the 2 million pounds of annual permethrin use is in non-agricultural settings (33). The law requires EPA registration of pesticides used in the US. Although registered for use in a variety of areas, including use in fabric impregnation, assessment of potential permethrin exposure during field uniform treatment is limited. Considerations for pesticide exposure are pesticide formulation, packaging, spraying technique, quantity sprayed, and climatic conditions (77). Exposure potential is the first step in understanding present hazards.

Permethrin is regulated according to use. When used on crops and other large areas, including during uniform spray treatment, permethrin is considered a restricted use pesticide. It is a general use pesticide for applications around the home for pest control. When used as a topical treatment for scabies and lice, permethrin is considered a pharmaceutical, and therefore regulated by the U.S. Food and Drug Administration (US FDA) (92).

Simultaneous dermal exposure measurements, air sampling, and biological monitoring is necessary to fully understand exposure and dose. Several air sampling

methods have been established; however, few methods are available for dermal exposure assessments.

2.17 BIOLOGICAL MONITORING

The most comprehensive knowledge of permethrin exposure is possible when dermal exposure measurements, air sampling, and biological monitoring are conducted simultaneously to determine full-body exposure. Many studies speculate that biological monitoring (biomonitoring) is necessary in assessing exposure (25; 75) (90). Monitoring permethrin concentrations in blood or blood products such as plasma offer several advantages. When using blood, it is possible to monitor the parent compound, which eliminates some uncertainty of relying on metabolites. However, pyrethroids can be determined in blood for only a few hours after exposure due to their quick metabolism and elimination (80). Leng and Lewalter found pyrethroids were detectable in blood only one-hour post-exposure, with concentrations rapidly decreasing over the first 24 hours (79). Leng *et al*. demonstrated the benefits of urine compared with blood, to provide adequate exposure information from multiple routes of pyrethroid exposure (78). Urine is advantageous over plasma because it is non-invasive and relatively easy to collect. Biomarkers can be indicators of multiple routes of exposure to a chemical. They also reflect differences between individuals with respect to absorption and metabolism.

However, biomonitoring is not without drawbacks. The major challenges of biomonitoring are the uncertainties and variabilities. Intra- and inter-subject variability makes an inference to a population difficult. With studies relying on biomonitoring, sample sizes are often small and very specific in their exposure criteria (14). There is uncertainty in the portion of the absorbed compound that is eliminated. Also, exposure

does not always lead to effect, making it critical to understand the metabolism of the chemical of concern. Confounding factors are a valid consideration when using biomonitoring if the biomarker or metabolite is derived from several exposure sources (66) (29).

Urine is non-invasive and relatively easy to collect for use in biomonitoring. Though urine is replenished, the output varies by the individual based on hydration and activity levels, which can also affect the concentration of metabolites available for analysis. The metabolites of permethrin are cis-3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*cis*-DCCA), trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-(1-cyclopropane) carboxylic acid (*trans*-DCCA), and 3-phenoxybenzoic acid (3-PBA). *Cis*-DCCA, *trans*-DCCA, and 3-PBA are not specific but are metabolites of other pesticides. The 3-PBA metabolite is a general group consisting of 10 of the 18 pyrethroids registered for use in the US (29), including but not limited to permethrin, cypermethrin, deltamethrin, allethrin, resmethrin, and fenvalerate (12). *Cis*-DCCA and *trans*-DCCA are more specific and found in several pesticides, including permethrin, cypermethrin, and cyfluthrin (12). Measuring all three common metabolites from each sample provides information on the exposure source. Knowledge of the metabolism and excretion patterns of permethrin are necessary to collect samples at an appropriate time that will accurately capture the metabolites in urine.

Exposures to permethrin result in the presence of three metabolites in urine, 3-PBA, *cis*-DCCA, and *trans*-DCCA. Because of the higher potent insecticidal activity of the *cis*- isomer than the trans, more of the trans- metabolite is found in the urine (19). However, the presence of a metabolite in urine does not indicate adverse health effects.

Rather, measurable amounts of metabolites, especially of cis- and trans- DCCA, show baseline exposures that may occur in the general public and may reveal populations with heightened exposures or risks of exposures.

It is possible to distinguish between oral and inhalation versus dermal uptake with separation of *cis-* and *trans-* DCCA isomers in urine during biomonitoring. A ratio of *trans: cis* greater than one indicates the primary route of entry was inhalation, oral, or both (80). A ratio of less than one indicates a dermal absorption was more relevant.

2.18 Dermal Exposure Sampling Techniques

Dermal exposure occurs through direct contact with chemicals, indirect contact with surfaces, clothing, or residues, and through the air. There are several studies to show dermal exposure is a significant, if not dominant, pathway of exposure among pesticide workers (43; 50; 77). Skin exposure to chemicals will contribute to the overall body burden and can lead to localized responses. Dermal exposure depends on processes and individual behaviors and can have high levels of variability among individuals.

Sampling methods for dermal exposures are not developed as much as sampling for exposure via the inhalation or ingestion routes (27). Several dermal exposure techniques are accepted, including surrogate skin and removal sampling, but limitations and assumptions must be recognized.

Several methods capture dermal exposures using material over the skin as a surrogate. These include patch sampling, dosimeter gloves, and whole-body sampling. Surrogate skin is a passive sampling method used to estimate the amount of contaminants deposited on the skin through a collection material and analyze the collection material for the contaminant. However, limitations exist because the absorbency of the collection

material does not have the same characteristics as skin and may lead to potential over- or underestimation of exposure.

Whole-body sampling (WBS) provides valuable information and is the most frequently used when assessing pesticide exposure (10). Even more so than with the use of dosimeter gloves, distributional assumptions are not required, and absorbed dose for the entire body can be extracted (43). Whole-body sampling does have disadvantages. By nature of covering the whole body, whole body sampling can be cumbersome. Therefore, participants may not cooperate fully, especially when the weather is hot or if they are required to wear long pants or tops. Another consideration is that the extraction of the contaminant requires a large amount of solvent. Lastly, all body regions, or even specific areas within a particular region, do not absorb equally (81), which can be an assumption in WBS. For example, a leg may have a large amount of contaminant absorbed on the calf but very little may absorb via the upper or inner thigh. When looking at the amount absorbed to the leg body region, differences within the region (calf versus upper inner thigh) are not distinguished, and the two areas do not share identical absorption characteristics.

2.18.1 Patch Sampling

Patches may be placed above or below clothing but should have a non-permeable material on the backside. The non-permeable layer prevents the contaminant from being held against the subject's skin and to prevent the patch from absorbing sweat, instead of the potential contaminant of interest. An assumption of patch sampling is uniform exposure across the entire body. Another assumption is the absorbency of the patch material is close to the absorbency of skin, which often leads to an overestimation of

absorption and dermal exposures. With the limited consensus on the significance of dermal exposures to pesticides, patch sampling can provide valuable data when paired with biomonitoring, which is what will be done during this study with permethrin.

The use of patch sampling is common in pesticide exposure studies, and the WHO, EPA, and Organization for Economic Co-operation and Development (OECD) have standardized methods for this type of sampling (132) (32; 101). Patch sampling uses the placement of absorbent material at different small skin surface locations on the body. The anatomical locations represent a fraction of the body for potential exposure and applied across all exposed skin. The level of exposure for each skin area covered by a patch is typically related to the skin surface area of an anatomical region (10).

2.18.2 Dosimeter Gloves

Another example of surrogated skin sampling is the use of dosimeter gloves, which share many of the assumptions and limitations of patch sampling. Due to the size of gloves, they hold an additional challenge, requiring a significant amount of solvent to extract a solute from the material. Also, due to the awkward nature of gloves, while performing intricate tasks that require dexterity, dosimeter gloves may not be worn for the entire sampling period. However, dosimeter gloves will be used to assess dermal exposures to permethrin during a field treatment of military uniforms with permethrin.

Dosimeter gloves are made from an absorbent material and worn either over (potential exposure) or under protective gloves (to test the reliance of the personal protective equipment). They are commonly used to estimate exposure during pesticide applications (100). A benefit of dosimeter gloves is distributional assumptions are not required since the absorbed dose is for the entire anatomical region. However, dosimeter

gloves raise challenges when donning and doffing of the garments, and chemical extraction from the material requires a lot of solute (43). Frequent changing of dosimeter gloves is an important task to avoid saturation. Wearing of the dosimeter gloves during the entire sampling time, regardless of the level of participation in the event, may also prove difficult and uncomfortable to the subjects. During this study, 100% cotton dosimeter gloves will be worn over the protective gloves. They will be worn by all participants of the field treatment of uniforms for the duration of the treatment process.

2.19 AIRBORNE DISPERSION

It is essential to know if a potential exposure pathway to permethrin during the spray treatment of uniforms exists. Therefore, it is critical to gain an understanding of permethrin drift characteristics in the vicinity of spraying for consideration in the pathway completion. A concern about permethrin dispersion is unnecessary exposure to the Working Party during the field treatment of military uniforms with permethrin. Tests involved in measuring the dispersion of permethrin include quantitative and qualitative droplet collection, meteorological recording, active air sampling, and observations.

The size of droplets used in a spray will impact the efficiency of the chemical applied. The use of permethrin for mosquito abatement relies on small droplets that will disperse in the air and provide the furthest coverage practical. Droplet size is impacted by evaporation during dispersion, and small drops may not deposit on the intended target as air movement carries them. At the same flow rate, reducing the diameter of a droplet results in an increased number of droplets that are more susceptible to drift and air movements (63). Dispersion of pesticides following spraying may last from a few minutes to an hour (74). However, the specific chemical properties of permethrin and the

composition of the spray suggests low expected concentration in ambient air (137). During the spray treatment of military uniforms, it is not beneficial for the spray to disperse. Safeguards are taken, including spray nozzles that produce larger and heavier droplets, and wands that are pointed down and directly at the uniforms. Also, emulsions are found to give sprays that are coarser than water-soluble formulations, which results in decreased spray drift.

Given the use of permethrin for spray treating uniforms and the presence of the Working Party in the vicinity of the spray, this research will explore the dispersion of permethrin and its potential to produce a complete exposure pathway.

2.19.1 Area Air Sampling

Spray drift during pesticide applications can be measured in a number of ways, including collection of ground deposition (103), sedimentation of pesticide on surfaces (16; 49; 94) and active air sampling onto adsorbent material (128). OSHA Versatile Sampler Tubes (OVS-2) that contain a glass fiber filter (GFF) and two sections of XAD-2 adsorbent are the evaluated method by the OSHA Organic Methods Evaluation Branch (98). Using XAD-2 tubes with active air sampling accumulates both gas and particle phase compounds.

Air sampling provides information for the assessment of potential exposures to a wide gamut of airborne hazards. There are regulatory authorities that set limits for exposure to substances deemed hazardous to human health. The limits set include RELs, PELs, military exposure limits (MEL), but are typically based on personal sampling, not environmental sampling. However, this does not mean that limits cannot apply when

using environmental sampling, or that it is not useful to identify hazards using area air sampling.

A well designed and controlled experimental approach that will provide information about the dispersion of permethrin is useful for identifying potential exposure risks to personnel. Characteristics and conditions of the spray, including the size of particles, the concentration of the spray, environmental factors, sampling time, and duration, are preliminary considerations.



Figure 2.6. OSHA Versatile Sampler, OVS-2 tube (116)

2.19.2 Rotating Impactor Sampling

To understand the tendency of permethrin aerosol to drift in the vicinity of the uniform spray event, a means to capture and evaluate the movement and transport of the spray is necessary. There are three rotating impactors, the Hock impactor, RotoRod impactor, and the Florida Latham Bond (FLB) impactor, for field droplet sizing and volumetric measurements (16; 24). The FLB sampler is a rotating impactor developed to quantify the amount and the droplet size characteristics of airborne sprays. It consists of the base that contains a small motor, powered by four AA batteries, to spin an arm at a velocity of 5.6 m/sec. On the end of each arm are two, one-inch magnesium oxide (MgO)

coated slides. Slides coated with MgO are better designed for water-based formulations, such as the technical grade permethrin used during the spray treatment of military uniforms, because they are more volatile and prone to evaporation than oil-based formulations. Water-based formulations impact the MgO coated slides and leave a permanent impaction on the slide. Even if the droplets shrink or evaporate, the impaction can be precisely measured (21). The FLB sampler uses small collection surfaces, rotated at sufficiently high speeds where capture efficiencies are high and not significantly affected by wind speed. The wind does not affect because the rotation speed is faster than the expected wind speed (16). Since there is limited information on the specific spray used for uniform treatment, the volume median diameter of spray droplets is not known. They are expected to be larger than 30 um, which is more common for mosquito control sprays designed for drift to assist in distribution and coverage (16). However, there is some concern that the high velocity may shatter the larger droplets used for uniforms spray, which may affect collection efficiency.

The height of the collection surface is another consideration in capturing the dispersion of permethrin in the vicinity of the field uniform spray event. Because of the larger droplet size and the downward pointed spray wand, the height of the collection should not be too high as to be above the potential spray. However, collection too close to the ground will capture dispersion that is not likely to pose an exposure risk to personnel.

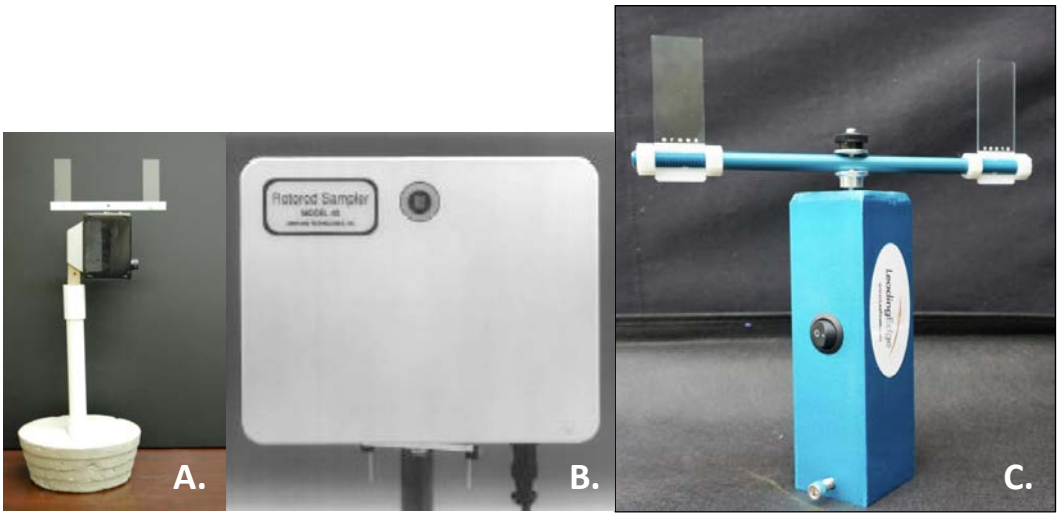


Figure 2.7. Three rotating impactors, A. the Hock (38), B. RotoRod (113), and C. the FLB (76)

2.19.3 Meteorological Conditions

Wind speed and direction, temperature, humidity (%), and sunlight intensity may affect the dispersion of permethrin and the rate that droplets fall onto the uniforms or drift to the surrounding area. It was found that the volume median diameter of droplet size decreased by 30-50% as the wind speed in a wind tunnel was increased from 45 to 63 m/sec as well as farther downwind deposition (49). Even though the large droplets of permethrin spray are pointed downward toward the uniforms, wind considerations may be significant when conducting uniform spray events. Humidity affects droplet evaporation, which may alter suspension time in the air. Because vapor pressure will increase with humidity, though unexpected, an increase in humidity has the potential to influence potential inhalation risks (aerosols, spray droplets, dust, and vapors). Temperature affects evaporation, which may reduce the size of the droplets and, therefore, the potential dispersion of the permethrin spray.

Chapter 3. Materials and Methods

3.1 Study Subjects

Sampling took place over two days, 11 March and 20 March 2020, sampling days one and two, respectively. This study enrolled participants from NEPMU5 and the USS *Comstock*, who requested the uniform treatment. Assigned personnel consisted of certified pesticide Applicators and Sailors not trained in the uniform treatment process. All supporting personnel were eligible to participate and deemed healthy by their unit, as indicated by assignment to this task. The lead researcher met with all eligible participants, described the study and potential risks, and then 13 of the 15 eligible participants signed the informed consent. The protocol and ICD were approved by the USUHS Institutional Review Board (IRB) before fieldwork initiation (IRB Number: USUHS.2019-032). Personnel disclosed if they were or might have been pregnant at the time of sampling, as they would not participate in the uniform spraying event. Personnel were asked to disclose if they took medications containing ibuprofen, or if they participated in spraying of uniforms during the previous three days so that circumstances could be recorded; there were no disclosures. One participant left the study after the baseline sample was collected.

All subjects were assigned a random number that maintained subject confidentiality. During sampling, media was collected from individuals and was identified using a standardized naming convention that supported confidentiality while permitting differentiation of individual samples. The names of the study participants were

not used to identify any samples collected during this study. Identifying numbers used on samples were for analysis purposes only.

3.2 SITE SET-UP

Sampling occurred during a routine field mass spray treatment of uniforms on Naval Base San Diego. Uniforms were laid out in-line by the Working Party, in a fashion to fit the most uniforms into the available space while also minimizing the ground exposed between uniforms (Figure 3.1). Each line was divided into three zones. The first 25% of the spray line (zone A) and the last 25% of the spray line (zone C) were expected to have the highest airborne concentrations and present the highest opportunity for permethrin exposure. Increased exposure potential may be present in the last 25% of the spray line, as the Applicator may have permethrin remaining and may over-saturate, to expend the mixed solution. It was assumed the middle 50% of the spray line (zone B) would have the most homogeneous spraying of uniforms.

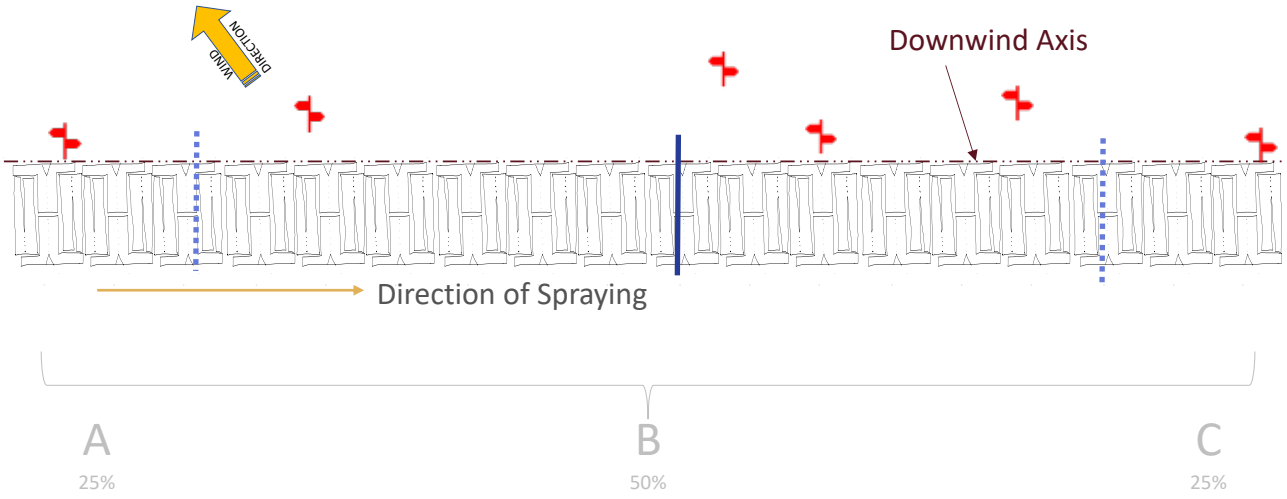


Figure 3.1. Site set-up, with uniforms in-line

Five-ounce bottles of technical grade, 40% permethrin (NSN: 6840-01-334-2666), were mixed with 2-gallons of water and used for uniform treatment. An AG sprayer with a 20-gallon tank manufactured by PBM Supply & Manufacturing, Inc., was used (Figure 3.2). It was placed on a pull cart and powered by a vehicle battery. Long pants and sleeves, gloves, a respirator, and eye protection were worn by the Applicators as prescribed by the pesticide worksheet in Technical Guide 14 (6).



Figure 3.2. AG Sprayer pulled behind the Applicator

3.2.1 Environmental Sampling

Nine air sampling rigs were designed, using half-inch polyvinyl chloride (PVC) pipe (Figure 3.3), to collectively mount the two air sampling methods discussed in detail in section 3.3.2. The rotating impactor was mounted on half-inch PVC pipe, inserted on the bottom of the motor. Using this mount, the horizontal rotating arm on the FLB sampler measured 23 inches from the ground. On the other vertical pole, the air sampling media was mounted at 38 inches from the ground. These heights were selected to

simulate the breathing zones of the Working Party as they bent over to flip, straighten, and collect the uniforms (Figure 3.4).

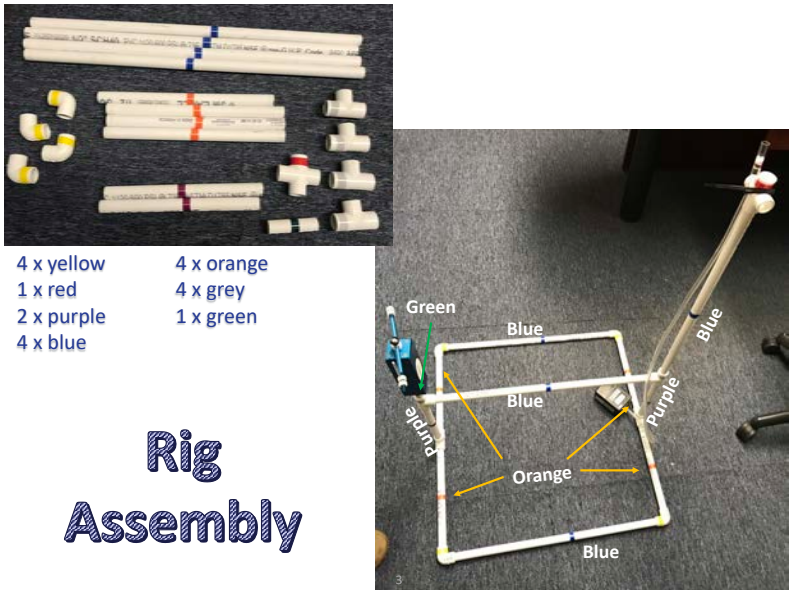


Figure 3.3. Air Sampling Rig Assembly



Figure 3.4. OVS-2 tube with pump and FLB rotating impactor on a sampling rig

Applicators operated upwind from the uniforms, and sampling rigs were placed downwind of the uniforms, and therefore downwind of the spray efforts. The rig orientation standard (Figure 3.5) is the rotating impactor oriented on the left, and the taller pole for the area air sampler on the right. The reference point corner was placed at the randomly selected location. The number of sampling points per line was determined by using a ratio of one point per 16 uniforms, with a maximum of 10 samples per uniform line.

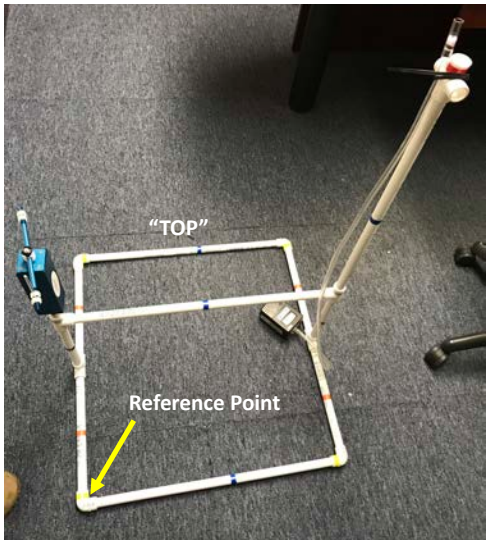


Figure 3.5. Rig orientation standard

Air sampling points were placed using a stratified random sampling approach (52). Locations within strata were identified using a random-digits table (71), using feet as measurements. To allow for comparison between the first and last 25% of uniforms, the sampling points within zone C were a mirror of sampling points in zone A, (A=C). The sampling locations rigs were randomly placed in zone B along the downwind axis, not to exceed three feet from the downwind axis and not to be in the spray plane. Distance from the downwind axis was systematically determined, a priori. The distances

were based on previously published work (21; 38; 39; 60; 127). Rigs were placed, a priori, each at one, two, and three feet from the downwind axis.

On the first day of sampling, there were 120 uniforms for treatment, which were laid out in two parallel lines of 60 uniforms each, with 7.5 feet between the lines. The two lines of uniforms were considered one iteration because of their proximity. Twenty-five percent of 120 uniforms are 30 uniforms, or 60 feet long. The first two-digit number between zero and 60 was used for placement of rig within zone A, along the downwind axis (Table 3.1). Fifty percent of 120 uniforms are 60 uniforms or 120 feet. The first three-digit number from zero to 120, from the last number used, determined placement of the first rig in zone B. The rigs were not moved between the morning (front) and afternoon (back) iterations. Twenty-five bottles of permethrin and 50 gallons of water were used to treat both sides of 120 uniforms.

Table 3.1. Rig Placement

Day	Iteration	Zone	Rig	Location (feet)	Distance from downwind axis
1	1 and 2	A:	1	21	0
		B:	2	104	1
			3	69	1
			4	95	2
			5	120	2
			6	80	3
			7	13	3
		C:	8	21	0
2	3 and 4	A:	1	6	0
		B:	2	20	1
			3	41	2
		C:	1	6	0
2	5 and 6	A:	1	6	0
		B:	2	20	1
			3	41	2
		C:	4	6	0

On the second day of sampling, there were 63 uniforms for treatment, which were laid out in two parallel lines of 31 and 32 uniforms each, with 75 feet between the lines. The separation between lines allowed them to be treated as separate events; iterations three and four for treatment of the first side, and iterations five and six for the treatment of the backside of the uniforms. Twenty-five percent of 32 uniforms are eight uniforms, or 16 feet long. The first two, two-digit numbers between zero and 16, was used for placement of one rig within zone A, along the downwind axis (Table 2). The rig placement in zone A was mirrored in zone C. Fifty percent of 32 uniforms are 16 uniforms or 32 feet. The next two, three-digit number from zero to 32, determined

placement of the rigs in zone B. The rigs were not moved between the iterations. Eight bottles of permethrin and 16 gallons of water were used to treat both sides of 63 uniforms.

3.2.2 Ambient Weather

Ambient weather conditions were measured to understand how conditions affected permethrin in the air during field spraying. The WS-2902 Wi-Fi Osprey Solar Powered Wireless Weather Station was purchased from Chickadee Solutions (Figure 3.6). It is a self-contained, solar-powered, weather monitoring station that reports weather conditions, including temperature (degrees Fahrenheit (°F)), humidity (%), wind speed (mph) and direction (cardinal direction), and sunlight intensity (lumen per square meter (Lux)). Variability was reduced by placing the weather station on the same surface for the duration of sampling.



Figure 3.6. The Ambient Weather WS-2902 Wi-Fi OSPREY Solar Powered Wireless Weather Station

3.3 SAMPLING

3.3.1 Biological Sampling

Urine samples were collected to measure metabolites *cis*-DCCA and *trans*-DCCA. Urine samples from study participants were collected at baseline to establish if individuals had exposures to permethrin before commencing uniform spraying and to determine initial *cis*-DCCA and *trans*-DCCA metabolite concentrations from other sources such as ibuprofen use. Samples were collected at a minimum of 4 hours post the start of spraying. The samples are pending analysis at the Biomedical Instrumentation Center (BIC) at USUHS in Bethesda, Maryland. Sample storage and shipment protocols were established based on previously published work (71; 78; 129). All samples were stored and shipped at -20°C. Sample preparation protocol followed that described in Olsson, A.O., et al. (2004), with some slight modifications (99). Full laboratory analysis of urine is detailed in Appendix A. The permethrin metabolite DCCA was further analyzed to separate and quantify the isomers, *cis* and *trans*-DCCA.

3.3.2 Patch Sampling

Potential dermal exposures were measured using dosimeter gloves and patch sampling. Patches were made from alpha-cellulose paper and backed with aluminum foil, as recommended by the WHO, EPA, and Organization for Economic Co-operation and Development (OECD) (106; 129; 130). Alpha-cellulose paper was purchased from University Products, Holyoke, MA. The aluminum foil backing prevented permethrin from being held against the skin of a study participant as well as prevented the paper from absorbing sweat from the study participant.

Dermal patches were placed on the right forearm of each individual, half-way between the elbow and the bend of the wrist, on the underside of the forearm (Figure 3.7). The collection area was 4 inches by 4 inches, with approximately 2 inches above and below the sampling area for attachment. If the individual was wearing long-sleeves, the alpha-cellulose paper backed with aluminum foil was fixed on top of the clothing in the same location. Patches were affixed to each person before the start of uniform treatment and collected upon completion of each sampling iteration. The CIHL in Norfolk, Virginia, analyzed the total permethrin in the patch samples using OSHA Method 70 via SOP GC-59 with a limit of quantification of 0.8 µg per sample.



Figure 3.7. Dermal patch on right forearm constructed of alpha-cellulose paper; 100% cotton dosimeter gloves worn over the protective gloves

3.3.3 Dosimeter Glove Sampling

The second method used to sample potential dermal exposure to permethrin during a uniform spray event was dosimeter gloves. One-hundred percent cotton, 200 grams per square meter (g/m2) fabric weight, dosimeter gloves were purchased from

Gaxcoo, West New York, NJ. The gloves were one size. The dosimeter gloves were worn by study participants over their protective gloves (Figure 3.7) and were used to determine potential personnel exposure when they handled the recently sprayed uniforms. The dosimeter gloves remained on participants for the entire iteration, even when not actively engaged in touching uniforms or handling permethrin.

ALS Laboratories in Salt Lake City, Utah, analyzed the total permethrin in dosimeter gloves using NIOSH Method 9204 (Draft) by desorbing the gloves in isopropyl alcohol for the initial dilution. Dosimeter gloves samples were shipped at -20°C to the lab. ALS is an AIHA-LAP (American Industrial Hygiene Association- Laboratory Accreditation Program) accredited lab that serves as the primary method development contract lab for NIOSH.

3.3.4 Air Sampling

To measure and identify areas of potential inhalation exposures, area air sampling monitors were set up in the vicinity of spray operations. Measurements of permethrin concentration in the air were conducted following OSHA Method 70. Air was actively drawn onto the OSHA Versatile Sampler (OVS-2) tubes by an air pump.

OVS-2 tubes were purchased from SKC and designed with two filters in series to collect both vapors and aerosols. OVS-2 tubes are divided into a sampling section that consists of cleaned XAD-2 resin and a glass fiber filter that remains open to the environment and pointed downward to avoid particulates from settling onto the filter, and a backup section. Flexible plastic tubing (44 inches) was attached to the backup section of the OVS-2 tube. Two models of air pumps were used for sampling, the AirChek® XR5000 personal air sampler pumps, model 210-5000 by SKC, Inc., powered by

rechargeable lithium-ion battery packs, and the AirChek® universal sample pumps, model 224-44XR by SKC, Inc., powered by rechargeable nickel-metal hydride battery packs. The pumps were pre-calibrated at 1.0 L/min, to within 5% of the flowrate, and post-calibrated to ensure flowrate drift was not greater than ±5% (96). The pumps ran 5 min before spraying commenced and continued for 5 minutes after spraying ceased. After spraying, the pumps were turned off, and the OVS-2 tubes were collected, stored, and shipped at -20°C in compliance with OSHA Method 70. The Navy Comprehensive Industrial Hygiene Laboratory (CIHL) in Norfolk, Virginia, analyzed the OVS-2 tubes following OSHA Method 70. Results were reported as concentrations in micrograms per cubic meter (μg/m3) with a limit of quantification of 0.4 micrograms (μg).

FLB impactors were powered with 4-AA batteries. The 6V DC 60 Hz motor provided rotation at 686 revolutions per minute (RPM). Two one-inch glass slides with Magnesium Oxide (MgO) coating on one side were rotated to capture the permethrin droplets. Water-based formulations such as technical grade permethrin when used for mass spray treatment of uniforms, are more prone to evaporation than oil-based formulations. The MgO coated slides allow for measurement of the initial droplet impression, even after the droplet size decreases by evaporation. The MgO coated slides are mounted on a horizontal plane in a vertical position, approximately 18.5 centimeters apart, rotating at a velocity of 5.6 meters per second (m/sec) (21). The velocity of the FLB was higher than the wind encountered in the field during spraying (highest observed during sampling was 5.4 mph or 2.4 m/sec), eliminating the effects of wind speed. Collection of droplets with the impactors function under the principle that a small collection surface rotated at high speeds will effectively capture droplets (21).

FLB impactors are a way of collecting and measuring droplet density, size, and distribution. The droplet density was calculated using MgO coated glass slides on the rotating impactor for analysis on DropVision®, Leading Edge, Fletcher, NC. DropVision® is a compound microscope with a built-in, hi-resolution digital imaging processor that electronically captures images of the glass slide after droplets are deposited on the surface (Figure 3.8). This technology can eliminate background objects, coalesced droplets, and nonqualified droplets. Microscopy measurements across the full traverse of the slide, including the edges, provided the droplet density per unit area. DropVision® can measure droplet sizes as small as one pixel (<1 micron). Having the volume of permethrin and particle sizes quantified around the spray event is important for determining dermal and respiratory risks.

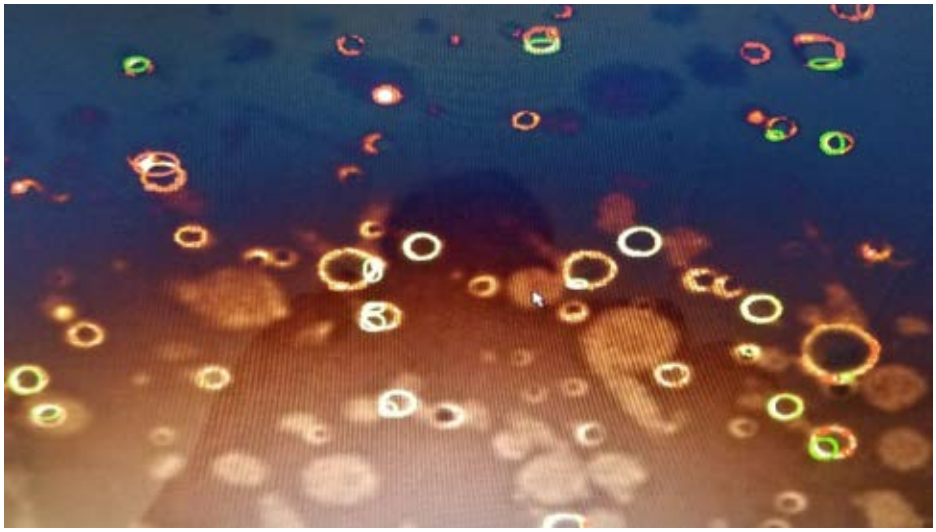


Figure 3.8. DropVision® slide picture. Valid droplets are discerned from background noise and highlighted in green; droplets in red are not included in the analysis.

3.4 STATISTICAL METHODS

Data were analyzed using Microsoft® Excel for Mac (version 16.37, copyright 2020, Redmond, WA) and STATA/IC 15.1 for Mac (STATA Corps LLC, College Station, TX) to obtain descriptive statistics and to perform inferential statistics and correlations. Statistical analysis was used to address each of the research questions: 1) what is the measure of absorbed pesticides in urine resulting from exposure via all routes during field uniform spray treatment using 40% permethrin; 2) using dosimeter gloves (pairs) and dermal patch sampling, what is the dermal exposure to permethrin; 3) What is the dispersion of permethrin in the vicinity of spray applications when measured with the FLB impactor and the area air sampler; 4) what is the concentration of permethrin in the vicinity of spray applications when measured with an area air sampler.

3.4.1 Statistical Methods of Urine Data

Due to the cease lab operations order issued in response to the COVID-19 pandemic, urine samples are currently held in proper storage waiting for lab operations to resume so that the metabolite analysis can be performed. The following describes how data will be handled once it becomes available.

Two two-way repeated measures Analysis of Variance (ANOVA) will be used to compare the mean difference and interaction between two independent variables on the dependent variable. The first repeated measures ANOVA will compare role (Applicator or Working Party) and time of sample collection (baseline, 4-hr after spraying starts, and 12-24-hr after spraying starts) to the total DCCA measured in urine (Table 4). The second repeated measures ANOVA will compare isomer (*cis*- and *trans*-) and time (baseline, 4-hr, 12-24-hr) (Table 5) to understand if there was an interaction between the two factors on the dependent variable.

3.4.2 Statistical Methods for Dosimeter Gloves and Dermal Patch Data

Study participants wore dosimeter gloves and dermal patches for the duration of a spray treatment of uniforms, and descriptive statistics were used to measure the central tendency and variability of potential dermal exposures. A repeated-measures ANOVA was used to compare the mean difference between the Applicators and the Working Party versus the two measures contributed by each person, patches and gloves.

3.4.3 Statistical Methods for Permethrin collected in the Air

All results from the OVS-2 tubes were reported with values less than the LOQ. Statistical analysis was not conducted using the OVS-2 tube results.

Results from the FLB impactor slides were summarized using descriptive statistics to show the droplet size distributions and droplet densities per slide. Then, comparisons between the location of the sampling points (zone and distance from the downwind axis), and the droplet size distribution and the droplet density were made using four one-way ANOVA tests. Each one-way ANOVA compared one categorical independent variable with one dependent variable with the assumption of similar variances across the groups. This was verified using Bonferroni's post-hoc test to adjust for multiple comparisons.

Chapter 4: Permethrin in the Air During a Field Spray Treatment to Military Uniforms

ABSTRACT

Permethrin is used throughout military services for added protection from disease vectors. It has proven to be an effective product since the 1980s and endured searches for new products and process improvements. Though permethrin has been around the military for entire careers, no known exposure assessment for personnel involved in field spray treatment of uniforms has been conducted. This study aims to quantify permethrin in vicinity of a uniform spray event to quantify potential inhalation, dermal, and indirect ingestion exposures. The collection of aerosol and vapor phases of permethrin followed OSHA Method 70. Rotating impactors were used to collect and measure droplet density, size, and distribution as an indication of dermal and indirect ingestion risks. DropVision® system analyzed the number of drops per slide and provided volume distributions of slides. Four one-way analysis of variance (ANOVA) tests compared sampling locations to average droplet span and density and showed no significant difference (p=0.9262) in mean droplet densities between zones. However, there was a difference in droplet size distribution, using the span between zones (p=0.0206). Differences in the mean span (distribution width) between the zones, despite no difference in the number of droplets, indicated the sizes of the droplets vary by zone. An Applicator behavior or aspect of the equipment producing this difference. Neither mean droplet density (p=0.2056) or mean span (p=0.1958) had statistical difference when collected at 0, 1, 2, and 3 feet off the spray line. Multiple variables influence a mass spray treatment of uniforms with permethrin, such as human behaviors, and performing a spray

during high humidity may increase the dispersion of permethrin droplets. The distances from the spray line used in this study showed permethrin persists in the air up to 3 feet, and further studies are required to determine permethrin persistence beyond that distance.

4.1 INTRODUCTION

Permethrin is an insecticide with many uses. It can be sprayed in neighborhoods for mosquito abatement, applied to crops for protection from pests, used on people as a lotion to treat scabies and lice, on pets to deter ticks and fleas, and sprayed on clothing, including US military uniforms. Permethrin has been used in the US military since the 1980s to provide added protection for servicemembers against disease vectors.

Permethrin is odorless and ranges from colorless to yellow and ranges in physical characteristics from solid to an oily liquid. It acts by interfering with brain functions and with sodium channels of the sensory nerves of mammals and insects. In insects, the voltage-gated sodium channels are disrupted, delaying their inactivation, leading to premature opening and increased impulses, causing tremors, paralysis, and death. It is a broad-spectrum pesticide that targets pests and has the desired properties of a short half-life, low mammalian toxicity, and useful general insecticidal properties. Human exposure to permethrin is possible through ingestion, inhalation, and dermal routes of exposure (68).

Pyrethroids were first formulated in 1949, and through research and development, four generations of pyrethroids were manufactured, each with increased photostability and lower acute mammalian toxicity. Permethrin was developed in 1973 as the first pyrethroid with adequate photostability for use in agriculture but was not registered for agricultural use until 1979 by the EPA (73).

Mosquito abatement and crop applications utilize sprays with small droplets that disperse away from the upward or outward pointed spray source. The US EPA describes spray drift as the airborne dispersal of a pesticide onto undesirable, or non-target sites, during or immediately after the application or use of the pesticide (30). The spray treatment of uniforms with 40% permethrin necessitates the deposition of droplets onto uniforms with a downward pointed wand. The unique techniques employed for the specific purpose of uniform treatment call for conditions that affect spray drift be understood and minimized to reduce potential exposure to permethrin by personnel in the vicinity of the spray efforts. The potentially exposed population during a field uniform spray event typically includes the Applicator and Working Party (WP), which is defined as the personnel who handle uniforms, to include positioning on the ground, flipping for drying, and removal of uniforms after the treatment evolution. Both populations, the Applicators and Working Party, work in the vicinity of spraying where permethrin may persist in the air. The distance of travel and concentration of permethrin in the air in the vicinity of uniform application are not well understood. Many studies investigate environmental and equipment factors affecting spray drift. Miller et al. (2011) found droplet size distribution, the height of spray, and the wind speed to influence spray drift (85); Nuyttens et al. (2006) found weather conditions, such as temperature, wind speed, relative humidity, and atmospheric stability influence spray drift (95); and, Hassen et al. (2013) showed that application practices, such as nozzle type, droplet size, spray height, and spray pressure all influence spray drift (60).

Spray drift during pesticide applications can be measured in several ways, including the collection of ground deposition (103), sedimentation of pesticide on

surfaces (16; 49; 94) and active air sampling onto adsorbent material (128). OSHA versatile Sampler Tubes (OVS-2) that contain a glass fiber filter and two sections of XAD-2 adsorbent is the evaluated method by the OSHA Organic Methods Evaluation Branch (98). Active air sampling using XAD-2 tubes allows for collecting both gas and particle phase compounds.

The Florida Latham Bond (FLB) impactor is a rotating impactor used for field droplet sizing and volumetric measurements (16; 24). The FLB sampler was developed to quantify the number of droplets and droplet size characteristics of airborne sprays. It consists of the base that contains a small motor, powered by four AA batteries, to spin an arm at a velocity of 5.6 m/sec. On the end of each arm are two, one-inch magnesium oxide (MgO) coated slides. Slides coated with MgO are better designed for water-based formulations, such as the technical grade permethrin used during the spray treatment of military uniforms, because they are more volatile and prone to evaporation than oil-based formulations. Water-based formulations impact the MgO coated slides and leave a permanent impaction on the slide. Even if the droplets shrink or evaporate, the impaction can be precisely measured (21). The FLB sampler uses small collection surfaces, rotated at sufficiently high speeds where capture efficiencies are high and not significantly affected by wind speed. The wind does not affect FLB sample collection because the rotation speed is faster than the wind speed (16). The volume median diameter of spray droplets is not known since there is limited information on the specific spray used during uniform treatment. Droplets during uniform treatment are expected to be larger than 50 μm, which is the maximum size for mosquito control sprays designed for drift to assist in

distribution and coverage (16). This study will investigate concentration and size characteristics of permethrin in the air in the vicinity of a routine uniform spray event.

4.2 MATERIALS AND METHODS

Sampling occurred during a routine field mass spray treatment of uniforms on Naval Base San Diego. Uniforms were laid out in-line by the Working Party, in a fashion to fit the most uniforms into the available space. Personnel minimized the ground exposed between uniforms to avoid permethrin droplets from returning to the air as a rebound from the hard surface (Figure 4.1). Each line was divided into three groups. The first 25% of the spray line (zone A) and the last 25% of the spray line (zone C) were identified as areas of higher exposure potential. Increased exposure potential may be present in the last 25% of the spray line, as the Applicator may have permethrin remaining and may over-saturate, to expend the mixed solution. It was assumed the middle 50% of the spray line (zone B) would have the most homogeneous spraying of uniforms.

4.2.1 Sampling

Nine air sampling rigs were designed, using half-inch polyvinyl chloride (PVC) pipe (Figure 4.2), to collectively mount the two air sampling methods, the OVS-2 tube and FLB rotating impactor. The rotating impactor was mounted on half-inch PVC pipe, inserted on the bottom of the motor. Using this mount, the horizontal rotating arm on the FLB sampler measured 23 inches from the ground. On the other vertical pole, the air sampling media was mounted at 38 inches from the ground. These heights served as an approximation of the breathing zone for the Working Party because most time was spent bending over to flip, straighten, and collect the uniforms. The rig was oriented with the

rotating impactor oriented on the left, and the taller pole for the area air sampler on the right. The reference point corner was placed at the randomly selected location. The number of sampling points per line was determined by using a ratio of one point per 16 uniforms, with a maximum of 10 samples per uniform line.

Air sampling rigs were placed using a stratified random sampling approach (52). Locations within strata were identified using a random-digits table (78), using feet as measurements. To allow for comparison between the first and last 25% of uniforms, the sampling points within zone C were a mirror of sampling points in zone A, (A=C). The sampling rigs were randomly placed in zone B along the downwind axis, less than three feet from the downwind axis and avoiding the spray plane. Distance from the downwind axis was systematically determined, a priori. The distances were based on commonly sampled distances in previously published work (21; 38; 39; 60; 129). Rig placement determinations were made, a priori, to include one, two, and three feet from the downwind axis. Five-ounce bottles of technical grade, 40% permethrin (NSN: 6840-01-334-2666), were mixed with 2-gallons of water and used for uniform treatment. An AG sprayer with a 20-gallon tank manufactured by PBM Supply & Manufacturing, Inc., was used (Figure 4.3). It was placed on a pull cart and powered by a vehicle battery.

On the first day of sampling, there were 120 uniforms for treatment, which were laid out in two parallel lines of 60 uniforms each, with 7.5 feet between the lines. The two lines of uniforms were considered one iteration because of their proximity. Twenty-five percent of 120 uniforms are 30 uniforms, or 60 feet long. The first two-digit number between zero and 60 was used for placement of rig within zone A, along the downwind axis (Table 4.1). Fifty percent of 120 uniforms are 60 uniforms or 120 feet. The first

three-digit number from zero to 120, from the last number used, determined placement of the first rig in zone B. The rigs were not moved between the morning (front) and afternoon (back) iterations. One-hundred twenty-seven ounces of permethrin and 50 gallons of water were used to treat both sides of 120 uniforms

On the second day of sampling, there were 63 uniforms for treatment, which were laid out in two parallel lines of 31 and 32 uniforms each, with 75 feet between the lines. The separation between lines allowed them to be treated as separate events; iterations three and four for treatment of the first side, and iterations five and six for the treatment of the backside of the uniforms. Twenty-five percent of 32 uniforms are eight uniforms, or 16 feet long. The first two, two-digit numbers between zero and 16, was used for placement of one rig within zone A, along the downwind axis. The rig placement in zone A was mirrored in zone C. Fifty percent of 32 uniforms are 16 uniforms or 32 feet. The next two, three-digit number from zero to 32, determined placement of the rigs in zone B. The rigs were not moved between the iterations. Forty-one ounces of permethrin and 16 gallons of water were used to treat both sides of 63 uniforms.

Sampling with OVS-2 media measured permethrin concentration in the air and was conducted following OSHA Method 70. Air was actively drawn onto the OVS-2 tubes by an air pump. OVS-2 tubes were purchased from SKC and designed with two filters in series to collect both vapors and aerosols. OVS-2 tubes are divided into a sampling section that consists of cleaned XAD-2 resin and a glass fiber filter that remains open to the environment and pointed downward to avoid particulates from settling onto the filter, and a backup section. Flexible plastic tubing (44 inches) was attached to the backup section of the OVS-2 tube. Two models of air pumps were used for sampling, the

AirChek® XR5000 personal air sampler pumps, model 210-5000 by SKC, Inc., powered by rechargeable lithium-ion battery packs, and the AirChek® universal sample pumps, model 224-44XR by SKC, Inc., powered by rechargeable nickel-metal hydride battery packs. The pump flow rates were set to 1.0 L/min and post-calibrated to ensure the flowrate drift did not change by more than ±5%. The pumps ran 5 min before spraying commenced and continued for 5 minutes after spraying ceased. After spraying, the pumps were turned off, and the OVS-2 tubes were collected, stored, and shipped in compliance with OSHA Method 70. The Navy Comprehensive Industrial Hygiene Laboratory (CIHL) in Norfolk, Virginia, analyzed the OVS-2 tubes and reported concentrations in micrograms per cubic meter (μg/m3) with a limit of quantification of 0.4 μg.

FLB impactors were powered with 4-AA batteries. The 6V DC 60 Hz motor provided rotation at 686 revolutions per minute (RPM). Two one-inch glass slides with MgO coating on one side were rotated to capture the permethrin droplets. Water-based formulations, such as technical grade permethrin used for mass spray treatment of uniforms, are more prone to evaporation than oil-based formulations. The MgO coated slides allow for measurement of the initial droplet impression, even after the droplet size decreases by evaporation. The MgO coated slides are mounted on a horizontal plane in a vertical position, approximately 7.3 inches apart, rotating at a velocity of 5.6 meters per second (m/sec) (21). The velocity of the FLB was higher than the wind encountered in the field during spraying (highest observed during sampling was 5.4 mph or 2.4 m/sec), eliminating the effects of wind speed. Collection of droplets with the impactors function under the principle that a small collection surface rotated at high speeds will capture droplets and demonstrate airborne concentrations effectively (21).

FLB impactors are a way of collecting and measuring droplet density, size, and distribution. The droplet density was calculated using MgO coated glass slides on the rotating impactor for analysis on DropVision®, Leading Edge, Fletcher, NC. DropVision® is a compound microscope with a built-in, hi-resolution digital imaging processor that electronically captures images of the glass slide after droplets are deposited on the surface (Figure 4.4). This technology can eliminate background objects, coalesced droplets, and nonqualified droplets. Microscopy measurements across the full traverse of the slide, including the edges, provided the droplet density per unit area. DropVision® can measure droplet sizes as small as one pixel (<1 micron). Having the volume of permethrin and particle sizes quantified around the spray event is important for determining dermal and respiratory risks.

4.2.2 Statistical Methods

Data were analyzed using Microsoft® Excel for Mac (version 16.37, copyright 2020, Redmond, WA) and STATA/IC 15.1 for Mac (STATA Corps LLC, College Station, TX) to obtain descriptive statistics and to perform inferential statistics and correlations.

4.2.2.1 Statistical methods for OVS-2 Tubes

There were thirty-six OVS-2 tubes submitted to the lab for analysis; all results were reported with values less than the LOQ. Statistical analysis was not conducted using the OVS-2 tube results.

4.2.2.2 Statistical Methods for FLB Impactor Slides

Comparisons between the location of the sampling points (zone and distance from the downwind axis), and the average span and the droplet density was made using four one-way ANOVA tests. Each one-way ANOVA compared one categorical independent variable with one dependent variable with the assumption of similar variances across the groups. This was verified using Bonferroni's posthoc test to adjust for multiple comparisons.

NECE provided a DropVision® analysis of the FLB rotating impactor slides. The measured droplets' density and size were determined by analyzing each slide in DropVision®, programmed to stop statistical processing at 500 droplets per slide. One hundred photographs were taken of each slide, and the number and size of droplets were tabulated. The FLB impactors captured particles on the slides, but they were not measured using radius and diameter, as those would be inaccurate for particles that are not perfect spheres. Volume distribution (Dv) are values based on relative size to other droplets on the slide. Droplet volume was measured using volume-weighted size distribution, Dv0.1, Dv0.5, and Dv0.9. The Dv values represent the diameter, "D," with volume distribution weighting, "v," and the percent of the sample below the particle size expressed as a decimal. The relative Dv values work on the assumption that the particle shapes among the captured droplets are similar in shape and composition (71). Dv0.5 may be referred to as the median particle size by volume, or volume median diameter (VMD). It represents the maximum particle diameter, in which 50% of the sample volume is below that diameter (38). DropVision® analyzes each slide as a data set. The Dv0.5, Dv0.1, and Dv0.9 are included in the output, along with the number of droplets, or

droplet density. However, using a relative scale of size, central values such as mean, median, or the average particle size, does not accurately represent droplet distribution. Therefore, the span (Equation 1) was used to quantify the distribution width and indicate the size and shape of spray droplets.

$$\text{Span} = \frac{(Dv0.9 - Dv0.1)}{Dv0.5} \qquad (1)$$

Dv0.9 = 90% of sample is below this diameter with volume weighting
Dv0.1 = 10% of sample is below this diameter with volume weighting
Dv0.5 = Median particle size by volume; 50% of sample is below this diameter with volume weighting

Span is a way to quantify the distribution width that captures the distribution represented by the Dv0.1, Dv0.5, and Dv0.9 data (71). Since there were two slides per FLB impactor, the two values for each Dv0.1, Dv0.5, and Dv0.9 were averaged for each sampling point diameter volume (Figure 4.5). The averaged values were used to find the span value for each sampling point.

4.3 RESULTS AND DISCUSSION

4.3.1 OVS-2 tubes

The values below LOQ from the OVS-2 tubes were not surprising. The air sampling pumps were calibrated to a flow rate of 1.0 L/min ±5%, as recommended by OSHA method 70. However, due to the short uniform spray duration, and as a result, short sampling times, the recommended air volume of 60 L was not met for most samples. The volume of air during each of the iterations was between 17.80 L and 79.2 L (mean: 49.05 L).

The CIHL-Norfolk target analyte recovery (123.6%) in the laboratory control sample (LCS) exceeded the quality control (QC) requirements of 80-120%. The high percent of analyte recovery is expected to result in an overestimation of the actual sample concentration. Permethrin in 100% of samples were <LOQ may indicate the high bias identified in the lab also applied to the samples. Because the analyte was not detected, no additional action is required. The lab reading exceeding the QC indicated high quantitation bias, and sample values are likely an overestimate of the concentration, but results are considered valid. The results obtained were all non-detect, indicating values below 0.4 μg/sample, even with the high bias.

Due to 100% of the values reported below LOQ, this study suggests that permethrin in the air around a spray treatment may not present an adverse health risk. When urine samples are analyzed, there will be further indications of permethrin exposures through measured metabolites, and the isomeric ratio will reveal the route of exposure.

4.3.2 FLB Impactors

The overall means and statistical error measured at Dv0.1= 7.40 (0.49), Dv0.5 = 13.63 (0.97), and Dv0.9 = 28.12 (2.72). The number of droplets per slide ranged from zero (11 slides) to 512 (9 slides) droplets with an average of 147 drops per slide and a standard deviation of 180.7. The size distribution, Dv0.1, Dv0.5, and Dv0.9, of permethrin spray around the uniform mass spray event could have a direct influence on the spray properties, such as the efficacy of delivery. The overall Dv0.5 (Dv0.5= 13.63 μm) was small, indicating a droplet size that more closely resembled spray used from a ULV sprayer (5-25 μm) (130). ULV droplets remain in the air column and move to the

intended target, such as crops or the bodies of vectors, staying the air until the droplets evaporate. However, uniform treatment practices aim droplets toward a target for immediate descent. DropVision® analysis output includes histograms with the droplet size and densities per slide (Figure 4.6).

The first one-way ANOVA was conducted by comparing the average number of droplets per slide from each FLB impactor (droplet density) to the zone (Figure 4.7). The one-way ANOVA indicated a failure to reject the null hypothesis (p-value = 0.9262) of similar droplet densities between zones the Bonferroni posthoc test confirmed significance for all comparisons. The null hypothesis was not rejected. The second one-way ANOVA compared the average span for each sampling location to the zone (Figure 4.8). Results from the one-way ANOVA suggest rejecting the null hypothesis of similar spans means in favor the alternative that the mean span is different between zones. The Bonferroni post hoc test shows comparison between zones A vs. B (p = 0.026) was significant, but the comparison between zones B vs C (p=1.00) and A vs. C (p= 0.051) were not significant. This suggests the span in zone A is different than B and C, but zones B and C are not statistically different.

Distance from the downwind axis was a second spatial factor measured to determine the dispersion of permethrin droplets in the vicinity of the uniform mass spray treatment event. A third one-way ANOVA was used to compare the droplet density and the distance from the downwind axis (Figure 4.9). The p-value (p=0.2056) suggested failure to reject the null hypothesis that the mean droplet density is similar, regardless of how far the FLB impactor was from the downwind axis. The Bonferroni post hoc test confirmed the one-way ANOVA, and the null hypothesis was not rejected. The fourth

one-way ANOVA was used to compare the span and the distance from the downwind axis (Figure 4.10). It also suggested failure to reject the null hypothesis, which was confirmed by the Bonferroni posthoc test.

FLB impactors measured droplet span and densities along the spray zones and within three feet from the spray line (distance). When comparing the droplet densities among the spray zones, there was no evidence to reject that the means were similar. The similarity in droplet density along the zone indicates there is no difference in number of droplets between the first and last 25% of uniforms and the middle 50%. However, when comparing the span among the zones, there was evidence that zone A was statistically different than zones B and C, but zones B and C were not statistically different. When comparing zones A and C, the *p*-value was 0.051. Differences in the mean span (distribution width) between the zones, despite no difference in the number of droplets, indicated the sizes of the droplets vary by zone.

Differences in the droplet size distribution could be attributed to behaviors of the Applicator such as starting or stopping the spray more frequently which may cause spray bursts. There may also be indications that the Applicator reached more uniform saturation, causing residual spray to be reflected from the wet uniform back into the air. Perhaps the AG sprayer pressure was not consistent for the duration of the spray line. Another potential cause of the different sized droplets could be perhaps the AG sprayer was not properly sealed after refilling of the tank with solution, which could cause a difference in pressure from before and after the refill. The cause of the difference of droplet sizes between the zones may be relevant to the potential exposures faced by personnel during a uniform spray event that warrants further investigation.

The distance from the downwind axis included 0, 1, 2, and 3 feet, which was established a priori based on the assumed decay of droplets as the distance from the spray source increases. The one-way ANOVA tests compared the distance to droplet density and the span failed to reject that the means of both droplet measurements were the same among all distances from the spray source. This indicated that within three feet from the spray source, the permethrin spray and droplets did not have decay in size due to evaporation or when descending toward the ground. Knowing that permethrin persists in the air similarly up to 3 feet around the spray source is an important characteristic to note for the protection of personnel during a spray event. An investigation into distances beyond 3 feet could help quantify the area around the spray source that poses increased exposure risk to personnel during a spray event, as the amount of permethrin is expected to diminish at some distance from the spray area.

4.4 Conclusions and Recommendations

Multiple variables influence a mass spray treatment of uniforms with 40% permethrin. Weather, personal behaviors and nuances in Applicator techniques influence the spraying which can lead to further dispersion of droplets. More frequent training and familiarization with the equipment used during a spray may help reduce the variability of spray during a mass treatment. Specific Standard Operating Procedure across the DoD for small jobs (where no Working Party is used) and large jobs (where uncertified Working Party personnel are used) as well as more clear instructions to provide further measurable elements to reach adequate saturation level of uniforms, and emphasis on adequate time for uniforms to dry before handling and thus reduce exposure variability among service members. The distances off the spray line used in this study showed

permethrin persists in the air similarly up to 3 feet around the spray source. An investigation into air sampling at distances beyond 3 feet could help quantify the area around the spray source that poses increased exposure risk to personnel during a spray event.

4.5 ACKNOWLEDGEMENTS

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4.6 DISCLOSURES

The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.

This research protocol was reviewed and approved by the USUHS institutional review board (IRB) (USUHS.2019-032) in accordance with all applicable Federal regulations governing the protection of humans in research.

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Figures

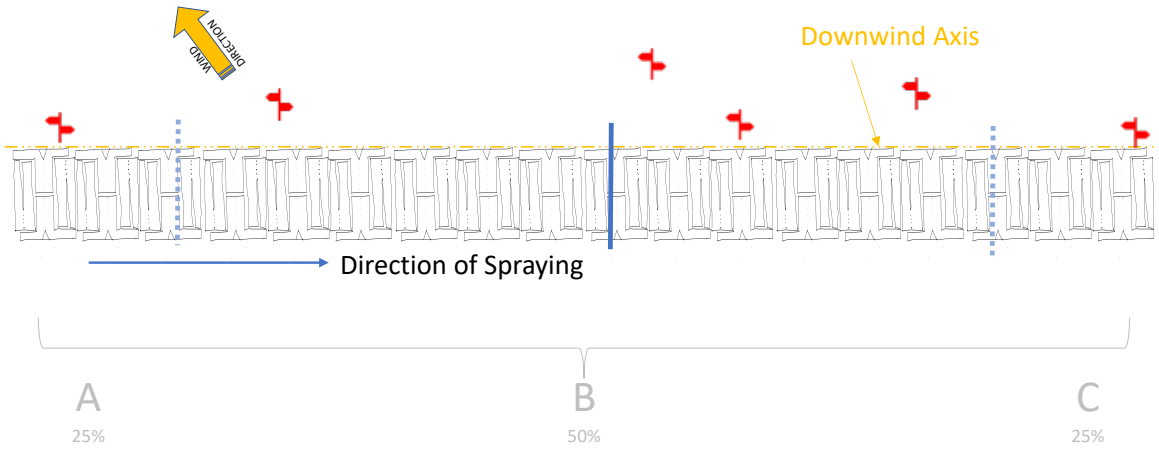


Figure 4.1. Site set-up, with uniforms in-line

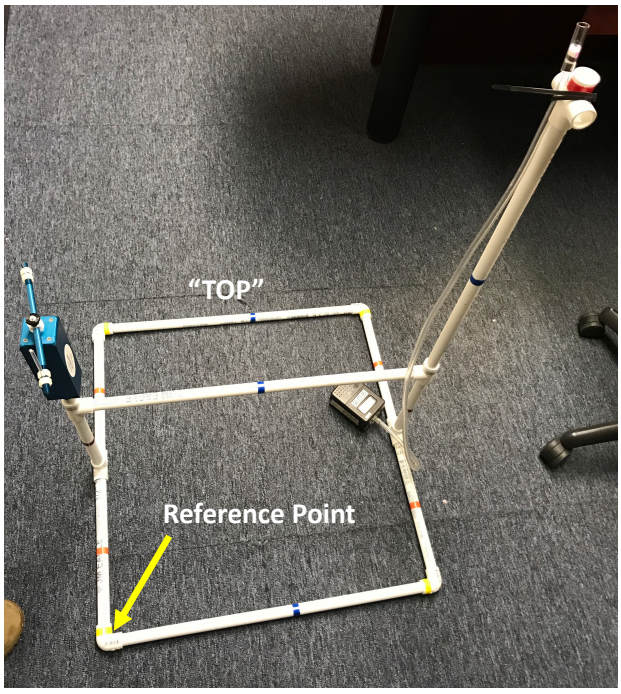


Figure 4.2. Air Sampling Rig Assembly



Figure 4.3. AG Sprayer

Table 4.1. Sampling Rig Placement

Day	Iteration	Zone	Rig	Location (feet)	Distance from downwind axis
1	1 and 2	A:	1	21	0
		B:	2	104	1
			3	69	1
			4	95	2
			5	120	2
			6	80	3
			7	13	3
		C:	8	21	0
2	3 and 4	A:	1	6	0
		B:	2	20	1
			3	41	2
		C:	1	6	0
2	5 and 6	A:	1	6	0
		B:	2	20	1
			3	41	2
		C:	4	6	0

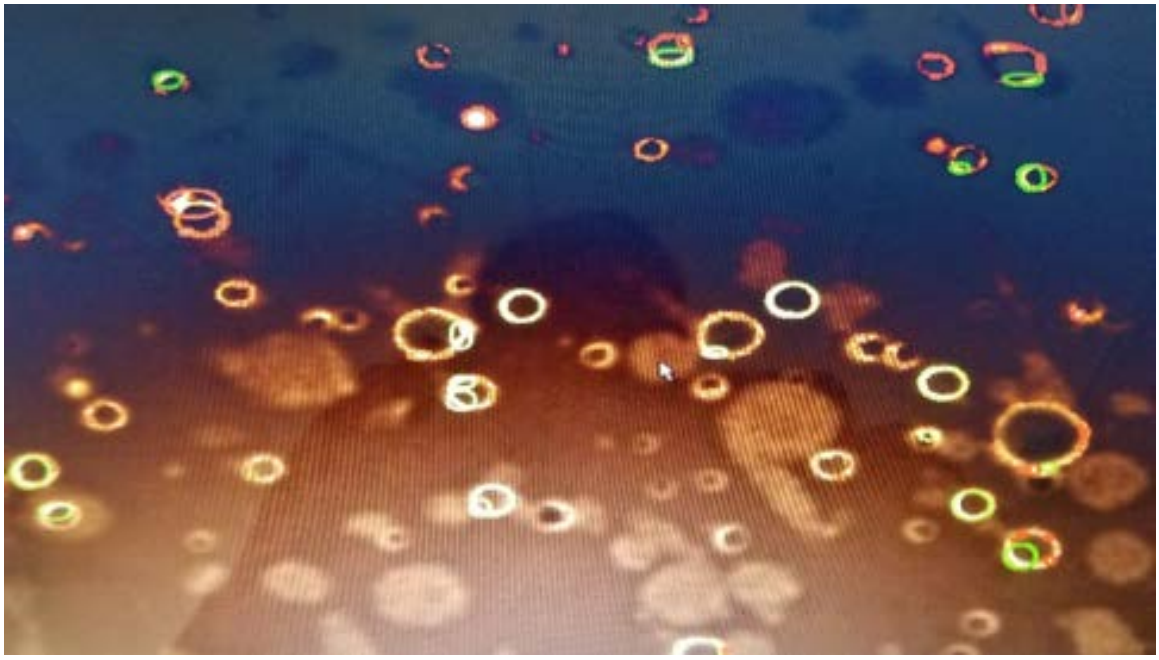


Figure 4.4. Slide on DropVision®. Valid droplets are discerned from background noise and highlighted in green; droplets in red are not included in analysis.

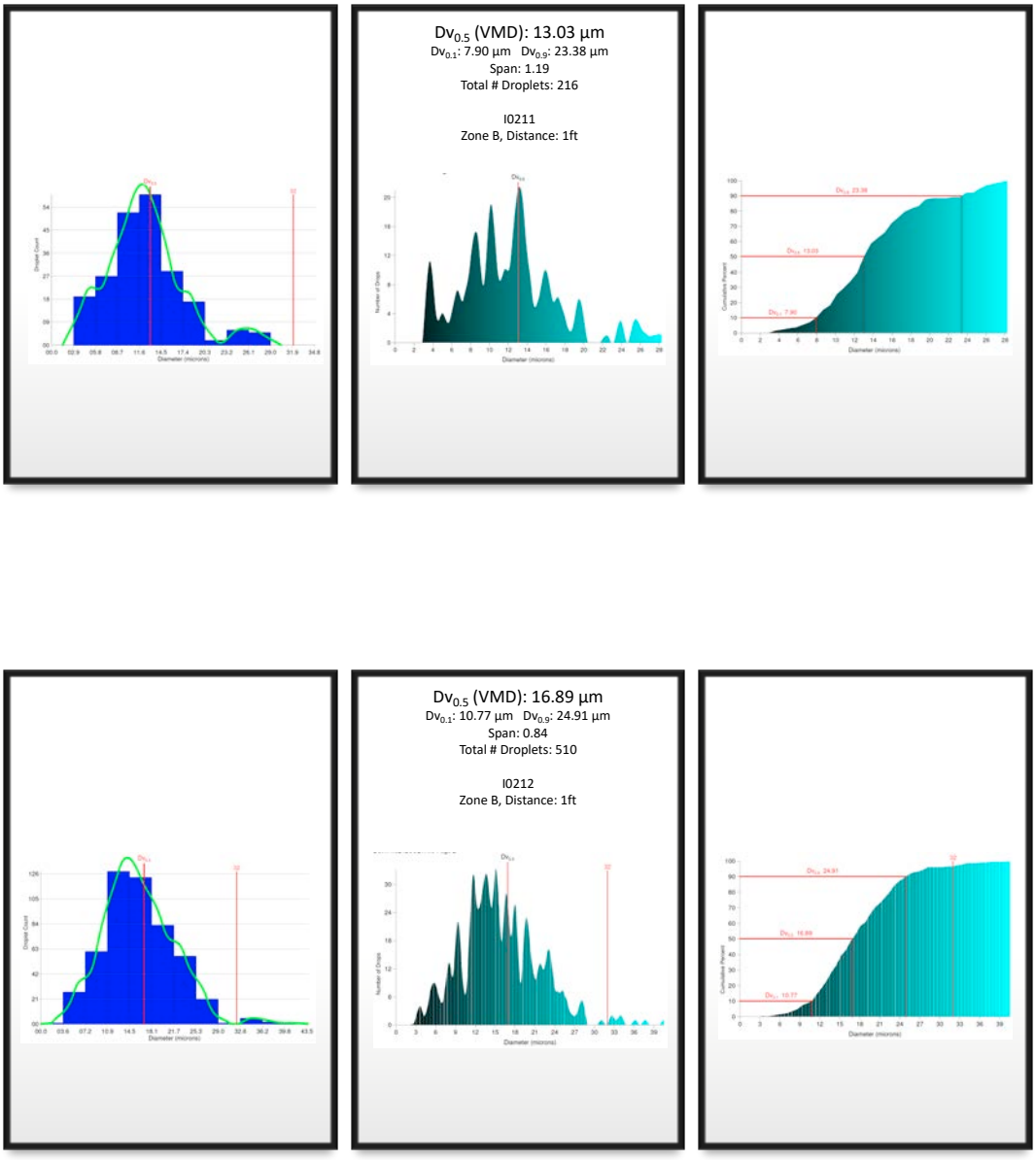


Figure 4.5. Two slides collated during iteration 2, rig 1. Co-located slides were averaged for that sampling point

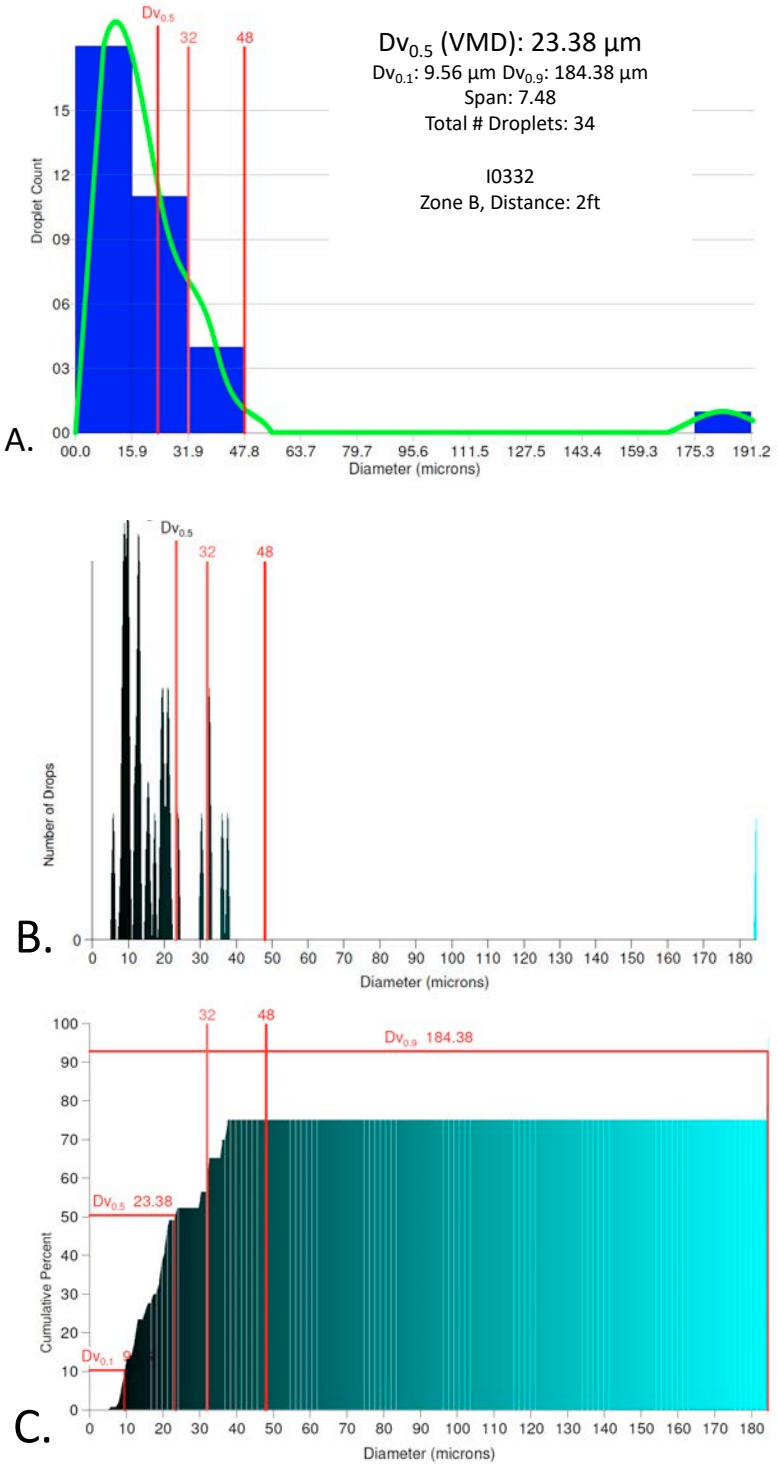
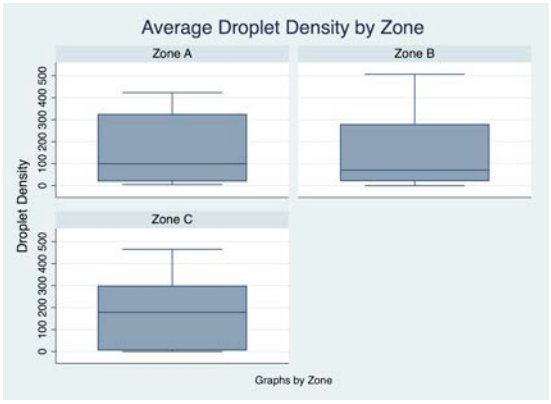


Figure 4.6. DropVision® output with A) particle size distribution, B) span by droplet density, C) cumulative droplet size. Markers signifying 32 and 48 are inherent to DropVision® with no consequence on the permethrin dataset of this study



Summary of avg_nmb_drps

zone	Mean	Std. Dev.	Freq.
A	162	176.16782	6
B	156.825	164.59066	20
C	187.66667	179.58498	6
Total	163.57813	164.17433	32

Analysis of Variance

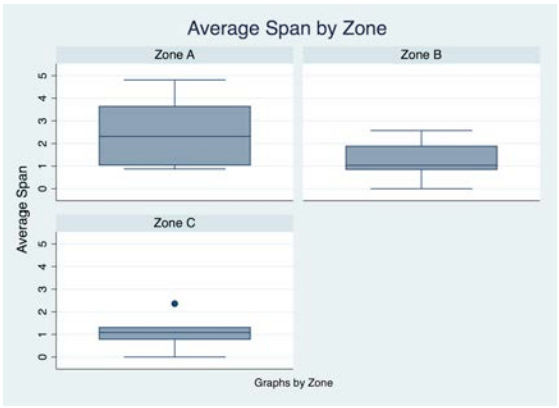
Source	SS	df	MS	F	Prob > F
Between groups	4408.58385	2	2204.29193	0.08	0.9262
Within groups	831140.971	29	28660.0335		
Total	835549.555	31	26953.2114		

Bartlett's test for equal variances: chi2(2) = 0.0768 Prob>chi2 = 0.962

Comparison of avg_nmb_drps by zone
(Bonferroni)

Row Mean-Col Mean	A	B
B	-5.175 1.000	
C	25.6667 1.000	30.8417 1.000

Figure 4.7. One-Way ANOVA comparing the droplet density to zone



Summary of avg_span

zone	Mean	Std. Dev.	Freq.
A	2.50	1.67	6
B	1.25	0.70	20
C	1.10	0.77	6
Total	1.46	1.05	32

Analysis of Variance

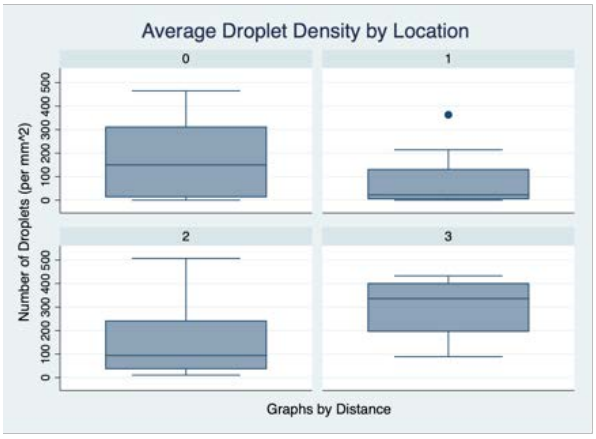
Source	SS	df	MS	F	Prob > F
Between groups	8.09033388	2	4.04516694	4.45	0.0206
Within groups	26.3371089	29	.908176169		
Total	34.4274428	31	1.11056267		

Bartlett's test for equal variances: chi2(2) = 7.6089 Prob>chi2 = 0.022

Comparison of avg_span by zone
(Bonferroni)

Row Mean-Col Mean	A	B
B	-1.25 0.026	
C	-1.39 0.051	-0.15 1.000

Figure 4.8. One-Way ANOVA comparing the span to zone



actual distance	Summary of avg_nmb_drps Mean	Std. Dev.	Freq.
0	174.83333	170.13515	12
1	85	133.135	8
2	157.8125	169.74202	8
3	298.5	149.87217	4
Total	163.57813	164.17433	32

Analysis of Variance

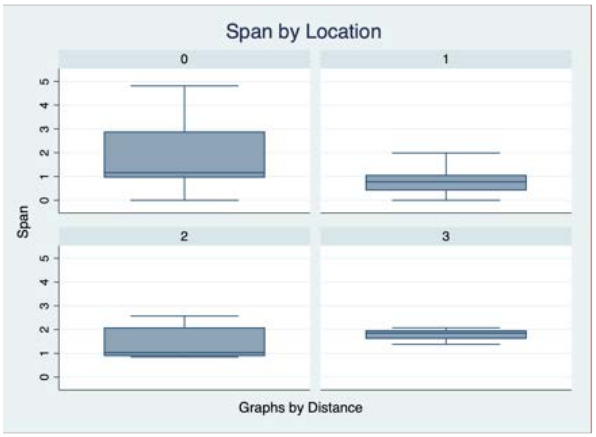
Source	SS	df	MS	F	Prob > F
Between groups	123997.919	3	41332.6398	1.63	0.2056
Within groups	711551.635	28	25412.5584		
Total	835549.555	31	26953.2114		

Bartlett's test for equal variances: chi2(3) = 0.5407 Prob>chi2 = 0.910

Comparison of avg_nmb_drps by actual distance (Bonferroni)

Row Mean-Col Mean	0	1	2
1	-89.8333 1.000		
2	-17.0208 1.000	72.8125 1.000	
3	123.667 1.000	213.5 0.224	140.688 0.964

Figure 4.9. One-Way ANOVA comparing droplet density to distance from downwind axis



actual distance	Summary of avg_span Mean	Std. Dev.	Freq.
0	1.80	1.44	12
1	0.81	0.61	8
2	1.43	0.71	8
3	1.79	0.29	4
Total	1.46	1.05	32

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	5.22818617	3	1.74272872	1.67	0.1958
Within groups	29.1992566	28	1.04283059		
Total	34.4274428	31	1.11056267		

Bartlett's test for equal variances: chi2(3) = 11.3892 Prob>chi2 = 0.010

Comparison of avg_span by actual distance (Bonferroni)

Row Mean-Col Mean	0	1	2
1	-0.99 0.254		
2	-0.37 1.000	0.62 1.000	
3	-0.01 1.000	0.98 0.766	0.36 1.000

Figure 4.10. One-Way ANOVA comparing span to distance from downwind axis

Chapter 5: Permethrin: A multi-route exposure assessment of human exposure during field treatment of military uniforms

ABSTRACT

For 30 years, the US military has relied on permethrin impregnated into field uniforms for protection against biting arthropods and exposure to arthropod-borne diseases. Most personnel have worn uniforms treated with permethrin and sprayed products containing permethrin. A mass spray treatment with 40% permethrin is a technical service provided by the Department of Defense preventive medicine units. During these operations, there is potential for personnel conducting the spray (Applicator) as well as supporting workers who are not actually spraying but are in the area and have contact with the uniforms during operation (Working Party, uncertified personnel who handle uniforms, including positioning on the ground, flipping, and removing uniforms) to be exposed to permethrin. Though the expected risk to population is low because they should wear protective gloves, an exposure risk assessment has not been conducted previously.

This multi-route exposure assessment occurred during a routine field mass spray treatment of uniforms. Urine samples were collected to measure metabolites *cis*-DCCA and *trans*- DCCA. Two-way repeated-measures Analysis of Variance compared the mean difference and interaction between the role (Applicator or Working Party) to the DCCA measured in urine. The second repeated-measures ANOVA compared isomer (*cis*- and *trans*-) and time (baseline, 4-hr after the start of spraying, and 12-24-hr after the start of spraying) to understand if there was an interaction on the dependent variable.

Dermal exposures were measured using dosimeter gloves and a patch worn on the right forearm of study participants. Where data about dermal exposures comes from a routine uniform spray event, care is needed to estimate the mean permethrin measured in patches because there was a high percentage of censored data. A repeated-measures ANOVA showed the average dermal exposure as measured with patches and gloves was not statistically different (p-value= 0.8340) for the Applicators and Working Party.

Similar potential dermal exposures may be reason to establish training and PPE requirements for the Working Party during a mass spray uniform treatment. This study suggests the Working Party should be considered along with the Applicators in risk assessments and published guidance and that PPE requirements should be for all personnel.

5.1 INTRODUCTION

Most people are aware they can purchase un-restricted pesticides from a local store; restricted-use pesticides require a state or federal license or certification to obtain, and are not available for purchase by un-certified users. However, it may not be common knowledge that pesticides are not limited to insecticides and herbicides. Pesticides include any substance or mixture intended for preventing, destroying, or repelling any pest. It includes herbicides to control weeds and unwanted vegetation, fungicides to inhibit or kill mold and mildew, and disinfectants to inactivate or destroy microorganisms. Permethrin is a type of insecticide that can be applied as a lotion to treat scabies and lice and sprayed on clothing, including US military uniforms. In 1979, in collaboration with the US Department of Agriculture (USDA), the DoD began evaluating the use of permethrin for clothing treatments. Application to field uniforms

was initially assessed in 1984 and approved for factory treatment of uniforms in 1990. Permethrin treatment is now the standard vector treatment option for many uniforms across the DoD (Figure 5.1). The 40% permethrin concentrate used in the DoD is classified as Restricted-Use and can only be used by DoD Certified Applicators.

Exposure to permethrin is possible through ingestion, inhalation, and dermal routes of exposure (68). There is no consensus on which exposure route is most significant (57). However, ingestion of permethrin is believed to be the primary exposure route for the general population (29; 62). Toxicokinetic studies have shown that dermal exposures to pesticides contribute minimally, and inhalation or oral exposures contribute the highest opportunity for exposure (28; 72; 125). However, studies by Atabila, Phung, Sadler, Connell, and Chu (2019), MacFarlane, Carey, El-Zaemay, and Fritschi (2013), Vitali, Protano, Del Monte, Ensabella, and Guidotti (2009), Van Hemmen and Brouwer (1995), and Bradberry, Cage, Proudfoot, and Vale (2005) suggest dermal exposure may be a major route among agricultural pesticide applicators (11; 17; 81; 122; 125).

Permethrin is odorless and ranges from colorless to yellow and physical characteristics range from solid to an oily liquid. Permethrin is a broad-spectrum pesticide that targets pests, including those of interest to public health, such as mosquitoes and fleas. According to Chrustek et al. (2018), permethrin acts by interfering with brain functions and sodium channels of the sensory nerves of mammals and insects (23).

Human health effects to permethrin can be local or systemic. Health effects are dependent on the route of exposure, dose, and duration, as well as individual metabolic and excretion responses (112). Symptoms of acute exposures may include reversible

effects that subside after the exposure stops, such as runny nose or cough. Chronic exposures to permethrin may induce hyperexcitability and whole-body tremors. The impact of long-term acute exposures needs further investigation. Between 2010 and 2020, there has been an increasing number of studies that investigate the concern for possible male reproductive effects (57; 84).

Biological monitoring is a way to measure the absorption of a chemical of interest via multiple routes of exposure. Compared to measuring personal exposures to environmental concentrations of a chemical with only personal breathing zone, area air sampling, and dermal concentrations, biomonitoring is more expressive of the actual risk of adverse health effects, as it measures the absorbed dose experienced by a worker. Biomonitoring also accounts for interpersonal differences between individual absorption and metabolic rates.

Dermal exposure can occur through direct contact with a chemical, indirect contact with surfaces, clothing, or residues, and through the air. Skin exposure to a chemical will contribute to the overall body burden and can lead to localized responses. There is agreement among studies that dermal exposures depend on processes and individual behaviors and can have high levels of variability among individuals (11; 81; 107; 108; 110; 122).

Respiratory exposures to permethrin are challenging to accurately measure, due mainly to the lack of homogeneity in atmospheric concentration levels and individual differences. Variables, such as wind, sunlight, humidity, as well as droplet size and pressure of the spray, affect the behavior of pesticides. Each individual has different

inhalation and exhalation performance that affects the amount of permethrin delivered into the body as an exposure.

The US military has relied on permethrin for protection against biting arthropods and for prevention of arthropod-borne diseases since the 1990s. For almost 30 years, most personnel have worn uniforms spray-treated with permethrin. Without an assessment of potential permethrin exposures during a uniform spray, we cannot confidently assess exposure risk.

Uniform treatment is a technical service offered by Department of Defense (DoD) preventive medicine units, such as the Navy Environmental Preventive Medicine Units (NEPMU) (located both within and outside the US) and the Navy Entomology Center of Excellence (NECE) in Jacksonville, FL for units preparing for contingency operations. Field treatment of uniforms requires an Applicator to spray permethrin to uniforms. Field treatment also requires a Working Party to layout, flip, and collect uniforms that have been saturated with the concentrated permethrin solution. The Working Party are personnel assigned to assist by the unit requesting the uniform treatment and are typically enlisted personnel. It may be assumed that since the Working Party is just laying out uniforms and flipping them over, they do not have any significant exposure during these tasks. Though the expected risk to this population is low because: wearing protective gloves is expected and because participation in uniform spray events occurs on a non-routine basis, a risk assessment has not been conducted previously. This study sought to quantify Applicator and Working Party dermal, inhalation, or ingestion exposures from handling wet uniforms and working near uniform spray. It is essential to understand

potential permethrin exposure risks to personnel operating within the DoD, so adequate precautions are taken to protect their health and well-being.

5.2 Materials and Methods

5.2.1 Study Subjects

Sampling took place over two days, 11 March and 20 March 2020, sampling days one and two, respectively. This study enrolled participants from NEPMU5 and the USS *Comstock*, who requested the uniform treatment. Assigned personnel consisted of certified pesticide applicators (Applicators) and Sailors not trained in the uniform treatment process (Working Party). All supporting personnel were eligible to participate and deemed healthy by their unit, as indicated by assignment to this task. The lead researcher met with all eligible participants, described the study and potential risks, and then 13 of the 15 eligible participants signed the informed consent. The protocol and ICD were approved by the USUHS Institutional Review Board (IRB) before fieldwork initiation (IRB Number: USUHS.2019-032). Personnel disclosed if they were or might have been pregnant at the time of sampling, as they would not participate in the uniform spraying event. Personnel were asked to disclose if they took medications containing ibuprofen, or if they participated in spraying of uniforms during the previous three days so that circumstances could be recorded. There were no disclosures regarding pregnancy, use of ibuprofen, or spray participation. One participant left the study after the baseline sample was collected.

All subjects were assigned a random number that maintained subject confidentiality. During sampling, media was collected from individuals and identified using a standardized naming convention that supported confidentiality while permitting

differentiation of samples. The names of the study participants were not used to identify any samples collected during this study. Identifying numbers used on samples were for analysis purposes only.

5.2.2 Site Set-up

Sampling occurred during a routine field mass spray treatment of uniforms on Naval Base San Diego. Uniforms were laid out in-line by the Working Party, in a fashion to fit the most uniforms into available space and to minimize the ground exposed between uniforms (Figure 5.2). Each line was divided into three groups. The first 25% of the spray line (zone A) and the last 25% of the spray line (zone C) were identified as areas of higher exposure potential. Increased exposure potential may be present in the last 25% of the spray line, as the Applicator may have permethrin remaining and may over-saturate, to expend the mixed solution. It was assumed the middle 50% of the spray line (zone B) would have the most homogeneous spraying of uniforms.

Five-ounce bottles of technical grade, 40% permethrin (NSN: 6840-01-334-2666), were mixed with 2-gallons of water and used for uniform treatment. An AG sprayer with a 20-gallon tank manufactured by PBM Supply & Manufacturing, Inc., was used (Figure 5.3). It was placed on a pull cart and powered by a vehicle battery. Long pants and sleeves, gloves, a respirator, and eye protection were worn by the Applicators as prescribed by the pesticide worksheet in Technical Guide 14 (6).

5.2.3 Biological Sampling

Urine samples were collected to measure metabolites *cis*-DCCA and *trans*-DCCA. Urine samples from study participants were collected at baseline to establish if individuals had prior exposures. Samples were collected at a minimum of 4 hours post

the start of spraying. The Biomedical Instrumentation Center (BIC) at USUHS in Bethesda, Maryland, analyzed the urine samples. Sample storage and shipment protocols were established based on previously published work (71; 78; 129). Sample preparation protocol followed that described in Olsson AO, et al. (2004), with some slight modifications. Full laboratory analysis of urine is detailed in an email (S. Moran 2020, personal communication, 29 April 2020). The permethrin metabolite DCCA was further analyzed to separate and quantify the isomers, *cis* and *trans*-DCCA.

5.2.4 Patch Sampling

Potential dermal exposures were measured using dosimeter gloves and patch sampling. Patches were made from alpha-cellulose paper and backed with aluminum foil, as recommended by the WHO, EPA, and Organization for Economic Co-operation and Development (OECD) (106; 129; 130). Alpha-cellulose paper was purchased from University Products, Holyoke, MA. The aluminum foil backing prevented permethrin from being held against the skin of a study participant as well as prevented the paper from absorbing sweat from the study participant.

Dermal patches were placed on the underside of each individual's right forearm, half-way between the elbow and the bend of the wrist (Figure 5.4). The collection area was 4 inches by 4 inches, with approximately 2 inches above and below the sampling area for attachment. If the individual was wearing long-sleeves, the alpha-cellulose paper backed with aluminum foil was fixed on top of the clothing in the same location. Patches were affixed to each person before the start of uniform treatment and collected upon completion of each sampling iteration. The CIHL in Norfolk, Virginia, analyzed the total permethrin in the patch samples using OSHA Method 70 with a limit of quantification of

0.8 μg per sample. Censored data was handled to reduce bias, but not lessen the power of the study by replacing values below the LOQ with LOQ/√2, or 0.5657 μg/sample.

5.2.5 Dosimeter Glove Sampling

The second method used to sample potential dermal exposure to permethrin during a uniform spray event was dosimeter gloves. One-hundred percent cotton, 200 grams per square meter (g/m2) fabric weight, dosimeter gloves were purchased from Gaxcoo, West New York, NJ. The gloves were one size. The dosimeter gloves were worn by study participants over their protective gloves (Figure 5.4) and used to determine potential personnel exposure when they handled uniforms. The dosimeter gloves remained on participants for the entire iteration, even when not actively engaged in touching uniforms or handling permethrin.

ALS Laboratories in Salt Lake City, Utah, analyzed the total permethrin in dosimeter gloves using NIOSH Method 9204 (Draft) by desorbing the gloves in isopropyl alcohol for the initial dilution. Dosimeter gloves samples were shipped at -20°C to the lab. ALS is an AIHA-LAP (American Industrial Hygiene Association- Laboratory Accreditation Program) accredited lab that serves as the primary method development contract lab for NIOSH.

5.2.6 Statistical Methods- Urine

Due to the cease lab operations order issued in response to the COVID-19 pandemic, these samples are currently held in proper storage waiting for lab operations to resume so that the metabolite analysis can be performed. The following describes how data will be analyzed once it becomes available.

Two two-way repeated-measures Analysis of Variance (ANOVA) will be used to compare the mean difference and interaction between two independent variables on the dependent variable. The first repeated-measures ANOVA will compare role and time of sample collection to the total DCCA measured in urine. The second repeated-measures ANOVA will compare isomer (*cis*- and *trans*-) and time (baseline, 4-hr after spraying started, and 12-24-hr after spraying started) to understand if there was an interaction between the two factors on the dependent variable.

5.2.7 Statistical Methods- Dermal

Study participants wore dosimeter gloves and dermal patches for the duration of a spray treatment of uniforms, and descriptive statistics were used to measure the central tendency and variability of potential dermal exposures. A repeated-measures ANOVA was used to compare the mean difference between the Applicators and the Working Party versus the two measures contributed by each person, patches and gloves.

5.3 Results and Discussion

5.3.1 Urine

Descriptive statistics describe the absorbed exposure to permethrin measured through metabolites in the urine of study participants. A baseline urine sample identified if there was exposure before the start of the study. The second urine sample was collected at least 4 hours after the spray started. Study participants from day one donated a third sample at 12-24 hours after the start of spraying. Study participants from sampling day two were not available for the 12-24-hour urine sample donation. There were 29 urine samples collected; 7 from Applicators and 22 from Working Party. The samples were collected from the three sampling times, 13 samples were collected at baseline, 12

samples from 4-hours (one Working Party participant was lost back to their unit), and 4 samples at 12-hours after spraying started. Results will be summarized to understand the central tendency and variability of exposures (Table 5.1).

A two-way repeated-measures Analysis of Variance (ANOVA) will be used to compare the mean difference and understand if there was an interaction between the two independent variables, role and time of sample collection, on the total DCCA measured in urine (Table 5.2). Several assumptions are associated with two-way repeated-measures ANOVA. The first assumption met was the total DCCA was measured on a continuous level. The second assumption was the independent variable is categorical (Applicator and Working Party) and independent. Three personnel participated in both sampling days but were assigned new numbers and treated as a new subject. There were nine days between sampling, which is adequate time for all permethrin exposures resulting from a uniform spray event to be excreted from the body.

A second two-way repeated-measures ANOVA (Table 5.3) compared two independent variables, isomer (*cis*- and *trans*-) and time (baseline, 4-hr, 12-24-hr) to understand if there was an interaction between the two factors on the dependent variable. The ratio of *trans:cis* is suggestive of the primary route of exposure among each study participant. A ratio of *trans:cis* >1 suggests inhalation or ingestion as the primary exposure route while *trans: cis* <1 suggests dermal is the primary exposure route.

Permethrin was measured in three urine samples from each study participant at time points intended to measure absorbed permethrin through the course of its metabolism and excretion from the body. The 12-24-hour urine sample was not collected after the second day of spraying which may result in unquantified excretion of

metabolites. Also, the small number of uniforms treated and the separation of the uniforms into iterations resulted in a short spray time, and therefore exposure times that may not adequately represent a routine uniform spray event exposure.

Although results from urine sampling are not currently available, the data may provide additional insight into the questions asked in this study. This study showed potential dermal exposures between the Applicators and the Working Party were not significantly different. The Applicators wear respirators and have more guidelines on how to perform tasks and what PPE they should wear. Once data is available, comparing the absorbed permethrin between the Applicators and the Working Party may indicate a need for the Working Party to wear comparable PPE or receive training on the mass spray event. Urine samples may indicate if a particular exposure route prevailed during the process and if the exposure routes are similar between the Applicators and the Working Party.

5.3.2 Dermal

This study investigated if there was sufficient evidence to conclude a difference in the average potential dermal exposure based on the role during the mass spray treatment of military uniforms with permethrin. Twenty-six pairs of gloves were submitted to ALS Laboratory for analysis with a range of detected permethrin from 45 to 120,000 μg/sample, a mean of 15,958.77 μg/sample, and a standard deviation of 24,649.48. Out of the 26 samples, six were from Applicators and 20 from Working Party personnel.

Twenty-six patches were submitted to CIHL-Norfolk. Six patches were from Applicators, and 20 from Working Party. Seventeen of the 26 (65%) samples were reported below the LOQ (LOQ= 0.8 μg). Three of the six patches from Applicators were

below LOQ and 14 of 20 from Working Party. Censored data was substituted with LOQ/√2, or 0.5657 μg/sample to reduce bias and to prevent reducing the power of the study by otherwise removing the values from the dataset. Permethrin detected in the patches had a range of values between 0.5657 (LOQ/√2) and 45 μg/sample, a mean of 4.61 μg/sample, and a standard deviation of 10.84. Patch samples had a high percentage of censored data that was handled with replacement, which may lead to results that are higher than actual potential exposures. Also, the CIHL-Norfolk target analyte recovery (143.5%, 140.0%, 157.2%) in the laboratory control sample (LCS) exceeded the quality control (QC) requirements of 80-120%. Permethrin detected <LOQ in 65% (17/26) of samples may indicate the high bias was also applied to the samples, but the analyte was not detected, and therefore, no additional action is required. The lab reading exceeding the QC indicated high quantitation bias, and sample values are likely an overestimate of the concentration, but results are considered valid.

A repeated-measures ANOVA was used to compare the mean difference between the Applicators and the Working Party who contribute two measures, patches and gloves. The repeated-measures ANOVA (Figure 5.5) suggests there is a difference in the mean value of exposure when comparing the patches to the gloves ("type" *p*-value= 0.0119). When comparing the Applicators and Working Party, their average values, either the gloves or the patches, were not statistically different ("role" p-value= 0.8345). The interaction term suggests the average value of exposure is similar between the Applicators versus Working Party in patches as in gloves was not statistically different ("type#role" *p*-value= 0.8340). The Mann-Whitney U non-parametric test was also performed because the assumption of normality was not met. Results were similar to the

repeated-measures ANOVA, showing this parametric test was robust against departures from the normal distribution assumption in this study.

Patch samples provided no evidence to reject that dermal patch means were different between the Applicators and Working Party and suggest an alternative. Person *08*, who served on the WP on the second day of sampling, showed the highest value (45 μg/sample) and the third-highest value (8 μg/sample) of permethrin absorbed on a patch, during iterations 5 and 3, respectively. The reason for elevated levels of permethrin on the dermal patch by the same person may have been personal behavior. It was not noted that this person was outwardly negligent with the permethrin spray. Perhaps they handled uniforms in such a manner where saturated material was close to their patch, indicating potentially heightened dermal exposure. Person *06*, who served as the Applicator during iteration 5, had the second-highest value (36 μg/sample) of permethrin on the dermal patch, but the iteration three patch value was below the LOQ. There were nine patch values reported above LOQ, of which the two highest values occurred during iteration 5, even though it had only 32 uniforms per line and the shortest sampling time. Because of the fewer uniforms during this iteration, it took less time to spray each side. The high values of permethrin measured in patches may imply uniforms were more saturated when the Applicator instructed the Working Party to flip and collect the uniforms compared to the other iterations.

Dermal samples collected from the Working Party spanned both iterations 3 and 4 by moving between the lines. The dermal sample data reported for the Working Party during iteration 5, was the Working Party that functioned for both iterations 5 and 6. The

Working Party moved between the uniform lines, whereas Applicators for each iteration remained assigned only to their uniform line, therefore, to their iteration.

Though not required of the Working Party through an SOP or on the safety label, personnel participating in a uniform treatment typically wear nitrile gloves, so it is not expected the levels of permethrin found in dosimeter gloves in this study will represent actual absorbed dose. However, since PPE is not required for the Working Party, this study sought to investigate potential exposure without consideration of PPE. The findings that potential dermal exposures in gloves were not significantly different between the Applicators and the Working Party were informative to the importance of allowing uniforms to dry before handling.

This study showed similarities in potential exposures between the Applicators and the Working Party. Applicators may have direct hand contact with permethrin, with transfer to the patches through indirect contact. The Applicators may also havecontact permethrin residue from the hose touching their arm as they maneuvered through their tasks. As they sprayed the uniforms, permethrin in the air may drift, which may present both an inhalation and dermal exposure source during this process. The Working Party has different responsibilities than the Applicators, but exposures were shown to be similar. The most considerable contribution of dermal exposure to the Working Party likely occurred during flipping uniforms before the uniforms were dry.

Flipping or collecting saturated uniforms is avoidable exposure. Working Party personnel can wear short sleeves, leaving their arms, neck, face, and even their hands exposed. The patch sampling in this study showed that dermal exposure is possible on the Working Party's forearms, equal to the potential exposure faced by Applicators. The

statistically significant difference in means between gloves and patches can likely be attributed to an indirect and direct transfer. During data collection, it was the Working Party who were observed directly touching the saturated uniforms before the uniforms had time to dry completely. Indirect permethrin transfer for the Applicators could have occurred from the wand or transfer during the mixing of the solution. The mean for the gloves was much higher for both roles.

During the repeated-measures ANOVA comparison between the gloves and patches, the difference in the two sample sizes likely affected the test. There were 26 patches and 26 pairs of gloves (total of 52 samples). Twelve of those samples were from Applicators, and 40 were from Working Party. Using G*Power and assuming a large effect size between the groups (Cohen's d = 0.8), there would need to be at least 26 samples from each group (42). The actual effect size was calculated in STATA and found the average exposure between roles differed by 0.06 standard deviations (Cohen's d = 0.06), which would require 4,362 samples from each group to find a significant result. Dermal sampling for this study was underpowered, even to detect a large effect size. Despite a small sample size and low power, this investigation provides valuable information regarding potential dermal exposures to permethrin in an understudied population.

5.4 Conclusions and Recommendations

Findings from the urine samples have the potential to provide invaluable information. Not only will the results show absorbed dose from individuals, but potentially the route of exposure. Absorption is influenced by factors that include route, duration, and extent of exposure; and further influenced by the rate of uptake by the

body, which varies by individual. When the current COVID-19 pandemic restrictions are lifted, further discussion on findings from the urine sample analysis will be added. Results will direct recommendations on the way forward with this line of research that will provide the necessary knowledge for the protection of our service men and women from exposures during mass spray treatment of uniforms with 40% permethrin concentrate. The samples are stored frozen at the lab for analysis. Pyrethroid metabolites are shown to remain stable when stored at -21°C for more than a year (78).

The average dermal concentration for Applicators and Working Party, regardless of patch or glove measurement technique, was not statistically different. The similar potential exposures may be a reason to reconsider the training and PPE requirements during a mass spray uniform treatment. If the goal is maintaining exposure as low as possible, this study suggests that long sleeves and pants and protective gloves should be the minimum required PPE. Most importantly, controls for the Working Party should be considered in the same risk assessment and in the same SOLs as the Applicators.

Service members have many occupational and environmental exposures, and characterization is difficult because of the transient nature of a military career. For example, accurately establishing similar exposure groups (SEGs) and understanding exposure profiles proves difficult when personnel move to new duty stations every 2-4 years, deploy to combat zones, are tasked with jobs outside of their career field, and have duties that range across various environments.

However, short term exposure assessments can fill in some of the knowledge gaps to better understand potential exposures in specific scenarios. The field treatment of uniforms is one such scenario that has taken place in the military since 1990 and

permethrin application techniques and uniform material has evolved since that time. Gaining an understanding of potential exposures that may occur during uniform treatment with permethrin is highly relevant to preventive medicine units currently conducting uniform sprays. Also, there is future relevance as new products become available for use on uniforms and as the safety label on uniforms is reconsidered for re-treatment of factory pre-treated uniforms, increasing the need for field uniform treatment evolutions.

5.5 ACKNOWLEDGEMENTS

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5.6 DISCLOSURES

The opinions and assertions expressed herein are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University or the Department of Defense.

This research protocol was reviewed and approved by the USUHS institutional review board (IRB) (IRB Number: USUHS.2019-032) in accordance with all applicable Federal regulations governing the protection of humans in research.

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FIGURES

Uniform Permethrin Treatment Matrix 12/4/2019

Uniform	Field Method			Factory	
	Aerosol	IDA	2-Gallon	InsectShield	Warmkraft
Marines					
Marine Corps Combat Utility Uniform (MCCUU)					
Marine Corps Tropical Combat Uniform (MCTCU)					
Flame Retardant Combat Ensemble (FRCE)					
Enhanced FRCE					
Nomex					
Navy					
Navy Working Uniform (NWU II) (desert)					
Navy Working Uniform (NWU III) (green)					
Nomex					
Blue Coveralls					
Improved Flame Resistant Variant Coverall (IFRV)					
Air Force					
Air Force Operational Camoflage Pattern (OCP)					
ABU (Airman Battle Uniform)					
RABU (Ripstop ABU)					
Nomex Flight Suits					
FR ABE (Airman Battle Ensemble)					
Army					
Operational Camoflage Pattern Army Combat Uniform (OCP-ACU)					
Improved Hot Weather Combat Uniform (IHWCU)					
Fire Retardant ACU (FR ACU)					
Army Combat Pant (ACP)					
Army Combat Shirt (ACS)					
Army Aircrew Combat Uniform (A2CU)					
Nomex					
Joint Special Operations Capable					

Grey - Not Evaluated
Amber - Under Evaluation
Red- NOT Approved
Green - Approved for Treatment

Approved for Treatment means that the garment treated by the company has been tested to ensure that the garment is in compliance with the EPA labeled initial treatment concentration of 0.52% w/w. Second, the factory treated garments are then laundered IAW AATCC Method #135-204 and subsequent samples are taken at established time points based on the Service and garment. These samples are then tested for permethrin residual in the fabric and also sent to the USDA CMAVE laboratories for bite protection testing. In order to ensure that the garment is in compliance with the EPA labeled initial treatment concentration EPA label-required permethrin concentration in the garment and also provide the required % of bite protection at established time points.
For example, in the case of a factory treated ACU, a garment must have 0.52% w/w +/- 10% permethrin in the fabric after initial treatment prior to laundering. The garment must also provide 90% bite protection after 50 launderings. Failure to meet either or both of these criteria means the garment is NOT APPROVED for treatment by that method or company.

The 6 oz aerosol spray may be applied to uniforms not previously treated by the factory, IDA or 2 Gallon Sprayer. May not be applied to uniforms containing Flame Retardant.
Impact on infrared (IR) not known, so may not be suitable for combat or hostile environments.

Figure 5.1. AFPMB uniform Permethrin Treatment Matrix

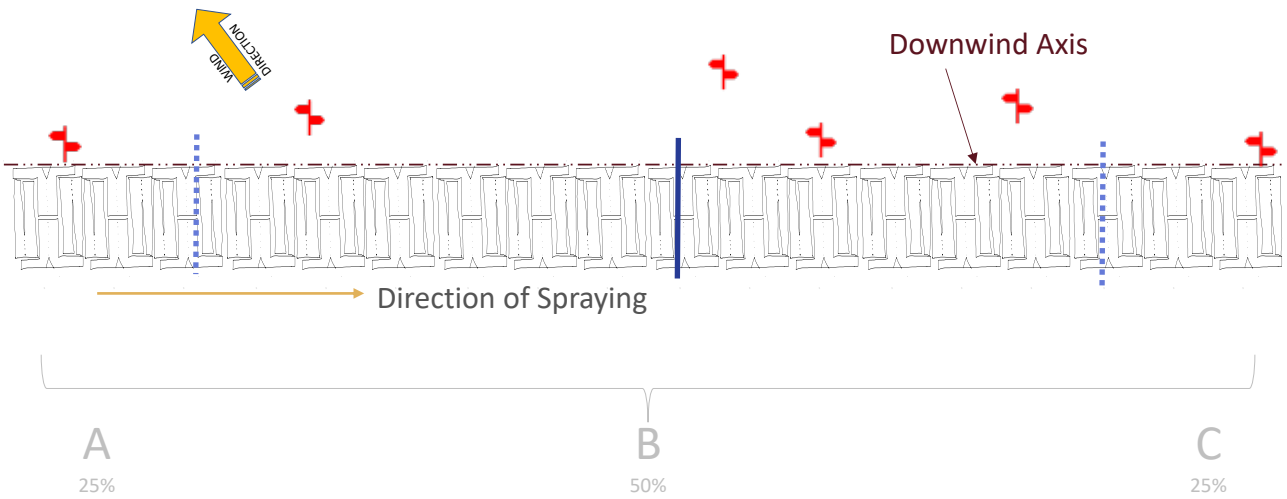


Figure 5.2. Site set-up, with uniforms in-line



Figure 5.3. AG Sprayer pulled behind the Applicator



Figure 5.4. Dermal patch on right forearm constructed of alpha-cellulose paper; 100% cotton dosimeter gloves worn over the protective gloves

Table 5.1. Summary of DCCA measured in urine

	Role	OBSERVATIONS (n)	MEAN	MEDIAN	ST. DEV.	MIN.	MAX
App	Baseline	3					
	4-hr	3					
	12-24-hr	1					
WP	Baseline	10					
	4-hr	9					
	12-24-hr	3					
TOTAL	n_{base}= 13 $n_{4\text{-}hr}$= 12 $n_{12\text{-}24\text{-}hr}$= 4	29					

Table 5.2. DCCA in urine to time and role

TWO-WAY REPEATED MEAURES ANOVA			
	App	WP	Total
Baseline			
4-hour			
12-24-hour			
Total			

Table 5.3. DCCA isomers in urine over time

TWO-WAY REPEATED MEAURES ANOVA			
	Cis-	Trans-	Total DCCA
Baseline			
4-hour			
12-24-hour			
Total			

Number of obs =	52	R-squared	=	0.1804	
Root MSE =	17772.8	Adj R-squared	=	0.1292	

Source	Partial SS	df	MS	F	Prob>F
Model	3.337e+09	3	1.112e+09	3.52	0.0218
role	13942982	1	13942982	0.04	0.8345
type	2.158e+09	1	2.158e+09	6.83	0.0119
type#role	14030710	1	14030710	0.04	0.8340
Residual	1.516e+10	48	3.159e+08		
Total	1.850e+10	51	3.627e+08		

Figure 5.5. Dermal repeated-measures ANOVA

Chapter 6: Conclusions and Future Studies

Mass spray treatment of uniforms with 40% permethrin presents inhalation and dermal exposure risks for Applicators and Working Party personnel. Dermal, inhalation, and total exposure routes were investigated for Applicator and Working Party personnel. Exposures may be reduced through PPE, training, and distancing. Conclusions and future studies for pesticide treatment of uniforms and discussion of limitations and lessons learned for the present investigation are presented in this chapter.

6.1 Biomonitoring and Analysis

Biomonitoring for permethrin metabolites in urine samples is a means to detect overall absorbed dose of permethrin by an individual, grounded on independent absorption, metabolic, and excretion patterns that have proved useful for measuring pesticide exposures (129). There are concerns that results may underestimate the total biological burden since there are trace amounts of metabolites excreted in feces. Previous studies measured urine from subjects with chronic exposures at much higher doses than faced by subjects in this study (78).

6.2 Dermal Sampling and Analysis

This study showed there are potential exposures to permethrin by personnel participating in a field spray treatment of uniforms via multiple routes. Potential dermal exposures could be a relevant exposure route if PPE is not worn appropriately. Hands are a possible major route of entry for permethrin. While less, other dermal routes of entry, such as the forearm, also appeared to have contributed to the overall dermal exposure. Urine sample results will provide additional information useful in identifying the actual

dose absorbed by personnel will provide information regarding the dominant exposure route.

Patch sampling results had 65% of the samples reported below the LOQ. This high level of censored data and use of replacement may have resulted in lower dermal values than actually occurred and need to be considered. The patch sampling was valuable since Working Party is not required to wear long sleeves during a field uniform treatment event. Dermal exposure to the arm is possible without proper protocol that governs all personnel involved.

Dermal exposures are difficult to quantify because of intra- and inter-personal variation of absorption, metabolism, and excretion of metabolites. Dermal exposures measured using surrogate skin methods, such as gloves and patches, may over- or under-estimate dermal absorption, based on what was collected. The use of urine to compliment dermal sampling can provide a comprehensive understanding of factors influencing pesticide absorption. Urine sample results are not available at this time, limiting analysis critical to the full reach of this study. However, once available, the data will be useful in identifying the absorbed dose and identifying the route of exposure related to the dermal sampling completed during this study.

6.3 Air Sampling and Analysis

The OVS-2 tubes resulted in 100% of the samples to be below the LOQ and limited the use of data in any statistical analysis. This study set out to compare multiple routes of exposure and to understand the amount of permethrin in the air around spray activity. Without data from the OVS-2 tubes, comparisons between the OVS-2 and FLB impactors were not possible. Increasing the flow rate to 2L/min and increasing the

number of uniforms treated during each iteration would increase the sampling time and amount of permethrin sprayed during each iteration. It would also increase the total air volume sampled with a better likelihood of detecting permethrin in the air. Even if the samples from the OVS-2 tubes had permethrin values above the LOQ, the OVS2 and the FLB rotating impactor are point samples. They may not be an accurate picture of the pesticide plume, as it will likely not be homogenous throughout the sampling area.

There is an indication that meteorological conditions, especially humidity, influence permethrin spray. However, absolute pressure was measured for this study. Absolute pressure is not a good indication of weather changes; instead, it is more a function of altitude. It would have been more informative to measure relative humidity to understand if there was a correlation between changing weather on permethrin dispersion.

6.4 FUTURE STUDIES

Future studies should examine the relationship between permethrin in the air and personal air sampling on individuals. While air sampling results in the current study were less than the lab's LOQ, findings of future studies conducted for longer spray periods may indicate the need to require respirators for all personnel on-site, and indicate greater distancing of Working Party personnel from active spray applications. Longer sampling times through the treatment of more uniforms per iteration may provide a more accurate snapshot of real-world potential exposures, as a division of uniform lines into independent treatment groups is unlikely. Increasing the flow rate of the air pumps to 2L/min to compensate for the short sampling times may be better to capture permethrin in the air and remains inside OSHA Method 70 constraints.

FLB impactor measured permethrin droplet density and size around the spray area, which may indicate dermal exposure. If droplets are present on surfaces surrounding the spray area, there is potential for personnel to face dermal exposures through touching the surface, and indirect ingestion through the hand-to-mouth transmission. Future studies should increase the distance from the spay line beyond three feet, to determine the distance where droplets are detected and to detect droplet decay. Advisement for personnel to remain outside of that area during spraying unless wearing a respirator is possible based on the distances and droplet characteristics. Results detected on the rotating impactor during this study showed a statistical difference in the mean number of droplets along the zones of the uniform spray line. Understanding the cause of this difference could shed light on Applicator behaviors and mitigation to unnecessary potential exposures. DropVision measured the impression of the water-based droplet on MgO coated slides and can remove background interferences. Adding a fluorescent to the spray captured in the slides increases accuracy and takes out the question of background images. Though fluorescence is not practical for the mass spray treatment of uniforms, the characteristics of spray used for this treatment is not as well understood as ULV spraying. Future studies should investigate the spray characteristics employed in a uniform spray event to understand the large droplet characteristics and decay behaviors.

6.5 SUMMARY

This study investigated multi-routes of exposures, which holds relevance in understanding potential exposures and realized exposures to balance the required PPE and conduct a risk assessment. The potential dermal exposures were not significantly different between the Applicator and the Working Party, and yet only the Applicator is

required to wear PPE. The findings from this study confirm the importance of establishing a DoD standard operating protocol, with specific PPE prescribed for all personnel, a minimum distance for personnel to remain from the spray when not actively working, as well as the optimal uniform saturation level. An easily understood and consistently employable description of processes to reach this saturation level will help reduce variability in the treatment of uniforms, and potentially the potential to exposures.

If changes to permethrin safety label permit re-treatment of factory pre-treated uniforms, understanding potential exposures to all personnel becomes more relevant across the services as there will be increased demand for field uniform treatment evolutions. There is on-going research for new products with increased protection from disease vectors and decreased risk to service members. As new products become available, studies should investigate the potential multi-route exposures faced by all personnel involved during real-world uniform mass spray treatment.

Appendix A

MEMORANDUM FROM Correspondence with Dr. Sean Moran, Biomedical Instrumentation Center, Uniformed Services University of the Health Sciences

SUBJECT: Experimental Section, urine analysis for Permethrin/DCCA

(Paragraph listing chemicals used (and manufacturer); I should also detail the making of stock solutions (concentrations and solvents) and storage conditions.)

The sample preparation protocol followed that described in Olsson, A.O., et al. (2004) (99), with some slight modifications. To **2 mL** of human urine, 25 uL of the **yyy** internal standard (**xxx** ng/mL in **yyy(solvent)**, **xxx** nmoles) was added. Prior to Solid Phase Extraction (SPE) the sample was acidified with **1.5 mL** of ~175 mM acetate buffer (310 uL glacial acetic acid, 0.97 g of sodium acetate in 100 mL water, pH adjusted with HCl to **4.0**). An SPE cartridge (Oasis HLB 3 cm^3, Waters, Milford, MA) was pre-equilibrated with 1 mL of methanol followed by 1 mL of 1% acetic acid using a vacuum manifold. The sample was loaded onto the SPE cartridge using syringe pressure; it was subsequently washed with 1 mL of a solution of 5% methanol/1% acetic acid using the vacuum manifold and then dried for an additional 30 seconds on the manifold. To elute the sample from the SPE cartridge, 1.5 mL of methanol was pushed through the cartridge using a

syringe and collected. This elution volume was dried in a **Speed Vac (need to add make and manufacturer)** and reconstituted w/ 100 uL of **starting HPLC solvent** for LCMS injection. **(Note: since DCCA is not glucuronidated, the β-glucuronidase-catalyzed hydrolysis step of the published sample prep. procedure (Olsson, et al. (2004) was skipped. Also 2 mL of acetonitrile was not added to the methanol elution volume prior to drying.)**

The LCMS system consisted of an Agilent 1200 series HPLC system interfaced to a Turbo V ESI source on a SCIEX Q-Trap 4000 mass spectrometer; nitrogen and dry air for the mass spectrometer was obtained from a Peak Scientific (**location**) model **AG-11** gas generator. A YMC-Triart C18 HPLC column (**2 X 100** mm, **3** um) was used at a flow rate of 200 uL/min. with a temperature of 30°C. The HPLC buffers were A = 100% water and B = 100% acetonitrile, both acidified w/ 0.1% formic acid. The HPLC gradient started at **50%** B and ramped to 100% B in **10** min.; the column was held at 100% for **4** min. for flushing, and then ramped back down to the initial solvent composition (**50%** B) and held for 8 min. before starting the next injection. The mass spectrometer was operated in negative ion mode with the following Source/Gas parameters: CURtain gas (CUR) = **15**, **yyy** (IS) = -4500V, TEMperature (TEM) = **450**°C, nebulizer gas (GS1) =

40, desolvation gas (GS2) = 30. The Multiple Reaction Monitoring (MRM) transitions from 207.2 Da to 35.3 Da for the analyte (d_0-DCCA) and from 210.2 Da to 35.3 Da for the IS (d_3-DCCA) were monitored with the following Compound parameters: Declustering Potential (DP) = -50V, Entrance Potential (EP) = -10V, Collision Energy (CE) = -30V, Collision cell eXit Potential (CXP) = -5V. The injection volume was 10 uL. The LCMS system was controlled and the data was collected and analyzed using Analyst 1.7 software (SCIEX, Toronto, Canada).

Calibration standards and Quality Control (QC) samples were made from 2 mL of pooled urine that was filtered through a 0.2 um filter and spiked w/ the appropriate amount of analyte and IS and then processed as above for actual samples. The concentrations used for the calibration curve were 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 ug/mL DCCA; two QC concentrations were 0.075 and 1.5 ug/mL.

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