CHARACTERIZATION OF TRANSFLUTHRIN EMISSIONS OVER TIME IN AN ENCLOSED SPACE OVER A RANGE OF DISCRETE TEMPERATURES

by

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DEDICATION

I dedicate this master's thesis to my friends and classmates who helped me navigate the process, and to my loving girlfriend who supported me and kept me sane throughout. I couldn't have done it without you.

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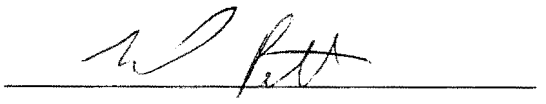
[Signature] 

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August 5th, 2014

ABSTRACT

CHARACTERIZATION OF TRANSFLUTHRIN EMISSIONS OVER TIME IN AN ENCLOSED SPACE OVER A RANGE OF DISCRETE TEMPERATURES

LT Merrick S. Pettebone, Masters of Science in Public Health, 2014

Thesis directed by: CDR Michael E. Stevens, Jr., Assistant Professor, Department of Preventive Medicine and Biometrics

The Armed Forces Pest Management Board (AFPMB) has expressed interest in using the commercially available pyrethroid, transfluthrin, as a spatial repellent in ongoing efforts to protect military personnel from vector-borne diseases. Transfluthrin is currently used in malaria endemic areas as an indoor residual spray. It is effective at significantly reducing the number of bites from mosquitoes. However, little is known about the actual amounts of transfluthrin in the air when effective repellency/bite reduction occurs, and if those concentrations are of concern to human health.

The purpose of this research is to characterize airborne transfluthrin concentrations, when a known amount of transfluthrin liquid is applied to fabric and then subjected to a range of discrete temperatures. A 17cm by 20 cm ($340cm^2$) piece of transfluthrin treated fabric was placed in a temperature-controlled, enclosed space simulating a tent or hut. Air samples were taken at multiple locations within the space

over an 8-hour day to track the movement of transfluthrin through the space. Experimentation was conducted at four temperatures (27-50)°C.

This research has demonstrated several relationships with the airborne concentration of transfluthrin. It increases as temperature and height are increased, it decreases as the distance from the source increases, and within the enclosed space used in this study, transfluthrin was well below the levels of current occupational exposure guidelines.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1: Introduction

Background and Significance

History is full of incidences in which diseases wiped out entire cities or defeated armies (8; 30; 33; 82; 88; 92). The role insects have played as vectors for these diseases was not understood until the late nineteenth century when a team led by Ronald Ross discovered mosquitoes could transmit malaria from infected patients to non infected patients (30). Many breakthroughs by other scientists soon followed, including the connection of mosquitoes to yellow fever transmission by U.S. Army Major Walter Reed and his team working in Cuba in 1900 (92).

Vector borne diseases have contributed more to worldwide morbidity rates throughout recorded history than all other causes combined (34). This held true until the early 1900's when the discoveries by Ronald Ross, Walter Reed, William Gorgas, and many others enabled public health officials to enact measures capable of reducing disease incidence rates (30; 34; 92). Malaria, dengue, and yellow fever, all of which are mosquito borne diseases, have seen a resurgence over the past three decades. This resurgence is at least partly due to a dramatic increase in vector resistance to currently used insecticides (86). As a result, the U.S. Armed Forces Pest Management Board (AFPMB) is investigating new insecticides and strategies, including the use of non-lethal spatial repellents (8; 82). Some promising work by Nicole Achee's group in 2012 showed repellency could be achieved at much lower concentrations than required for vector mortality (3). Additional work is desperately needed as the World Health Organization (WHO) estimates nearly half of the world's population is infected with at least one type

of vector-borne pathogen (34). Malaria is the most common and wide spread mosquito borne disease. In 2010 alone, there were 219 million new cases; 660,000 of which resulted in deaths (17).

Brief History of Insecticides and Repellents

The first attempts to repel insects were quite rudimentary out of necessity. Our ancestors most likely stuck close to cooking fires hoping the smoke would keep the insects away. In addition, our ancestors likely followed the example of the animals around them by spreading mud and dust over their skin. This layer of dirt would have provided a partial physical barrier to the insects (29).

The earliest recorded use of pesticides is in the time of Homer around 1000 B.C. when people would burn brimstone (sulfur) as a fumigant (29). In the first century A.D. Pliny the Elder recommended a wide variety of substances, including the use of gall from a green lizard (67). With the exception of sulfur, botanicals have been the type of pesticide in use the longest. Extracts from tobacco, chrysanthemums, legumes, and even citrus peels have been providing some level of deterrence to mosquitos for centuries (29). However, once it was discovered mosquitoes were not only annoying, but actually made people sick, interest in widespread application was heightened and synthetic forms of natural pyrethrins and other compounds were pursued.

Dichloro-diphenyl-trichloroethane (DDT)

Dichloro-diphenyl-trichloroethane (DDT) (see Figure 1.1) was first synthesized in 1874 (52), but it wasn't until 1939 that its insecticidal properties were discovered and DDT became the first of the modern synthetic insecticides (16; 24). DDT was found to be

a contact axionic nerve poison which shared a mode of action with pyrethrum and pyrethroids (83).

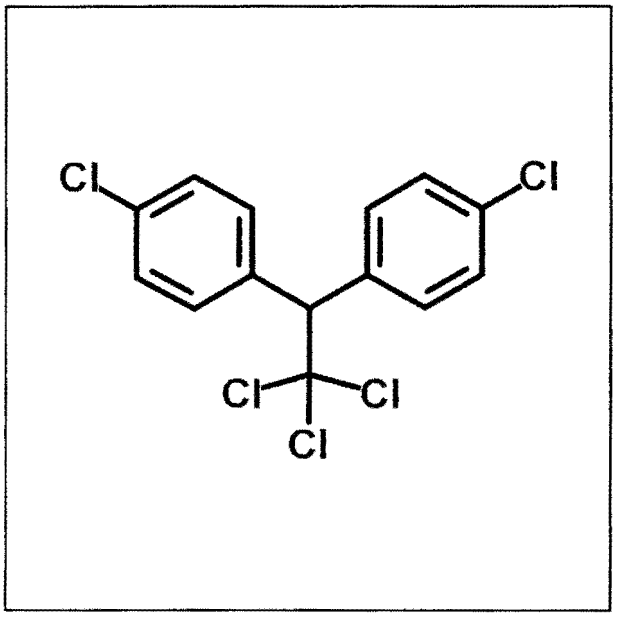


Figure 1.1: Structural Diagram of DDT (72).
Molecular Weight = 354 AMU (72). Melting Point = 107-110°C (78). Boiling Point = 260°C (78). Density = 0.99 g/cm (78). Vapor Pressure = 2.1x10-4 Pa @ 20°C (78). Solubility in Water = 0.001-0.004 mg/L (9).

In both vertebrates and invertebrates, DDT and pyrethroids interfere with the voltage gated sodium channels in the neurons (20). When a neuron is stimulated, the voltage gated sodium channel opens. This allows Na^+ ions to enter the cell (see Figure 1.2). The inside of the neuron becomes positively charged in relation to the space within the synaptic gap creating the "action potential" used to transmit an impulse to the next neuron (71). Each of these depolarization events usually lasts two to three milliseconds (39).

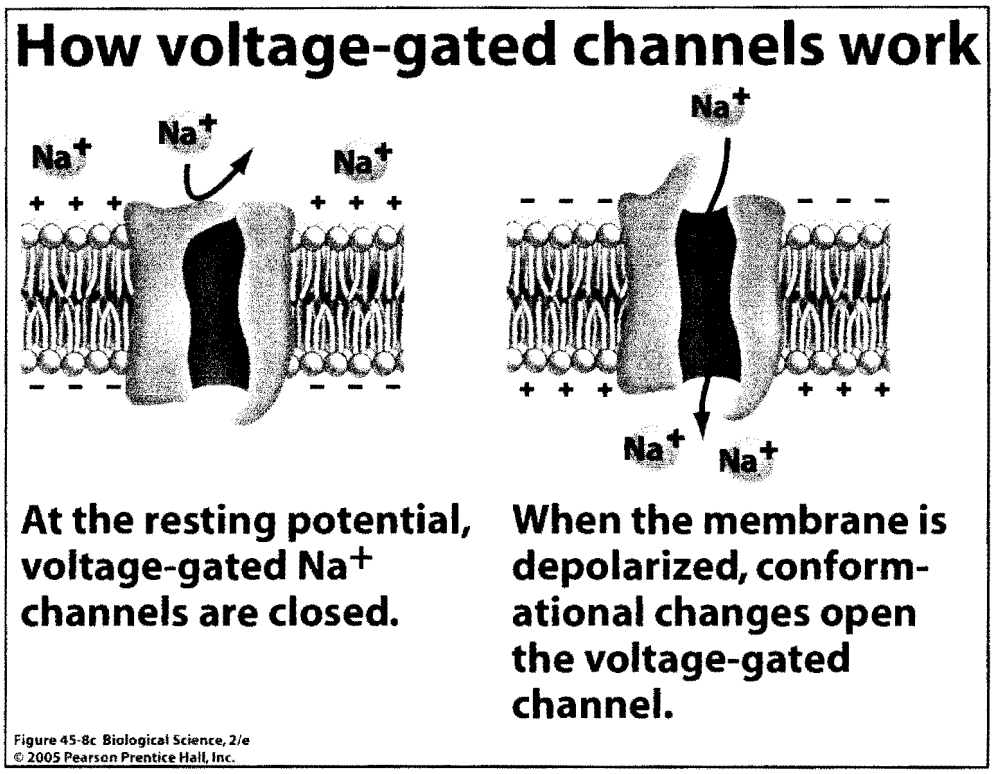


Figure 1.2: How voltage-gated channels work (1).

DDT, pyrethrins, and pyrethroids, bind to the voltage gated sodium channels causing the channels to stay open significantly longer (20). The length of time the sodium channels are open is dependent upon the chemical used, and ranges from 20 milliseconds up to 200 milliseconds, or even minutes (71). This persistent inward flow of Na^+ ions causes repetitive discharges of the neuron after only one stimulation (96). These repetitive discharges result in tremors, prostration, and seizures (71).

During World War II DDT was used to control malaria, typhus, body lice, and plague. It's effectiveness was shown when the incidence rate for malaria cases among the troops fell from 400,000 in 1946 to almost zero in 1950 (52). DDT was also used extensively on the home front. Small aircraft sprayed DDT over farm fields, forests, towns, and even entire cities as a method of pest control (16).

There were a variety of reasons for this wide spraying approach. DDT was being used in efforts to control a wide range of pests including spruce budworm, Dutch elm disease, typhus, spider mites, and malaria (16). Most importantly, DDT was cheap, long lasting, and effective (52).

By the late 1950's, problems with the heavy widespread use of DDT were becoming a concern. The persistence in the environment was considered by some scientists to present a problem. Given DDT's highly lipophilic nature, the concern was based on its potential accumulation in the fatty tissue of wildlife, which may lead to adverse health effects in wildlife and was postulated to lead to potential human health issues (24).

While the concerns about animal accumulation had some validity, no human deaths or cancers have been attributed to the compound (11; 66). The application of DDT has proven to be a tremendous benefit to human health worldwide. The Global Malaria Eradication Program that started in 1955 successfully eliminated malaria from Europe, North America, the Caribbean, and parts of Asia and South-Central America (84). Over the past decade, its use on the continent of Africa has slowly and judiciously been re-introduced due to the millions of malarial deaths associated with discontinuing the use of DDT in previous decades (46). However, as target insects have demonstrated DDT-resistance in the past in areas of extensive compound application (requiring higher and higher concentrations of DDT in the spray), the need to continue to expand the number of effective pesticide/repellent compounds exists (19).

Pyrethrum and Pyrethroids

The extract from crushed chrysanthemum flowers (pyrethrum) has proven to be very effective in repelling and/or killing insects. Pyrethrum actually consists of six insecticidally active esters collectively known as pyrethrins (see table 1.1) (71). These pyrethrin extracts are highly viscous liquids that are sensitive to oxidation, have high boiling points, and are difficult to store for long periods (38). As a result, historically pyrethrins have been expensive and supplies were dependent upon the growing season and the weather. Therefore, during World War II, one of the highest priorities was to discover a way to synthesize a pyrethroid (artificial pyrethrin). The first one was successfully produced in 1949 (85).

A potential limitation when using natural pyrethrum is its short shelf life when exposed to ultra violet light. The first synthetic pyrethroid synthesized specifically to have a reduced photosensitivity was permethrin in 1973 (45). Due to this development, synthetic pyrethroids now have half-lives of greater than 30 days, last longer than natural pyrethrum, and do not have to be applied as often (71). Unlike DDT, which can accumulate in the soil for decades, the synthetic pyrethroids will accumulate for only weeks or months before decaying (85). This allows farmers, or pest control personnel to spray once or twice a season without the concern of environmental buildup.

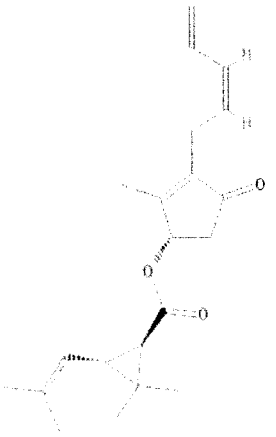
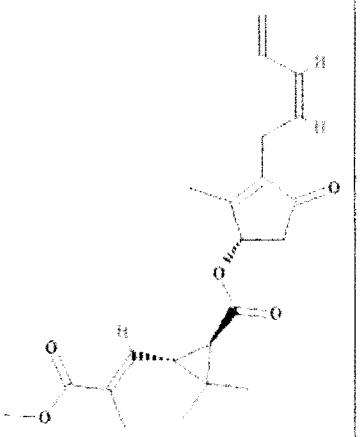
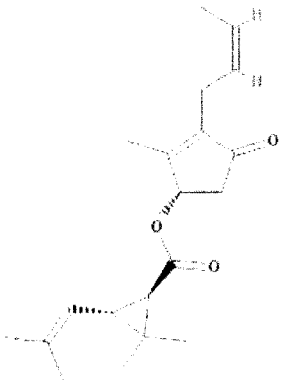
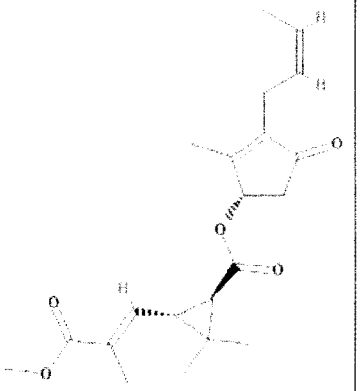
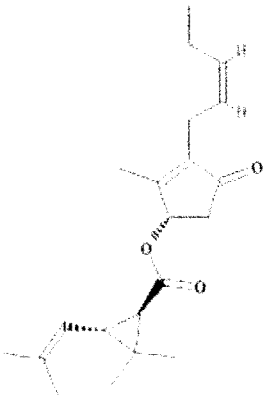
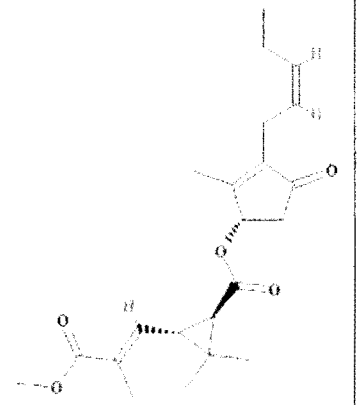
Pyrethrin I		Pyrethrin II	
Cinerin I		Cinerin II	
Jasmolin I		Jasmolin II	

Table 1.1: Pyrethrins.
The six insecticidally-active esters (pyrethrins) that make up pyrethrum (49; 71).

DDT, pyrethroids, and pyrethrins are all part of a group of neurotoxic chemicals that share a distinctive mode of action (discussed previously in DDT section) (71). Uptake of pyrethroids can occur in an organism either by absorption, ingestion, or inhalation. Typically, the most likely route is via dermal absorption. The highly lipophilic properties of pyrethroids allow for quick penetration of the skin. Diffusion along the cells of the epidermis is main distribution route to the central nervous system (CNS) once it is in the body (54).

There are currently over 3,500 pyrethroids registered with the U.S. Environmental Protection Agency (EPA) (87). Now into the fourth generation of synthetic pyrethroids, chemists continue to work on improving resistance to UV light and minimizing the effects on non-target species (29). These improvements have resulted in pyrethroids that are thought to be less toxic to birds and mammals than the pesticides they replaced, such as DDT (85). According to Elliot *et al.* in the Annual Review of Entomology, the new pyrethroids are also an order of magnitude more insecticidally active than DDT (22).

Spatial Repellents

The traditional methods of protecting people in their homes from vector borne diseases have focused on insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) (3). These methods have significantly decreased the disease burden in many countries; however, the effectiveness of some of these compounds is diminishing as insecticide resistance to these compounds increases (58). This increase in insecticide resistance is the direct result of both ITNs and IRS depending on delivering a lethal dose upon contact with the vector (3). Within every population there is genetic diversity. Within any given insect population, that diversity will allow a few individuals to be

resistant to any one pesticide. Once the insect population is exposed to an insecticide, the majority will die. However, those individuals with the innate resistance will survive and reproduce, passing on their resistance to the next generation. As time goes on, each generation within that insect population will have a higher percentage of individuals whom are resistant to the insecticide, eventually causing the insecticide to be all but useless (91).

The goal of a spatial repellent is not to kill the vector, but to repel it by irritating it or incurring some other non-lethal effect upon exposure to the compound. A benefit to this approach is even the most susceptible vectors live to reproduce. With all of the vectors surviving to reproduce and not just the naturally insecticide resistant ones, there is no resulting increase in the percentage of vectors who are resistant to the repellent (58). In addition, spatial repellents are effective at a much lower concentration than what is required to achieve a lethal exposure (3). This lower concentration, and a potentially different mechanism of action, allows for the possibility of using chemicals previously only used as pesticides as repellents (41).

Spatial repellents are able to achieve this goal of behavior modification by functioning in the volatile phase. Mosquitoes have been shown to avoid enclosures with an airborne concentration of DDT at only 51 parts per trillion as compared to the average lethal airborne concentration of 1% or 10 billion parts per trillion (57; 93). Such low requirements for the amount of insecticide/repellent in the air have several benefits. The lower amount of insecticide/repellent in the air results in lower exposures to the inhabitants of the structures and costs are reduced, as less chemical is needed per unit area. With direct contact between vector and insecticide/repellent no longer required, the

treated material can be anything from window curtains to a floor mat. This would increase use of the products, as they would now be multi-functional (58).

The spatial repellent properties of DDT were noted in the 1940's; however, little research on its repellency effects was conducted, as it was believed that vector mortality was the critical goal, not vector deterrence (58). In recent years however, as resistance to current insecticides has increased, so has interest in spatial repellency (3). Researchers have found several chemicals that have spatial repellency properties. One of the most favorable of these is transfluthrin (74).

TRANSFLUTHRIN

Transfluthrin is a 4th generation pyrethroid that has been in use since 1996 (74). Like the rest of the pyrethroids, it is an insecticidally-active ester compound (see figure 1.3) (71). Transfluthrin is used in commercial pest management around the world. It is also available in several countries in consumer products such as combustible coils (23). Manufacturers of transfluthrin recommend the commercial application of transfluthrin be conducted in unoccupied spaces due to the risk of adverse human health effects from inhalation or skin contact (23; 81).

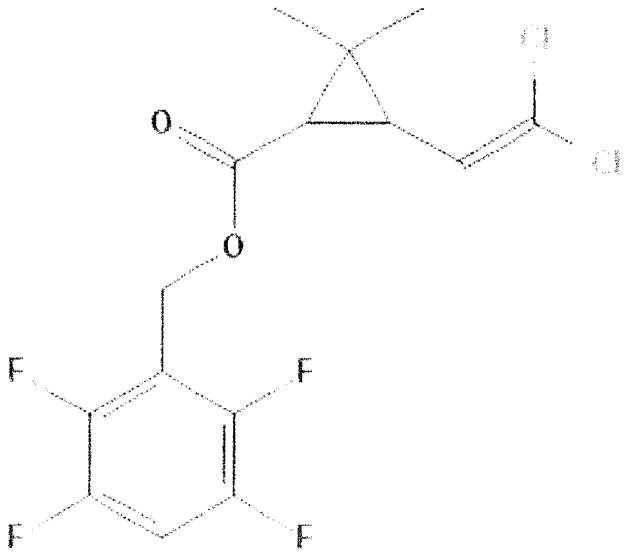


Figure 1.3: Structural Diagram of Transfluthrin (49)
Molecular Weight = 371 AMU (94). Melting Point = 32°C (94). Boiling Point = 250°C (74). Density = 1.5072 g/cm @ 23°C (74). Vapor Pressure = 9.0x10-4 Pa @ 20°C (94). Solubility in Water = 0.057 mg/L (94).

Human Health Effects

No studies have been conducted on humans to determine the signs and symptoms of an over exposure to transfluthrin. However, studies indicating human health effects resulting from exposure have been conducted with chemicals of the same class of compounds as transfluthrin (pyrethroids) (42; 47). Additionally, studies conducted on rats have shown profuse salivation, choreoathetosis, increased startle response, and terminal chronic seizures (71). The Material Safety Data Sheet (MSDS) for transfluthrin warns of potential skin and respiratory tract irritation (81). The list of reported signs and symptoms from exposed individuals is as follows: burning facial sensation, itchy face sensation, tingling face sensation, paresthesia, skin irritation, mucosal irritation, respiratory tract

irritation, headache, dizziness, nausea, epigastric pain, vomiting, anorexia, fatigue, twitching muscles, salivation, fluid in lungs, runny nose, and convulsion (70). These signs and symptoms are similar to typical nerve agent or organophosphorus poisoning (14; 32). The recommended treatment for transfluthrin poisoning is decontamination with soap and water, and supportive measures to treat the symptoms (2; 70).

Long-term exposures to humans can come from dietary, environmental, and occupational sources. The United States Environmental Protection Agency (USEPA) has evaluated long-term pyrethroid dietary exposure and found the estimated worst-case lifetime average daily exposure to be 0.117 micrograms (μg) per kilogram of body weight. This exposure is estimated by the EPA to not increase the cancer rates in the general US population (90). The major environmental route for pyrethroid exposure is through ultra-low volume (ULV) spraying techniques. These techniques result in aerosol clouds that are designed to stay airborne. The World Health Organization, along with others, has determined the short and long term risks to humans from ULV applications of transfluthrin to be negligible (36; 68; 94).

RESEARCH PURPOSE

Vector-borne disease has always had a devastating effect on armies during war. During the Mexican-American war, Ulysses S. Grant noted in his memoirs, "It was very important to get the army away from Vera Cruz as soon as possible, in order to avoid yellow fever, or vomito, which usually visits that city early in the year, and is very fatal to persons not acclimated". Of the 13,000 U.S. soldiers who died over the course of the Mexican-American War, only about 1,700 succumbed to combat related injuries. The other 90% died of disease, the majority being from yellow fever (88).

War may have changed over the years, but the threat of vector-borne diseases has not. The United States Department of Defense (DoD) Armed Forces Pest Management Board (AFPMB) has an initiative called the Deployed War-Fighter Protection (DWFP) research program. The purpose of this program is to "develop and validate novel methods to protect United States Military deployed abroad from threats posed by disease-carrying insects" (8). In the past the DoD has had a strong working relationship with the United States Department of Agriculture (USDA) Agricultural Research Service (ARS). This collaboration has produced many well-known insect-fighting products such as DEET and permethrin-impregnated fabrics (82). Currently the AFPMB (in conjunction with the Navy Medical Research Center (NMRC), the Walter Reed Army Institute of Research (WRAIR), and the USDA) is interested in researching and potentially deploying spatial repellent compounds such as transfluthrin to better protect deployed military personnel.

Recent efforts to investigate the use of transfluthrin for passive spatial repellency have been reported in the literature. In 2004, Argueta *et al.* studied novel transfluthrin-impregnated paper strips (7). In 2012, Ogoma *et al.* worked with novel transfluthrin-treated hessian (a type of canvas) strips (61). Both groups observed over 80% reductions in mosquito bites during their studies, yet no attempts were made to measure airborne concentrations of transfluthrin produced from their respective passive approaches (7; 61). Given the promising results reported by these researchers, and since previous studies have not characterized passively emitted transfluthrin in air, the goal, objective, and aims of this research are as follows:

Objective

The goal of this study is to characterize airborne concentrations of transfluthrin, a spatial repellent for mosquitoes, emanating from novel transfluthrin treated canvas strips at discrete temperatures, distances, and heights. The objective is to provide quantitative results of passively emitted airborne concentrations at various distances and heights from the pre-treated source material over a range of discrete temperatures. These data could then be linked with future entomologist-led vector behavior studies at different concentrations to assist in determining the most effective application method and concentration for vector repellency.

Aims

The following specific aims provide the framework necessary to accomplish the goal and objective of the study.

1) Over a range of discrete temperatures, determine the airborne concentration of transfluthrin at multiple distances and heights over time from a passive pre-treated source in an enclosed space using an established Occupational Safety and Health Administration (OSHA) sampling method (59).
2) Use descriptive and inferential statistics to assess differences, if any, in airborne concentrations over time and temperature at each of the various heights and distances from the repellent source material.

Chapter 2: Literature Review

As the concept of using passively emitted insecticides as spatial repellents is relatively new, there is a growing amount of research focused on the effectiveness of this approach on bite deterrence. However, there is not much in the literature on the topic of effective airborne repellent concentrations. Characterizations of airborne levels of actively emitted transfluthrin have been reported in the literature (55; 64; 69). In addition, efforts have demonstrated that passively-emitted transfluthrin from various substrates such as fabric or paper can repel mosquitoes, but the airborne concentrations were not measured (7; 61). While no one as of yet has tried to measure the airborne levels of passively emitted transfluthrin, several studies found DDT (a similarly sized molecule) was passively emitted in measurable levels (37; 57; 63).

Actively Volatized Transfluthrin

Combustible Mosquito Coils

Mosquito coils have been used for years to repel mosquitoes in attempts to provide for a disease free sleep environment throughout Asia, Africa, and South America (43). The acceptance of coil use, and likely effectiveness in reducing disease, is evidenced by the estimated 32 billion coils purchased every year (43). While the effectiveness in repellency when burning pyrethroid-treated coils has been known for years to be more than anecdotal, until 2001, no one had investigated how much active ingredient was actually being released into the air.

In 2001, Ramesh *et al.* analyzed the emissions from a burning mosquito coil containing 0.03% transfluthrin inside a 3.0 m x 3.5 m x 2.5 m study room. Air samples of

100 cm^3 were collected from various locations and times from within the study room and analyzed using gas chromatography-electron capture detection (GC-ECD). This work showed detectable airborne concentrations of transfluthrin in the low parts-per-billion by volume range (0.0134 ppm_v, or 13.4 ppb_v) (69).

As a result of work by Ramesh *et al.*, researchers established a 'ballpark' high end value for effective airborne repellency concentration of transfluthrin (69). This information can be used to formulate both more effective coils, and coils that expose the users to lower levels of airborne insecticide.

Kerosene Lamps

In 2002, Pates *et al.* studied two active methods of using kerosene lamps to increase the mass of transfluthrin emanated into the air. The first method was to mix the transfluthrin directly into the kerosene fuel for the lamp. The resulting reduction in mosquito bites was statistically insignificant regardless of how much transfluthrin they mixed into the kerosene.

The second method involved using the kerosene lamp to heat vegetable oil with 0.1% and 0.5% transfluthrin mixed into it. This approach resulted in bite rate reductions of 50-75% and >90% over a period of 4 hours, respectively. The researchers did not report airborne concentrations of transfluthrin for either method, but the second method importantly confirmed again that airborne transfluthrin effectively repels mosquitoes (64).

Electro-Vaporizers

In 2011, Nazimek *et al.* set out to determine the airborne concentration of transfluthrin from an electro-vaporizer. The researchers used Baygon brand electro-

vaporizers produced by S.C. Johnson, which uses both transfluthrin-containing liquid and gel inserts as the respective active ingredient materials. These electro-vaporizers are designed for indoor use and plug in like a small night-light. The manufacturer states that they are not to be used in non-ventilated rooms, or in rooms in which there are small children or very sensitive persons as skin irritation, itching, or coughing may result (10).

The electro-vaporizers were run for 6 hours, during which air samples were collected continuously at a rate of 0.5 L/min, for a total of 180 L per sample. The samples were then analyzed using a gas chromatograph (GC) with an electron capture detector (ECD) attached. The results indicated that the highest mean airborne concentration sample yielded an average airborne transfluthrin concentration of 2.4 $\mu g/m^3$ (0.00016 ppm_v, or 0.16 ppb_v) (55).

PASSIVELY EMITTED TRANSFLUTHRIN

Multilayer Paper Strips

In 2004, Argueta *et al.* studied the spatial repellency of 2,000 cm^2, multilayer paper strips, impregnated with 200 mg of transfluthrin (0.1 mg/cm^2). The study looked at the ability of these treated paper strips to repel *Aedes albopictus* mosquitoes under outdoor conditions. The researchers found the transfluthrin treated strips could repel over 80% of mosquitoes for over a month, relative to non-treated controls (7).

Hessian Strips

In Tanzania in 2012, Ogoma *et al.* evaluated the ability of transfluthrin-treated hessian strips (a burlap type material made from fine sisal fibers and locally used in cereal storage bags) to repel laboratory raised *Anopheles arabiensis* mosquitoes in an outdoor, naturally ventilated net tunnel measuring 60 m x 2 m x 2.5 m. The researchers

treated a 4.0 m x 0.3 m (12,000 cm^2) strip of hessian sacking with 10 ml of transfluthrin resulting in a mean concentration of 1.25 mg/cm^2.

After hanging the treated 12,000cm^2 strip in a perimeter around an individual seated in a chair, malaria-free mosquitoes were released. Trials were conducted on four consecutive nights each month, for six months, during which time the treated hessian strips were stored in uncovered bins at ambient temperature indoors (61). Their findings suggested freshly treated strips reduced the mosquito bite rate by >99%. And more importantly, they found that the treated strips provided >90% protection for the full 6 month length of the study (61).

Environmental conditions within the experimental netting enclosure were not discussed. However, the rearing conditions within the nearby insectary were listed as 28-29 °C, with 70-80% relative humidity (61). The insectary was constructed on a greenhouse frame with mosquito netting walls and a polyethylene roof (31). As both the experimental tunnel and the insectary had mosquito netting for walls, it can be assumed that the temperature and relative humidity were similar.

Passively Emitted Airborne Concentrations

No published study was identified which focused on measuring and characterizing airborne concentrations of transfluthrin from passively emitting sources. However, several studies have investigated the airborne concentration of passively emitted DDT (3; 13; 37; 57; 63). The DDT molecule is similar in structure and size to transfluthrin, resulting in similar volatilities and has demonstrated spatial repellency properties (23; 35; 72).

Passive DDT Emissions from Treated Fabric

In a paper by Martin *et al.* published in 2013, the airborne DDT concentrations emitted from treated polyester fabric in both laboratory and field environments were evaluated. As the passively emitted DDT was expected to be found in very low concentrations in the air, thermal desorption (TD) tubes were utilized to avoid solvent background affecting analytical detection at these low levels. The TD tubes were attached to low flow personal sampling pumps (100 mL/min lab, 200 mL/min field) to collect each of the samples. Sample analysis was conducted using a gas chromatograph (GC) paired with a mass spectrometer (MS) (57).

The GC-MS results yielded detectable and quantifiable amounts of DDT in the air both in the laboratory setting and in a field setting. The mean airborne concentration in the laboratory environment was approximately 29 $\mu g/m^3$, while the mean airborne concentration from the field setting was approximately 1.17 $\mu g/m^3$. The mass loading for both laboratory and field experiments covered the range of 0.09-2.0 g/m^2. The temperature and relative humidity in the laboratory were 26-31°C and 10-20% respectively. The field environment had temperatures of 22.4-29.2°C and relative humidity of 72-99% (57).

The determination of airborne DDT concentrations by Martin *et al.* was part of a larger study concurrently being conducted by Achee *et al.* (3; 57). The overall goal of the larger study was to quantify the chemical concentration in a treated air space that elicits a spatial repellent response in a vector population (3). This study showed a 70% reduction in the number of mosquitoes entering huts containing DDT treated fabric panels (2 g/m^2). The 70% reduction in the number of mosquitoes entering the hut, combined with the

measured 1.17 $\mu g/m^3$ of DDT in the air, suggests that DDT is an effective spatial repellent at ppt_v airborne concentrations (3; 57).

The higher concentration of 29 $\mu g/m^3$ found in the laboratory environment has several potential causes and implications (57). The laboratory conditions were measured at the start of each day. The temperature was between 26-31°C, and the relative humidity was reported at 10-20%. The mean field collection temperatures were all 25-26°C (57). With a potential temperature difference of up to 6°C, the difference in measured airborne concentration may be partially due to the higher laboratory temperature resulting in a higher vapor pressure for the DDT(13). In addition, while both laboratory and field experiments used identically treated fabric, the sizes of the fabric and the volume of the spaces were drastically different. The laboratory chamber had a volume of $0.0284m^3$, and a $0.3097m^2$ piece of treated fabric (57). With a mass loading of 2 g/m^2, this translates into 21.8g of DDT for every cubic meter of air in the chamber. The field experiments used huts with volumes of $50m^3$ and $19.8m^2$ of treated fabric (57). Using the same mass loading of 2 g/m^2, the huts had only 0.792g of DDT per cubic meter of air. As a result, the laboratory test chamber had 27.5 times the amount of DDT proportionately.

The mean relative humidities under field conditions were 83-86% (57). This is drastically different than the 10-20% relative humidity in the laboratory environment. The Tenax-TA used in the sampling is the preferred adsorbent for use in high relative humidity environments, but even it is affected by the high moisture content of the air (76). This results in lower concentrations being reported than actually exist. The implication being that the airborne concentration of DDT in the huts during the study were most likely higher than the 1.17 $\mu g/m^3$ reported (57).

Residual DDT Levels

The previously discussed studies evaluated airborne concentrations of DDT from freshly treated fabric sources (3; 57). Van Dyk *et al.* and Singh *et al.* both evaluated levels of DDT which may be bioaccumulated, found as metabolic by-products and/or exist as residual concentrations in the living environment. Van Dyk *et al.* collected leafy vegetables, chicken samples (muscle, fat, and liver), human serum, indoor air, floor dust, outside soil, and potable water. All samples were collected two months after DDT was used for indoor residual spray treatments. DDT was detected in all indoor air samples, with a mean concentration in such samples of 3.9 $\mu g/m^3$ (37).

Singh *et al.* evaluated residual airborne concentrations for eight months after DDT was used for indoor residual spray treatments. The indoor air samples revealed airborne concentrations of DDT that ranged from 1.0-14.6 $\mu g/m^3$. The samples also indicated that after an initial concentration decrease, at the end of the eight-month sampling period, the airborne concentration was measured at 5.9 $\mu g/m^3$ (63).

Comparison of Laboratory vs. Field Emission Rates of DDT

In his unpublished thesis completed at Uniformed Services University of the Health Sciences in Bethesda, Maryland, Brown compared DDT emission rates in the laboratory environment to emission rates in a field environment, as well as measured the air change rate using a CO_2 decay method. The laboratory work was conducted in thermal micro chambers over a range of temperature to try to predict what would be found in the field (13).

The fieldwork was conducted using two experimental huts in Thailand. The huts were equipped with screen windows and doors. These screens allowed for natural

ventilation, with a mean air change rate measured at 7.21 air changes per hour. Samples were collected at five locations within both huts. There was no significant difference in the results from the samples between the two huts. Within each hut, the results indicated airborne concentrations of DDT from 1.1-2.1 $\mu g/m^3$ (13). These findings were consistent with the airborne concentrations found by other researchers using similar mass loading of DDT (37; 63).

PHYSICAL AND CHEMICAL CHARACTERISTICS OF TRANSFLUTHRIN

Transfluthrin is a 4th generation pyrethroid that has been in use since 1996 (74). Like the rest of the artificial pyrethroids, it is an insecticidally active ester compound (figure 1.2) (71). It has a molecular weight of 371 g/mol, a density of 1.51 g/cm^3 at 23°C, a boiling point of 135°C at 0.1 mm Hg, and a vapor pressure of $9.0x10^{-4}$ Pa at 20°C (23; 74; 81). Table 2.1 compares the some of the physical and chemical characteristics of transfluthrin with several common insecticides and repellents. Many of the characteristics of transfluthrin are similar to the other insecticides listed in the table. However, while the vapor pressure of transfluthrin in lower than that of the insect repellent DEET, it is higher than those of the other insecticides listed.

	Transfluthrin	Permethrin	DDT	DEET
Empirical Formula	$C_{15}H_{12}Cl_2F_4O_2$[74]	$C_{21}H_{20}Cl_2O_3$[80]	$C_{14}H_9Cl_5$[72]	$C_{12}H_{17}NO$[73]
Molecular Weight (g/mol)	371.2[74]	391.3[53]	354.5[72]	191.3[73]
Appearance	Colorless crystals[74] or brown liquid[65]	Colorless crystals or yellow liquid[53]	Colorless crystals[9]	Light yellow liquid[79]
Density	1.5072 g/cm^3 at 23°C[74]	1.190-1.270 g/cm^3 at 20°C[80]	0.99 g/cm^3[78]	0.998 g/cm^3[79]
Vapor Pressure	9.0x10^{-4} Pa at 20°C[94]	2.87x10^{-6} Pa[53]	2.13x10^{-4} Pa at 20°C[78]	0.34 Pa at 25°C[79]
Melting Point	32°C[74]	34-39°C[25]	107-110°C[78]	-45°C[4]
Boiling Point	250°C at 760 mm/Hg[74]	220°C at 0.05 mmHg[25]	260°C[78]	111°C at 1 mmHg[79]
Solubility in Water	0.057 mg/L[74]	0.0055 mg/L[53]	0.001-0.04 mg/L[9]	2-3 mg/ml[15]

Table 2.1: Physical Characteristics of Several Insecticides / Repellents (4; 9; 15; 25; 53; 65; 72-74; 78-80; 94).

Transfluthrin is used in commercial pest management globally against flies, mosquitoes, and cockroaches. It is also available in several countries in consumer products such as combustible coils (23). Manufacturers of transfluthrin recommend the commercial application of transfluthrin be conducted in unoccupied spaces due to the risk of adverse human health effects from inhalation or skin contact, such as skin, eye, or respiratory tract irritation (23; 81).

Method of Action

Transfluthrin is considered an axonic poison (29). Even though it is a nerve poison, it is not a cholinesterase inhibitor like organophosphorus or carbamate insecticides (51). Transfluthrin, along with rest of the pyrethroids, is part of a group of neurotoxic chemicals that share a distinctive mode of action which was discussed in the DDT section of Chapter 1 (71). Transfluthrin can enter an organism in many different ways, but the highly lipophilic properties of transfluthrin allows for quick penetration of the skin. Diffusion along the cells of the epidermis is main distribution route to the central nervous system (CNS) once it is in the body (54).

Human Exposures

Research by Baygon suggests transfluthrin's action on mammals is 1,000 to 10,000 times weaker than on insects (74). However, human over exposure is possible through ingestion, inhalation, and dermal exposure. Review of the published scientific literature contains no reports of transfluthrin poisoning, and contained no reported clinical cases of acute pyrethroid poisonings of any kind until pyrethroids started being used in China in 1982 (26). Since then, overexposures to pyrethroids have been reported from occupational exposures, accidental exposures, and suicide attempts around the world (2; 5; 21; 26; 56; 75).

Occupational over exposures to pyrethroids are usually the result of not complying with safe handling techniques while spraying or during handling of the liquid pyrethroids, resulting in significant dermal absorption (26; 75). These exposures typically result in burning or itching sensations of the face, which can be accompanied by

dizziness (26). Symptoms usually disappear on their own within 24 hours, and all disappear within 2 days (2; 75).

Reported cases of accidental over exposure were mostly due to ingestion of pyrethroids, generally resulting in digestive related symptoms. Symptoms included epigastric pain, nausea, and vomiting. Some of the ingestive poisonings resulted in temporary comas (26).

The most severe over exposures were suicide attempts in which people intentionally consumed pyrethroids. The literature did not contain any references to successful suicide attempts involving pyrethroids, but two unsuccessful tries were discussed as case studies (2; 21). Initially, one of the patients presented with only mild throat pain and epigastric discomfort. After receiving a gastric lavage, the patient was transferred to intensive care for monitoring. During the first 24 hours the patient's condition seemed to improve, but then she developed a cardiac conduction disturbance. She was provided with supportive care, and after 3 days, her cardiac issues resolved themselves. At the 4-week follow up, there were no signs or symptoms of cardiac issues or any other sign of her pyrethroid ingestion (21).

The other case study present in the literature involved a 20 year old man who presented with abdominal pain, vomiting, dizziness, convulsions, altered mental state, elevated heart rate, increased respiratory rate, semi-dilated pupils, hypoxia, and pulmonary edema. The patient was placed on a ventilator and given supportive care. His condition gradually improved, and after four days he was weaned off the ventilator. His remaining complaints of mild headache, dizziness, and fatigue all improved and he was discharged from the hospital on the 6^{th} day (2).

The complete list of symptoms of overexposure to transfluthrin include burning, itchy, or tingling sensation; parasthesia; skin or mucosal irritation; respiratory tract irritation; headache; dizziness; nausea; epigastric pain; vomiting; anorexia; fatigue; twitching muscles; salivation; fluid in lungs; runny nose; and convulsions. All of which are consistent with neurotoxin poisoning. There is no antidote for transfluthrin poisoning. The theoretical lowest lethal oral dose of pyrethrum is 750 mg/kg in children and 1000 mg/kg in adults (56). Treatment involves decontamination (such as washing of the skin and gastric lavage) and symptomatic and supportive care (70). Human experience and some experimental work conducted on human volunteers and a guinea pig model show that non-lethal effects of pyrethroid poisoning are reversible and result in no permanent change (5).

Exposure Guidelines

Within the United States, there are three places to turn for exposure limit regulation and/or guidance in regard to transfluthrin. The Occupational Safety and Health Administration (OSHA) provides legally binding standards. The American Conference of Governmental Industrial Hygienists (ACGIH) and the National Institute for Occupational Safety and Health (NIOSH) provide non-binding recommendations and guidelines. These three groups have the common goal of protecting workers, but they are distinctly separate organizations with different purposes.

The ACGIH is a "private, not-for-profit, nongovernmental corporation whose members are industrial hygienists or other occupational health and safety professionals dedicated to promoting health and safety within the workplace" (6). ACGIH does not set legally binding standards. It reviews the current literature in order to issue suggested

guidelines called Threshold Value Limits (TLVs). These TLVs are recommendations and not legally binding (6).

NIOSH (a United States governmental organization) also looks at the most current science to make recommendations for exposure limits. NIOSH issues Recommended Exposure Limits (RELs) based upon its findings. Just like the ACGIH TLVs, the NIOSH RELs are recommendations and are not legally binding (50).

OSHA on the other hand, does issue legally binding limits. These limits are called Permissible Exposure Limits (PELs) (50). However, due to the fact that PELs are legally binding, any changes must go through the legislative process. As a result they are perpetually slightly outdated. Another significant difference is that ACGIH and NIOSH do not need to consider technical or economic feasibility when making recommendations whereas OSHA is required to do so (6; 50).

In the case of transfluthrin, or any of the other synthetic pyrethroids, there are no regulatory standards or guidelines listed. The accepted practice for establishing exposure limits for pyrethroids (as recommended by the ACGIH and NIOSH) is to use the standard developed for pyrethrum (28). The TLV, REL, and PEL for pyrethrum, and therefore transfluthrin, are all 5 mg/m^3, or 0.33 ppm_v (6; 28; 50). These guidelines are developed with the intent of determining what level of exposure a worker can be exposed for 8 hours a day, 5 days a week, for 40 years of work life, without experiencing any negative health outcomes (6). For transfluthrin, the expected adverse health issues from long-term over exposure are liver damage and irritation to the lungs and the respiratory tract (6).

The United States military also uses Military Exposure Guidelines (MEGs). These MEG's are used to determine acceptable exposure levels to troops while deployed. Some

potential exposures to troops may be only a couple of hours in duration, a couple of days, or a year due to extended deployment periods. As a result, MEGs are issued for 8-hour, 14-day, and 1-year durations of expected exposure (89).

As with TLVs, RELs, and PELs, there is no MEG specifically for transfluthrin. Therefore, just like with the TLVs, RELs, and PELs, the MEG for pyrethrum is used in its place (28). The MEGs for pyrethrum, and therefore transfluthrin, are shown in table 2.2 (89).

Duration of Exposure	MEG	Risk Severity	Basis of MEG
8-hour	5.0 mg/m^3	Negligible	TLV_TWA
14-day	1.2 mg/m^3	Negligible	TLV_TWA
1-year	1.2 mg/m^3	Negligible	Adjusted TLV

Table 2.2: Military Exposure Guidelines (MEGs) for transfluthrin (89).
MEGs allow commanders in the field a method to assess risks to their troops in an operational environment as they make tactical decisions.

Chapter 3: Methodology

Method

The concentration of airborne transfluthrin released from the treated hessian strips was determined using an established OSHA air sampling method (OSHA Method 70) under controlled conditions. There are no validated sampling and analysis methods developed specifically for transfluthrin, or the other synthetic pyrethroids. The accepted practice is to use the pyrethrum method (see Table 3.1) (28).

Sample Method	OSHA Method 70 (Pyrethrum)
Sampling Media	OSHA Versatile Sampler (OVS) tubes containing XAD-2 resin (OVS-2)
Air Flow Rate	1.0 L/min
Sample Time	60 minutes
Total Sample Volume	60 L
Analytical Method	Gas Chromatograph – Flame Ionization Detector (GC-FID)

Table 3.1: Details of sampling method (59).

Set-Up

Greenhouse & Chamber

The research was conducted within an empty, temperature-controlled greenhouse at the U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) facility in Beltsville, Maryland. This space was chosen because it was similar in size to both single room dwellings and tents used by the military for work and sleeping (see Figure 3.1).

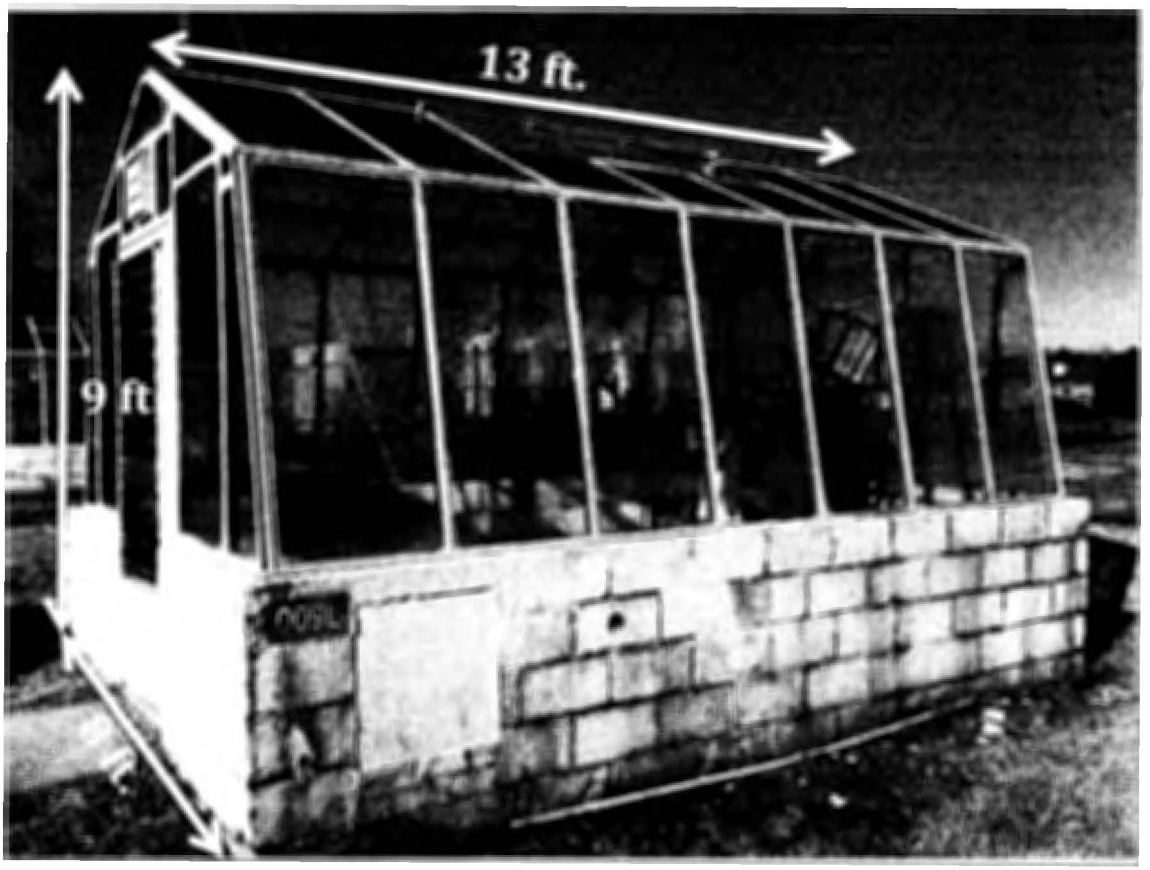


Figure 3.1: Small greenhouse used for sampling.
This small greenhouse was located at the USDA Agricultural Research Services, Agricultural Research Center in Beltsville, Maryland.

The greenhouse was equipped with a natural gas heater. The heater was operational, but its blower caused turbulence within the air space of the greenhouse each time it turned on. Therefore a chamber was constructed within the greenhouse to provide a static environment. The chamber measured 4ft (1.22m) wide by 7ft (2.13m) tall by 11ft (3.35m) long, for a total chamber volume of $308ft^3$ ($8.72m^3$). The chamber was constructed on a platform to allow air from the greenhouse to circulate underneath it, and all sides of the chamber had at least 1ft (0.3m) clearance to allow the surrounding air to circulate and warm it evenly. The chamber was constructed of lumber and 6-mil clear plastic sheeting.

Source Material

The source material consisted of transfluthrin-treated hessian strips. Hessian strips are a burlap-type material made from grain storage bags (61). These burlap-like strips were treated with a combined transfluthrin, detergent, and water solution (10ml transfluthrin, 90ml liquid detergent, and 400ml water) and were prepared by the USDA laboratory in Jacksonville, Florida. The resulting treated strips contained 15.07g of transfluthrin at 0.29g per $100cm^2$. The treated 263cm by 20cm ($5260cm^2$) hessian strip was cut into 17cm by 20cm ($340cm^2$) sections each containing 0.9 grams of transfluthrin. The treated $340cm^2$ hessian strips were stored in a refrigerator until use.

In the Ogoma study, 400cm by 30cm strips of treated hessian sacking were used in a 60m by 2 m by 2.5m netting tunnel. For this work, a fresh 17cm by 20cm piece of treated hessian strip was hung at one end of the experimental chamber at the beginning of each sampling day. The strip was hung at a height of 1m, centered left to right within the chamber.

Air Sampling Points

The sampling points were distributed throughout the chamber at 1m intervals horizontally from the source material. Samples were collected at horizontal distances of 1, 2, and 3 meters from the source. These sample points and the source material were all located at a height of 1m above the floor. In addition, at the 2m distance, samples were collected at the floor (height of 0m) and ceiling (height of 2m). This arrangement allowed for the comparison of airborne concentrations at the 3 distances, and the 3 heights.

Figure 3.2 is the view from the top of the chamber looking down. It shows the spacing between the source material and sample blocks. It also shows that the source

material and sample blocks were positioned down the center of the chamber. Figure 3.3 is the view from the side of the chamber, which displays the corresponding distances and heights for both the source material and the sample blocks.

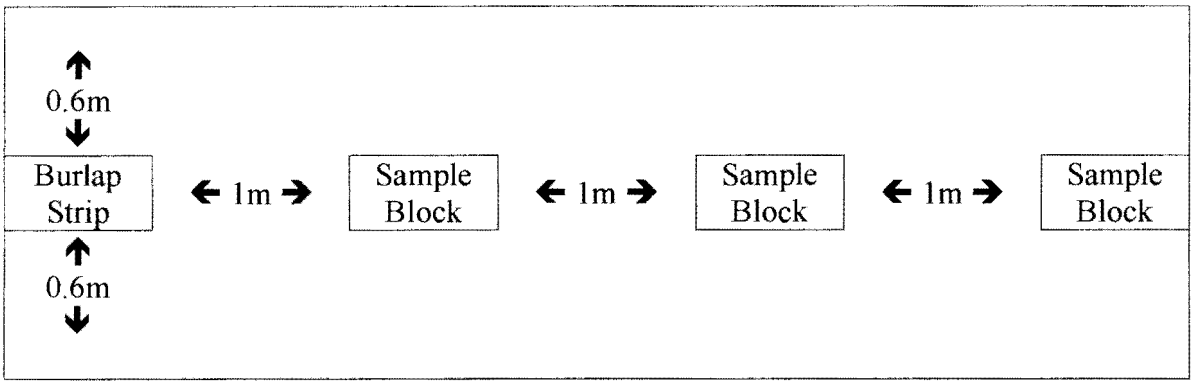


Figure 3.2: Top view of experimental layout.
The top view of the experimental layout indicates the distances between the sample blocks and the source material. All sample blocks, and the source material were positioned along the center of the chamber.

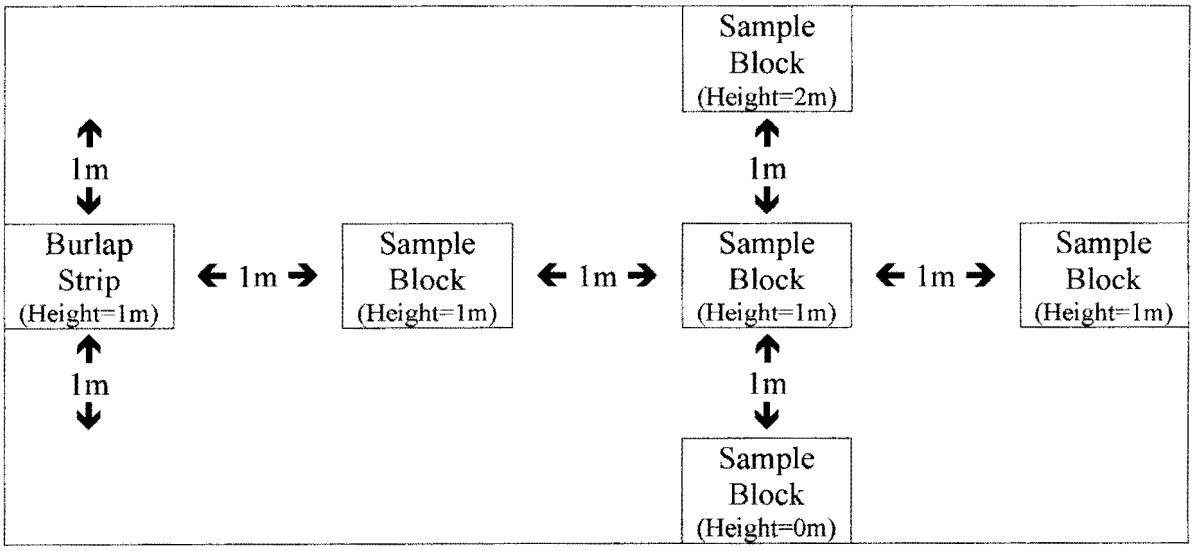


Figure 3.3: Side view of experimental layout.
The side view of the experimental chamber indicates distances and heights between sample blocks and source material.

Sample Blocks

In order to collect multiple sequential samples from each sampling location, sample blocks were created to hold up to 10 samples each (see Figure 3.4). Before each sampling day, the OVS-2 tubes for each sampling point were attached to the appropriate piece of Tygon tubing and placed into the corresponding hole in the block. This allowed sequential samples to be taken without the need to enter the chamber by switching the air pumps from one tube to another outside the chamber.

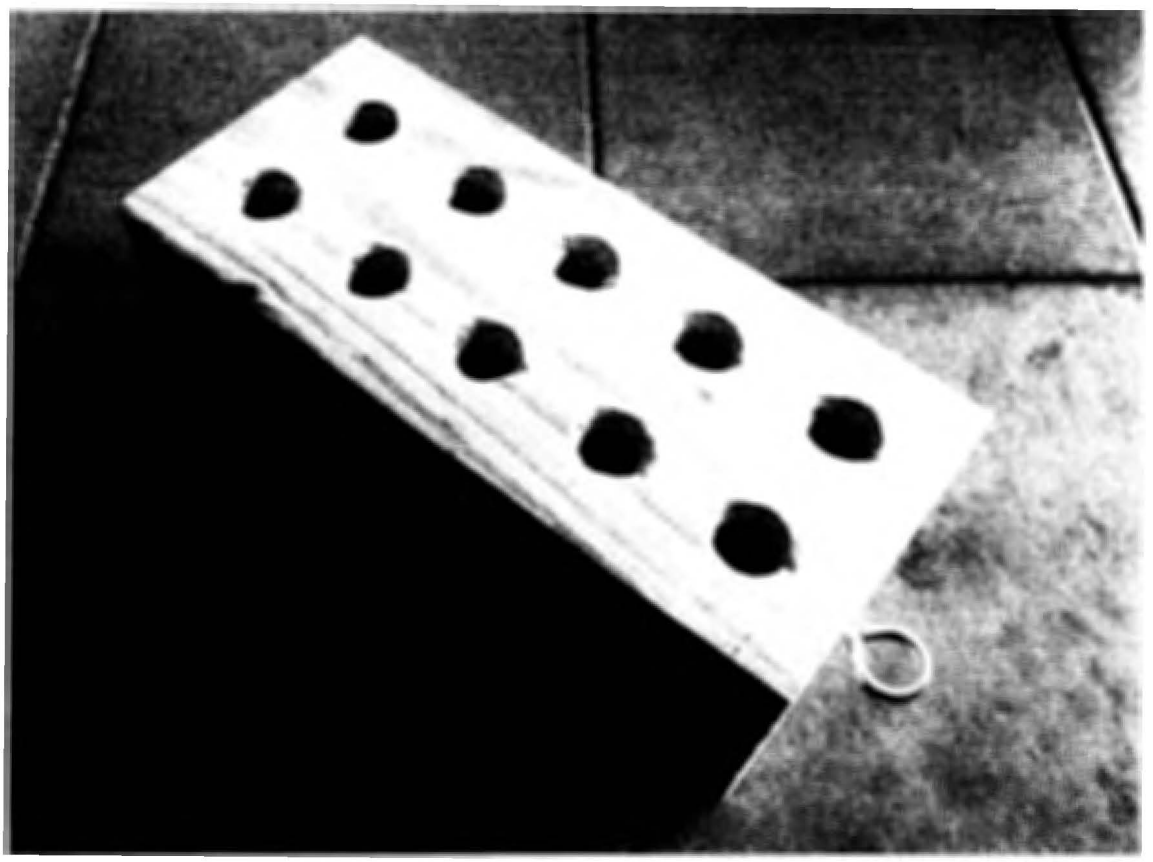


Figure 3.4: Example of a sample block.
Each sample block was designed to hold up to 10 OVS-2 sample tubes. This enabled multiple samples to be taken at each location during a run.

Air Pump Location

Due to the distinct temperature drop every time the door to the greenhouse was opened, it was determined the air sampling pumps should be located not only outside the chamber, but outside the greenhouse as well. To this end, first a tent, and then a shed were erected next to the greenhouse. The Tygon tubing was run out of the greenhouse from an air vent, through insulated ducting, and into the heated tent. Once in the tent, the multitude of Tygon tubes were labeled, color coded, and run to an air sampling pump stand created for this specific purpose (see Figure 3.5). The air sampling pumps used were Gillian 5000's (800-5000 cc/min)(Sensidyne, Clearwater, Florida) (77). The pumps were calibrated before every run, and a post-run calibration check was performed at the end of each day. All calibrations were conducted volumetrically with a DryCal Defender 510-M (Bios International, Butler, New Jersey) (12).

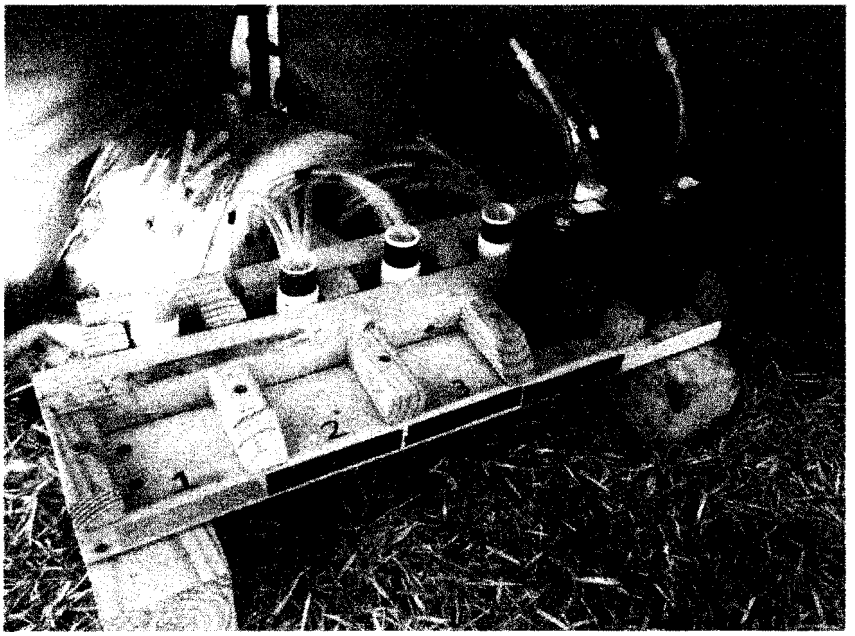


Figure 3.5: Air sampling pump stand.
Color-coded and labeled air sampling pump stand located in tent adjacent to greenhouse. The color-coding and numbering system reduced the possibility of accidentally connecting the wrong tygon tube when changing samples during a run.

SAMPLING PLAN

8-Hour Sampling Day

Due to the wide variation in the times people may be working or sleeping in huts or tents containing transfluthrin-treated hessian strips, an 8-hour sampling day was selected. Additionally, utilizing an 8-hour day provided an opportunity to study the movement of transfluthrin over time through the chamber, both from a distance and height perspective from the source material. It was expected airborne concentration levels of transfluthrin would start low and then rise over the course of the sampling day.

The 8-hour length of time as the length of time also matches the typical 8-hr occupational exposure limit standards for most chemicals. Samples collected and analyzed in this study allowed an 8-hour exposure to be calculated, with subsequent comparison to the OSHA PEL and ACGIH TLV. Exposures from this study were not expected to approach or exceed standards, the ability to quantitate over this period allowed an easy comparison to established regulatory standards for a similar compound (pyrethrum).

Per the OSHA method, each sample was collected at a flow rate of 1 L/min for 60 minutes (59). Due to logistical restrictions, it was decided to collect 5 samples from each sampling point over the 8-hour day. These samples were collected during hours 1, 2, 4, 6, and 8 of each sampling day (Table 3.2 shows visually how the samples were spread out over time). No sampling took place during hours 3, 5, and 7. This timing scheme was developed in an attempt to evaluate any potential initial increases in airborne concentration and to characterize the directional movement and airborne concentration over the entire chamber over the 8-hour sample day.

Hour #	1	2	3	4	5	6	7	8
Sample #	1	2	X	4	X	6	X	8

Table 3.2: Visual representation of sample timing.
This visual representation shows sample collection times over each 8-hour run. A total of (5) - 1h samples were collected from each sample point during each run.

Sample Locations

In order to assess the various airborne concentration levels of transfluthrin throughout the chamber, sample points were needed at multiple locations. The simplest, and most straightforward design was to place the sampling positions in a cross or plus configuration (see Figure 3.6). The comparison of results from sample points 'Near', 'Center', and 'Far' allowed for analysis of airborne concentration gradients over distance from the source material. And the comparison of results from sample points 'Top', 'Center', and 'Bottom' allowed for analysis of concentration gradients relative to height.

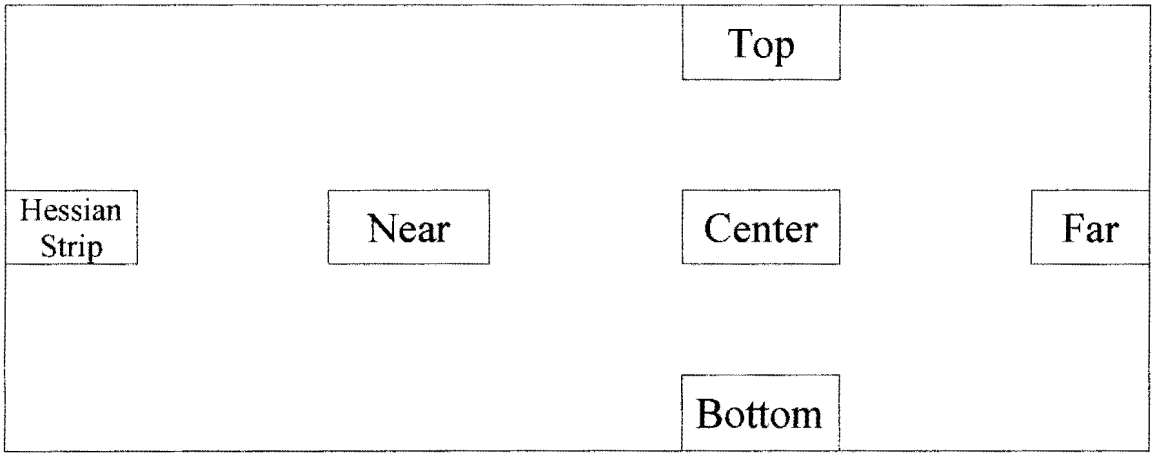


Figure 3.6: Side view of experimental layout.
This side view of the experimental sampling chamber indicates the sample point positions. The "plus" shaped pattern enabled comparisons of airborne concentrations over both distance from source and height above the floor.

Sampling Temperatures

To enable comparison of airborne transfluthrin concentrations over a range of temperatures, discrete temperature points were required. The low end of the temperature range of interest was 25°C (77°F). The temperatures used were 27°C, 33°C, 42°C, and 50°C (+/- 2°C). Temperature was monitored continuously and recorded every 60 seconds with a Hobo Data Logger (+/-0.21°C)(Onset, Bourne, Massachusetts)(62).

Sample Blanks and Passive Samples

The analytical method required one sample blank per 8-hour sampling day (59). The blank is never exposed to the experimental environment and is used to detect potential contamination of the samples from other sources. In addition, as no entry into the greenhouse and sampling chamber was made during the 8-hour sampling day to avoid affecting the airborne transfluthrin concentration levels, an assessment of the potential impact of leaving uncapped 1-hour samples in the chamber for the entire day was desired. This was due to the ability of the sorbent to potentially passively adsorb transfluthrin from the air after the 1-hour active sampling period was complete. To document and quantify any such passively adsorbed transfluthrin, one OVS-2 tube per run was uncapped and placed in the 'Near' sample block. No Tygon tubing was attached to, or air pulled through this passive sample.

Combined Sampling Plan

For each 8-hour sampling day during which successive 1-hour samples were taken, samples were collected at each of five locations within the chamber. At each of these locations, 1-hour samples were collected during hours 1, 2, 4, 6, and 8, for a total of five 1-hour samples per location. In addition, during each 8-hour sampling day there was

a passively exposed sample and one sample blank. The five samples for each of the five locations, plus the passive and blank samples, resulted in twenty-seven samples per 8-hour day. With four temperatures, each collected in triplicate; the resultant total of samples collected was 324.

Temperature Spikes

During the preliminary work to determine how consistent the thermostat within the greenhouse was, it was observed that everyday there was a midday spike in temperature of roughly 2°C. Once the test chamber was constructed within the greenhouse, the midday temperature spike increased to almost 10°C. Covering the greenhouse with a green 16x20ft general purpose tarp from Home Depot, and hanging a blue 6x8ft Home Depot general purpose tarp inside the southerly facing end of the greenhouse corrected this issue. The tarp inside the southerly facing end of the greenhouse also helped reduce the severity of the temperature drop resulting from opening the greenhouse door.

Cold Chamber

Subsequent to the tarp corrections to reduce overheating of the test chamber, it was observed that the chamber inside the greenhouse was consistently 5°C cooler than the rest of the greenhouse. There was observed to be adequate space on all sides of the chamber, including beneath it, for the warm air from the greenhouse to keep it at temperature. However, there was discovered to be insufficient air circulation around all sides of the test chamber. The hot air from the heater was blowing down one side of the chamber directly at the thermostat. Therefore, only one half of the greenhouse was being kept at temperature. This resulted in the side of the chamber away from the heater

exhaust being exposed to considerably cooler air. This issue was resolved by hanging two HDX 20in high velocity floor fans from the ceiling and placing one Lasko 16in oscillating stand fan on top of the heater. The fans were able to increase circulation within the greenhouse sufficiently to eliminate the temperature differences.

Post Sunset Warming

Once the mid-day temperature spikes and inadequate circulation issues were resolved, it was discovered that the temperature within the chamber was still gradually cooling by 5°C throughout the afternoon and into the evening. It was also observed once the sun set, the temperature within the chamber would slowly increase back to the desired set point. The thermostat was mounted to a dark piece of plywood. This plywood would absorb a thermal load from the sun and radiate the heat to the thermostat mounted on the other side of it. Covering a white plastic sign with aluminum foil and placing it between the greenhouse glass and the dark plywood resolved the issue. After resolving the radiant heat issue with the thermostat, the thermostat was able to function properly (within +/- 2°C of set point temperature) at all times.

The Destructive Forces of Nature

After six of the twelve sampling days were completed, nature interrupted the research by dumping 12 inches of snow on the research site and destroying the tent in which the air sampling pumps and other equipment were located. While all equipment was undamaged, a new environmentally controlled enclosure was needed before sampling could resume. This obstacle was overcome by purchasing a small 3ft by 7ft shed from Home Depot. This sturdier enclosure provided a space in which the air

sampling pumps could operate within their designed temperature range, therefore allowing research to resume.

Chapter 4: Results

Statistical Analysis

The transfluthrin airborne concentration level results were analyzed using SPSS Statistics software version 22. The alpha chosen to signify statistical significance was 0.05. The distribution of airborne concentration levels in parts per trillion (ppt_v) was not normally distributed. Once the airborne concentration levels were log_{10} transformed the data approximated a normal distribution (see figure 4.1). All statistical analysis was completed using the log_{10} of the airborne concentrations. The analysis of the data used four methods: graphically, one-way ANOVA with Tukey Post Hoc test, univariate ANOVA, and Kruskal-Wallis non-parametric tests.

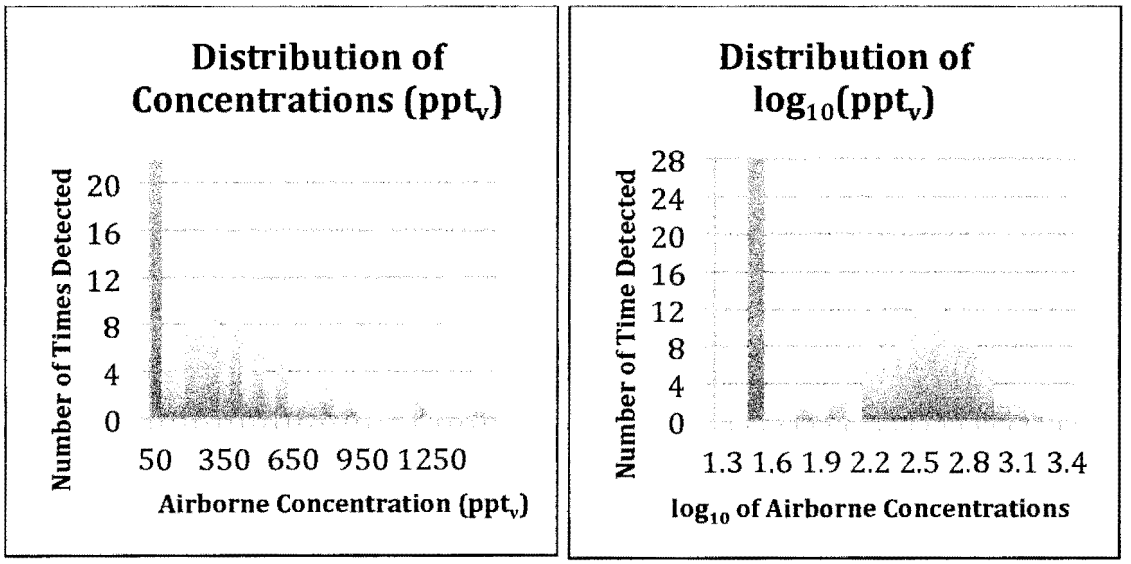


Figure 4.1: Comparison ppt_v vs. $log_{10}(ppt_v)$.
Comparison of airborne concentration level distribution in ppt_v vs. log_{10} of ppt_v. The large spikes at 50 ppt_v, and at 1.5 log_{10} are the results of the large percentage of non-detectable samples.

Sixty-nine percent (208/300) of the analyzed samples were found to be below analytical detection limits (57 ppt). These non-detects do not necessarily indicate the sample was transfluthrin-free, only that the results fell below the analytical sensitivity level of the instrument used in the analysis. All 75 samples collected at 27°C resulted in non-detects; therefore the data from the three 8-hour sampling days at this temperature were excluded from the statistical analysis. Following the guidelines for statistical treatment of non-detects in environmental sampling from the United States Environmental Protection Agency, (EPA) fifty-nine percent (133/225) of the remaining samples were replaced with values equal to one half the limit of detection (60).

TIME

Airborne concentration levels of transfluthrin were not significantly different when analyzed over time. This held true both for each of the individual sampling locations (Near, Top, Center, Bottom, and Far) and for the chamber as a whole. The "bottom" sample location was observed to experience the most change over time, which would seem to suggest an association between concentration and time. However, the effect was not statistically significant (see Table 4.1). Graphing the mean airborne concentrations for the chamber as a whole does not suggest any impact in concentration over time (see Figure 4.2). The univariate analysis of variance indicates combining "time" with "temperature", "distance", or "height" causes the other condition to no longer have a significant impact on concentration (see Table 4.2).

Sample Location	Significance (p-value)
Near	0.489
Top	0.936
Center	0.763
Bottom	0.360
Far	0.837

Table 4.1: One-way ANOVA of Concentrations Over Time, Split by Location. One-way ANOVAs were conducted for each sampling location within the chamber, comparing concentrations over time. No location had a significant change in concentration during the 8-hour runs.

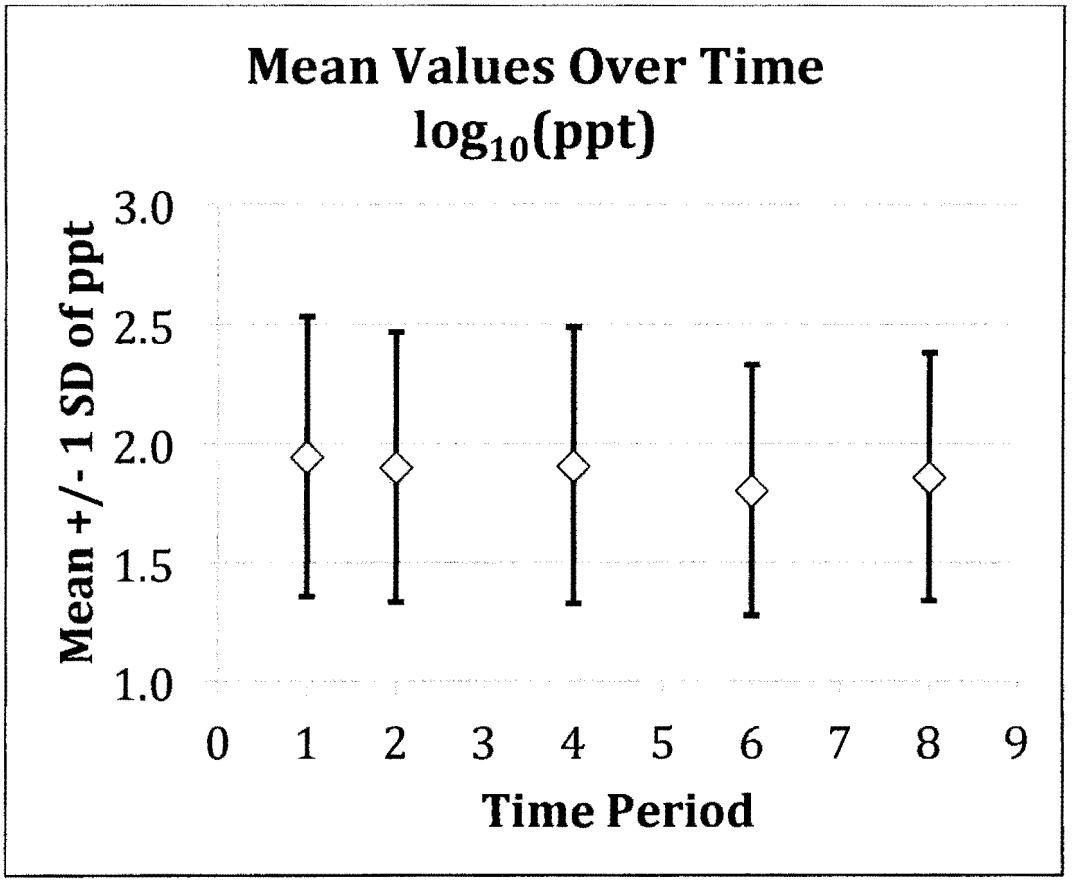


Figure 4.2: Mean Airborne Concentration Over Time.
The analysis of the data comparing the sample time periods to each other showed no significant difference (p=0.721) in airborne concentrations ($\log_{10}(ppt_v)$) over time.

Dependent Variable: Log_{10} of ppt_v

Source	Significance (p-value)
Time	.776
Time * Temp	.727
Time * Distance	.577
Time * Height	.488
Time * Temp * Distance	.850
Time * Temp * Height	.382

Table 4.2: Univariate Analysis of Variance- Time Displayed.

The analysis of the impact time had on airborne concentrations of transfluthrin showed that neither time, nor any combination of factors that took time into account, had any significant effect.

TEMPERATURE

Airborne concentration levels of transfluthrin were significantly different when analyzed over the range of discrete temperatures. At three of the five sampling locations (near, top, and center) the differences in airborne concentrations at each temperature were statistically significant at p=0.000. For the other two locations (bottom and far), the differences in airborne concentrations over the range of temperatures were not statistically significant, with p-values of 0.441 and 0.110, respectively (see table 4.3). The graph of the mean concentrations at each temperature suggests a direct correlation between temperature and concentration (see Figure 4.3). The result of a univariate analysis of variance comparing airborne concentrations at each of the discrete temperatures confirms the correlation between airborne concentrations of transfluthrin and temperature (p=0.000) (see Table 4.4).

Sample Location	Significance (p-value)
Near	0.000
Top	0.000
Center	0.000
Bottom	0.441
Far	0.110

Table 4.3: One-way ANOVA of Concentrations Over Temperature.
One-way ANOVAs were conducted for each sampling location within the chamber, comparing concentrations at each temperature. Significant changes were found at the Near, Top, and Center locations (p=0.000). However, the Bottom, and Far locations did not show significant changes (p=0.441 & p=0.110)

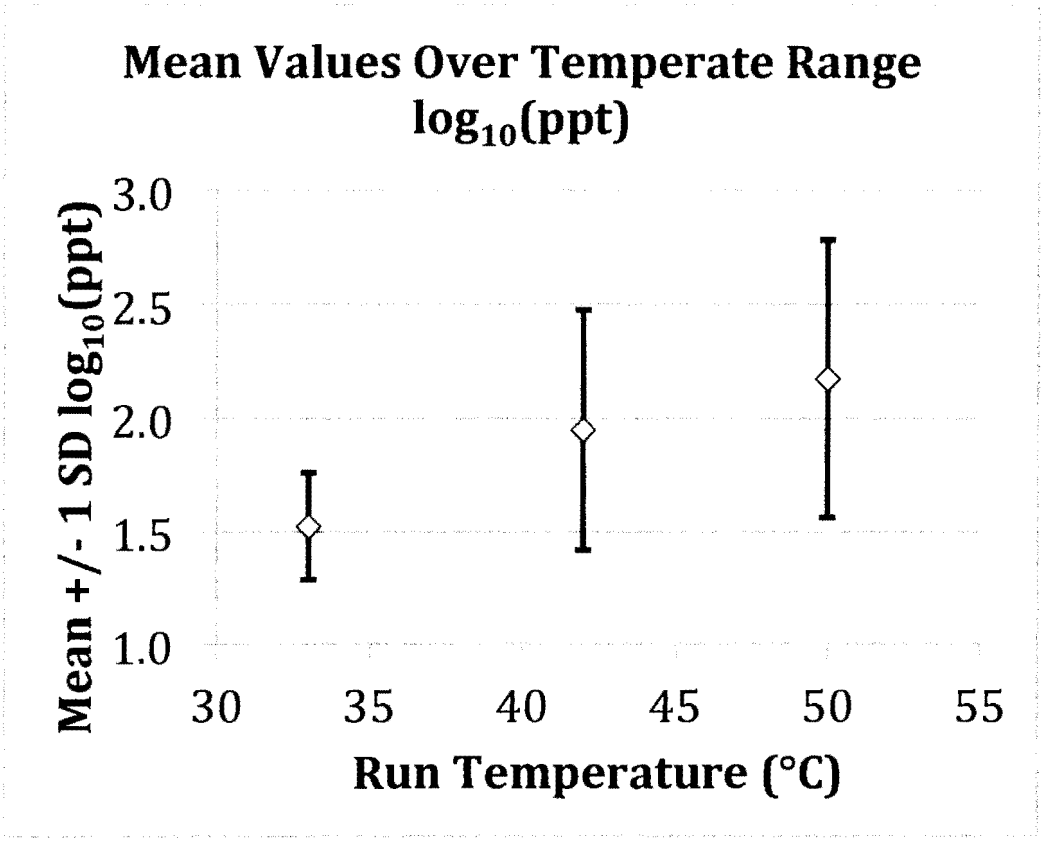


Figure 4.3: Mean Airborne Concentrations Over Discrete Temperatures.
Comparison of the means of $\log_{10}(ppt_v)$ at 33°C, 42°C, and 50°C. The analysis showed a clear correlation between increased temperature and increased airborne concentrations (p=0.000).

Dependent Variable: Log_{10} of ppt_v

Source	Significance (p-value)
Temp	.000
Time * Temp	.727
Temp * Distance	.000
Temp * Height	.001
Time * Temp * Distance	.850
Time * Temp * Height	.382

Table 4.4: Univariate Analysis of Variance – Temperature Displayed.

The analysis of the data evaluating the impact of temperature on airborne concentrations show that temperature, and any other factor combined with temperature (except time) have significant impacts on airborne levels of transfluthrin.

DISTANCE

Airborne concentration levels of transfluthrin were significantly different when analyzed over distance from the source material. At two of the three temperatures analyzed (42°C and 50°C), the differences in airborne concentrations over distance were statistically significant with p-values of 0.000 for both (see Table 4.5). There were no statistically significant differences in airborne concentration over distance at 33°C (p=0.628). The findings suggest an inverse correlation between distance from the source material and airborne transfluthrin concentration levels (see Figure 4.4). The result of a univariate analysis of variance comparing airborne concentrations at each distance confirms the significant decrease in concentrations over distance (p=0.000) (see Table 4.6).

Temperature	Significance (p-value)
33°C	0.628
42°C	0.000
50°C	0.000

Table 4.5: One-way ANOVA Over Distance, Split by Temperature

One-way ANOVAs conducted for each temperature, evaluated the impact of distance on concentration levels. At the cooler temperature (33°C) there was no significant difference between distances (p=0.628). However, at the higher temperatures (42°C & 50°C) distance did have a significant impact on concentration (p=0.000). The disagreement between the temperatures is likely due to the high number of non-detects at 33°C.

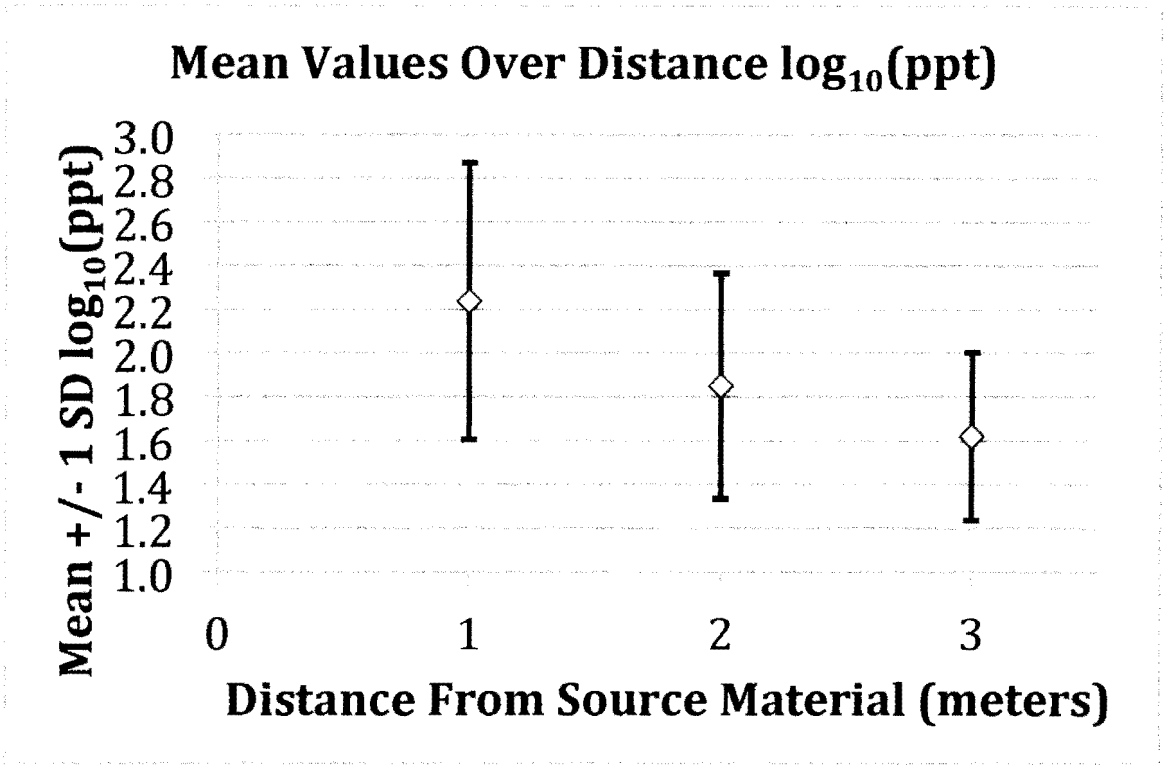


Figure 4.4: Mean Airborne Concentration Over Distance

Comparison of the means of $\log_{10}(ppt_v)$ at 1, 2, and 3 meters laterally from the source material. The analysis of the data comparing each of the distances to each other showed a clear inverse correlation between increasing distance and decreasing airborne concentrations (p=0.000).

Dependent Variable: Log_{10} of ppt_v

Source	Significance (p-value)
Distance	.000
Time * Distance	.577
Temp * Distance	.000
Time * Temp * Distance	.850

Table 4.6: Univariate Analysis of Variance – Distance Displayed.
The univariate analysis of variance shows that both distance and any other variable combined with distance (except time) have significant impacts on airborne concentrations of transfluthrin.

HEIGHT

Airborne concentration levels of transfluthrin at 50°C were significantly different when analyzed over height above the floor (p=0.002). However, this did not hold true at the lower temperatures. At 33°C and 42°C, the ANOVA resulted in p-values of 0.198 and 0.440 respectively (see Table 4.7). Neither of which is statistically significant. The disparity in results at different temperatures is likely due to the high number of non-detects at the lower temperatures making accurate analysis of the lower temperatures difficult. The graph of the mean concentrations for all temperatures at each height does not visually suggest a correlation between height and airborne concentrations of transfluthrin (see Figure 4.5). The univariate analysis of variation, however, suggests significant differences in airborne concentrations from one height to another do exist(p=0.023) (see Table 4.8). Further analysis of the data indicates the airborne concentrations of transfluthrin at heights of 1 and 2 meters are not statistically different from each other. However, the airborne concentration of transfluthrin at a height of 0 meters (on the floor) is significantly lower than the other two heights. These differences are made evident when comparing homogeneous subsets of the data (see Table 4.9).

Temperature	Significance (p-value)
33°C	0.198
42°C	0.440
50°C	0.002

Table 4.7: One-way ANOVA Over Distance, Split by Temperature

One-way ANOVAs conducted for each temperature, evaluated the impact of height on airborne concentration. At the cooler temperatures (33°C & 42°C) height had no significant impact on concentrations (p=0.198 & p=0.440). However, at the highest temperature (50°C), height did have a significant impact on concentration (p=0.002). The incongruence between temperatures is likely due to the high rate of non-detects at lower temperatures.

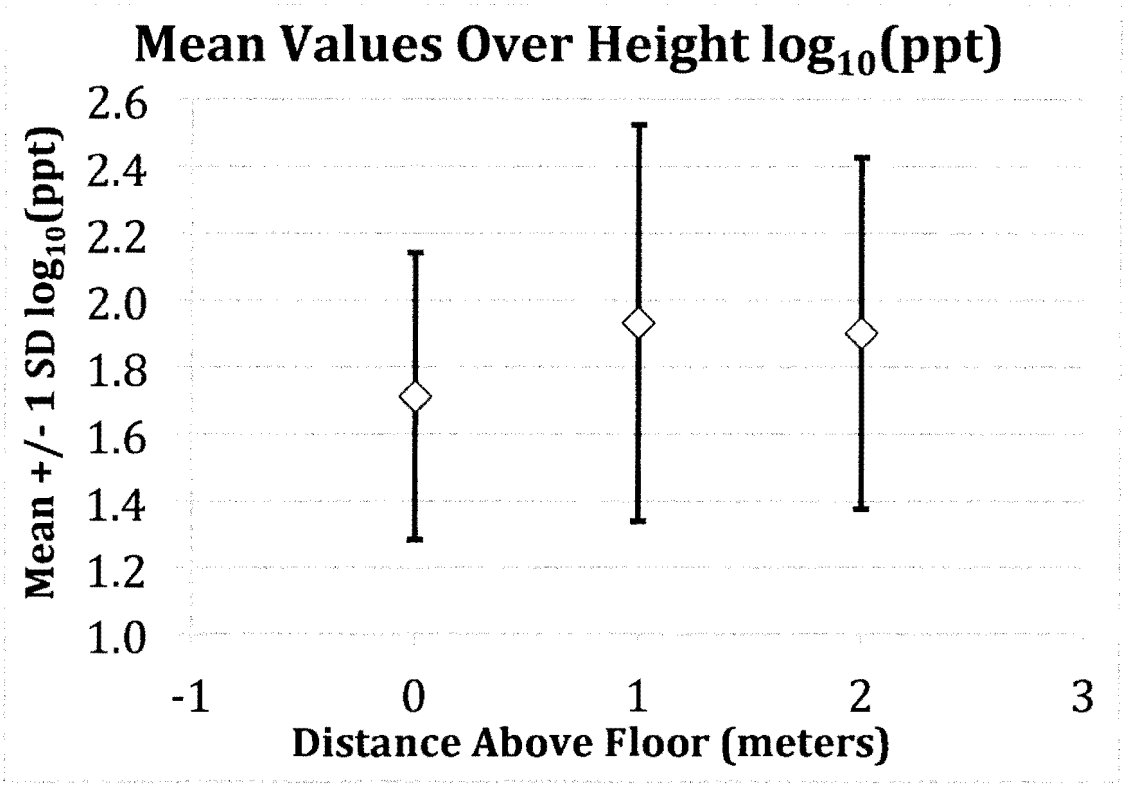


Figure 4.5: Mean Airborne Concentration Over Height.

Comparison of the means of $\log_{10}(ppt_v)$ at heights of 0, 1, and 2 meters above the floor does not suggest any significant differences visually. However, a one-way ANOVA comparing each of the three heights showed a significant difference (p=0.036).

Dependent Variable: Log_{10} of ppt_v

Source	Significance (p-value)
Height	.023
Time * Height	.488
Temp * Height	.001
Time * Temp * Height	.382

Table 4.8: Univariate Analysis of Variance – Height Displayed.

The univariate analysis of variance shows that both height and temperature have significant impacts on airborne concentrations.

Tukey HSD[a,b]

Height Above Floor	N	Subset 1	Subset 2
On Floor	45	1.7226	
1 Meter From Floor	135		1.9401
2 Meters From Floor	45		1.9094
Sig.		1.000	0.911

Means for group in homogeneous subsets are displayed.
Based on observed means.
The error term is Mean Square (Error) = 0.162

a. Uses Harmonic Mean Sample Size = 57.857

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 4.9: Homogeneous Subsets of Concentrations by Height.

Comparing the homogeneous subsets show where the significant difference is in airborne concentration of transfluthrin. The concentrations at heights of 1 & 2 meters above the floor are statistically the same, while the concentration at a height of 0 meters (on the floor) is significantly lower.

Chapter 5: Discussion

This research attempted to characterize the movement of transfluthrin in the airborne environment within an enclosed space when emitted passively from treated source material. To properly evaluate the behavior of transfluthrin in the air, several specific factors were investigated. These factors were time, temperature, distance from the source of transfluthrin, and height.

Time

In evaluating the effects of time on airborne concentrations of transfluthrin, several decisions had to be made. Based on the proposed ideas by the Armed Forces Pest Management Board (AFPMB) to use passively emitted transfluthrin source material in work places and sleeping tents, an 8-hour sampling day was selected. The duration of each air sample was defined by the validated OSHA sampling method 70 which was determined to be the most appropriate for the compound (as no established method specifically for transfluthrin exists), which is 60 minutes in length (59). With each sample being 1 hour in length, the sampling frequency was established as one sample at each location, every hour, for the duration of each 8-hour sampling day. Logistical issues forced the number of samples to be reduced to five, 1-hour samples, spread over each 8-hour sampling day.

The finding of no significant differences in airborne concentration levels between time periods suggests two important things. The first is that transfluthrin seems to rapidly permeate the chamber space. This suggestion appears to be supported as the sample results from hour 1 (minute 0 to minute 60) were not statistically different from the

sample results from hour 8 (minute 420 to minute 480). This meets the American Society for Testing and Material (ASTM) standard of three consecutive time periods without significant differences for establishing equilibrium (13). This implies once an effective mass loading of transfluthrin is established and loaded onto a material, it could provide near-immediate effectiveness as a spatial repellency device upon hanging the source material/device in the desired space. The second important point regarding these statistically insignificant findings is a consistency and stability in airborne concentration levels. Once a steady state airborne concentration level is achieved, protection of the people inside the space is provided for the duration of the workday/night if this airborne concentration level has been determined through mosquito studies to be a level that produces an effective spatial repellency.

There were no evident differences when visualizing the airborne concentration levels of transfluthrin between samples from hour 1 through hour 8 at each respective location, relative to the same location point, but statistically significant differences were observed when comparing the different locations within the chamber. While the number of data points for each of the five sampling locations was too small to conduct a statistical analysis for each location, a clear pattern emerged from the data. Within the first hour, transfluthrin had spread to every part of the chamber according to the airborne concentration levels measured at the various sampling points in the chamber. Sampling points close to the source material produced higher concentration levels of transfluthrin than those farther away from the source. As time progressed, the airborne concentration levels at the locations close to the source material remained steady (suggesting concentration equilibrium at the locations nearest to the source), while the airborne

concentration levels at the farther points increased with each subsequent sampling period. This pattern suggests that the transfluthrin contained in the source material rapidly spreads throughout the chamber in a diffusive manner, eventually equilibrating with the environment over a relatively short timeframe.

TEMPERATURE

The discrete temperatures used in this study were selected on the basis of varying diurnal temperatures and extreme temperature points which deployed service members in different locations abroad may potentially encounter. U.S. military and civilian personnel are deployed or stationed all over the world in climates that range from arctic conditions in Alaska, hot and humid in the Philippines, to the desert conditions of the Middle East. Many places have significant temperature fluctuations between day and night. Personnel need to be protected from vector borne diseases 24 hours a day. Any potential spatial repellent would need to work over a wide range of temperatures. The low temperature was selected to be 25°C as it is a common night or winter temperature, it is considered standard work environment temperature, and there is growing evidence that mosquitoes are less active and less infectious at temperatures below 25°C (27; 48). The high end of the temperature range to be tested was determined by the capabilities of the natural gas heater and the desire to replicate the extreme temperature encountered in areas such as the Middle East (53.3°C recorded in Kuwait in 2010) (97). The sample runs were conducted at 27°C, 33°C, 42°C, and 50°C (+/- 2°C). The +/-2°C level of consistency was only achievable after taking into account and correcting the issues discussed in the "methodology" chapter.

The findings of significant differences in airborne concentration levels of transfluthrin at each of the four selected temperatures were consistent with previous work on volatilization rates of other pesticides (18; 40). The vapor pressure of a pesticide is a major factor in determining the volatilization rate. Increases in temperature increase the effective vapor pressure and therefore the volatilization rate (40).

The research suggested a correlation between increasing temperatures and increased airborne concentration levels of transfluthrin. Disease carrying vectors live in a wide variety of temperature range. The *Anopheles* mosquito, which is responsible for transmitting malaria, lives comfortably between 17-33°C (44).

The cooler it is, the more protective clothing people can comfortably wear. As the temperature increases, and comfort drives people to wear fewer protective layers and to leave windows open to allow for a cooling breeze, the protective concentration of transfluthrin in the air increases. In a fortuitous way, as people may increase their vulnerability to disease-carrying insects in warmer temperatures, the airborne concentration of transfluthrin will be higher as well, thus likely providing increased repellency against these pests.

Distance

The findings of significant differences in airborne concentrations of transfluthrin at different distances suggest the potential for overlapping areas of protection (see Figure 5.2). Not every tent may require a transfluthrin-containing device or source within it if the effective spatial repellency area of the compound extends beyond the tent containing it. It may be enough to protect every other tent, and the remaining tents and surrounding areas may be adequately protected by the overlap. Future studies correlating airborne

concentrations to mosquito behavior are required to determine how wide, high, and directional the protective "bubble" actually is, and what spacing is required to provide protective overlapping areas.

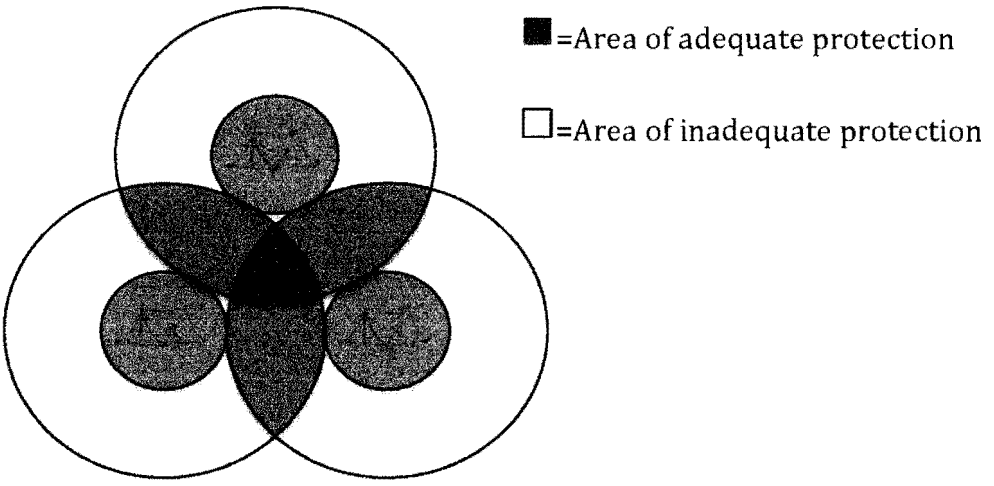


Figure 5.2: Potential overlapping areas of protection.
In a camp or village setting, structures tend to be closely spaced. Each structure protected by a spatial repellent would also be surrounded by a bubble of lower concentration that would provide inadequate protection. The overlapping areas of these larger bubbles have the potential to reach sufficient concentration levels to be protective.

HEIGHT

The findings suggest a clear pattern of movement and delineation of airborne concentrations by height. As the transfluthrin emanates from the treated source material at a height of 1 meter, it was observed to move laterally and upward. As a result, the highest concentrations were found at height of 1 and 2 meters above the floor. Transfluthrin was detected at a height of 0 meters (floor height), but at significantly lower

concentrations. The implication of this finding is the best protection would seem to be provided at heights of 1m and above. While active, personnel may have exposed skin (sleeves rolled up) but would be within the range of higher concentration (see Figure 5.3). At night, while sleeping on the floor (height of 0.0m) or on a cot (height of 0.45m), personnel would be in the area of lower concentration, but would likely have the additional protection of bed netting (95). This lower concentration would also allow sleeping personnel to minimize their inhalation exposure to airborne transfluthrin.

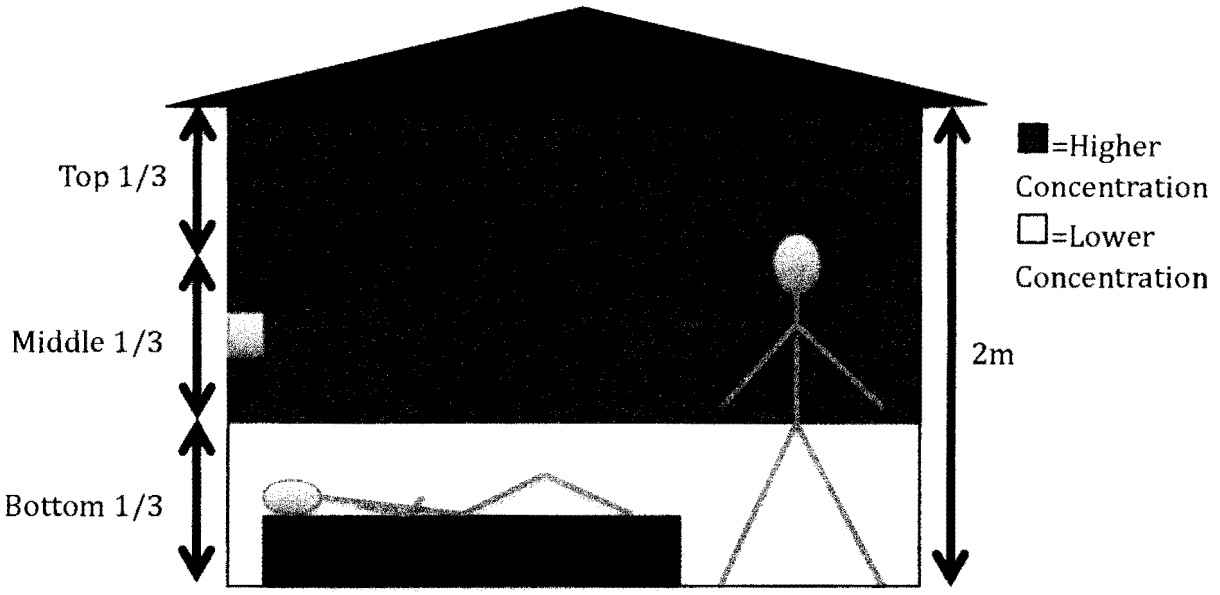


Figure 5.3: Airborne concentration levels by layer.
The data suggests that as transfluthrin moves through still air, that it progresses laterally and upward. As a result the highest concentrations of airborne transfluthrin are located at heights equal to, and above the source material.

OTHER FACTORS OF NOTE

In addition to the factors of time, temperature, distance, and height already discussed, two other factors played a major roll in determining the airborne concentrations of transfluthrin detected. These factors are the amount of transfluthrin

loaded onto the source material and the surface area of said source material. During this research, both factors were kept consistent to enable comparisons between sampling days. But the potential impact of both factors must be understood.

Mass Loading

During the Ogoma *et al.* study each strip was loaded with 15.07g of transfluthrin (61). As discussed earlier in the methodology section, this research used strips containing only 0.9g of transfluthrin each. If both masses were applied to sufficiently large, and identical pieces of hessian cloth, and these strips were placed into identical testing chambers in which it was possible to achieve equilibrium, logic dictates different results would be expected. If a theoretical 1% of the transfluthrin from each strip were required to be airborne to achieve equilibrium, then one test chamber would have 0.15g of transfluthrin spread throughout the chamber volume, while the other would only have 0.009g of transfluthrin in the air. This distinction is important when trying to compare research conducted by different groups.

Surface Area

Continuing the comparison of the Ogoma *et al.* study with this research, surface area must be accounted for. Ogoma et al. applied 15.07g of transfluthrin to hessian strips 12,000cm^2 in size resulting in a concentration of 0.0012g of transfluthrin per cm^2 (61). This research used strips of 340cm^2 containing 0.9g of transfluthrin resulting in a concentration of 0.0026g of transfluthrin per cm^2, or just over double the concentration of the Ogoma study. If a direct comparison study were conducted comparing airborne concentrations resulting from an identically sized strip with each concentration, the

expected results would show the higher strip concentration resulting in a higher airborne concentration.

The correlation of higher concentrations on fabric to higher airborne concentrations only holds true to a point. Any given piece of fabric only has a certain amount of surface area to which transfluthrin can be placed. Once the surface is saturated with transfluthrin, any additional transfluthrin will form a new layer covering the lower one. The resulting covered layer is unable to volatize, as it has no contact with the air. The result of this complicated relationship between the concentrations of transfluthrin on fabric to airborne transfluthrin makes it hard to compare research conducted by different groups to each other. But an understanding of the impact must be taken into account when designing future research.

Chapter 6: Conclusions / Future Research

The results of this study suggest temperature, distance from source, and height above the floor, all have significant impacts on the airborne concentration of transfluthrin. Time did not appear to have an effect on concentration. These results suggest transfluthrin may be an effective choice for use as a fast-acting spatial repellent against disease-carrying pests. However, future studies are required to determine the mass loading and surface area of source material that will yield airborne concentrations for effective repellency as well as airborne concentrations that will provide an acceptable margin of safety for human health.

Many locations with endemic vector-borne diseases are geographically located in tropical or sub-tropical areas having high, or greatly fluctuating levels of relative humidity (RH). This study controlled RH levels to below 40% in an attempt to focus on the impact of ambient temperature. A dehumidifier was utilized to keep the RH below 40% for all sample runs. Future research should evaluate the effects of RH on passive emission rates of transfluthrin as it may have a significant impact on the airborne concentration levels, and thus the effective spatial repellency of the compound(40).

Few tents or huts are sealed tightly, resulting in most having some level of air exchange. Many even have screen windows to allow for a cooling breeze. Previous research has shown the typical air change rate in a typical Thailand hut with screen windows to be 6-9 complete air changes per hour (13). This research looked at airborne concentrations in an enclosed space with still air and an air change rate of 0.03 air changes per hour, or about 1 complete air change per day. Future work should be

conducted to evaluate airborne concentration levels in spaces over a range of reasonable air change rates.

This study was conducted at temperatures between 27°C and 50°C. Potential vectors for disease live comfortably at temperatures lower than 27°C (44). Future research should include cooler temperatures such as 20°C, or even 15°C. If the trend suggested by this study continues to lower temperatures, the airborne concentrations of transfluthrin at these cooler temperatures will require modifying the sampling method used, or require using a different sampling method completely, given the likely trace amounts of transfluthrin mass within the airborne space.

Occupational Safety and Health Administration sampling method 70 calls for the samples to be collected at 1 liter of air per minute, for 60 minutes (59). Future studies should consider increasing the flow rate and/or increasing the sampling time. Doubling the sample time to 120 minutes would cut the limit of detection in half. This increase in sampling time would reduce a researcher's ability to track changes in concentrations over smaller time periods. However, the number of non-detect samples would be greatly reduced. Increasing the collection rate from 1 liter per minute to 2 or 3 liters per minute is also a possibility, as long as the pressure drop effects within the sampling tubes are evaluated. If a higher flow rate could be used, then the researcher could continue to track, and perhaps enhance the tracking, of changes in airborne concentrations over time by examining changes every 30-60 minutes. Non-OSHA methods may also provide additional sampling options.

This study collected 1-hour samples successively over 8-hour sampling days. When the chamber as a whole was evaluated, there were no significant changes in

concentration over time. Individual locations did demonstrate changes, indicating the entire chamber volume had not reached equilibrium during the 8-hour time day (see Time section of chapter 5 and Figure 5.1). Future research should evaluate airborne concentrations of transfluthrin over a longer time period, such as 24 hours. Larger spaces designed for living in deployed environments, such as medium and large tents, should also be used in evaluating the spread of transfluthrin within spaces over time.

The potential variations in mass of transfluthrin and surface area are nearly infinite. Future research should evaluate the effects of keeping the mass consistent while changing the surface area, and the effects of changing the mass while keeping the surface area consistent. This information would help guide future work into establishing the most efficient, and cost effective, method of delivering desired airborne concentrations for use as a spatial repellent or insecticide.

A multi-disciplinary approach to future research would greatly enhance the level of knowledge and help create a more complete picture in regards to the potential use of transfluthrin as a spatial repellent. Future work involving both mosquito behavioral studies and airborne concentration characterization of transfluthrin over a range of temperatures would allow entomologists and pest control personnel to determine the most judicious mass amounts required for spatial repellency over desired spaces and timeframes while public health/occupational health personnel such as industrial hygienists could compare measured airborne concentration values to human health guidelines developed for specific pyrethroids or similar classes.

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