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**Creating Safe & Effective Inherently
Mosquito Repellent Clothing**

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IN PARTNERSHIP WITH PATAGONIA, INC.

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Abstract

Patagonia is an outdoor clothing and gear company that has a need to create inherently mosquito repellent clothing in order to provide protection from bites and diseases to their customers, who often can be found hiking and fishing. Though many competing brands have accomplished this goal using hazardous chemicals like permethrin, Patagonia has chosen to invest additional time and resources to find and discover a natural solution with limited harm to both human health and the environment.

In order to meet Patagonia's needs in terms of effectiveness and safety, we looked to nature to both understand the mosquito, and figure out how other organisms repel it. The main mechanisms that mosquitoes use to locate humans are CO₂ detection, odor detection, and heat detection. Our options discuss combinations of methods to prevent or interrupt these detection mechanisms.

A key challenge we concentrate on is the contradictory need for something volatile, but something that can last long term. To put simply, the solution will be inherently volatile because the repellent must find the mosquito before the mosquito finds the human. However, most of these natural volatile compounds, such as those found in essential oils, are no longer effective after a matter of a few hours.

This project explores a number of methods for creating a textile treatment that would be allow us to take advantage of environmentally-friendly repellents such as natural oils and benign synthetic compounds, which are often volatile. One of the most promising options is to use a permanent cyclodextrin treatment on the garment, and provide an complementary repellent spray, which the customer can use regularly to refresh the garment's repellency. Other options are slight derivatives of this concept, such as a detergent to refresh repellency, as well as other textile binding options such as nanoemulsion to confer long-lasting repellency.

Additionally, we explore other possible physical barriers to enhance repellency, such as the use of treated netting, or materials that block heat.

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Introduction

Mosquitoes often seem like just a nuisance, but they pose as serious threat to public health in the United States and abroad through the diseases that they transmit. Patagonia, an outdoor clothing company that values sustainability and eco-friendliness, wanted to create a solution to protect its customers from this threat. Its customers often purchase the products for wear during activities such as hiking, fishing, and camping. Inherently mosquito repellent clothing would be a valuable addition for these consumers, as their hobbies often put them at risk for bites.

Mosquitos are the most infamous among insects for their abilities to transmit a number of deadly diseases; there are over 3500 species, and different subtypes carry different diseases. To pair down this huge variation, this project was oriented around two types: the *Anopheles* and the *Aedes aegypti*. These two species of mosquitoes, in particular, spread diseases that are of utmost concern. The *Anopheles* is a genus of mosquito that contains a number of species capable of spreading malaria, a serious and sometimes deadly disease that affected over 200 million individuals in 2015, according to the World Health Organization. The *Aedes aegypti* is a species of mosquito that spreads Zika, Dengue Fever, and Chikungunya. The public health agencies advise avoidance of mosquitoes in preventing outbreaks of these diseases, as there are no vaccines available yet.

This challenge is especially difficult considering that most industry standard products are either harmful to the environment, or have been associated with human health issues. DEET, which is commonly used as a bug spray, has come under scrutiny in recent years due to hazards to both the environment and health. Health Canada put it on the *Cosmetic Ingredient Hotlist*, banning it for use in cosmetics. Similarly, permethrin is commonly used on clothing applications, such as a L.L. Bean's *No Fly Zone* line. However, there is a great deal of concern regarding its toxicity in water environments.

Rather than follow this industry trend, Patagonia came to Greener Solutions to find mechanism that limited these negative health and environmental implications without sacrificing effectiveness. The type of solution would also need to fit well into the overall company mission to "Build the best product, cause no unnecessary harm, use business to inspire and implement solutions to the environmental crisis." Considering both the original assignment and Patagonia's overall mission, our group developed a more specific goal of creating a non-toxic, environmentally-benign, and long-lasting mosquito repellent mechanism that could be applied to clothing.

Moving forward, we investigate how mosquitoes find and bite humans in order to use bio-inspiration to find a solution that works effectively for a long period of time without causing harm to human health or the environment.

Approach

Mosquitoes have evolved, over millions of years, alongside humans and other mammals to seek out blood as a protein source with which the females use to develop their eggs. This strong link between reproduction and the successful blood hunt has led mosquitos to develop very sophisticated homing mechanisms, orienting on a variety of chemical and physical cues: moisture, visual contrast, carbon dioxide (CO₂), odor and heat. Biologists have long thought that CO₂ provides the most important and longest-range sensory cue (Gillies 1980). Exhaled by humans at a concentration of roughly 4%, this provides a 100-fold increase over ambient concentrations (0.03-0.04%) with which the mosquito uses to locate its prey. The question regarding how insects detect CO₂ has been studied for decades (Willis and Roth 1952; Stange and Stowe 1999; Kwon et al. 2007), and scientists have only recently uncovered the neurological and molecular pathways utilized to detect this ubiquitous product of metabolism. This knowledge could provide an exciting avenue to effectively and harmlessly evade a mosquito's detection.

Indeed, recent research aimed at blocking the *Ae. Aegypti* mosquito's CO₂-receptor, rendering the subject invisible to the blood-seeking mosquito, has shown promise (Turner et al. 2011; Tauxe et al. 2013). Anandasankar Ray and his research group at UC Riverside found a series of small organic molecules that strongly induce prolonged responses in the cpA neurons of

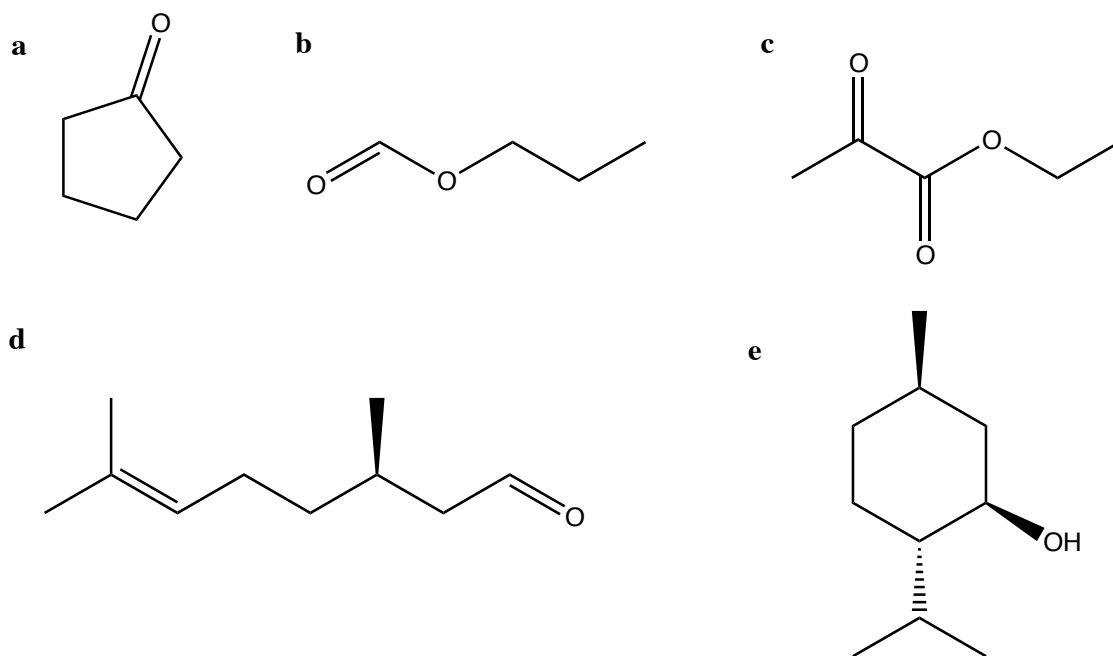


Figure 1: Molecules **a-c** (cyclopentanone, propyl formate, and ethyl pyruvate, respectively) were shown to induce a strong response in the cpA neuron of *A. gambiae* and *A. aegypti* mosquitos. Molecules **d, e** (citronellal and menthol, respectively) were shown by Dr. Ray's Kite Company to also block the mosquito's CO₂ receptor

A. gambiae and *A. aegypti* mosquitos, the neurons responsible for CO₂ detection. Furthermore, these organics share common functional moieties, ketones, esters, and hydroxyls, providing insight into the binding site of the neuron. So although the exact organic molecules used by Ray *et. Al.* carry multiple human and environmental hazards, these functional groups provide a pathway to identifying structurally similar chemicals without hazards to block the cpA neuron without causing unnecessary harm (See Figure 1).

These results show exciting promise to effectively shut off the mosquito's CO₂-detecting apparatus; however, new research shows the female *Ae. Aegypti* uses multi-modal sensing to her advantage, simultaneously and independently detecting various cues to distinguish a human blood host from other surroundings, and compensates for any single impaired sensing mechanism by relying on the others (Van Breugel et al. 2015). First detection appears to occur via the CO₂ plume of the blood host; mosquitos display their characteristic "cast and surge"

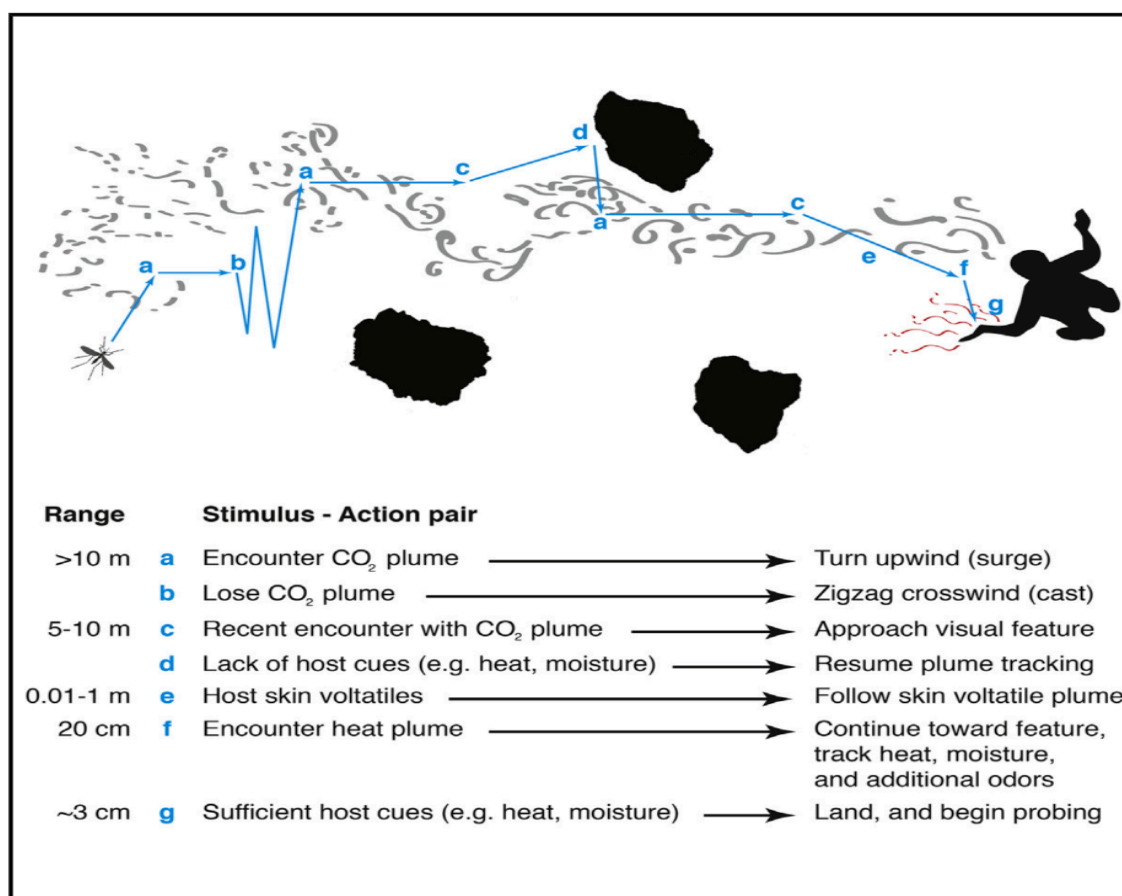


Figure 2 (Van Breugel et al. 2015): A visual graphic of a mosquito's path as she hones in on a blood source. Her multi-faceted approaches to detecting a human allow differentiation between the target warm-blooded mammal and other non-targets such as trees and rocks.

behavior, turning towards the higher gradient of CO₂, at concentrations as low as 500 ppm and distances of up to 50m (Van Breugel et al. 2015). And while detection of CO₂ heightens the mosquito's response to visual, olfactory, or thermal cues, these pathways occur independently, not iteratively. In other words, should a mosquito happen to visually detect a warm, proximal object without first detecting a CO₂-plume, it may still decide to land (See Figure 2). Thus simply shutting off the CO₂-detection mechanism will not create a satisfactory repellent. Indeed, the sophisticated and multi-pronged detection mechanisms "renders mosquitoes' host seeking strategy annoyingly robust." (Van Breugel et al. 2015)

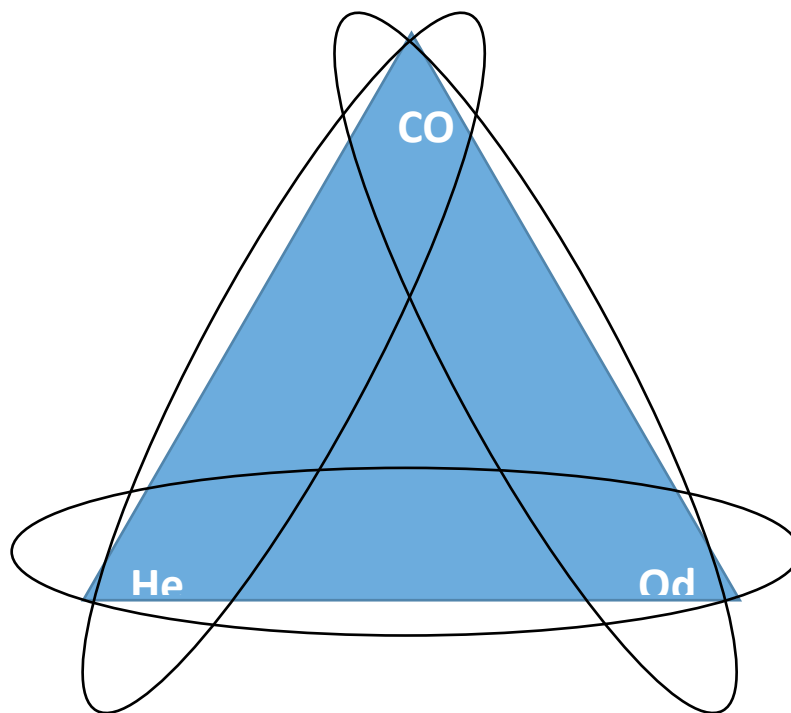


Figure 3: A triangle of detection, showing pictorially the three primary means of detection blood-seeking mosquitos use to find their host. Blocking any pair of these three means effectively deters mosquitos from landing.

Further work out of the Howard Hughes Medical Institute, aimed more at finding effective repellence schemes than simply understanding host-seeking behavior, genetically

altered the *AaegGr3* gene of *Ae. Aegypti*, responsible for a subunit of the CO₂-receptor, to test directly the lack of CO₂ detection against a control, non-mutated group (McMeniman et al. 2014). They further experimentally isolated body heat and olfactory cues via a model human arm with variable temperature and human odorant to study the mosquito's response to these various stimuli. The authors showed that in the presence of CO₂ but absent the odorants, the non-mutated group only blood-fed when the blood was heated to 37°C, not 26°C. Conversely, the mutated group (which cannot detect CO₂), rarely blood-fed even at 37°C. Furthermore, in the presence of odorants and CO₂, blood kept at ambient 26°C attracted only the non-mutated mosquitos. However, in the presence of both warm blood (37°C) and human odorant, both the mutated and non-mutated groups alike regularly blood-fed. Thus the authors concluded that any pair (See Figure 3) of stimuli acts to sufficiently attract the female mosquito to land and blood-feed. Tied together with Van Breugel's work, a visual camouflage could in principle act as a substitute, or supplement, to the stimuli triangle below. In effect masking the visual contrast a mosquito senses could prevent the mosquito from approaching a potential host close enough to detect its heat signature. While low-contrast colors, and even camouflage, have been shown to induce avoidance of a blood target by mosquitos (Gibson and Torr 1999), and this strategy should be considered a supplementary deterrent, the strategies discussed herein will focus on more sophisticated means of deterring mosquitos

In order to design a successful repellant, we must include multiple channels capable of masking a pair from of points from the triangle, preferably blocking all three vertices. Dr. Ray and his colleagues have already shown promise in blocking the detection of CO₂, as discussed above. Molecules that effectively block odor-receptors have also been studied, as will be discussed below.

Heat blocking may prove the most difficult vertex to achieve. A solution of simply containing heat with a mylar shield or something analogous could work temporarily, but will act as an oven, allowing no thermal radiation to escape, rendering the subject very uncomfortable, especially during activities such as hiking. Furthermore, heat would simply escape through the holes in the material, be they for feet or hands, etc. Thus the mosquito would see a huge thermal signature on these exposed appendages.

Taking an alternative view of heat, it is formally a form of electromagnetic radiation, with wavelengths in the infrared region of the spectrum. Approximating a human body as a black body with a temperature of 37°C, the center wavelength of its black-body spectrum sits at 9,500 nm. Thus, in order to mask heat detection, one must somehow divert or convert radiation from 7,000-14,000 nm so as not to reach the mosquito. Several research efforts are currently underway in search of such a technology (Clausen et al. 2014; Wang et al. 2014; Zhou et al. 2011; Hososhima et al. 2015), using metamaterials to artificially bend the light or using long-wavelength upconverting or downconverting materials to convert this mid-infrared radiation into other wavelengths not detectable as a heat signature. However these technologies are currently extremely nascent, currently struggling to mask even a few human cells, let alone an entire human. Indeed, the military's extreme interest in hiding its soldiers' and weaponry's heat

signatures from infrared cameras will eventually drive this technology to become commonplace, but at this time we do not see blocking heat a viable route in the near or even medium term.

In the short to medium term, blocking both CO₂ and odor receptors presents a viable option to repelling mosquitos. We will focus on the synergism of blocking CO₂ and odor receptors for the remainder of this report.

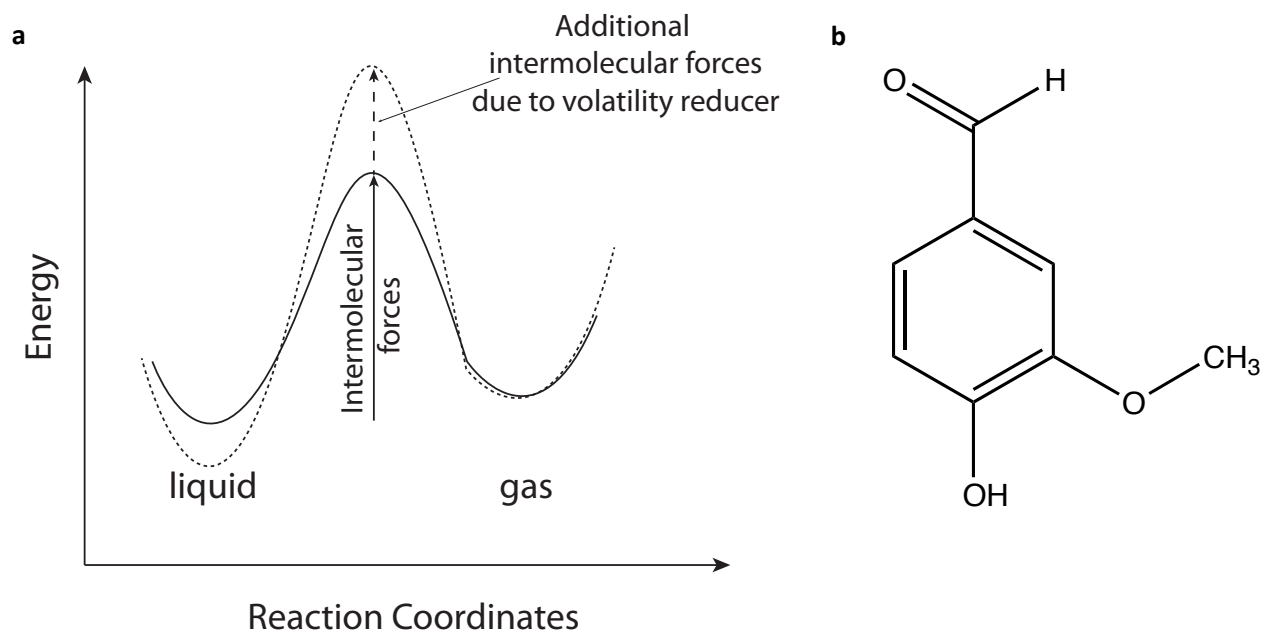


Figure 4: **a.** A reaction diagram of evaporation. Two energy wells depict the liquid and gas phases, and an energy barrier depicts the intermolecular forces. These forces can be increased with addition of a volatility reducer **b.** The structure of vanillin. Notice the many functional groups: aldehyde, hydroxyl, and ether.

Repellency and Volatility

Defining mosquito repellency, whereby a substance or action deters or prevents a mosquito from landing on a blood-host, imposes an inherent contradiction juxtaposing the goal of creating a permanently repellant material against the imposed requirement of an air-borne chemical. In essence, a repellant must reach the mosquito before the mosquito reaches the warm-blooded host, in order for said repellant to protect the host from the mosquito. This requirement imposes a limitation on the amount of time any repellant be effective against mosquito bites. In other words, any chemical that deters a mosquito from landing must do so from a distance, and therefore must volatilize to span said distance. The chemical will, regardless of its rate of volatilization, eventually need to be replenished. This imposes a finite limit on the

length of effective time for any given mosquito repellent.

This is not to say we must simply accept the natural volatility of our proposed repellents. It has long been known that the addition of certain chemicals can greatly reduce the volatility of a target molecule. Volatilization is the act of transitioning from either the liquid phase or the solid phase into the gas phase, via evaporation or sublimation, respectively. For our purposes we will focus on evaporation, as this is a closer approximation of our system than sublimation. A molecule may evaporate when it possesses sufficient kinetic energy to overcome the liquid-phase intermolecular forces. Molecules in the liquid and gaseous phases of any substance are continuously interchanging, creating a dynamic equilibrium. The intermolecular forces of a substance determine where this equilibrium is held; they are essentially a measure of how strongly the molecules stick to one another. Thus substances without strong intermolecular forces are more volatile than those with weak intermolecular forces, at a given temperature. This insight provides a route to decreasing the volatility of a chemical: mix in another chemical with strong intermolecular forces. The additional compound will stick to the compound of interest, thus increasing the overall intermolecular forces of the mixture, and thereby decreasing volatility (See Figure 4, part **a**). Several compounds have been shown to significantly decrease the volatility of a target compound, as this technique is widely used in odor control and delayed fragrance release (Hedges 1998), as well as increasing the effectiveness time of insect repellents (Khan, Maibach, and Skidmore 1975), among other things. Of specific interest to this work is the use of vanillin, the primary flavor component to natural and synthetic vanilla extract, and cyclodextrin, a three-dimensional ring of dextrose molecules which can encapsulate a host molecule, reducing volatility and thereby increasing the effective time of our proposed mosquito repellents (Khan, Maibach, and Skidmore 1975).

The mechanism of action for vanillin is not widely reported; however, a mechanism can be easily deduced. Vanillin has an extremely low vapor pressure: just 0.0031 – 0.0056 mm Hg at 20°C, in part because of its extensive and diverse functional groups, as noted in Figure 4, part **b**. These functional groups allow for an extensive range of intermolecular interactions, from hydrogen bonding to induced dipole interactions, which not only reduces the vapor pressure of vanillin itself, but also reduces the volatility of any nearby molecules in a mixture of substances, through the mechanism explained above. And it is this reduced volatility which leads to much longer effectiveness times when added to a mixture of mosquito repellents, up to a 176% increase in some cases (Khan, Maibach, and Skidmore 1975). Thus by increasing intermolecular forces, vanillin reduces volatility and greatly increases effectiveness time.

Strategies

Repellents

There are a wide variety of chemicals available now that are considered to be repellent to mosquitoes. In order to narrow down our options, we took into account repellent mechanism, health and environmental hazards, and efficacy. Compounds that were not effective, or had any serious concerns pertaining to health or the environment were ruled out. Refer to the evaluation section of this report (page 15) for more specific details on these chemicals.

Oil Based Option

This option would exclude any synthetics, and combine the most effective oils to block CO₂ and odor sensing. The three oils we chose were **PMD**, **geraniol**, and **lemongrass**. The PMD and geraniol block odor sensing, while the lemongrass oil blocks CO₂ detection, creating the dual protection necessary for an effective repellent. Vanillin would also be included in this option, as it has been proven to increase protection time when combined with essential oils (Hill, 2007).

Combination – Oils & Synthetics

Another option would be to include synthetic repellents, specifically Picaridin and IR3535, in the mixture with the essential oils and vanillin. The synthetics we researched would be more effective in combination with the oils, since they only protect via odor blocking. However, the synthetics tended to be among the most effective, and would likely be a worthwhile chemical to include.

Note: Literature often noted surprising variety in effectiveness as different combinations of oils, additives, and other chemicals were mixed. Different combinations would likely be worth experimenting with given available resources.

Applications

Plant-based repellents have been used for generations in traditional practice as a personal protection measure against host-seeking mosquitoes. Recently, commercial repellent products containing plant-based ingredients have gained popularity among consumers due to the health risks associated with the industry standards, DEET and Permethrin. However, the mechanism of action of plant-based oils lies in their volatility, which provides a vapor barrier than can deter or repel mosquitoes from coming into contact with the host (Songkro et al, 2011). This represents an inherent contradiction because, due to their volatile nature, essential oils demand frequent reapplication to maintain potency (Bhupen et al, 2013). Thus, our group was charged with the challenge of finding a strategy that served to increase the protection time of these compounds by hindering their volatility, while still maintaining enough volatility to effectively create the vapor shield. What we needed was a controlled release mechanism that could be applied to textiles to create a technical fabric with mosquito repellent capability.

Two application techniques came from our extensive review of the existing literature on the topic of controlled release mechanisms and mosquito repellency:

1. Microencapsulation using cyclodextrin
2. Nanoemulsion technology

Additionally, it was nearly consensus in the literature that the addition of vanillin (5%) served to slow the release and increase protection time.

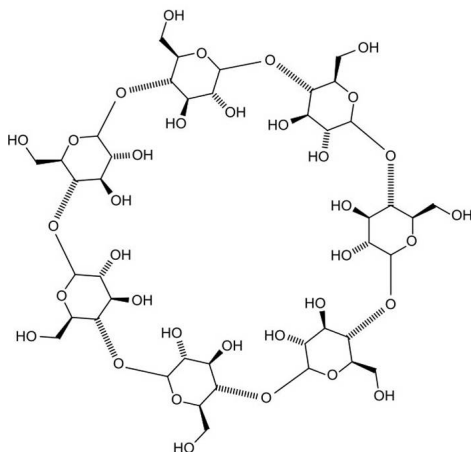
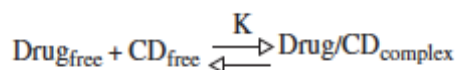


Figure 5: Microencapsulation using β -Cyclodextrin (β -CD):

One method to control the release of volatile repellent agents is use of inclusion forming compounds like β -cyclodextrin. Cyclodextrins are large 3D sugar molecules (cyclic oligosaccharides) with a hydrophilic outer ring surface and hydrophobic core. This allows it to incorporate a variety of functional agents, including our repellent oil extracts, in what are called “host-guest complexes” in a 1:1 ratio with the given equilibrium equation:



They can be fixed to textiles by way of chemical bonding using cross linking agents in the presence of a catalyst. Reagents, including one of three polycarboxylic cross-linking agents, include:

- PCA: 1,2,3,4-butanetetracarboxylic acid (BTCA)
- PCA: citric acid (CTR)
- PCA: polyacrylic acid (PAA)
- Catalyst: sodium dihydrogen hypophosphite (NaH_2PO_2)

BTCA showed the highest reactivity of the three crosslinking agents, necessitating the lowest reaction time for attaining a definite grafting rate.

This slows and allows for control of the release rate of the volatile oils, thereby increasing mosquito protection time. Many types of CDs including α , β and γ -CD and the hydroxypropylated and methylated β -CD derivatives have been tested, but we find β -CD to be the most feasible and effective for adherence to textiles. It is widely used in many industries including medical, pharmaceuticals, cosmetics, and food due to its low cost and wide range of application. β -CD is commonly used in topical applications as it does not cause skin irritation (Songkro et al, 2011). For these reasons, β -CD was selected for the inclusion complex with our repellent solution in an attempt to reduce chemical volatility and prolong protection time.

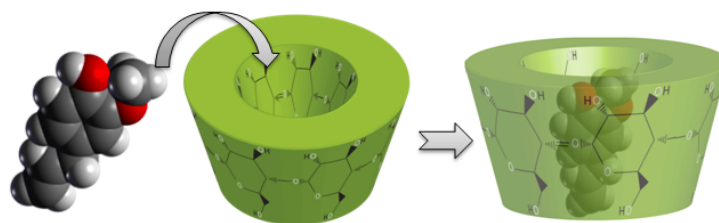


Figure 6. Inclusion Complex

The crosslinking technique allows for permanent bonding of cyclodextrin fabrics to the textile. Grafting occurs through formation of a crosslinked copolymer between the PCA (polycarboxylic acid) and CD molecule. The copolymer does not covalently bond to the polyester (PET) fabric, but rather physically adheres to or becomes entangled into the fibrous network, resulting in grafting that is resistant to washings and permanent. This is due to the fact the PCA cannot react with the PET because polyester does not carry any free reactive groups. This is not the case for wool and cotton, where this technique *does* result in chemical bonding. Grafting rate depends on 1) temperature of curing 2) time of curing and 3) ratio of PCA/CD (Martel et al, 2002). However, it should be noted that this technique has only been testing on non-woven fabrics. Further testing should be done to determine efficacy of crosslinking techniques on woven fabrics, so as to incorporate this technique with the physical protection provided by a woven material.

Table 1. Feasible Interactions between β -CD and some textile fibres [14].

Parameter	Cotton	Wool	PES	PA	PAN	PP
Ionic interactions	-	+	-	+	+	-
Covalent bonds	+	+	-	+	-	-
Van der Waal forces	-	-	+	+	+	-
Crosslinking agents	+	+	+	-	-	-
Graft polymerisation	+	+	+	+	+	+

+ = possible, - = not possible, PES-polyester, PA-polyamide, PAN-polyacrylonitrile, PP-polypropylene.

Figure 7: Interactions between β -CD and textiles

Studies have been conducted that confirm a permanent grafting of CD and CD derivatives using the following method:

“Fabrics were impregnated by the aqueous solution that contained the reactants, roll-squeezed, dried and thermofixed (at variable temperature and time) and finally washed several times with warm water, until water was clear. Raw and treated samples were dried 30 min at 104 °C before being weighted” (Martel et al, 2002).

While we are unsure of the exact mechanism of release, we believe that body heat as well as physical movement allows for slowed release the essential oil “guest” molecule from the temporary cyclodextrin “trap”.

One important note of using this method is that binding of CDs tend to increase the weight of the garment by approximately 1.5 times. “In the most drastic conditions, the weight increase of the fabrics due to the graft reaction could reach 25–30%-wt” (Martel et al, 2002). We are unsure of the extent to which this will affect the breathability of the product.

Additionally, this application design would allow for incorporation of other desired compounds such as a fragrance molecule, and might help to entrap odours from sweat molecules.

We propose that β -cyclodextrin treated textiles are a feasible way to obtain long lasting and reloadable mosquito repellent fabrics. “These cavities can be emptied during the washing process. Empty cavities can be reloaded with padding, dipping or spraying” (Martel et al, 2002).

We propose that this reloading process be done in one of two ways:

1. A specially formulated spray with a mixture of safe, effective, repellent compounds.
2. Similarly, a detergent, which reloads CD inclusion complexes with successive washings.

The inclusion of cyclodextrins into textiles is well established for a variety of uses, requiring minimal additional research. This process requires classical finishing equipment, the use of non-toxic chemicals, and does not involve any organic solvent. Studies have shown the versatility and effectiveness of this method using both natural fibers that carry reactive chemical function, as well as neutral fibers such as polyester (PET). We believe that this method is feasible for near immediate implementation, though further research should be done into for ideal formulation of spray and detergent reloading solutions.

Encapsulation using Nanoemulsion technology

Nanotechnology refers to the systematically arranged functional structures which consist of particles with size-dependent properties. The advantage of nanomaterials in textiles is that it creates functionality without affecting breathability, texture, or comfort of the garment.

“Nanotechnology can provide high durability for fabrics as they have a large surface area to volume ratio and high surface energy, thus presenting better affinity for fabrics and leading to an increase in durability of the function” (Bhatt & Kale, 2015). Additionally, nanotechnology can play an important role in introducing new and permanent functions to fabrics.

Though this inclusion technique represents a relatively new technology in the textile industry, it is currently in use for a variety of applications including hydrophobicity, antibacterial properties, conductivity, anti-wrinkle properties, antistatic behavior, light guidance and scatter (Yetisen et al, 2016). Our research indicates the use of this technology is a plausible method to incorporate mosquito repellent compounds into textiles with increased longevity and permanence. Furthermore, nanomaterials offer wide application potential to create garments that can sense and respond to external stimuli via electrical, color, or physiological signals (Yetisen et al, 2016). We believe this technology might also include potential for novel repellency techniques such as pyroelectric sensing. For the scope of this examination, we will focus on the use of this technology for inclusion of repellent compounds. We propose that this would be a feasible, but longer term solution for Patagonia to explore.

The most common approach to formulating nanoemulsion is through high-energy emulsification, compared to low-energy emulsification, due to its ease for large-scale production and low cost. High energy emulsification is done by using high shear stirring, high pressure homogenizers, and ultrasound generators. Studies have been conducted to assess stability, release kinetics, and repellent efficacy using essential oil nanoemulsions composed of various repellent compounds. One study showed efficacy of nanoemulsion particles using citronella oil (Nuchuchua et al, 2009). Aqueous dispersions of nanoemulsion, composed of 20% essential oil, 75% glycerol, and 5% emulsifier (Montanov 82), were created using the following method:

“The oil mixture was dissolved in melted emulsifier at 45°C. To obtain nanoemulsion, oil phase was dispersed in the hot aqueous phase under stirring condition at 200 rpm, 50°C for 5 min before high-speed stirring at 16,500 rpm for 3 min to obtain pre-emulsion... The pre-emulsion was then passed through a high-pressure homogenizer for five cycles at pressure of 1,500 bars. After homogenization the produced oil in water nanoemulsion was cooled down to room temperature to obtain the nanoemulsion prepared with high-pressure homogenization.”

The study concluded that the nanoemulsion droplet size and its composition influence the release rate of essential oils. The smaller droplet size resulted in better physical stability, higher release rate, and longer mosquito repellent activity (Nuchuchua et al).

These emulsions can then be applied to textiles using various application techniques such as the Layer by Layer (LBL) technique or a conventional pad-dry method. One study conducted by Bhatt and Kale looked at mosquito repellency (species unidentified) in textiles treated with chrysanthemum oil nanoemulsions, applied to clothing using the LBL technique. This method requires repeated dipping with two polyelectrolyte solutions (one cationic, one anionic) combined with the essential oil nanoemulsion solution to obtain desired number of Proton Exchange Membrane (PEM) layers. The study found repellency rate increased with decreasing size of nanoemulsion particles, increased oil nanoemulsion concentration, and increased number of PEM layers. Treated fabrics showed up to 80% mosquito repellent efficiency after 25 washes (Bhatt & Kale, 2015).

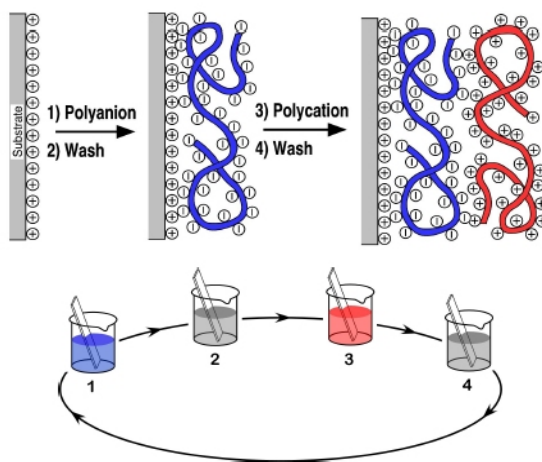


Figure 8: PEM Formation using Layer-by-Layer technique. Research indicated that the greater the numbers of layers, the longer durability of repellency in treated fabrics.

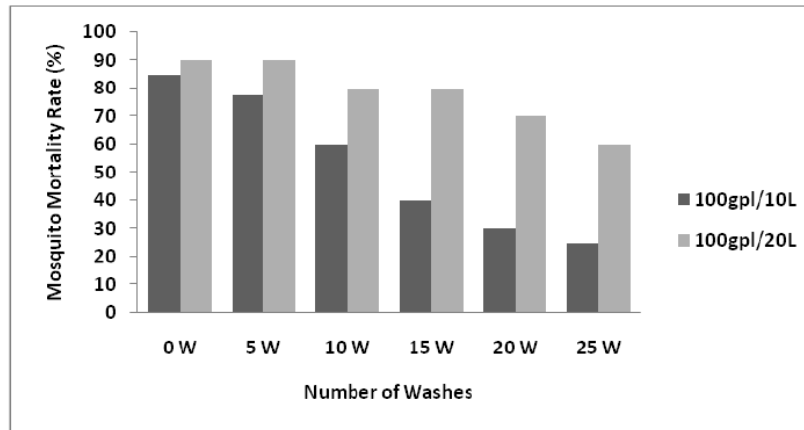


Figure 2: Mosquito Mortality Rate of Washed Samples Treated with 100 gpl Chrysanthemum Oil Nano Emulsion

Figure 9: mosquito repellency rate after successive number of washes. Graph compares 10 PEM layers (dark gray) to 20 PEM layers (light gray) at constant concentration (100 g/L) of chrysanthemum oil (Bhatt & Kale).

Design

In addition to the repellent and its application, which are the main components of an effective and long lasting solution, there are a number of simple design components that could be also incorporated to improve efficacy. These approaches are not chemical based, but physical add-ons that

- **Net:** A net that covers the wearer's face could be used in order to create a physical protective barrier. This would be something that could either be zipped into place, or rolled back into the hood.
- **Patch/Strips:** Rather than applying a treatment such as cyclodextrin to the whole garment, Patagonia could apply the treatment to smaller sections (like strips going across), or a detachable patch. This could potentially cut costs, and allow for more flexibility in design for the rest of the garment.
- **Colors:** Mosquitoes have been found to have a more difficult time finding humans wearing low-contrast colors, such as dark green in a forest setting (van Bruegel et al, 2015). Choosing camouflaging colors for the mosquito-repellent line could be an easy way to add efficacy.
- **Weave:** A tighter weave creates a stronger barrier that makes it more difficult for mosquitoes to bite.
- **Heat:** Masking the heat that a human emits would be a great way to help cloak someone from mosquitoes, but the technology is not ready yet. The United States military is currently working on this concept, so it may be something that is available in a number of years.

Evaluation

Human Health & Environmental Hazard Analysis

In this section we summarize our hazard assessment of the plant based oils, synthetics and additives that are incorporated in our proposed strategies above. To assess the hazards of our solution, we created an evaluation framework for health and environmental toxicity. Our hazard analysis table contains 7 health endpoints and 2 environmental endpoints. The health endpoints include carcinogenicity, reproductive effects, endocrine disruption, eye irritation, skin irritation, acute mammalian toxicity and respiratory irritation. The environmental endpoints we used to evaluate environmental hazards include biodegradability and acute toxicity. Health and environmental hazards that were prioritized when deciding the chemicals that would be incorporated in the repellent include carcinogenicity, reproductive effects, and if the chemical is a high skin and eye irritant, aquatic toxicity and biodegradability.

First we will discuss our methods of hazard assessment, then specifically discuss different health and environmental hazards for each chemical. Below is our hazard table that represents the health and environmental hazards for all the chemicals we considered and encountered during our research. Our descriptive analysis of the health and environmental hazards are pertaining specifically to the proposed repellent strategy.

Methods:

Initially, we used the Pharos Project to screen our chemicals of interest (plant based oils, synthetics, and additives) against authoritative lists to identify the associated health and environmental hazards. If the chemical was not included in the Pharos Project, we proceeded to look into authoritative lists such as PubChem, ChemIDPlus, Epi Suite etc. We searched additional sources for health and environmental hazards including Material Safety Data Sheets (MSDS). After looking into those various resources, our search also included reading scientific literature to fill data gaps.

In order to standardize each hazard that had a GHS classification, we used the GreenScreen for Safer Chemicals Version 1.3 Hazard Criteria by Clean Production Action. The GreenScreen for Safer chemicals is a widely used method for comparative chemical hazard assessment that can be used to identify chemicals of high concern, and eventually assist individuals in making informed decisions of health and environmental hazard. The benefit of GreenScreen is that it allowed us to take GHS classifications, scientific research data, and information gathered from MSDS and other authoritative lists, to be categorized into hazard ratings based on low, medium, high and very high. We categorized hazards for each chemical into these groups. The hazard table (Table 1) is also coded by color and number for our hazards; 1 being lowest hazard, and 4 being very high.

We concluded data gaps when there was no valid information represented in any of the methods discussed above. In some instances, sources reported that since the chemical is not used on a large scale, studies assessing for our endpoints of interest have not been conducted.

Compounds		Health Endpoints							Environmental Endpoint	
Group	Chemical	Carcinogen	Reproductive	Endocrine Disruption	Eye Irritant	Skin Irritant	Acute Mammalian Toxicity	Respiratory Irritation	Biodegradability	Aquatic Toxicity
Standard	Permethrin	1	4	3	3	2	3	3	1	4
Oils	Geraniol	1	1	1	3	3	1	-	1	3
	Limonene (outrule)	1	-	-	4	3	-	-	-	-
	Lemongrass Oil	1	-	-	4	3	1	1	1	1
	Peppermint Oil	-	2	3	3	3	1	1	1	3
	Thymol	1	2	-	4	4	3	2	2	2
	Wintergreen Oil	-	-	2	3	3	2	2	4	2
	Eugenol (Clove Oil Extract)	-	-	-	3	3	2	3	2	2
	Catnip Oil (Nepetalactone)	-	-	-	1	2	-	2	-	-
	PMD	1	1	1	3	1	1	1	1	1
	Citronella Oil (java type)*	3	1	1	3	3	2	1	-	2
Synthetics	IR3535	1	2	1	3	1	1	2	3	-
	Picardin	1	1	-	1	1	4	1	-	2
	Undecalactone	1	1	1	3	3	1	2	1	2
Additives	Vanillin	2	-	1	2	-	2	2	1	2
	Cyclodextrin	1	2	-	2	2	-	2	1	1
	Citric Acid	1	-	1	4	3	1	2	1	1
	1,2,3,4-butane tetra carboxylic acid	-	-	-	3	-	2	-	-	-
	polyacrylic acid	2	2	-	2	-	-	2	4	1
	disodium hydrogen phosphate	-	-	-	3	2	4	2	4	1
	sodium dihydrogen hypophosphite	-	0	-	-	0	-	-	-	2

Table 1: Health and environmental endpoints of plant based oils, synthetics, and additives.

Hazard Key				
Very High	High	Medium	Low	No Data

Health and Environmental Evaluations – Plant Based Oils

This section will summarize the health and environmental hazards for the specific chemicals that are presented in our final solution

- PMD (Lemon Eucalyptus Oil)
- Geraniol
- Lemongrass
- IR3535
- Picaridin
- γ -Undecalactone

Geraniol | Geraniol is a acyclic monoterpene-alcohol, and its distribution is included in bergamot, carrot, coriander, lavender, lemon, lime, nutmeg and more. It is the primary component of rose oil, palmarosa oil, and citronella oil (java type) (PubChem 1). The health hazards for Geraniol were classified under Japan's GHS system, Germany's FEA, and New Zealand's GHS for the health endpoints of carcinogenicity, eye irritation, skin irritation, acute mammalian toxicity, and the two environmental endpoints of biodegradability and aquatic toxicity. For geraniol oil, based on Japan's list of hazardous chemical, this compound meets the Globally Harmonized System criteria for a non-carcinogen, high skin irritant, and high aquatic toxicity. Based on New Zealand's list of hazardous chemicals, geraniol meets the GHS criteria for low acute mammalian toxicity, and high irritation to the eye. The German FEA also reported that Geraniol has a low biodegradability element. Since reproductive effects and endocrine disruption were not reported in the authoritative lists or Pharos Project, we researched the MSDS which concluded geraniol as a low health hazard for reproductive effects and endocrine disruption. When summarizing the total hazard for this compound, we grouped chemicals into low, medium or high overall hazard and rated geraniol as medium.

PMD | *p*-Menthane-3,8-diol, also known as PMD, is a monoterpene spent product that comes from the distillation of the Australian lemon-scented gum tree leaves (Carrol 2006). The U.S. Centers for Disease Control (CDC) have endorsed PMD in their list of non-DEET mosquito (Carrol 2006).

According to the EPA, studies showed no treatment related signs of developmental and mutagenicity toxicity (US EPA, PMD). However, PMD is considered a corrosive eye irritant when used as a technical product, and causing temporary eye damage when used as an end use product (US EPA, PMD). There was no supporting literature of the concentrations that lead to corrosive eye irritation response. While PMD is not classified as a skin sensitizer in the United States, it is classified as a Sensitizer (S) according to ECHA standards (US EPA, PMD). Lastly, PMD has shown no oral toxicity. When considering different environmental hazards, aquatic toxicity and persistence is of high concern for our strategy since the clothing will be washed and chemicals will enter wastewater. According to the GreenScreen chemicals criteria, PMD has a low persistence in water. Additionally, the EPA did not indicate any significant exposure to birds, fish, invertebrates, or any other non-target organisms. Overall, PMD is a promising compound because it is the only plant-based repellent that has been advocated for use by the

CDC in endemic areas, demonstrated clinical efficacy to prevent malaria, and poses no risks to human health according to the EPA (US EPA, PMD).

Lemongrass | Lemongrass oil was considered to have low health hazards for carcinogenicity, acute mammalian toxicity, and respiratory irritation. According to Pharos, The German FEA classified lemongrass oil as a low environmental hazard for biodegradability and aquatic toxicity, and is classified as a potential concern for hazard to waters. Based on the European Unions list of hazardous chemicals, lemongrass meets the GHS criteria for a high skin irritant and very high eye irritant, and based on the New Zealand list of hazardous chemicals it meets the GHS criteria for low acute mammalian toxicity. In order to understand respiratory effects, we researched the MSDS for lemongrass oil and identified it as a low respiratory irritant. For reproductive and endocrine health effects, more research was needed and we had to conclude insufficient data.

Health and Environmental Evaluations – Synthetics

IR3535 | IR3535 (3-[N-Butyl-N- acetyl]-aminopropionic acid, ethyl ester) has been used in Europe for more than 20 years. In 1999, IR3535 was registered for use in the United States. It is currently used as an insect repellent for application to human skin and clothing to repel mosquitos, flies, and ticks (WHO Report). Based on New Zealand's list of hazardous chemicals, IR3535 meets the GHS criteria as a high eye irritant. Other endpoints were not classified with GHS, but further research through MSDS and other authoritative lists helped us determine the health and environmental hazards. The World Health Organization (WHO) report, *WHO Specification and Evaluations for Public Health Pesticides: ETHYL BUTYLACETY-LAMINOPROPIONATE*, concluded that the main hazards relate to eye and skin irritation. However, they state that the skin irritation effects were mild when observed in animals, and the effects were not observed in humans. While IR3535 meets the GHS criteria for a high eye irritant, the WHO report concluded that the eye irritation risks were considered acceptable and preventable (WHO Report). Further, they concluded no long-term toxicity or carcinogenicity studies (WHO Report). Medium reproductive toxicity of IR3535 was reported in the *Regulation (EU) n°528/2012 concerning the making available on the market and use of biocidal products for IR3535* (Substance Eval, 2013).

Picaridin | Picaridin, or Icaridin in Europe (KBR 3023, 2-[2-hydroxyethyl]-1-piperidinecarboxylic acid-1-methylpropyl ester), is a synthetic compound developed in Europe in the 1990s, and have been available in the United States since 2005 (Diaz). When gathering our data, we observed that Picaridin had relatively lower hazards for each endpoint compared to other compounds on the hazard table. For the health endpoints, Picaridin was concluded to have low or none observed carcinogenic effects by the EPA based on dermal exposure. Reproductive toxicity studies were conducted in a two-generation reproductive study in rats (National Pesticide Center Picaridin 2009). When rats were administered 50,100, or 200 mg/kg of Picaridin, they did not show any clinical effects from treatment. There was no evidence of toxicity found, except for acanthosis and hyperostosis in the skin. They concluded that 200mg/kg chronic exposure of Picaridin to rats skin did not result in reproductive toxicity (Astroff, 1999). The Environmental Protection Agency's New Pesticide Fact Sheet for Picaridin reported that Picaridin is of

relatively low acute toxicity, moderate acute oral and dermal toxicity, and practically non-toxic for primary eye and skin irritation (Kegley 2016). The National Pesticide Information Center reported that Picaridin's K_{oc} value implies that it will adsorb and be moderately mobile to sediments and suspended solids in the water column, and that the bio concentration factor of 10.4 suggests it will not bio concentrate in aquatic organisms (National Pesticide Center Picaridin 1999). No information of groundwater contamination was found (National Pesticide Center Picaridin 1999). Not included on our health and environmental hazards is the terrestrial organism toxicity. Further, based on the results of the studies used for the EPA New Pesticide Fact Sheet, they concluded Picaridin can be considered as non-toxic to birds (Kegley 2016). Lastly, there was insufficient data to conclude endocrine disruption health hazards and biodegradability environmental hazards (National Pesticide Center Picaridin 1999).

γ -Undecalactone | γ -Undecalactone is considered a high skin and eye irritant, and medium respiratory irritant, according to GHS criteria. It is a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Registered Pesticides. Based on Germany's list of hazardous chemicals, γ -undecalactone is considered to be a low concern for biodegradability and a low hazard to aquatic life. However, γ -undecalactone was categorized under the "screening" list for aquatic toxicity, based on their assessment of the quality of the information available. While there is no translation to a hazard rating, the H-phrase indicated for undecalactone is H412, which translates to "Harmful to aquatic life with long lasting effects." According to the MSDS for γ -undecalactone, it is considered to have low reproductive hazard, low endocrine disruption hazards, and low acute mammalian toxicity (Sigma Aldrich 2007).

Efficacy & Feasibility

In order to determine overall feasibility of each compound used in our proposed repellent, we analyzed the health hazards, environmental hazards, and efficacy of the chemical. In this section we will discuss our evaluation of the third pillar of evaluation, **efficacy**. We will also discuss how we considered both efficacy and hazard assessment into overall feasibility. Therefore, in addition to analyzing the chemicals health and environmental hazards, the efficacy of the chemicals as a mosquito repellent was also researched.

The literature has shown that there is no standard method of how "repellency" is used in various studies of testing the efficacy of different mosquito repellents against various mosquito genera. Table 2 identifies the mechanism of the compound, whether it is CO₂ blocking, an odor repellent or unknown mechanism. The table also considers protection time against the mosquito of interest, % protection, and if the compound is on the market. The table also indicates how we summarized the hazard for each chemical – either low, medium, or high. After collecting data on the efficacy end points, and determining an overall hazard level, we grouped compounds into a low, medium or high feasibility category to being used as a repellent that meets both Patagonia's goal and mission. We will first discuss various efficacy findings of the plant based oils, synthetics, then additives. The values shown on the chart are crude protection times without the volatility controlling additives. We anticipate the protection to be higher when our application strategies for decreasing volatility are implemented. The chemicals that we conclude can be implemented into a short-term repellency solution contain both CO₂ and odor blocking properties.

Table 2. Efficacy summary of repellent options.

Compounds		Efficacy					Feasibility
Group	Chemical	Mechanism	Protection Time	% Protection	On the Market?	Hazard	
Standard	Permethrin	Pesticide		100%	Yes	High	Low
Oils	Lemongrass Oil	CO2 Blocking		78.8	Yes	Low	High
	Peppermint Oil	CO2 Blocking		100%	Yes	Medium	Medium
	Wintergreen Oil	CO2 Blocking		Unknown	Yes	Medium	Medium
	Clove Oil	Unknown		100%	Yes	Low	High
	δ -Undecalactone	Unknown		91%	Yes	Low	High
	Catnip Oil	Unknown		80%	Yes	Unknown	Low
	Geraniol	Odor Blocking		75% Protection	Yes	Low	High
	PMD	Odor Blocking		"Maximal"	Yes	Low	High
	Citronella Oil	Odor Blocking		100%	Yes	High	Low
Synthetics	IR3535	Odor Blocking		100%	Yes	Low	Medium
	Picaridin	Odor Blocking		"Moderate"	Yes	Low	High

Key Protection Time	>4 High	1-4 Medium	<1 hr Low	Unknown
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Efficacy Review - Plant Based Oils

PMD, Oil of Lemon Eucalyptus, p-methane 3,8-diol | The repellent spray that will be applied to the cyclodextrin treated clothing consists of Lemon Eucalyptus Oil (PMD). Overall, the active ingredients of plant based oils are often highly volatile, leading to a short protection time against the mosquito (7). However, PMD is an exception because it has a “lower vapor pressure than volatile monoterpenes found in most plant oils, and it provides a very high protection from a broad range of insect vectors over several hours” (Maia et al, 2011). In addition to PMD being less volatile, there are a variety of studies that have compared efficacy to DEET. In one study, repellent efficacy was assessed for the plant *Corymbia Citriodora*, which consists of the repellent compounds: citronellal, PMD, citronellal and limonene. When 30% of PMD was applied topically, there was a 96.88% repellency percent protection from mosquitos for 4 hours, and when 20% of PMD was applied topically in a separate study there was a 100% protection identified against the *Ae. Aegypti* for 120 minutes (Maia et al, 2011). In another study that used the Environmental Protection Agency (EPA) protective efficacy scale, PMD had a moderate level of protection against the *Anopheles* mosquitoes, compared to DEET which had a minimal level of protection against the *Anopheles* mosquito (Diaz, 2011). Maia et al., in *Plant Based Insect Repellents: A review of their efficacy, development and testing*, concluded that PMD can be recovered from the distillation of *E. Citriodora* levels, and is widely available in the genus *Cymbopogon* plants (Maia et al, 2011). In addition, these plants are widely cropped in many malaria endemic countries, where it used for essential oil production (Maia). In addition, Carroll et al. in *PMD, a registered botanical mosquito repellent with DEET-like efficacy*- reviews PMD’s performance against *Aedes*, *Anopheles*, *Culex*, and *Ochlerotatus* mosquitos. This study also presents laboratory findings of subjects comparing DEET and plant based alternatives, and concludes its value for public health applications. In table 3, extracted from Carrol et al.’s paper, summarizes PMDs performance in relation to DEET against mosquitos of various species. Overall, our literature research of PMD efficacy, as well as our summary on the health and environmental hazards of PMD, suggest that PMD formulations are for the most part either equal or great than the performance of lower concentrations of DEET (Carrol, 2006). Overall, our research on the literature of PMD efficacy, as well as our summary on the health and environmental hazards of PMD, we classified it in the high feasibility group to be used in the plant-based spray.

Table 3. PMD, a Registered Botanical Mosquito Repellent with Deet-Like Efficacy (Carrol, 2006)

Type of test	Mosquito	% AI ¹ and relative repellency			Dosing	Reference
		PMD	vs.	Deet		
Laboratory	<i>Anopheles gambiae</i>	50	=	20	0.68 vs. 0.48 g/cm ²	Trigg and Hill (1996)
Field	<i>An. funestus</i> and <i>An. gambiae</i>	50	=	50	"Adequate coverage"	Trigg (1996a)
Laboratory	<i>An. gambiae</i>	20 ²	>	7	"Small measured" PMD 25% lower ³	Hill (1998)
Field	<i>An. arabiensis</i>	71	=	15	"Normal practice"	Govere et al. (2000)
Field	<i>Ochlerotatus taeniorhynchus</i>	20	=	25	1 g/650 cm ²	Barnard et al. (2002) ⁴
Field	<i>An. darlingi</i>	30	>>	15	3 ml/lower leg	Moore et al. (2002)
Laboratory	<i>Aedes albopictus</i>	20	=	15	1 g/650 cm ²	Barnard and Xue (2004)
	<i>Culex nigripalpus</i>	20	=	15	1 g/650 cm ²	Barnard and Xue (2004)
	<i>Oc. triseriatus</i>	20	=	15	1 g/650 cm ²	Barnard and Xue (2004)
Laboratory	<i>Aedes aegypti</i>	20	≅	10	1 g/600 cm ²	This study
		20	≅	30	1 g/600 cm ²	This study
Field	<i>Oc. melanion</i> and <i>Ae. vexans</i>	20/26	=	20	1 and 1.5 g/600 cm ²	This study

¹ AI, active ingredient; concentrations are rounded up to the integer.

² Presswell, personal communication.

³ Based on ED₉₀.

⁴ Barnard et al. (2002) indicate that they tested Repel OLE lotion with PMD at 40%, but Barnard and Xue (2004) alter this value to 26% for both studies. However, our records and those of Wisconsin Pharmacal (Wundrock, personal communication) indicate that Repel OLE lotion was only produced with 65% PMD within a 30% OLE fraction, for an absolute concentration of 19.5% PMD (here rounded to 20%). Values of 40% would refer to the OLE concentration in the spray, in which PMD is again at 65%, for an absolute concentration of 26% PMD in the spray.

Geraniol | Geraniol Oil is grouped as a chemical with odor repellent properties. We will discuss various efficacy studies of geraniol against mosquitos, the protection time, and reasons that we grouped it in the **high** feasibility group. Citronella consists of **geraniol (18–20%)**, limonene (9–11%), methyl isoeugenol (7–11%), citronellol (6–8%), and **citronellal (5–15%)**. In one study, *Efficacy of the Botanical Repellents Geraniol, Linalool, and citronella against mosquitos*, Muller et al. found that the geraniol, applied via a diffuser, was about twice as effective as the linalool diffuser, and five times as effective as the citronella diffuser in protection against the *Ae. aegypti* indoors. In the same study, and the one highlighted in the efficacy table 2 above, geraniol was found to have 75% protection for a medium protection time against the mosquito. The study also concluded that there were 355 feeding attempts near the citronella diffuser, 74 feeding attempts near the linalool diffuser, and 38 attempts near the geraniol diffuser (Muller 2009).

Lemongrass Oil | Lemongrass oil is categorized as a compound with CO₂ blocking properties. We will discuss three studies in which lemongrass oil showed high protection against mosquitos. Maia et al. presents an overview of repellent plant efficacy in their literature review. In this overview of repellent plant efficacy from the literature review table (Maia et al, 2011), lemongrass oil was researched in USA, South Africa, and Bolivia high protection against mosquitos was found. In two field studies conducted in Bolivia, lemongrass oil was found to have a 74% protection against *An. darlingi* for 2.5h and a 95% protection against *Mansonia* spp. for 2.5 hours (Syed, 2008). When Lemongrass oil was tested by using a methanol leaf extract that was applied topically in a laboratory study, 78.8 % protection against *An. arabiensis* for 12 hours (Waka M). In another study when 100% lemongrass oil was applied topically, with vanillin, there was a 100% protection found against the *Ae. Aegypti* for 6.5 hours (Hill, 2007). The last finding indicates that the addition of vanillin increases protection time. Overall, our research on the literature of lemongrass efficacy against mosquitos, as well as summarizing lemongrass as a low hazard, led us to classify it in the high feasibility group to be used in the plant-based spray.

Efficacy Review - Synthetics

IR3535 | We will discuss three studies in which the efficacy of the repellent IR3535 was tested and compared with other industry standards such as, DEET. In one article, *Chemical and Plant Based Insect Repellents – Efficacy, Safety and Toxicity*, Diaz and colleagues compared the efficacy's and toxicities of synthetics and plant-derived insect repellents (Diaz). They reported that IR3535, when used in aerosols, lotions, and pump sprays at a 7.5-19% concentration, showed a moderate level of efficacy against the anopheline mosquito, maximal level of efficacy (up to 2 hours protection time) against the culicine (arbovirus) mosquito, and a moderate level of efficacy (up to 3 hours protection time) against ticks (Diaz). The table X in the appendix highlights the comparison of IR3535, Picaridin, PMD, Citronella, Permethrin, and DEET. Another study, published in 2010, researched the insecticidal, acaricidal and repellent effects of DEET- and IR3535-impregnated bed nets using a novel long-lasting polymer-coating technique (Faulde 2010). Faulde and colleagues observed the impregnated fabric by using the arm-in-cage test. They found that when IR3535 was applied at concentrations over 10 g/m², 100 percent repellency was measured by complete landing and biting protection, compared to when DEET was applied at concentrations above 3.7-3.9 g/m² (Faulde, 2010). The Journal of American Mosquito Control Association published, *Comparative Efficacy of IR3535 and DEET as repellents against adult Aedes aegypti and Culex quinquefasciatus*. This study also evaluated arm-in-cage laboratory evaluations of IR3535 and DEET, with 10% and 20% concentrations for each repellent. When time to first bite was observed for IR3535 (10% concentration), the mean protection time was comparable to DEET. At 20% there was a greater protection observed against the Ae. Aegypti for 3 hours, and 6 hours against the Cx. Quinquefasciatus. (Clik 2004). When IR3535 was observed through the use of four commercially available products, the mean protection time of the products containing IR3535 ranged from 1.5-2.83 hours against the Ae. Aegypti and 3.5 to 6.5 h for Cx. quinquefasciatus. Similar findings were concluded for the mean time to 2nd bite (Clik 2004). In addition to these studies discussed, our extended research on the literature of IR3535 efficacy, as well as summarizing IR3535 as a low hazard, led us to classify it in the high feasibility group to be used in the synthetic + plant based oil spray.

Picaridin | The exact mechanism of Picaridin is unknown, but studies have concluded that its vapor barrier extremely noxious to the mosquitos taste and olfactory senses – discouraging it from biting (Diaz 2016). Therefore, we categorized Picaridin as a compound with odor repelling properties. Diaz et al. also reported on efficacy of Picaridin against the anopheline and culicine mosquitos (Diaz 2016). They reported that when it was applied with lotions, pump sprays, and wipes at a concentration of 7-20%, a moderate level of efficacy was found against the anopheline mosquito, maximal level of efficacy against the culicine mosquito, and a moderate level of efficacy against ticks (Diaz 2016). This information can be found in the appendix, under table X. In another study, five treatments were used to compared efficacy of Picaridin versus DEET - two negative controls (ethanol), one technical grade DEET treatment used as a positive control, given that this repellent is considered as the golden standard), and two formulations of Picaridin at 10 and 20%. Their method of evaluation included observing human landing collections starting 30 minutes after treating the legs of 5 trained volunteers, from 5pm-12 am (Van Roey, 2014). In




their table presenting data on “Percent repellency with mosquito genera and mosquito species separately and for all mosquitoes and all repellents combined.” They reported that Picaridin 20% had extremely similar repellency (96.48%) against the anopheles’ mosquito as DEET (98.70). The same finding was observed against the *Aedes* spp., with Picaridin 20% repelling 97.86, and DEET repelling at 97.24% (Van Roey, 2014). Again, in addition to these studies discussed, our extended research on the literature of Picaridin efficacy against mosquitos, as well as summarizing Picaridin as a low hazard, led us to classify it in the high feasibility group to be used in the synthetic + plant based oil spray.

γ -Undecalactone | is a natural compound present in food sources such as edible fruits and dairy products (Menger et al, 2014). It is said to have a high binding affinity to AgOR48, inhibiting the mosquito’s olfactory sensor and thus repelling them without rendering toxic effects. It is considered to have low reproductive hazard, low endocrine disruption hazards, and low acute mammalian toxicity. A study by Menger et al. at the Laboratory of Entomology at Wageningen University and Research Center conducted a review of nine candidacy compounds against controls of DEET and PMD on repellency against *Anopheles gambiae* and *Aedes aegypti* mosquitos. Candidacy compounds included: 2-nonanone; 6-methyl-5-hepten-2-one; linalool; δ - decalactone, and δ -undecalactone. The study tested all compounds at 1% concentration. The results indicate that δ -undecalactone was the most effective compound of those tested in reducing number of *Anopheles* mosquito landings (91%), even compared to DEET (84%) and PMD (89%). δ -undecalactone (57%) also proved more effective than PMD (47%) in deterring landings of *Aedes aegypti*, and comparably effective to DEET (58%). The study concludes that lactones are highly promising repellents due to their low toxicity, pleasant-smell, and natural occurrence (Menger et al, 2014). We perceive γ -undecalactone to be particularly promising due to its comparative effectiveness against the two mosquito types of particular concern, as well as its low human and environmental toxicity. Another study showed efficacy of δ -UDL in reducing house entry of malaria vectors, and concluded that “delta-undecalactone is a novel repellent that showed higher effectiveness than the established repellent PMD,” (Menger et al, 2014). The compound’s use in mosquito repellent products is currently patented under the authors of this paper, Takken and Van Loon. The feasibility and efficacy of the compound in conjunction with our previously stipulated application methods should be further tested before implementation.

Conclusion

Summary

The scope of the work for this semester's Greener Solutions project was to compile a body of research on the issue, and create an opportunity map for Patagonia, providing a range of solutions to choose from in both the long and short term. In considering parameters such as available research, possible hazard concerns, market status, and general efficacy, we grouped our solutions into long term and short term goals as follows:

			
Short Term	<ul style="list-style-type: none">• Oils: PMD, a• Synthetics: IR3535, Picaridin, γ-Undecalactone	<ul style="list-style-type: none">• Cyclodextrin + Spray• Cyclodextrin + Detergent	<ul style="list-style-type: none">• Visibility• Net• Weave• Patch
Long Term		<ul style="list-style-type: none">• Nanoemulsion	<ul style="list-style-type: none">• Heat

Repellent: All of the repellent options fall into the short-term category, since they are readily available on the market, and their use in this capacity is relatively well studied.

Application: Cyclodextrin is commonly used in textiles on the market, so the application strategies that involve cyclodextrin are feasible in the short term. We consider nanoemulsion to be a more long-term strategy for Patagonia, as there is still research that needs to be done before use of this method is feasible at scale.

Design: A number of the “add on” design options would be relatively easy to implement into any line, such as adding a face net or using colors with low contrast. The only option that would not be feasible at this time is the heat option, as there is no widely known method for masking heat on this scale at this time.

Limitations and Next Steps

Given that this course only lasted for one semester, and there were no resources for experimentation, there is still work to do in order to bring these proposals to completion. We have compiled some suggested “next steps” for Patagonia, so they will be able to pick up where we left off.

First, in terms of repellent efficacy, we have little knowledge on the necessary concentrations of the repellents that we proposed to be able to repel mosquitoes, nor how they work together. In numerous instances of academic literature, we found examples of certain combinations of oils working better or worse than expected based on the oils’ independent efficacies. This indicates that some testing should be done, within the groups of oils and synthetic chemicals we proposed as safe options, as to which combinations work best.

In terms of hazard analysis, the values we reported were general to the chemical, but not specific to the context of the application. For example, the repellent chemicals will not be ingested, or for the most part, applied directly to skin. This may affect how the hazards should be interpreted; Patagonia likely has more experience with the hazard analysis of clothing treatments and can carry the research forward from this point.

Additionally, some outstanding questions we have are:

- How long will the repellent be effective for?
- What will the garment smell like with these repellents sprayed on?
- How will the material handle issues such as temperature changes or body odor?
- How much will the treatment and spray cost?
- What are the most effective relative oil concentrations for the formulation of the spray and detergent?

Closing

The goal of this semester of Greener Solutions and our partnership with Patagonia was to create insect repellent clothing that is non-toxic, environmentally benign, and long-lasting. In order to accomplish this goal, we studied how mosquitoes locate their hosts, explored available methodologies for repellency and textile treatments, and curated a number of options from these learnings. The strategies, which are composed of repellents, application methods, and design-add ons, can be mixed and matched for the most effective, appealing, and safe solution to be produced.

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Appendix

Appendix Table #1 Chemical and Plant-Based Insect Repellents: Efficacy, Safety, and Toxicity: Available insect repellents: formulations, efficacy, safety, and toxicity (Diaz 2016)

Insect repellents (chemical names)	Formulations (strength %)	Efficacy against Anopheline (malaria) mosquitoes	Efficacy against Culicine (arbovirus) mosquitoes	Efficacy against ticks	Efficacy against flies and biting midges ("no-see-ums")	Toxicity and other adverse effects
DEET	Aerosols Lotions Pump sprays Wipes (5–100%)	++	+++	+	++	Potential neurotoxicity if applied under sunscreen. May damage plastic and some synthetic fabric clothing. Safe for cotton.
Picaridin (US) and Icaridin (EU) (2-(2-hydroxyethyl)-1-piperidine-carboxylic acid 1-methylpropyl-ester)	Lotions Pump sprays Wipes (7–20%)	++	+++	++	+++ High levels of protection up to 12 hours against <i>Amblyomma americanum</i>	Possible skin irritation. No damage to plastics or clothing.
IR3535 (3-[N-butyl-N-acetyl]-amino-propionic acid ethyl ester)	Aerosols Lotions Pump sprays Wipes (7.5–19.7%)	++	+++ EPA: up to 2 hours protection time for mosquitoes.	++ EPA: up to 3 hours protection time for ticks.	+++	Causes eye irritation. Potential toxicity if ingested or inhaled. May damage plastic and clothing.
Oil of lemon eucalyptus (p-menthane-3,8-diol)	Pump sprays (10–40%)	+++	+++ EPA: up to 2 hours protection	+++ EPA: up to 2 hours protection	+++	Potential skin irritation in atopic individuals.
Citronella (3,7-dimethyloct-6-en-1-ol) Natural plant oil obtained from <i>Cymbopogon</i> spp. grasses.	Bath oils Candles Lotions (0.5–20%)	+	+	0	0	May damage clothing. Potential eye irritation and skin irritation and allergies.
trans-3-(2, 2-dichlorovinyl)-2, 2-dimethyl-cyclo-propane-carboxylate Pyrethroid derived from dried crushed	Sprays for clothes insect nets sleeping bags boots (0.5%)	+++	+++	+++	+++	Irritation. Pyrethroid resistance is now developing in mosquitoes. No damage to plastics or clothing.
EPA, Environmental Protection Agency.						
Protective efficacy scale: 0, no protection provided; +, minimal level of protection; ++, moderate level of protection; +++, maximal level of protection.						

Appendix Table #2. Part of GreenScreen that was used to assess hazard levels. (PDF that will be included in next version)