

CHARACTERIZATION OF CHEMICAL BY-PRODUCTS RELEASED DURING  
COMBUSTION OF COMMERCIALY AVAILABLE METOFLUTHRIN-TREATED  
MOSQUITO COILS

by

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Thesis submitted to the Faculty of the  
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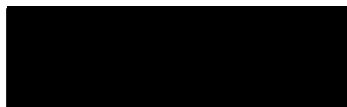


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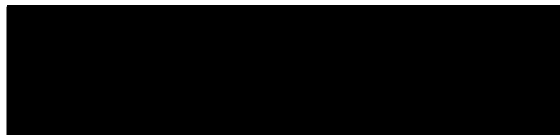
## **DEDICATION**

I would like to dedicate this thesis to my lovely wife, Aimee. You have given me the strength, support and encouragement to make it through this challenging endeavor. I love you with all my heart.

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## **ABSTRACT**

### **CHARACTERIZATION OF CHEMICAL BY-PRODUCTS RELEASED DURING COMBUSTION OF COMMERCIALY AVAILABLE METOFLUTHRIN-TREATED MOSQUITO COILS**

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The practice of burning mosquito coils has been around for nearly one hundred years, with its primary purpose to repel nuisance insects and prevent vector borne disease transmission. Globally, billions of mosquito coils are burned each year to control mosquitoes due to their effectiveness and inexpensive cost. Since their inception, mosquito coils have commonly contained naturally derived pyrethrin or synthetically formed pyrethroid insecticides as the active ingredient. However, the active ingredient represents only a small portion of the mosquito coil's total composition. The remainder of the coil's weight is comprised of dyes, fragrance and fillers, such as wood dust or coconut flour. Recently published field data also suggests that even untreated (blank) mosquito coils possess the ability to repel mosquitoes. This research consisted of attempting to characterize the various airborne chemical components generated from combusting these coils, in addition to metofluthrin. Blank mosquito coils were also

characterized to determine if any variances existed in the coil contents when compared to treated coils. Samples in this study were collected by burning mosquito coils within a non-air tight cylindrical combustion chamber contained within a laboratory chemical fume hood. Sample collection was conducted by using multiple types of solid phase microextraction (SPME) fibers with subsequent analysis using gas chromatography-mass spectrometry (GC-MS) instrumentation to identify the various gas phase compounds generated during combustion of the coils. This study identified the release of 72 chemical compound by-products in addition to the active pyrethroid ingredient, which included aromatic hydrocarbons, phenols, fatty acids, and aldehydes.

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# CHAPTER 1: INTRODUCTION

## BACKGROUND

Mosquito coils, burned by the billions annually, are used throughout the world, but are most commonly employed as a form of active mosquito control in the continents of Asia, Africa and South America (18). Mosquito coils are a commonly used mosquito control method in low-income communities due to their inexpensive cost (24).

Whether to prevent disease transmission or simply repel nuisance pests, the practice of burning mosquito coils has been around for nearly a hundred years (45). A mosquito coil can be defined as a spiral-shaped mosquito-repellent incense that can burn for six to 10 hours (11). Since their inception, mosquito coils have commonly contained pyrethrum or synthetic pyrethroids as the active ingredient. Once lit, the active ingredient is vaporized and released in a smoke cloud to provide protection against biting mosquitoes (36). Along with insecticidal characteristics, pyrethrum and pyrethroids also exhibit spatial insect repellent qualities. Opposed to killing the insect on contact, spatial repellents produce an insect repellency from a distance and inhibit the mosquito from locating its target vertebrate host (12). If an infected mosquito can enter a dwelling and feed on its host before the insecticide kills it, the insect may still be able to transmit a vector-borne disease. On the other hand, if the infected mosquito never enters the dwelling to feed because it is repelled by the release of the active ingredient into the air from the coil, the disease cannot be transmitted. However, the active ingredient only represents a very small portion, typically less than 0.2% by weight, of the mosquito coil's composition (20). The remainder of the coil's weight is comprised of dyes, fragrance and

fillers, such as wood dust or coconut flour (7).

Around 1890, Japanese businessman, Eiichiro Ueyama formulated the idea of kneading a mixture of starch and dried mandarin orange skin powders with pyrethrum into the shape of a incense stick (12). At the time, the most common manner to repel mosquitoes consisted of burning a mixture of pyrethrum powder and sawdust in a brazier. Ueyama's design successfully vaporized the pyrethrum powder. However the sticks burned too quickly, only lasting approximately 40 minutes, which was too short to provide any reliable mosquito-repelling action (12). It wasn't until 1895, when Ueyama's wife, Yuki, suggested elongating and thickening the sticks and forming them into a spiral to make them last longer (12). After several more years of experimentation, in 1902, Ueyama finally perfected a prototype that was ready for marketing (12). This final design consisted of cutting a stick of mosquito incense, then hand rolling the stick into a spiral shape. (12). This manual method of manufacturing continued until 1957, when the implementation of machine punching initiated the mass production of mosquito coils (12). Today, more than 29 billion mosquito coils are sold throughout the world (12).

Pyrethrum is a naturally occurring insecticide derived from the flowers of plants belonging to the genus *Chrysanthemem* and contains six insecticidal esters, referred to as pyrethrins (6; 10). Pyrethrum powder was commonly used as the active ingredient in mosquito coils. However it has become less desired in favor of more effective pyrethroids, synthetic analogs of the naturally occurring pyrethrins. First sought after to minimize dependence on naturally derived ingredients, pyrethroids have been found to provide a superior knockdown effect, where the target insects are rapidly paralyzed so

they cannot feed (6; 23). Pyrethroids are generally less toxic to mammals because they are rapidly metabolized and easily eliminated (13). Pyrethroids also demonstrate limited persistence (weeks, opposed to years) in soil limiting contamination of the environment (13). Additionally, pyrethroids are not easily dissolved in water and tend to be less susceptible to photo-decomposition (28).

The first pyrethroid, allethrin, developed in 1949 by Schechter, Green and La Forge, has led to the discovery of many new compounds that present greater thermal stability, while still maintaining low mammalian toxicity and minimal environmental contamination (33). One of the newest discovered pyrethroids is a compound known as metofluthrin (2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (*EZ*)-(1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-prop-1-enylcyclopropanecarboxylate) (Figure 1) (39). Originally registered

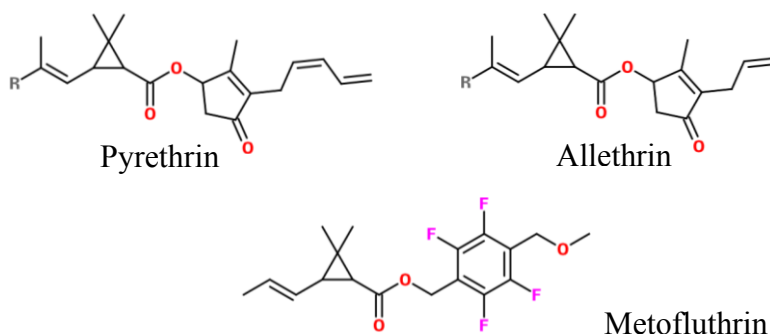


Figure 1. Chemical structure of (a) pyrethrin, (b) allethrin and (c) metofluthrin

in Japan in 2005, metofluthrin has demonstrated to be an extremely effective spatial mosquito repellent when applied as the active ingredient to mosquito coils (23).

Traditionally, the application of pyrethroids required the pesticide to be vaporized using a heat source, such as a mosquito coil or electric vaporizing mat (23). Sumitomo Chemical set out to develop a pyrethroid that would not only work as an active ingredient for conventional mosquito control application methods, like mosquito coils, but would also

volatize at room temperature to allow use without an applied heat source.

Throughout the history of mosquito coil use, the coils have always had the presence of an insecticidal (natural or synthetic) active ingredient. However, recently published field data suggests that even mosquito coils free of active ingredients possess the ability to repel mosquitoes. Achee *et al.* (2012) reported that the smoke from “blank” (pyrethroid-free) mosquito coils provided 48% deterrence of mosquitoes within an enclosed space, relative to using no intervention at all (1). This suggests that constituents of the smoke from the burning coil may also elicit mosquito spatial repellency effects. This finding calls into question the necessity of adding an active ingredient in mosquito coils to repel mosquitos. What is really repelling the pests...the active ingredient or some other compound released in the mosquito coil smoke?

#### **PUBLIC HEALTH SIGNIFICANCE**

Mosquito coils are frequently used overnight inside living areas (Figure 2) with little to no room ventilation and can burn for up to eight hours (18; 29). Unfortunately, it

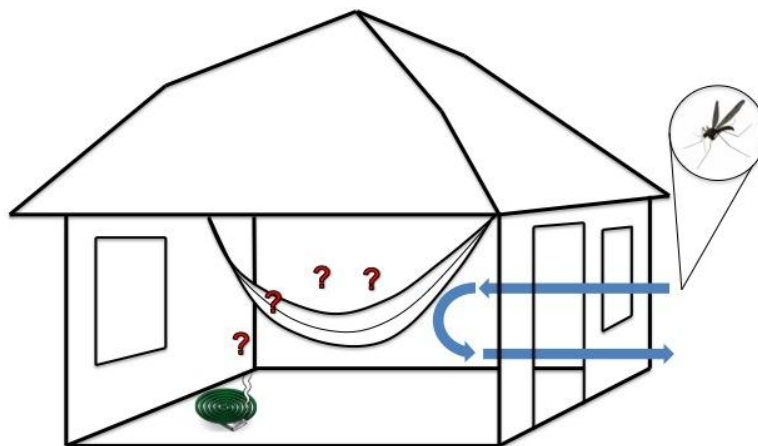


Figure 2. Conceptual model of mosquito coil use to repel nuisance (and potentially disease harboring) mosquitos from entering the living space of an occupied dwelling

is the combustion by-products of the coils that may pose a risk to human health and warrant a qualitative analysis. One estimate of a single overnight (eight-hour) exposure to mosquito coil emissions was equivalent to smoking more than 100 cigarettes (21).

Previous studies analyzing chemical by-products released during the combustion of mosquito coils have been conducted (7; 18; 20; 21; 46). However, these studies have focused only on quantifying levels of various pre-selected analytes following specific sampling methods. To expand on these previous studies and evaluate the potential health risks associated with exposure to smoke from metofluthrin-treated mosquito coils, a comprehensive characterization and qualitative analysis of the chemical by-products released during combustion of mosquito coils needs to be conducted.

#### **RESEARCH OBJECTIVES**

The purpose of this study was to characterize and compare the chemical compounds (other than the active ingredient) generated during the combustion of metofluthrin-treated and pyrethroid-free (blank) mosquito coils, to determine if these compounds may represent potentially hazardous chemical by-products, such as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and/or known human carcinogens, or chemical analytes that may also provide mosquito repelling qualities. This study also characterized and compared the physical properties (pre and post burn coil mass, airborne particulate matter mass, burn temperature and total burn duration) of treated and blank mosquito coils to determine if any of those parameters varied significantly between the different coils and resulted in the release of different chemical by-products. In addition, literature was reviewed to determine if any of the identified compounds are known to exhibit spatial repellent traits for mosquitoes.

## **RESEARCH QUESTIONS**

The following research questions were addressed, analyzed and answered during this study to evaluate variations between similar and different types of mosquito coils with respect to the physical characteristic and concentration of active ingredient.

### **Question #1:**

Does the chemical by-product profile generated during the combustion of a mosquito coil differ when compared to the chemical by-product profiles generated during the combustion of similar mosquito coils from the same manufacturer with the same listed concentration of active ingredient?

### **Question #2:**

Does the chemical by-product profile generated during the combustion of a blank mosquito coil differ from the profile generated during the combustion of a metofluthrin-treated mosquito coil from the same manufacturer?

### **Question #3:**

Do combustion temperatures vary between coils with the same and/or different listed concentration of the active ingredient? If so, does the varying combustion temperature result in the release of different chemical by-products, and at what specified temperatures?

### **Question #4:**

Does the combustion of mosquito coils generate chemical by-products known to present a risk to human health?

## **SPECIFIC AIMS**

The following Specific Aims were developed to answer the Research Questions listed above:

### **Specific Aim #1**

Characterize and compare the physical properties of treated (0.00975% metofluthrin, same manufacturer) and blank (same manufacturer as metofluthrin-containing coils) mosquito coils.

### **Specific Aim #2a**

Combust metofluthrin-treated mosquito coils (0.00975% metofluthrin, same manufacturer) in a controlled laboratory environment and qualitatively analyze and evaluate combustion by-products released using SPME for sample collection and subsequent GC-MS analysis.

### **Specific Aim #2b**

Combust blank mosquito coils (same manufacturer as metofluthrin-containing coils) in a controlled laboratory environment and qualitatively analyze and evaluate combustion by-products released using SPME for sample collection and subsequent GC-MS analysis.

## CHAPTER 2: LITERATURE REVIEW

### MOSQUITO COILS

Liu *et al.* (1987) measured the toxic effects following exposure to allethrin mosquito coils in rats. As part of this study, the physical properties of the mosquito coil and the volatile and semi-volatile chemical vapors, in addition to the particulate matter/smoke, emitted into the air during the combustion were characterized (20). Ground coils and coil ash were collected and quantified for content. A Soxhlet apparatus was used to extract and determine the level of active ingredient in the coil material, where the heavy metal concentration was analyzed using an atomic absorption spectrophotometer. Other organic compounds of the coil were collected in an impinger containing hexane and analyzed by a GC equipped with a flame ionization detector (FID). It was reported that the smoke emitted by the burning mosquito coil contained respirable particles coated with allethrin, along with significant amounts of heavy metals (cadmium and lead) and organic vapors (acids, phenols, carbonyls, alcohols, esters, and hydrocarbons) adhering to the particles as well. The study concluded that long-term inhalation exposure to the mosquito coil smoke could cause adverse health effects to the consumer.

Lazaridis and Löfroth (1987) evaluated the release of airborne particles containing a range of polycyclic aromatic hydrocarbons (PAH) in semi-enclosed spaces from heating two brands of insecticide (mosquito) coils (19). Coils contained 0.30% and 0.35% (by weight) pyrethrins and originated from Sweden and Greece, respectively. The coils were lit in a 15m<sup>3</sup> sealed room with no external supply air. Room air was only circulated by a small fan. Particulate matter samples were collected from burning coils using glass fiber filter media connected to a pump set at a flow rate of 10m<sup>3</sup>/h (166.67

L/min). The Ames test was used to evaluate the mutagenicity of smoke extracts. The authors conclude that the mutagenic activity exhibited in response to the exposure to particulate matter containing PAH demonstrates potential carcinogenic effects following exposure to mosquito coil smoke.

Chang and Lin (1998) utilized a 146.3L polypropylene burning chamber to analyze the smoke generated while burning 2 cm long pieces of mosquito coils containing the active ingredient allethrin (7). The airflow, temperature and humidity within the testing chamber were maintained constant during the burning of the mosquito coil pieces. Once the coils were ignited using a nickel chrome wire, various sampling methods were used to collect the respective particulates contained within the smoke. Glass fiber filters were used to collect particulate mass in the inhalable range (<100 microns in size) and total particulate samples were captured on a membrane filter in accordance with NIOSH Method 7400, while silica gel sorbent was used for collecting gaseous samples. The sampling identified the presence of formaldehyde, acetaldehyde and acrolein in gaseous phases, as well as adsorbed to the surface of particulate matter collected from the mosquito coil smoke. The amount of formaldehyde collected in the sample exceeded the ACGIH Threshold Limit Value - Ceiling (TLV-C) limit, while acrolein was detected near its TLV-C limit. Interestingly, the active ingredient, allethrin, was only detected in the particulate samples. This discovery lead the authors to speculate that these particulates may be too fine to adhere to insects; thus, they imply that allethrin may not serve well as an effective spatial repellent for mosquitoes.

Krieger, *et al.* (2003) conducted a chemical analysis of *d*-allethrin mosquito coils containing octachlorodipropyl ether (S-2) (18). Frequently found in pyrethrin or *d*-

allethrin mosquito coils, S-2 acts as a synergist ingredient. Hydrogen chloride and formaldehyde, formed from the combustion of S-2, can react in the “smoky air” from a mosquito coil to produce bis(chloromethyl)ether (BCME), a known lung carcinogen. Mosquito coils were manufactured in China and obtained from various retail outlets in Indonesia as well as Asian markets in southern California. The authors conclude by acknowledging that there is not enough knowledge to support safe use of mosquito coils containing S-2. In addition, they recommend that further study of the presence of BCME as a combustion product must be determined.

Liu *et al.* (2003) evaluated the potential human health implications of emissions associated with mosquito coils (21). This study characterized the emissions of four common brands of mosquito coils manufactured in China and two common brands produced in Malaysia. Although active ingredients were similar between the different brands, the base material varied by the coil’s country of origin. Sawdust was mainly used as the base material of the Chinese coils, whereas the coils originating from Malaysia were manufactured using coconut husks or shells. Emission rates were evaluated for specific by-product analytes produced from these filler materials. Fine particulate matter less than 2.5 micrometer in diameter ( $PM_{2.5}$ ), polycyclic aromatic hydrocarbons (PAHs), carboxyl compounds, and volatile organic compounds (VOCs) were collected during combustion of a mosquito coil in a  $0.15\text{ m}^3$  polyvinyl chloride experimental chamber. An eight-channel optical particle counter was used to quantify particulate matter concentrations. PAHs were extracted from the  $PM_{2.5}$  filters in a Soxhlet apparatus using dichloromethane. The PAH samples were then analyzed using a high-performance liquid chromatograph system with a fluorescence detector attached. A 2,4-dinitrophenyl

hydrazine-coated cartridge was used to evaluate the smoke for carboxyl compounds. Stainless steel traps Tenax TA were used to collect and evaluate VOCs. The study identified concentrations of fine (0.1 – 1.0  $\mu\text{m}$  in diameter) and ultrafine ( $< 0.1 \mu\text{m}$  in diameter) particles, PAHs, VOCs, and aldehydes, all of which may result in irritation of the respiratory system, that exceeded health-based regulations and guidelines. Based on these findings, the study's authors speculated that exposure to the smoke generated from these mosquito coils could pose both acute and chronic health risks to humans.

Zhang, *et al.* (2010) compared “products of incomplete combustion” (PIC) data from Liu *et al.* (2003) with the results of their independent study of newer mosquito coils to determine if there had been any product improvements regarding emission rates of PIC (46). The main purpose of this study was to compare a new “smokeless” brand of mosquito coil, which uses charcoal powder opposed to conventional materials like saw dust or coconut shells. However, for supplementary comparison purposes, four additional brands of conventional, smoke-producing mosquito coils were also evaluated and compared during this study. Coils were lit in a  $0.15\text{m}^3$  constant flow, filtered air, sealed chamber. Based on previous studies, emission rates were measured for particles with an aerodynamic diameter of  $\leq 2.5\mu\text{m}$  ( $\text{PM}_{2.5}$ ) mass, along with the total particle number. In addition, the gas-phase and particle-phase for 16 target polycyclic aromatic hydrocarbons (PAHs), 14 aldehydes and acetone, and 10 volatile hydrocarbons were also evaluated. Four separate sampling methods were employed for sample collection: 1) A real-time particle mass concentration monitor, a condensation particle counter and an eight-channel optical particle counter were used to measure  $\text{PM}_{2.5}$  the number and mass concentrations of the particles. 2) A quartz fiber filter followed by polyurethane foam

filled tube connected to a sampling pump was used to measure the gas-phase and particle phase of PAHs. 3) U.S. Environmental Protection Agency Method TO-11A was followed to measure for carbonyl compounds. 4) Standard charcoal tubes connected to a sampling pump were used to collect volatile hydrocarbons. The results of this study demonstrated that PM<sub>2.5</sub> emission rates of the smoke producing coils were comparable to coils analyzed in the Liu *et al.* (2003) study. However, the “smokeless” coils displayed PM<sub>2.5</sub> emission rate five to ten times less than the non-smokeless coils in this study, along with a two to three-fold reduction in formaldehyde (and other similar aldehydes). The remaining test parameter findings from this study were similar to findings from Liu *et al.* (2003).

#### **METOFLUTHRIN**

Matsuo *et al.* (2005) conducted various studies to evaluate the efficacy of metofluthrin for repelling various types of mosquito species (23). Several of the tests involved placing common house mosquitoes in 28m<sup>3</sup> and 30m<sup>3</sup> chambers containing mosquito coils treated with various concentrations of metofluthrin (ranging from 0.005 – 0.04%). Knockdown efficacy was evaluated and compared to coils containing 0.2% *d*-allethrin. In all incidents, the metofluthrin-treated coils demonstrated quicker knockdown time for 50% (KT<sub>50</sub>) of the test mosquito population than the *d*-allethrin coils. An additional field study was conducted to compare and evaluate the percent reduction of 0.005% metofluthrin-treated coils to 0.03% transfluthrin and 0.3% *d*-allethrin coils. As with the previous study, the metofluthrin coils performance was superior to the other test coils and produced a greater percent reduction of mosquitoes. This study demonstrated that metofluthrin is a very effective pyrethroid when applied as the active ingredient in

mosquito coils. In addition, because of the relatively high volatility when compared to other pyrethroids, metofluthrin also serves as a viable insecticide for other non-heated formulations as well. The authors did not address any characterization of the potential by-products that were released during the combustion of the metofluthrin-treated mosquito coils.

#### **NON-PYRETHROID SPATIAL REPELLENTS**

Synthetic insect repellants like *N,N*-diethyl-3methylbenzamide (DEET) and (1*S*,2'*S*)-2-methyl-piperidiny1-3-cyclohexane-1-1carboxamide (SS220) work extremely well to repel mosquitoes in both topical and spatial applications (8). Burning of citronella has demonstrated to be effective against mosquitoes and is recognized as an insect repellent by the United States Environmental Protection Agency (USEPA) and Centers for Disease Control and Protection (CDC) (16).

Additional studies have been conducted on evaluating the biting deterrence of mosquitoes when exposed to saturated and unsaturated fatty acids. Throughout Oceania, dried male inflorescences (clusters of flowers) of breadfruit are burned to repel flying insects, including mosquitoes (15). Jones, *et al.* (2012) evaluated crude extracts collected from male inflorescences collected of breadfruit trees in Hana, Maui, HI. More than 30 compounds were identified from the extracts that included terpenes, aldehydes, fatty acids and aromatics (15). Their study identified capric acid, undecanoic acid and lauric acid, all fatty acids, presented repelling qualities towards female *Aedes aegypti* adult mosquitoes. Furthermore, these three fatty acids were found to more effective mosquito repellents than DEET, which was used as a positive control for the study.

In India and parts of Africa, the burning of seed oil extracted from the *Jatropha curcas* plant is used to repel mosquitoes (5). Cantrell, *et al.* (2011) evaluated fatty acid and triglyceride extracts from *J. curcas* seed oil (5). Their study concluded that oleic, palmitic, linoleic and stearic fatty acid extracts deterred *Aedes aegypti* mosquitoes.

Ali *et al.* (2012) evaluating the biting deterrence of several saturated and unsaturated fatty acids against *Aedes aegypti* mosquitoes (4). The study evaluated and compared 12 saturated ( $C_{6:0}$  to  $C_{16:0}$  and  $C_{18:0}$ ) and 7 unsaturated ( $C_{11:1}$  to  $C_{14:1}$ ,  $C_{16:1}$ ,  $C_{18:1}$  and  $C_{18:2}$ ) fatty acids. Results of the study demonstrated that the mid-length chain ( $C_{10:0}$  to  $C_{13:0}$ ) saturated fatty acids provided the greater repelling characteristics. Three of the unsaturated fatty acids ( $C_{11:1}$ ,  $C_{12:1}$ , and  $C_{14:1}$ ) evaluated also exhibited notable mosquito repelling qualities. The study concluded that undecanoic, dodecanoic, 10-undecenoic and 11-dodecenoic acids demonstrated the greatest repelling abilities, when compared to DEET.

#### **SOLID PHASE MICROEXTRACTION**

Given the many potentially uncharacterized compounds that may be produced during the combustion of mosquito coils, traditional sorbent-based sampling and solvent-based analysis for each of the individual airborne compounds produced from coil burning may present a very time-consuming and expensive endeavor. In addition, the employment of established National Institute of Occupational Safety (NIOSH) methods for qualitatively/quantitatively identifying airborne contaminants requires prior knowledge of what is expected to be released into the air to ensure proper sorbent material selection and sampling pump calibration. Conversely, implementing the solid phase microextraction (SPME) sampling approach can minimize the number of different

experiments needed to conduct a comprehensive qualitative analysis, given its capability to collect a wide range of volatile and semi-volatile airborne compounds. The SPME technique is a solvent-free and passive sample preparation alternative to other conventional sampling approaches, which employs a sorbent-coated fiber to extract different analytes from an airborne environment (30). Opposed to using an analyte specific sorbent media attached to calibrated sampling pump, the SPME technique employs a passive sampling technique, requiring no sampling pump, that can sample for a wide range of airborne volatile compounds. The SPME fiber passively extracts the sample when the analytes dissolve and diffuse into the coating material (40). Fiber coatings can be selected to sample for a wide range of polar, semi-polar and non-polar organic compounds (34). Conventional sampling procedures are usually time-consuming, labor intensive, multi-stage operations (31). Each step in this process is susceptible to errors and loss of sampling material, especially in situations involving the analysis of highly volatile compounds (40). The SPME technique integrates sample extraction, concentration and purification into one step making it an inexpensive and rapid method to collect the sample (31). The SPME sample can be delivered directly into an analytical system, such as a gas chromatography-mass spectrometry (GC-MS) instrument, with no additional sample preparation. The SPME collection fiber can be inserted directly into the heated GC inlet and analyzed immediately after the sample is collected. Once the fiber has been removed from the GC inlet, it can be reused instantly with no further sample media preparation (40). Considering the likelihood that many different chemical compounds are released during the combustion of a mosquito coil,

the SPME technique can be a very effective and efficient method to use when conducting a qualitative analysis.

### **PARTICULATE MATTER (TOTAL DUST)**

In addition to the release of volatile and semi-volatile chemical vapors, mosquito coils also disperse dust particles from the combustion of the inert filler material used to make up the majority of the coil mass. Dust particles contained in smoke emitted from a mosquito coil as particulate matter has shown to elicit spatial repellent effects without the presence of an insecticidal active ingredient (1). Particulate matter generated from the combustion of organic filler materials repels mosquitoes by causing irritation to the mosquito's sensory organs (1). However, dust particles can be harmful to humans as well. The World Health Organization defines dusts as solid particles that range in size from below 1  $\mu\text{m}$  up to at least 100  $\mu\text{m}$  (44). Larger particles (greater than 30  $\mu\text{m}$ ) are deposited in the upper airway in the head (nose and larynx), where smaller particles (less than 10  $\mu\text{m}$ ) can travel to the alveolar region of the lungs, where gas exchange with the blood takes place (44). Heavy metals, like lead, cadmium and zinc, can be released from a burning mosquito coil and adhere to particulate matter emitted in the smoke produced during combustion (22; 32). The presence of these heavy metals attached to these particles can lead to health problems with the pulmonary, immune and central nervous systems in the body (17). Therefore reducing exposure to dust produced by a mosquito coil is essential to public health. There is no Recommend Exposure Limit (REL) for total dust set by NIOSH, however, the Occupational Safety and Health Administration (OSHA) has established a Permissible Exposure Limit (PEL) at 15  $\text{mg}/\text{m}^3$  and the American Conference of Governmental Industrial Hygienist recommends the Threshold

Limit Value (TLV) not exceed  $10 \text{ mg/m}^3$  (25). Dust particles are a major component of mosquito coil smoke and serve as the vehicle to transport the numerous potentially hazardous by-products released from the combustion of a mosquito coil. Mosquito coils are generally restricted to residential use, while exposure standards have only been established for the occupational setting. However, knowing the potential mass/concentration of dust generated by a mosquito coil and the associated health risks, health officials, along with industry, can strive to promote safer methods to manufacture and use mosquito coils.

#### **LITERATURE GAP**

Analytes released during the combustion of pyrethroid-based mosquito coils have actively been studied over the past 30 years. However, current literature has been focused mainly on the detection and quantification of specific analytes of potential human health consequences. No published studies were found that provide a complete characterization of chemical by-products released from mosquito coils that also may provide mosquito-repelling capabilities, like the release of fatty acids. Additionally, there was no data uncovered that focused on the release of specific analytes produced from the burning mosquito coils treated with metofluthrin. Therefore, a comprehensive, qualitative analysis is necessary to determine what (if any) additional potentially hazardous compounds are generated during the combustion of metofluthrin-treated mosquito coils. By implementing a passive, sorbent-free sampling technique like SPME, a number of previously unidentified combustion by-products, such as fatty acids, aromatics, and aldehydes, may be identified that in coil smoke that may pose risk to human health. Additionally, these chemical by-products may exert their own spatial

repellent qualities that could be further investigated. Current literature also fails to address the following questions: 1) Do combustion temperatures vary between coils with different concentrations of the active ingredient? 2) Do varying combustion temperatures between mosquito coils result in release of different chemical by-products? This study aimed to address these issues by identifying all detectable chemical by-products released from pyrethroid-based mosquito coils containing varying listed concentrations of the active ingredient, metofluthrin.

## CHAPTER 3: MATERIALS AND METHODS

This study consisted of two experimental analyses. The first experiment involved characterizing and comparing the physical properties (pre and post burn coil mass, airborne particulate matter mass, burn temperature and total burn duration) of metofluthrin-treated and blank mosquito coils to determine if confounding variables other than the presence of active ingredient affects the outcome of the chemical by-product profile generated during the combustion of mosquito coils. The second experiment employed a qualitative analysis of the components found within both treated and blank mosquito coils using analytical instrumentation to determine if there were differences in the chemical by-product profile generated when combusting different types of coils. Data collection and analysis for this study was conducted at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Beltsville Agricultural Research Center-West (BARC-West), Invasive Insects Biocontrol & Behavior Laboratory. No human subjects were exposed during any portion of the experiments.

### MATERIALS

#### Mosquito Coils

Mosquito coils were received in packages containing five pair of intertwined coils per pack (10 individual coils per pack). Coils were evaluated in this study included:

Treated Coils (0.00975% metofluthrin, SC Johnson & Co. Inc., Racine, WI) – Coils were spray dosed with a 0.00975% solution of metofluthrin.

Blank coils (untreated, SC Johnson & Co. Inc., Racine, WI) – Coils were manufactured in the same manner as the treated coils, but contained no active ingredient.

### **Filter Cassettes**

Particulate matter samples were collected using preloaded matched-weight GLA 5000 membrane polyvinyl chloride (PVC) filters (Cat. No. 225-8202, SKC Inc., Eighty Four, PA). The filters measured 37 mm in diameter and 5 $\mu$ m thick and were preloaded and sealed into the cassette in a controlled laboratory environment by the manufacturer. Matched-weight filters were selected because no filter pre-weighing or conditioning was required, which allowed for sampling to be made quicker and with greater accuracy (35). These filter cassettes contained two matched-weight filters, where the top filter collected the particulate matter and the bottom filter served as the control. Once sampling was completed, the filters were weighed again. The difference between the two filters represented the sample weight.

### **SPME Fibers**

Volatile and semi-volatile chemical vapors emitted into the air during the burning of mosquito coils were collected using three different SPME fiber types. SPME fibers were selected with respect to their affinity towards non-polar, semi-polar, and polar compounds (34). The fiber coatings included:

30 $\mu$ m Polydimethylsiloxane (PDMS) (Product # 57308, Sigma-Aldrich Co. LLC, St. Louis, MO) – Selected to sample for non-polar semi-volatile compounds with a molecular weights ranging from 80-500 atomic mass units (amu) (34).

75 $\mu$ m Carboxen/polydimethylsiloxane (CAR/PDMS) (Product # 57318, Sigma-Aldrich Co. LLC, St. Louis, MO) – Selected to sample for semi-polar gases and trace-level volatile compounds with a molecular weight ranging from 30-225 amu (34).

85 $\mu$ m Polyacrylate (PA) (Product # 57304, Sigma-Aldrich Co. LLC, St. Louis, MO) – Selected to sample for polar semi-volatile compounds with a molecular weight ranging from 80-300 amu (34).

### COMBUSTION CHAMBER DESIGN

A “smokestack” style combustion chamber (Figure 3a) was constructed using a piece of stainless steel ventilation duct material (8 in. diameter x 24 in.) with both ends left open. An eight-inch to six-inch reducer was placed on the top opening. A damper was installed and set open at a 45-degree angle in the smaller portion to reduce airflow escaping the chamber (Figure 3b). An inspection door (11 in. x 3.5 in.) was installed two inches from the bottom of the chamber. Fifteen solid phase microextraction (SPME) fiber sampling ports were mounted on the sidewalls of the chamber staggered one inch to



Figure 3. (a) “Smokestack” combustion chamber, (b) damper, and (c) sampling ports

the right, two inches to the left and one-half inch to the top and/or bottom of the adjacent port (Figure 3c).

#### **PRELIMINARY SAMPLING AND METHOD DEVELOPMENT**

Preliminary samples were collected and analyzed using GC equipped with a Flame Ionization Detector (5890 Gas Chromatograph, Agilent Technologies, Inc., Santa Clara, CA; 5890 Flame Ionization Detector, Agilent Technologies, Inc., Santa Clara, CA). Test coils were ignited and contained within the aforementioned open-ended cylindrical combustion chamber. Samples were collected with all three SPME fiber types at various exposure durations (1 min, 5 min, 15 min, 30 min, 60 min, 120 min, 240 min and 360 min). SPME fiber samples were then analyzed using the GC-FID. The inlet temperature was set at the recommended conditioning temperature for the respected SPME fiber. This enabled the maximum potential of compounds collected on the fiber volatilizing and entering the GC column, while reducing the risk of excessive wear and tear to the SPME fibers due to exposure to extreme temperatures. The oven of the GC was set at 40°C with an initial temperature ramp rate established at a constant 10°C increase per minute until the oven reach a maximum temperature of 300°C. This temperature range was selected to help ensure chemical compounds collected volatilized and entered the mobile phase in the GC column. Peak response and resolution were evaluated for each SPME fiber coating. Temperature ramp rate periods were adjusted accordingly to maximized peak separation and increase overall resolution (Table1). Fiber specific sampling periods were established to provide a sufficient peak response without compromising overall resolution due to potential co-elution of collected chemical compounds.

Table 1. GC inlet temperature and oven temperature ramp rate parameters

Fiber Coating	Inlet Temp. (°C)	Ramp Period	Oven Temperature Ramp Rates			Run Time (min)
			Ramp Rate (°C/min)	Next °C	Hold Time (min)	
PDMS	250	Initial	-	40	0.5	0.5
		1	5.0	95	0	6.0
		2	0.5	100	0	16.0
		3	5.0	140	0	24.0
		4	2.0	180	0	44.0
		5	5.0	220	0	52.0
		6	40	300	0	54.0
		Post	-	300	0.5	54.5
CAR/PDMS	300	Initial	-	40	0.5	0.5
		1	5.0	105	0	13.5
		2	0.25	106	0	17.5
		3	0.5	112	0	29.5
		4	2.0	120	0	33.5
		5	5.0	160	0	41.0
		6	40.0	300	0	45.0
		Post	-	300	0.5	45.5
PA	280	Initial	-	40	0.5	0.5
		1	10.0	80	0	4.5
		2	5.0	100	0	8.5
		3	0.25	104	0	24.5
		4	2.0	120	0	32.5
		5	5.0	180	0	44.5
		6	40	300	0	47.5
		Post	-	300	0.5	48.0

### SAMPLE SIZE

This is an exploratory study; using Lenth's *Java applets for power and samples size*, confidence interval for one mean analysis, a sample size of five (5) for each coil type analyzed was determined to be sufficient to estimate a 95% confidence interval with a margin of error of  $\pm 1.242$  standard deviations (Lenth, 2006-9).

### PHYSICAL CHARACTERISTICS

Coils were combusted and analyzed one at a time during this study. To begin, the mass of each coil was measured using a laboratory balance with a measurement tolerance

to 0.0001 grams and recorded prior to burning. The coil was situated into a terracotta bowl lined with a pre-weighed piece of aluminum foil (Figure 4), placed in the combustion chamber and ignited. All coil combustion was performed within a laboratory



Figure 4. Sample mosquito coil in aluminum foil lined ash collection bowl

chemical fume hood. Laboratory records reported that the flow rate of the hood was maintained at 80 cubic feet per minute with the sash opened to 18 inches. Throughout the coil combustion period, ash from the coil fell into the lined bowl and was collected on the foil. Once the entire length of the coil had been combusted, a process which typically lasted seven hours, the ash and the foil were weighed again to calculate the remaining portion of the mosquito coil that was not released into the atmosphere. On an hourly basis throughout the combustion period, the burn temperature of the coil was monitored and recorded. An infrared thermometer (Model # 412, Cooper-Atkins Corp., Middlefield, CT) equipped with a microneedle thermocouple (Model # 50209, Cooper-Atkins Corp., Middlefield, CT) was used to measure temperature of the coil. The

thermocouple tip was placed onto the hottest point of the coil, a blackened area between the unburned coil and the coil ash where smoldering was visually observed (Figure 5).



Figure 5. Illustration of the smoldering mosquito coil highlighting the region where the coil combustion temperature was measured

The combustion duration, start to finish, was monitored and recorded using a stopwatch and was initiated once the release of smoke was visually confirmed from the smoldering portion of the coil and ending when no more smoke was visible.

Ambient air samples were also collected to measure for the total mass of particulate matter released into the immediate atmosphere during the combustion of the mosquito coils. Sample collection and analysis was conducted in accordance with National Institute of Occupational Safety and Health (NIOSH) Method 0500 – *Particulates Not Otherwise Regulated, Total* (25). Three samples utilizing this method were collected for each mosquito coil sample run using pre-loaded, PVC filter cassettes connected to sampling pumps (Model AirChek XR5000, SKC Inc., Eighty Four, PA) operated at a flow rate of 1.5 liters per minute for one hour (Table 2). Once the sampling

train was assembled, the filter cassettes were inserted 10.5 inches into the combustion chamber through the exhaust opening and suspended inside (Figure 6). Two field blank

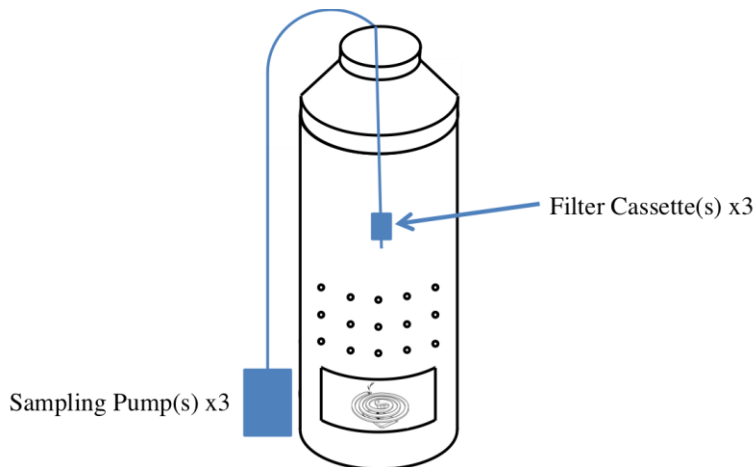


Figure 6. Filter cassette sampling train

samples (a total of 20) were also submitted for analysis for each mosquito coil combusted in the study (Table 2). The purpose of these blank samples was to help validate the integrity of the air samples during storage and transportation. Blanks were handled in the same fashion as the samples except that no air was drawn through them. The particulate matter samples were subsequently sent to the United States Navy Comprehensive Industrial Hygiene Laboratory (CIHL), Norfolk, VA, to be analyzed.

### Sample Collection

Mosquito coils were ignited and contained within the aforementioned open-ended cylindrical combustion chamber. The coil was allowed to burn for one hour before any sampling to allow sufficient time for the inner volume of the combustion chamber to achieve a state of equilibrium of the chemical vapors released by the burning coil.

Sample collection was conducted using three different SPME fiber coatings, PDMS,

CAR/PDMS and PA. Three SPME fiber samples were collected for each fiber coating (for a total of nine samples per coil) (Table 2). The fibers were each placed into a SPME fiber holder (Product # 57330-U, Sigma-Aldrich Co. LLC, St. Louis, MO) (Figure 7a) and inserted concurrently through sampling ports installed on the side of the combustion chamber. The CAR/PDMS and PA fibers were removed from the chamber after one hour of exposure to the mosquito coil smoke, while the PDMS fibers were exposed to the coil smoke for two hours. During the GC method development, the PDMS fibers demonstrated that a longer exposure period was necessary to elicit a sufficient signal response without interfering with peak resolution. The fume hood sash was closed during



Figure 7. (a) SPME fibers and holder, and (b) SPME fiber storage container

the SPME sampling period to limit potential contamination from air outside the hoods filtration system. Once sampling was completed, the SPME fibers were sealed, held at room temperature and stored in specifically designed storage containers (Cat. No. 57589-U, Chromline, Prato, Italy) (Figure 7b) until time of analysis (typically one to 23 hours). These containers incorporated a polytetrafluoroethylene (PTFE) sealing cup and a spring loaded sealing mechanism that protected the fibers from exposure to outside contaminants (9). In a study conducted by the manufacturer, CAR/PDMS SPME fibers were exposed to 14 volatile compounds then subsequently sealed in the storage

containers (9). This study demonstrated that less than 10% of the material was lost after 15 days while samples were held at room temperature. Once analysis was complete, fibers were returned to the storage containers to protect and ensure future sample integrity.

The SPME fibers were analyzed at the USDA Invasive Insects Biocontrol & Behavior Laboratory with a GC-MS instrument (6980N GC Network, Agilent Technologies, Inc., Santa Clara, CA; 5973 inert Mass Selective Detector, Agilent Technologies, Inc., Santa Clara, CA) using the fiber specific inlet temperature and oven temperature ramp rate parameters developed during preliminary testing. The GC inlet contained a 0.75 mm inside diameter (ID) straight/SPME liner (Cat. No. 21111 Agilent Technologies, Inc., Santa Clara, CA). A non-polar, low bleed DB-5 J&W GC column of 30 m length and 0.25 mm internal diameter and 0.25  $\mu\text{m}$  film thickness (Part. No. 122-5032, Agilent Technologies, Inc., Santa Clara, CA) was used. Each chemical compound identified through GC peak integration was verified through the use of the National Institute of Standards and Technology (NIST) Mass Spectral Database (version NIST 11, NIST, Gaithersburg, MD), which compared each analyzed sample compound against thousands of chemical mass spectra for probability matching. In addition, one background sample for each of the three fiber coatings was collected each day of sampling prior to lighting the sample coil for that day (Table 2). The background samples were collected inside the chemical fume hood using the same sampling durations that were developed for the active sampling period.

These methods were repeated using mosquito coils containing no insecticide, i.e. blank. The chemical by-product profile generated during the combustion of the blank

Table 2. SPME Fiber and Filter Cassette sampling schedule

Coil Type	SPME Fibers Samples						Filter Cassettes			
				Background						
	PDMS	CAR/PDMS	PA	PDMS	CAR/PDMS	PA	Total	Sample	Field Blank	Total
Treated Coils	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
Subtotal	15	15	15	5	5	5	60	15	10	25
Blank Coil	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
	3	3	3	1	1	1	12	3	2	5
Subtotal	15	15	15	5	5	5	60	15	10	25
Total	30	30	30	10	10	10	120	30	20	50

mosquito coils was compared to the results of the treated coils to determine if lack of active ingredient (and varying combustion temperature, if applicable) affected the presence of released chemical by-products.

### STATISTICAL ANALYSIS

Statistical analysis was performed in SPSS Statistics (version 22, IBM Corp, Armonk, NY). The Student's t-test was used to analyze data collected for the Physical Characterization (pre and post burn coil mass, airborne particulate matter mass, burn temperature and total burn duration) studies. An analysis of frequencies was conducted on the list of detected chemical compounds collected during the qualitative analysis study using the Fisher exact test. All study data was managed and graphs were plotted using Microsoft<sup>®</sup> Excel<sup>®</sup> for Mac 2011 (version 14.4.1, Microsoft Corp, Redmond, WA).

## CHAPTER 4: RESULTS AND DISCUSSION

### PHYSICAL CHARACTERISTICS

#### Mosquito Coil Mass

The mosquito coil mass study results (Table 3) indicated a significantly ( $p=0.061$ ) greater pre-burn mass (mean = 12.662 g) for the treated mosquito coils when compared to the blank coils pre-burn mass (mean = 12.316 g). However, there was no significant ( $p = 0.214$ ) difference between the post-burn mean masses of treated (mean = 0.291 g) and blank (mean = 0.296 g) coils, indicating that the treated coils loss a significantly ( $p=0.021$ ) greater percentage of the starting material (Figure 8).

Table 3. Pre and post-burn masses of metofluthrin-treated and blank mosquito coils

Coil Type	Pre-burn mass (g)	Post-burn mass (g)	% Lost
Treated	12.7257	0.3011	97.63
	12.7559	0.2833	97.78
	12.7797	0.2888	97.74
	12.3604	0.2915	97.64
	12.6905	0.2919	97.70
Mean $\pm$ S.D.	12.662 $\pm$ 0.172	0.291 $\pm$ 0.006	97.70 $\pm$ 0.064
Blank	12.1146	0.2939	97.57
	12.0898	0.2884	97.61
	12.0789	0.3001	97.52
	12.5218	0.2985	97.62
	12.7739	0.3006	97.65
Mean $\pm$ S.D.	12.316 $\pm$ 0.316	0.296 $\pm$ 0.005	97.59 $\pm$ 0.050

Limited information is known about the specific manufacturing processes that were involved in the production of the sample mosquito coils. Both coil types were produced and supplied from the same manufacturer. The treated coils were marked “spray treated with 0.00975% metofluthrin.” Assuming both the treated and blank coils were manufactured in the same manner, the amount of base material in both types of coils

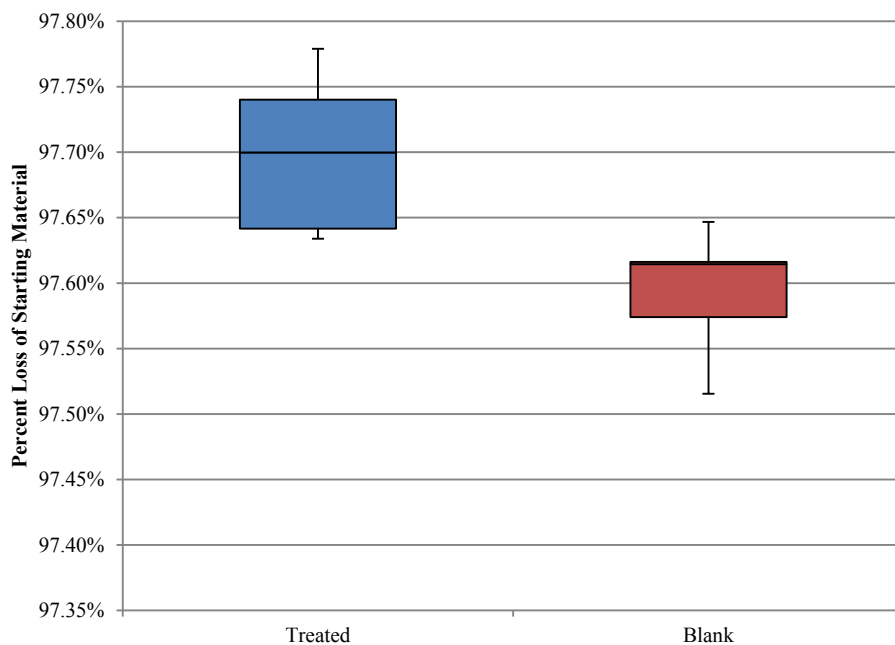


Figure 8. Percent loss of starting material comparison box-and-whisker plot for treated and blank mosquito coils

should be similar. If the treated coil mass was measured prior to spray treatment, no significance difference should exist in the amount of base material used in manufacturing each coil type. Once metofluthrin and the added fragrance are applied to the treated coils, it is presumed that the treated coil mass would be greater than a blank coil. The similarity between the post-burn masses suggests metofluthrin and the fragrance applied to the treated coil are volatilized during the coil combustion and released into the air. Those chemical compounds are either emitted as chemical vapors or would be found adhering to the surface of airborne particulate matter produced through combustion of the coil. It is not expected for metofluthrin and the added fragrance to be contained in the left over coil material that was collected during this experiment.

## Airborne Particulate Matter Mass

Analysis of the airborne particulate matter revealed that treated coil particle mass and concentration (mean = 2370.00  $\mu\text{g}$ ; mean = 26.33  $\text{mg}/\text{m}^3$ ) was slightly greater than mass and concentration (mean = 2182.67  $\mu\text{g}$ ; mean = 24.25  $\text{mg}/\text{m}^3$ ) of particles collected from blank coils (Table 4). However, there was no statistical difference ( $p=0.290$ ) between those results (Figure 9).

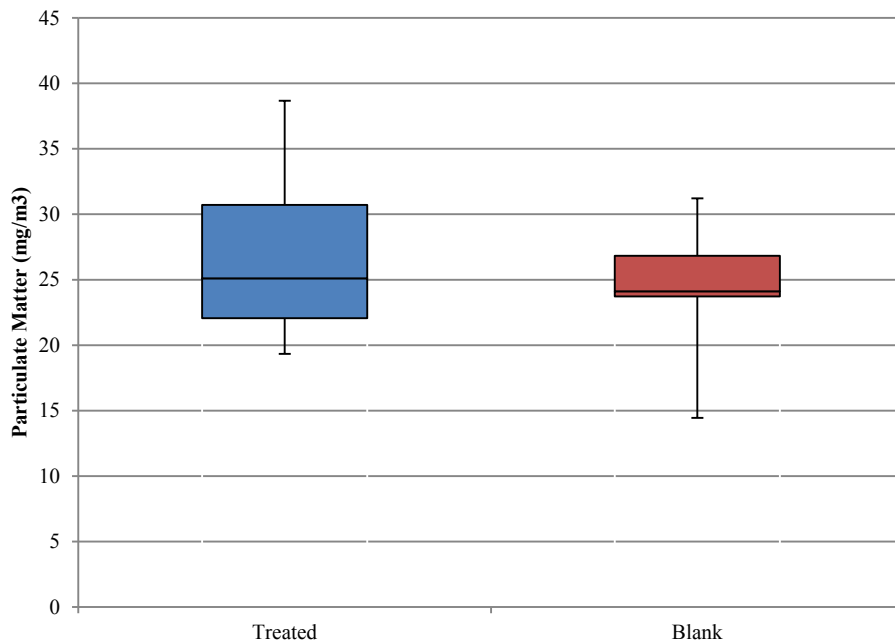


Figure 9. Airborne particulate matter mass comparison box-and-whisker plot for treated and blank mosquito coils

The data suggests that the presence of metofluthrin and/or added fragrance does not contribute to the overall mass of collected particulate matter. Although not significantly different, the increased mean total dust particle mass collected from the treated mosquito coils may be attributed to metofluthrin and/or the added fragrance adsorbing to the surface of the particle emitted into the surrounding ambient air. The

Table 4. Airborne particulate matter mass and concentration from metofluthrin-treated and blank mosquito coils

Coil Type	Air Volume (L)	<-----Results----->		
		Total Particle Mass ( $\mu\text{g}$ )	Concentration ( $\text{mg}/\text{m}^3$ )	
Treated	90	2320	25.78	
	90	2980	33.11	
	90	2040	22.67	
	90	1740	19.33	
	90	1740	19.33	
	90	1780	19.78	
	90	2620	29.11	
	90	1930	21.44	
	90	2910	32.33	
	90	2090	23.22	
	90	2190	24.33	
	90	2560	28.44	
	90	2910	32.33	
	90	3480	38.67	
	90	2260	25.11	
	Mean $\pm$ S.D.		2370.00 $\pm$ 524.800	26.33 $\pm$ 5.832
	Blank	90	2210	24.56
		90	2460	27.33
90		2130	23.67	
90		2280	25.33	
90		2170	24.11	
90		2810	31.22	
90		2660	29.56	
90		2150	23.89	
90		2170	24.11	
90		2560	28.44	
90		2150	23.89	
90		1820	20.22	
90		1300	14.44	
90		1400	15.56	
90		2470	27.44	
Mean $\pm$ S.D.			2182.67 $\pm$ 419.344	24.25 $\pm$ 4.659

pesticide and fragrance only represent a small portion of the total coil mass, but at the microgram level, it is realistic to suspect that some additional particle mass may be the result of their presence. The purpose of this study was to evaluate differences in total dust particulate matter production between the types of mosquito coils. The nature in which the experiment was carried out, it is difficult to directly correlate the sample mean mass collected to potential human exposure concerns. The samples were collected in a small volume confined space in close proximity to the source. This design does not accurately reflect a potential human exposure instance. A more extensive study involving collection of particulate matter in a larger living space sized environment would be recommended to determine the potential exposure risks to total dust generated from mosquito coils. This would allow issues like airflow and dilution of particulate matter to be taken into account when assessing a potential health risk. Furthermore, this study only determined particle mass and concentration, not the chemical make-up of the dust particles. Future studies should look to characterize and quantify the particle composition, looking for known potential human health concerns, like the presence of heavy metals (lead, cadmium, zinc, etc.).

### **Mosquito Coil Total Burn Duration**

The average total burn duration for the blank (mean = 427.80 min) coils was slightly longer than the burn duration for the treated (mean = 425.20 min) coils (Table 5). However, there was no statistical difference ( $p=0.779$ ) between burn durations of coil types (Figure 9).

The data from this study suggests that the presence of metofluthrin and/or added fragrance does not affect how long the coil will burn once ignited. Burn duration appears

Table 5. Total burn duration for metofluthrin-treated and blank mosquito coils

Coil Type	Burn Duration (min)
Treated	431
	416
	436
	398
	445
Mean $\pm$ S.D.	425.20 $\pm$ 18.485
Blank	436
	418
	427
	435
	423
Mean $\pm$ S.D.	427.80 $\pm$ 7.727

to be more correlated with the coil mass. When comparing the burn durations recorded for all sample coils, the coils with a greater mass of starting material tended to burn for a longer duration. This should be expected because there is more material available to burn before the coil would extinguish on its own.

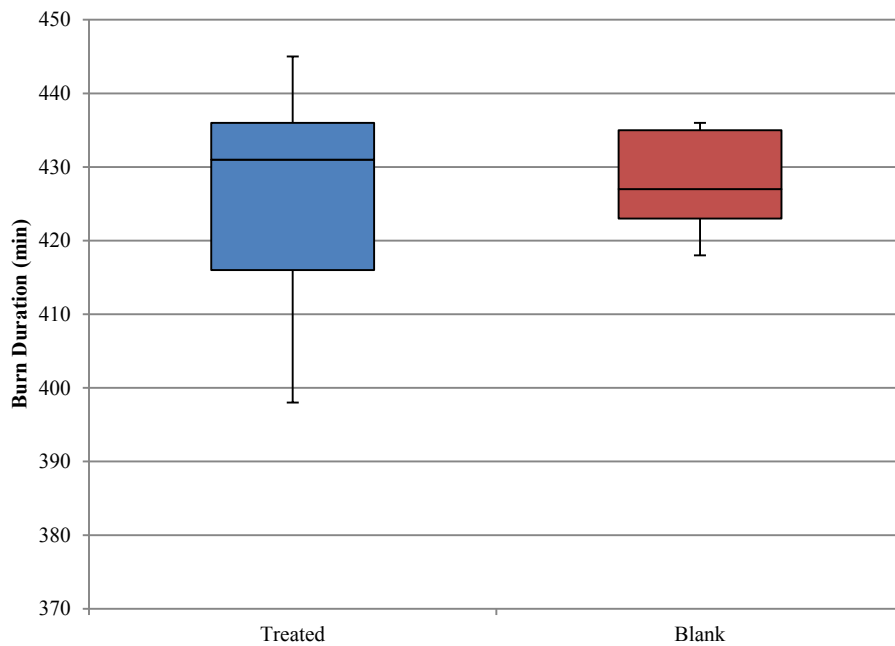


Figure 10. Total burn duration comparison box-and-whisker plot for treated and blank mosquito coils

### Mosquito Coil Burn Temperature

The mean burn temperature for the blank (mean = 321.03 °C) mosquito coils was slightly hotter than the burn temperature for the treated (mean = 316.43 °C) coils (Table 6). However, there was no statistical difference (p=0.242) between the burn temperatures of the different coil types (Figure 11).

Table 6. Mosquito coil burn temperature (°C) of metofluthrin-treated and blank mosquito coils

Coil Type	<-----Burn Temperature (°C)----->							Mean ± S.D.
	Initial	60 min	120 min	180 min	240 min	300 min	360 min	
Treated	280	332	354	301	278	304	351	314.29 ± 31.658
	265	330	311	304	316	319	316	308.71 ± 20.830
	310	306	311	306	329	324	343	318.43 ± 14.034
	326	332	303	335	311	325	322	322.00 ± 11.372
	301	321	315	310	326	314	344	318.71 ± 13.683
Mean ± S.D.								316.43 ± 19.119
Blank	334	340	337	317	335	331	334	332.57 ± 7.413
	320	337	317	342	321	342	318	328.14 ± 11.596
	287	329	319	312	324	312	320	314.71 ± 13.659
	304	309	303	329	309	313	324	313.00 ± 9.916
	326	311	324	318	319	300	328	316.71 ± 10.210
Mean ± S.D.								321.03 ± 12.856

The data suggests that the presence of metofluthrin and/or added fragrance does not affect the burn temperature of a mosquito coil. Recorded burn temperatures did vary between individual coils through out the total burn period. The coolest recorded temperatures were generally collected immediately after the coil was lit, before the coil was fully smoldering. After the first hour, the coils all exceeded combustion temperatures exceeding 300 °C. Every attempt was made to pinpoint the hottest region of each mosquito coil, however it was difficult to locate this region at times with the thermocouple tip. Any subtle movement of the thermocouple would sometimes result in

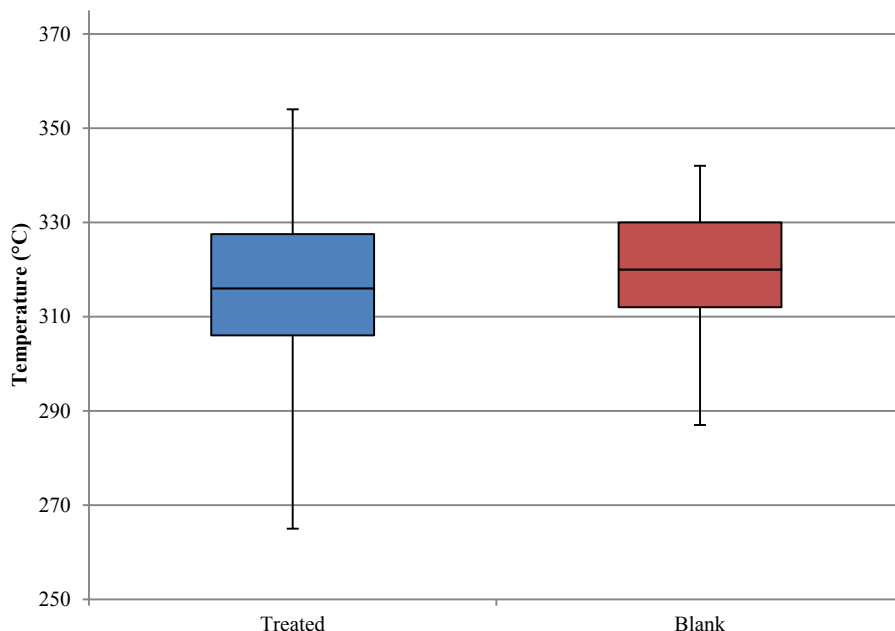


Figure 11. Burn temperature comparison box-and-whisker plot for treated and blank mosquito coils

drastic observed temperature changes (greater than 20 ° C at times). Due to the limitations of the temperature monitoring equipment, additional work should be completed using a more precise monitoring method to confirm no differences between the combustion temperature of different types of mosquito coils.

#### QUANTITATIVE ANALYSIS

The SPME fiber samples (n=120) yielded a total 6,492 individual peaks that exceeded a 3:1 signal to noise ratio. Using the NIST Mass Spectral database, 984 different chemicals were identified (both coil and background samples). Background samples (n=30) identified the presence of 189 different chemical compounds, which represented potential contaminants in the laboratory and chemical fume hood ambient air, were removed from the listing reducing the number of individual analytes detected from

the burning of mosquito coils to 795. Of these remaining chemical compounds, 219 analytes were detected in the baseline drift region of the evaluated chromatographs. This is an area of the chromatograph, towards the end of the sample run, where the GC baseline moves steadily (and generally rapidly) up scale (3). These compounds are generally detected as the result of column bleed from the GC column due to the higher GC oven temperatures associated with the end of the sample run. Consequently, those analytes detected in this region were also omitted from the total number of chemical compounds detected. An additional 267 were only detected once throughout all SPME fiber mosquito coil sample runs (n=90). The NIST 11 filtering algorithms have been comprehensively tested in an effort to avoid discarding a correct match, however the algorithm still uses “a rank peak in common” logic to attempt to identify the detected analyte (27). Because it is anticipated that the chemical compound profiles generated from similar mosquito coils should be comparable, those various compounds should be expected to be detected more than once throughout the sampling process. Therefore, chemical compounds only identified once were also excluded from consideration for evaluation, leaving a total of 309 different analytes for evaluation. Of the 309 remaining chemical compounds, 67 were rejected because the Match Quality of the compounds was less than 50. Match Quality values less than 50 indicates a substantial difference exists between the unknown and the reference chemical compound, making confirmation of the match uncertain (2). Match Quality values greater than 90 represents a very good match for the unknown compound. From the list of 242 compounds that had a Match Quality greater than 50, only 72 met the “very good match” criteria and were considered for further evaluation in this study (Figure 12).

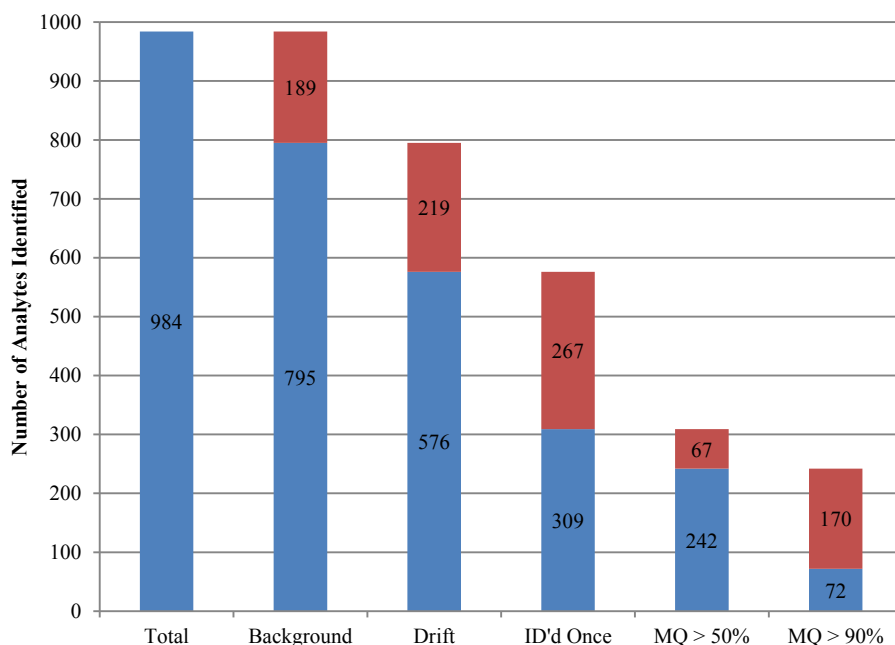


Figure 12. Bar graph illustrating number of detected analytes eliminated for specified reasons to derive the final count of compounds with a Match Quality greater than 90%

Those 72 analytes (Table 8) detected during this study represented 17 different chemical compound classifications (Table 7). Of the 72 analytes evaluated during this study, 39 (Table 8) were isolated from all 10 sample coils. Twelve additional chemical compounds (Table 8) were only isolated from one coil type (nine from treated coils and

Table 7. List of chemical compound classifications represented from the analytes detected during the burning of mosquito coils

Chemical Compound	<i>n</i>	Chemical Compound	<i>n</i>
Phenols	24	Furans	2
Aldehydes	9	Phenolic Aldehydes	2
Fatty Acids	8	Amides	1
Aromatic Hydrocarbons	6	Ethers	1
Ketones	4	Furfuryl Alcohols	1
Alkanes	3	Lactones	1
Alkenes	3	Phenylpropenes	1
Aromatic Carboxylic Acids	3	Weak Acids	1
Carboxylic Acids	2		

Table 8. List of chemical by-products collected during while burning mosquito coils

Chemical Compound	P <sup>1</sup>	Chemical Compound	P <sup>1</sup>
<b>Acetic acid</b> <sup>4</sup>	-	<b>3-Methoxycatechol</b>	-
Acetosyringone	1.000	<b>Methoxyeugenol</b> <sup>3</sup>	-
Apocynin	1.000	2-Methoxyhydroquinone	1.000
<b>Benzaldehyde</b> <sup>4</sup>	-	<b>4-Methoxyisonitrosoacetanilide</b>	-
Benzocycloheptatriene **	1.000	Methyl 10-methylundecanoate	1.000
<b>Benzofuran</b> <sup>2,4</sup>	-	<b>Methyl benzoate</b> <sup>4</sup>	-
<b>Benzoic acid</b> <sup>4</sup>	-	<b>Methyl dodecanoate</b> <sup>4</sup>	-
n-Butyl fumarate *	0.048	Methyl isomyristate	1.000
<b>Capric acid</b> <sup>4,5</sup>	-	<b>Methyl myristate</b> <sup>4</sup>	-
<b>Catechol</b> <sup>2,4</sup>	-	<b>5-Methyl-2-furaldehyde</b>	-
Cetane * <sup>4</sup>	0.167	<b>2-Methylbenzofuran</b>	-
<b>Chavibetol</b>	-	3-Methylcatechol	1.000
<b>trans-Cinnamaldehyde</b> <sup>4</sup>	-	4-Methylcatechol	1.000
Coniferyl alcohol	1.000	<b>3-Methylcyclopentane-1,2-dione</b>	-
Coniferyl aldehyde *	0.167	1-Methylnaphthalene <sup>4</sup>	1.000
m-Cresol <sup>3,4</sup>	1.000	<b>2-Methylnaphthalene</b> <sup>4</sup>	-
<b>o-Cresol</b> <sup>3,4</sup>	-	<b>Myristic acid</b> <sup>4</sup>	-
<b>p-Cresol</b> <sup>4</sup>	-	trans-13-Octadecenoic acid	1.000
<b>Cyclotene</b>	-	Oleic Acid <sup>4,5</sup>	1.000
2,6-Di-tert-butyl-4-sec-butylphenol ** <sup>3</sup>	0.444	1-Pentadecene *	0.048
3,4-Dimethoxytoluene	0.444	Phenanthrene <sup>3,4</sup>	1.000
<b>3-Ethyl-2-hydroxy-2-cyclopenten-1-one</b>	-	<b>Phenol</b> <sup>3,4</sup>	-
<b>4-Ethylguaiaicol</b> <sup>3</sup>	-	<b>o-Pyrocatechualdehyde</b>	-
<b>4-Ethylphenol</b> <sup>3</sup>	-	Styrene * <sup>2,4</sup>	0.167
Eugenol <sup>3,4</sup>	1.000	Syringaldehyde	1.000
<b>2-Furanone</b>	-	<b>Syringol</b> <sup>3</sup>	-
<b>Furfural</b> <sup>3,4</sup>	-	1-Tridecene <sup>4</sup>	0.524
<b>Furfuryl alcohol</b> <sup>4</sup>	-	2,6,10-Trimethyldodecane *	0.048
Gentisaldehyde *	1.000	<b>Vanillic acid</b>	-
<b>Guaiaicol</b> <sup>3,4</sup>	-	<b>Vanillin</b> <sup>4</sup>	-
Heptadecane *	0.167	Isovanillin	1.000
<b>trans-Isoeugenol</b>	-	o-Xylene * <sup>3,4</sup>	1.000
<b>Lauric acid</b> <sup>4,5</sup>	-	2,3-Xylenol <sup>3,4</sup>	1.000
Mequinol	0.444	<b>2,4-Xylenol</b> <sup>3,4</sup>	-
2-Methoxy-3-(2-propenyl)phenol <sup>3</sup>	1.000	<b>2,5-Xylenol</b> <sup>3,4</sup>	-
<b>2-Methoxy-4-vinylphenol</b>	-	3,5-Xylenol ** <sup>3,4</sup>	0.167

\* Chemical compound only detected metofluthrin-treated mosquito coils

\*\* Chemical compound only detected on blank mosquito coils

**Bold** indicates presence of the chemical compound detected from all sample coils (treated and blank).

P-statistic could not be calculated because the weighted group (chemical not detected) = 0. Such cases are invisible to the Fisher Exact Test

<sup>1</sup> Fisher Exact Test analysis with  $\alpha=0.05$  and one degree of freedom

<sup>2</sup> IARC Group 2B Carcinogen

<sup>3</sup> IARC Group 3 Carcinogen

<sup>4</sup> Health effects data available indicating inhalation as primary route of exposure

<sup>5</sup> Chemical compound previously identified to repel mosquitoes

three from blank coils). The Fisher Exact Test was used to compare and analyze the frequency that each chemical compound was detected between the sample coil types. In

the case of this study, it was hypothesized that the chemical profiles would vary between the different types of coils (treated vs. blank). In this case, a low p-value would suggest that there is a significant difference in the frequency of detection of the respective chemical compound (Table 8). A higher p-value indicates that it is more likely that the analytes would be represented on both coil types (Table 8).

The IARC has classified three chemical compounds (benzofuran, catechol and styrene as noted in Table 8) as “Group 2B: possibly carcinogenic to humans” (14). This classification is given in cases where there is “limited” evidence of carcinogenicity caused by exposure to the listed chemical in humans. Further evaluation is necessary to determine if mosquito coils can release a sufficient concentration of these analytes where exposure could increase human cancer risks. In addition, 18 of the 72 compounds identified have been classified by IARC as “Group 3: not classifiable to its carcinogenicity to humans” (Table 8) (14). This classification is noted in incidents when there is inadequate evidence to support risks of carcinogenicity in humans after exposure to the chemical. However, this does not imply that there is no health risk associated with Group 3 or unclassified chemicals. Mosquito coils pose an inhalation risk. There are adverse health effect reports available for 40 of the 72 chemical compounds (Table 8), listing inhalation as the primary route of exposure (26; 38). No information pertaining to potential human health concerns was uncovered for the remaining 32 detected analytes.

Eight different fatty acids were isolated. Previous studies have indicated that saturated and unsaturated fatty acids have been proven to provide spatial repellency of mosquitoes (4; 5; 15). Three of the detected fatty acids (capric acid, lauric acid, and oleic acid as noted in Table 8) have been indicated to possess the qualities to repel mosquitoes.

All three of these fatty acids have been classified by the Food and Drug Administration (FDA) as generally regarded as safe (GRAS) for human consumption and listed as legal food additives (37). Capric acid is naturally derived from animal fats, milk and certain plant oils, including palm and coconut oils (41). Lauric acid is also found naturally in palm and coconut oils and milk, as well (42). In mosquito coil production, coconut husks are commonly used fillers in mosquito coil manufacturing (7). Oleic acid is the primary fat in nut and seed oils, along with poultry, like chicken and turkey (43). Scientists have also discovered that oleic acid is secreted as a pheromone in bees and ants when they die or are threatened (43). A possible reason for this behavior serves to warn other in the colony to stay away, which results in a repelling affect.

## CHAPTER 5: CONCLUSIONS

Mosquito coils are a well-established and effective method to repel mosquitoes. Throughout the history of mosquito coil use, the coils have always included the application of a natural or synthetic insecticidal active ingredient. The active ingredients have been tested and proven to help control the presence of nuisance biting and potentially disease causing vectors. However, the active ingredient is not the only chemical compound released into the air during the combustion of mosquito coils. The filler material, which is combusted to release the active ingredient, also imparts beneficial qualities and potential concerns. While some of the inert ingredients may pose risk to human health, others possess spatial repellent qualities that bestow little to no risk to human health.

This study was broken into two different analyses. The first portion involved characterization and comparison of the physical properties of treated and blank mosquito coils. Parameters, like pre and post burn coil mass, airborne particulate matter mass, mosquito coil burn temperature and total burn duration, were evaluated to determine the incidence of any significant difference between the coil types. Of the four parameters evaluated, only the pre and post burn coil mass study illustrated a significant difference between the treated and blank coils. The pre burn mass was greater for the treated coils. This is most likely contributed to the application of metofluthrin and an added fragrance to the coils. The post burn masses of both mosquito coil groups were statistically the same, signifying that the active ingredient and added fragrances were released into the ambient air from the treated coils. The addition of metofluthrin and added fragrance did

not statistically affect the airborne particulate matter mass, coil burn temperature or burn duration for the treated coils.

A qualitative analysis of the chemical compounds (other than the active ingredient) generated during the combustion of treated and blank mosquito coils was conducted using SPME in conjunction with GC-MS in the second phase of this study. Through the use of SPME, a broad range of chemical analytes were able to be collected and analyzed for the purpose of identifying chemical compounds that could represent known risks to human health and/or analytes that have been reported to display mosquito-repelling qualities. This research study cataloged and compared the presence of 72 different analytes, detected during the combustion of metofluthrin-treated and blank mosquito coils. The Group 2B carcinogens (benzofuran, catechol and styrene) were isolated during this analysis. However, further evaluation is necessary to determine if these compounds are generated at quantities large enough to affect human health. The literature and prior research indicated that three of the fatty acids (capric acid, lauric acid and oleic acid) identified in this study possess the ability to elicit a mosquito repelling effect.

#### **LIMITATIONS**

The sampling technique that was used in this study does have limitations. Although SPME can detect for a wide range of chemical analytes, selectivity is dependent upon the sorbent material (coating) of the fiber. A fiber coating that is highly selective for non-polar chemicals, i.e. PDMS, will not readily detect polar compounds in a heterogeneous chemical environment (34). Although advantageous for a qualitative analysis study, SPME is not reliable for obtaining quantitative results. SPME is more

ideal for detecting chemical analyte presence. SPME can lay the foundation for future quantitative studies where specific NIOSH sampling methods can be employed to detect and quantify the amount of a specific chemical analyte in the environment. In addition, SPME has the capability to detect for chemicals that only exist at trace levels in the environment (30). However, in environments dominated by a select few chemical compounds, trace level chemicals may get lost in the background region of the chromatograph.

### **FUTURE RESEARCH**

This research served as a pilot study to characterize the chemical by-products from the burning of metofluthrin-treated mosquito coils. Future work should look to expand on the basic setup presented in the methodology chapter of this paper. Filter cassettes and SPME fibers were used to collect particulate matter and analytes released from mosquito coils in a very small volume combustion chamber. This setup was designed to capture the maximum number of potential chemical compounds released from a burning mosquito coil and does not necessarily represent a real world human exposure scenario. A qualitative analysis conducted in a larger room environment may lend more to discovering what chemical compounds are released at quantities large enough to potentially affect human health once the analytes have become dilute with ambient air. Considerations should be made to accomplish this nature of study in both a controlled and field type environment.

Only three SPME fibers were used in this study. The fibers chosen for this study were selected on the principle of trying to capture as many different chemical compounds as possible. Future work should employ the use of additional fiber coatings to sample for

more specific analytes. Samples can also be taken for shorter or longer periods of time to look for trace level compounds that may have been undetectable with the fibers used in this study.

This study only compared one concentration of active ingredient to coils containing no active ingredient. Future work should explore additional concentrations of the same active ingredient used in this study, as well as comparing metofluthrin-treated coils to coils containing different commonly used pyrethroid insecticides.

Following this qualitative analysis, a quantitative study can evaluate and conclude whether the known chemicals released during the burning of mosquito coils are generated in quantities large enough to affect human health. A quantitative analysis can also produce data that demonstrates the concentration of a sampled analyte over time and distance from the source by mapping chemical plume generated by the mosquito in a large room environment.

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