ABSTRACT

COLLINS, LYNDA ELLEN. Non –Chemical Insecticidal Textiles. (Under the direction of Dr. Behnam Pourdeyhimi and Dr. Marian McCord.)

Mosquito-borne malaria threatens 40% of the world's population, killing at least one million people each year. Efforts to control mosquito populations with chemicals and habitat elimination are often beyond the means of many nations. In addition, both mosquitoes and the diseases they carry are becoming resistant to common chemical and medical interventions. In most cases vaccines are not available. Novel insecticidal textiles were created by binding safe, food-grade diatomaceous earth to fabrics with three different structures including: a 100% polypropylene 200 holes per inch mosquito netting, a 100% cotton terry cloth, and a knit fabric of unknown fiber content with an unusual texture on one side similar to a shag carpet. Mortality in mosquitoes of 86.1% was seen 24 hours after the initial exposure of 15 minutes to the mosquito netting loaded with 98.5 gm DE/g fabric. No significant difference in mortality was seen between the different fabric structures. The mechanical nature of the killing mechanism should exclude cross resistance that has developed from the use of chemical insecticides.

Non-Chemical Insecticidal Textiles

by
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BIOGRAPHY

Lynda Collins received the Bachelor of Science in Computer Engineering from Graceland College, Lamoni, Iowa. She earned a Master of Industrial Design from North Carolina State University in 2005 and returned to complete a Master of Science in Textiles, Management and Technologies in 2008.

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1. Introduction

Mosquitoes are omnipresent in the environment worldwide and present unique challenges to humans and animals due to their ability to transmit diseases and produce large numbers of eggs that reach adult stage within 30 days. They also are a nuisance, limiting outdoor human and animal activity. Vector-borne mosquito diseases include such diseases as Dengue virus, West Nile virus, LaCrosse virus and Lymphatic Filariasis.

Of the mosquito-borne diseases, malaria is the most severe, infecting over 300,000,000 people worldwide and killing over 1 million people a year. Deaths due to additional complications (e.g., malaria anemia) are estimated to be an additional 190,000 to 974,000 people each year. 75% of malaria victims are children under the age of five, and those who survive may suffer lifelong neurological problems including blindness, weakness, speech problems and epilepsy[1].

Current methods of disease control may be short-lived since history has shown that living organisms develop resistances to chemical interventions. Lengeler found that mosquitoes are becoming immune to insecticides commonly used in their control.[2, 3] Plasmodium parasites, a family of four parasites carried by mosquitoes, any one of which can cause malaria, are also becoming resistant to the most commonly used drugs.[1, 2, 4, 5, 6] New and innovative ways to combat mosquito-borne diseases are needed.

2. Literature Review

2.1 Methods of Controlling Vector-Borne Diseases

There are three principle mechanisms currently used to reduce the impact of mosquitoes:

1. Immunization against the diseases that are transmitted by mosquitoes;
2. Control the target insect population using various agents (chemicals, natural predators, habitat elimination, etc.);
3. Prevent insect contact with potential hosts by the use of repellents or physical barriers;

Although a vaccine does exist for Yellow Fever, There are no vaccines currently available for most of the mosquito-borne diseases such as Dengue virus, West Nile virus, LaCrosse virus, and Malaria[7], leaving various forms of mosquito control the only viable methods of disease prevention.

2.1.1 Control of Mosquito Populations

An integrated regimen of larvae and adult mosquito control is most likely to produce effective and long-term control of mosquito-borne diseases[1, 7, 8]. The complex interaction between a host and the malaria parasite requires a mosquito to become exposed to a *Plasmodium* parasite and survive long enough for the parasite to mature into infectious sporozoites before malaria can be transmitted. *Plasmodium falciparum*, the most serious and prevalent malaria parasite in Africa, requires a minimum of 10 days to become infectious[1, 8]. Slightly shortening the life expectancy of the adult mosquito, which is between 2 and 3 weeks depending on species and weather conditions, prevents the maturation of *Plasmodium* parasites, producing an immediate

effect on the spread of malaria, protecting the entire community[8]. By comparison, larvae control is not as immediate and success is harder to measure. However, the most effective and most permanent solutions have been from larvae control through both habitat elimination and aggressive use of insecticides[8].

2.1.1.1 Habitat Control

Prior to WWII, efforts to control mosquito populations centered on controlling mosquito breeding grounds through a variety of approaches. These included introducing natural predators such as mosquito fish and bats, draining wetlands and other breeding sites, and making habitat uninhabitable by the use of oil or insecticides such as paris green[8], also known as copper acetoarsenite, a highly toxic copper salt[9], in standing water and small bodies of water. Currently, the most common approach is wide scale use of insecticides such as dichlorodiphenyltrichloroethane (DDT) to target adult mosquitoes by using insecticide impregnated bednets and spraying interior walls of dwellings[1, 7, 8].

The targeting of adult mosquitoes through the use of DDT and other insecticides has proven to be successful across Europe, Asia, and North America; however, malaria eradication proved unsuccessful in more challenging areas such as Africa due to poor health infrastructure and political climates and the presence of ideal breeding conditions for mosquitoes[8]. As pointed out by Fred Gould in the 2007 Triangle Malaria Symposium, malaria eradication in places like Europe and North America is a relatively simple task because the climate is not ideal for

mosquito breeding[8]. Success in wet, tropical environments is much more difficult. Success has been achieved in Brazil, despite favorable breeding conditions, but the use of a rigid regime of habitat control as opposed to relying solely on insecticides was critical. The limited mobility and the concentration of mosquito larvae make them ideal targets for control measures. Efforts concentrated on intense and regular inspection and treatment of all possible breeding sites, making individual inspectors personally responsible for success in their assigned regions. The Brazil project made extensive use of paris green, which is known to be highly toxic and would be unacceptable in most areas today. Success is dependent on large scale, systematic and long term effort that few developing nations can afford. Larvae control today could be a significant tool in malaria prevention, but it would depend on a combination of financial aid from wealthier nations, the development of new, safer insecticides and a concerted effort of the indigenous populations[8].

2.1.1.2 Biological Control

Advancements in the field of genetics are being used to find novel ways to control mosquitoes[10]. Mosquitoes rely on a combination of olfactory, visual, gastronomical and physical cues in host seeking. Disrupting the olfactory cues, which have been demonstrated to be of primary importance in host seeking behavior, could limit interaction between mosquito and host, thereby controlling disease transmission. In an effort to better understand the mechanism of host-seeking, H. Biessmann, et al., isolated and characterized the genes responsible for olfactory recognition in the *Anopheles gambiae* mosquito for the purpose of

facilitating the development of ways to interfere with host seeking behavior of *Anopheles* mosquitoes[11].

Over 70 years ago, Clay Huff demonstrated the genetic foundation of vector competence, the ability of a mosquito to host parasites, in mosquitoes. Huff showed that *Culex pipiens,* commonly known as the house mosquito, could be made more susceptible to an avian form of malaria by selective breeding. Eliminating vector competence in mosquitoes effectively eliminates the threat of malaria and other diseases. Recent advances in genomics have made it possible to develop molecular maps of several mosquito species, providing tools to identify specific genes responsible for susceptibility to parasite infection[12].

Gould, et al. have explored methods of introducing transgenes, bits of engineered foreign DNA, into mosquitoes to render them incapable of carrying the malaria parasites. Although success has been obtained in a laboratory environment, challenges remain in devising ways to introduce the genetic changes into the wild population. One possible approach makes use of transposon, or "jumping genes", in which a segment of transposon DNA is detached from the chromosome and reinserted into a random part of the genome by an encoded protein produced by the transposon. The cell repairs the hole left by the transposon, resulting in two copies of the altered DNA[13].

Another possible approach to introducing transgenes into the mosquito population is the use of homing endonuclease gene (HEG), which has the ability to make exact copies of itself onto

both branches of a chromosome. The HEG accomplishes this by encoding a protein to snip the twin of the HEG-containing chromosome in two. The cell repairs the twin using the HEG-containing chromosome as a template. If this is done in egg and sperm cells, the offspring inherit the HEG at nearly 100%. Although HEG's occur naturally in fungi, plants, bacteria and bacteriophages, they do not occur in insects or animals. It is a significant challenge to adapt the HEG to insects[13].

While biological controls hold promise, there are challenges that must be addressed. At present, working solutions are estimated to be ten years away[13]. There is public concern over the possible unintended effects of altering the wild population that may hinder the development of transgenic approaches. There is also concern about the possibility of successfully eradicating disease from an area which would prevent the human population from building immunity to the disease. If the disease were to adapt to the altered mosquito, it could be re-introduce to the human population and wide-spread epidemics might result, although the growing resistance of mosquitoes to pesticides has not produced rebound epidemics[13].

2.1.1.3 Chemical Controls

There are several classes of compounds that are commonly used as insecticides including organochlorines, organophosphates, carbamates and inorganic elements[9, 13, 14]. The number of suitable insecticides for the use of mosquito control is limited by the necessity of use inside human dwellings. DDT, an organochlorine insecticide, pyrethrins, a natural organophosphate insecticide derived from the chrysanthemum plant, and pyrethroids, synthetic insecticides that

are similar to pyrethrins, are commonly used to control mosquitoes because of their relative safety to humans. All have been proven harmful to non-target species such as birds and aquatic life[15]. Although DDT has been banned in many countries due to concerns about the toxic

Table 1: Insecticides and their mode of action[9]

Class of Compound	Insecticides	Action	Comments
Organochlorines	DDT, DDE, methoxychlor, ethylan, chlorobenzilate, Chlordane, Aldrin and Dieldrin	neurotoxin	Contain carbon, chlorine, and hydrogen, Very stable chemically so they persist in the environment, Resist metabolism and build up in fat tissues of animals and accumulate in the food chain
Organophosphates: Aliphatic-phosphoric acid derivatives	Malathion, trichlorfon, monocrotophos, dimethoate, dicrotophos, oxydemetonmethyl, disulfoton, dichlorvos, mevinphos, methamidophos, and acephate	Interfere with neuro-muscular junctions, causing rapid twitching and eventual paralysis	Organophosphates are the most dangerous insecticides to vertebrates, Used extensively in place of Organochlorines because they are chemically unstable and break down in the environment.
Organophosphates: Phenyl derivatives	Parathion, stirofos, profenophos, sulprofos, and isofenphos	Interfere with neuro-muscular junctions, causing rapid twitching and eventual paralysis	More stable than other organophosphates and are longer lasting
Organophosphates: Heterocyclic derivatives	Diazinon, azinphosmethyl, chlorpyrifos, methidathion, phosmet, and dialifor	Interfere with neuro-muscular junctions, causing rapid twitching and eventual paralysis	
Carbamates	Carbamate	Interferes with neuro-muscular junctions, causing rapid twitching and eventual paralysis	Have low dermal toxicity to mammals
Inorganic elements	Paris Green		copper acetoarsenite which is a highly toxic copper salt

effects on the environment and non-target species, it is still commonly used in malarious areas for mosquito control. Pyrethroids have largely been used to replace DDT in those areas where DDT is strongly discouraged, or banned, even though pyrethrooids are more dangerous to vertebrates[13] and can cause severe poisoning in infants[15]. Long term use and sporadic spraying techniques have produced mosquitoes that are resistant to many commonly used insecticides including DDT and pyrethroids, limiting their effectiveness and causing significant concern for future options for mosquito control[1, 7, 9, 14]. Due to the emerging acquired resistance to insecticides, extensive research is being done to develop new insecticides[16, 17].

Guille describes the use of two insecticides on one bednet allowing the use of a wider range of insecticides to address acquired resistance concerns. Less toxic residual pyrethroid (bifenthrin 50 mg/m^2 or deltamethrin 25 mg/m^2) was used on the lower half of the net and the more toxic carbamate (carbosulfan 300 mg/m^2) on the upper half, minimizing contact with the net user. Effectiveness of the net relies on the fact that mosquitoes tend to fly upward when confronted with a barrier. While test subjects using the nets did experience a reduced rate of blood feeding by mosquitoes and the kill rate was significantly greater for *Culex. quinquefasciatus*, the kill rate of *Anopheles gambiae* was no greater than with pyrethroid. In addition, 20% of test subjects reported possible side effects from the carbamate impregnated nets (headache or sneezing) raising the question of safety[16].

Azadirachtin (AZ) is the active ingredient in neem, a newer insecticide derived from a tropical tree in the Meliaceae (Mahogany) family. AZ is a steroid-like substance which causes

repellency, anti-feeding behavior, antioviposition (egg deposition), adverse changes in metamorphosis, decrease in fecundity and egg-sterility in many insect species at concentrations of as little as 2 to 4%. Relatively high concentrations of neem were needed to obtain mortality against *Aedes aegyti*, but when used with *Bacillus thuringiensis var isreallensis*, a bacterium used in control of budworms and gypsy moths, increased or synergistic effects were obtained. It was also found that crushed neem seed kernels and leaves could be used in temporary pools to control mosquito populations, although care is essential as extreme toxicity to neem has been observed in rainbow trout and bluegill sunfish. Chemical studies largely show neem to have no acute oral toxicity, no acute dermatological toxicity, and no acute eye irritant qualities. Young neem leaves are eaten in Burma and Thailand. Neem bark components are used in toothpaste in Europe and India and neem soap is used in India[18].

Neem is largely seen as an agent to control farm pests and is used on crops. Resource poor farmers can grow their own neem trees to synthesize their own insecticide, but neem takes longer to take effect than other chemical insecticides, which may frustrate farmers. Neem is also easily adversely affected by environmental factors such as rain and temperature and may need to be reapplied to crops frequently[17].

Despite the development of new insecticides, current techniques of using a single insecticide over long periods of time for mosquito control result in acquired resistance posing a significant risk of loss of effectiveness of insecticides[14]. It has been shown that using insecticides in

rotation or in a mosaic pattern (treating different areas in a village with different insecticides) decreases the incidences of acquired immunity in mosquitoes to insecticides.[14]

2.1.2 Preventing Mosquito/Human Contact

75% of all malaria victims are children under the age of five[1, 2]. In addition to risks faced by young children, pregnant women and their unborn children are at significant risk of health complications such as anemia caused by malaria [1, 2, 4]. Insecticidal-treated textile materials (ITM's) (e.g. bednets) have been shown to be an effective mechanism of vector control by killing mosquitoes via chemical transfer from the material, in addition to providing a physical barrier[1, 2, 4, 8]. The fact that adults living in malarious areas have developed immunities to *plasmodium* parasites caused some concern that the use of bednets would delay children from acquiring immunity to malaria and ultimately result in higher mortality. Studies on child mortality from all causes have been conducted and it has been shown that bednets significantly reduce child mortality, especially among infants, and reduce the occurrence of low birth weights among infants born of mothers who consistently use bednets. Elevated mortality is not seen in older children who were protected with bednets[3, 4, 19]. Additionally, mosquito borne diseases such as *Lymphatic filariasis*, which affects an estimated 120 million people, often occur in the same areas as malaria and are carried by the same mosquitoes. In many cases, the distribution of bednets is effective in preventing multiple diseases[20].

Although insecticide treated bednets are an important tool in controlling malaria and other mosquito-borne diseases, there are problems associated with their use. Until recently, nets lost

their effectiveness after four or five washings. Bednets are washed often to clean them of dust and urine from infants. Nets are often washed in natural bodies of water, which introduces insecticides to streams and rivers and wear the nets excessively causing holes due to abrasion during wash[2]. The insecticidal quality of nets is essential to their effectiveness as mosquitoes are killed on contact. Therefore they provide some level of protection to people both under and near the net even in the presence of holes.[16, 17] Nets must be retreated often to maintain effectiveness, but re-treating nets is labor intensive and expensive to the point of being beyond the means of many. The development of long lasting nets have been made possible by new textile treatment processes (microencapsulation) alleviating the need for retreating nets, but such nets are more expensive to produce, creating a tradeoff between longevity and initial investment .

Pyrethroids are the most common insecticide used in bednets and have caused severe poisoning in infants, who are less able to efficiently breakdown the toxin. Lesser symptoms have been observed in adults such as asthmatic breathing, sneezing, nasal stuffiness, headache, nausea, incoordination, tremors, convulsions, facial flushing and swelling, and burning and itching sensations[17]. Of particular concern is the susceptibility of infants and young children to both the effects of malaria and the toxicity of the insecticides used in nets.

Additional solutions are needed in the arsenal against mosquito-borne diseases. The ideal "next generation" of insecticidal textiles will rely solely on non-chemical, non-toxic, and risk-free insecticidal properties for use in clothing and bedding. These textiles will be impenetrable by

the insect and will protect the individual from bites. Additionally, the textiles will protect the broader community by disabling the mosquitoes' ability to transmit disease through multiple bites.

2.1.3 Non-toxic Insect Controls

It has long been known that inert dusts have a toxic effect on insects as seen by the fact that the Chinese have used dusts as insecticides for 4000 years, and animals such as birds and elephants take dust baths to deter insects[21, 22, 23, 24]. Mounting concerns over toxicity, acquired insecticide resistance and environmental consequences of chemical insecticides has resulted in a resurgence of interest in exploiting inert dusts for insect control[23, 24, 25]. Dusts that have been shown to have insecticidal properties[21, 23, 25] include silica gel, alumina, activated charcoal, feldspar, quartz, and diatomaceous earth (DE) as well as others[22, 23, 24, 25, 26]. Silica gel proves quite effective against insects, but it also has inhalation carcinogenic properties in humans. DE is somewhat less effective against insects, but because it is extremely safe and is recognized by the United States Food and Drug Administration (US FDA) as a food additive, it has received increased attention. Alumina and activated charcoal both show good efficacy against insects[23, 24, 25].

DE, the fossilized remains of unicellular algae called diatoms, consists primarily of amorphous silicon dioxide, which unlike crystalline silicon, is non-toxic and commonly used as a food additive in applications such as grain storage and de-worming[22]. There are different formulations of DE which have been optimized for insect control by adding binders that cause

it to adhere to both the insect and the grain, increasing the damage to the insect. To overcome the natural repellant properties of DE, bait in the form of sugars and starches are added to DE sold as insecticide to encourage insect interaction. Ingestion of DE by insects causes internal injuries to the insect. The addition of silica gel particles has been shown to increase the effectiveness of DE, but silica gel is recognized by the US FDA as a foreign substance in food. DE particles are sometimes coated with silica gel to satisfy the US FDA requirements while still maintaining increased effectiveness[21, 27].

DE has been shown to affect a variety of insects including insects found in stored grains, ants, roaches, honeybees and wasps[21, 23, 25]. While insect mortality caused from exposure to DE is generally high, efficacy varies significantly between formulations, insect species and even between different strains within species[24]. Rigaux et al. showed that different tolerances to DE existed within different strains of the same insect[25]. DE is not commercially used and acquired resistance from overuse is unlikely. Tolerant insects were found to move more slowly, limiting their exposure, and to avoid areas of high concentrations of DE. DE kills by dehydration and tolerant insects were found to lose water more slowly than susceptible insects; however, that was determined to be a consequence of a lower exposure rate to DE. The slower movement of tolerant insects suggests that they would be less likely to migrate to influence other populations through breeding[25].

2.1.3.1 Mechanisms of Death Caused by Diatomaceous Earth

Until the mid to late 1940's the exact mechanism of death was unclear, because the morphological structure of DE and other dusts are sharp and porous, and they are both

oleophilic and abrasive. Mortality could be caused by abrading the thin outermost layer of the cuticle, causing dehydration from fluid loss through lacerations, or by absorption and disruption of the outermost lipid layer of the cuticle, causing dehydration from the lack of a moisture barrier.

Wigglesworth et al. determined the primary cause of death to be dehydration from abrasion of the cuticle layer. Experiments showed scratches on the cuticles and weight loss or insects that were allowed to crawl across dust covered filter paper. The work was further supported by the lack of weight loss of dead insects that had been dusted without opportunity for abrasion. Jones findings contradicted those of Wigglesworth et al., showing that despite an increase in water loss in insects that were abraded; the primary mechanism of death was the disruption of the cuticular layer of an insect due to lipid absorption. Jones demonstrated that an essential variable was the area of contact of DE to the cuticle. The difference between live and dead insects found in the work of Wiggleswroth was explained by Jones to be the increased contact between DE and insect cuticle caused by the motion of live insects which encouraged dust particles to sift through protective hairs and settle onto the insect cuticle. The increased mortality among insects that experienced abrasion was explained by the increased surface area caused by the abrasion[22, 26]. Jones further supported this conclusion by the use of activated charcoal, a soft, nonabrasive, absorbent particle, which proved to cause significantly higher mortality than abrasive particles[26]. Today it is generally accepted that DE causes mortality by disrupting the cuticular waxes which cause desciccation[21, 23, 24, 25, 26, 27]. Wigglesworth, Jones, and Dowdy and Fields, who explored the synergism between heat and the use of DE, all found

that insects completely recover when given access to water or nectar, further supporting the conclusion that DE causes desiccation[22, 24, 26].

Jones further concluded that the particle size had an effect on insect mortality, showing that until the particle reached 5μm, a decrease in size increased mortality caused by an increase in cuticular contact, while particles less than 5μm decreased insect mortality as the smaller particles clumped on insect hairs, protecting the cuticle from dehydration.

2.2 Methods of Making Insecticidal Fabric

Interaction between net material and insecticide is complex and hard to predict. Cotton, which has a high moisture regain, absorbs more insecticide than polypropylene, which has no moisture regain. However, permethrin, lamdacyhalothrin and alphamethrin (but not deltamethrin) are less effective on cotton than on synthetic fibers[4], meaning the same concentration of these insecticides can be used on all fibers types. There is anecdotal evidence to suggest that deltamethrin produces greater side effects on polyethylene and polypropylene than on other fibers. The rate at which nets lose insecticide to evaporation and to wash is dependent on fiber composition, with polypropylene and polyurethane losing efficacy before cotton, but cotton is less durable and more easily damaged than the synthetic fibers[4].

Traditional nets require regular re-treatment with insecticide to maintain effectiveness. Methods of coating ITM's (dipping and spraying) result in decreased effectiveness in only four or five washings and require re-treatment every six months even without washing. There is difficulty in determining how often nets need to be re-treated as the frequency of actual washes

are exaggerated by end users who report nets are commonly washed every two weeks. Researchers quantifying the actual washes found that in a six-week period, most nets had been washed only once or twice[2]. Large differences in dosages occur when re-treating nets and difficulty exists in determining the uniformity of distribution of insecticide on nets as it is difficult to test the presence or dosage of insecticide on nets. Pigments that fade at the same rate of the insecticide have been considered, but no such pigment has been developed[2]. The spraying techniques used in re-treatment to alleviate the considerable effort required to retreat bednets require more insecticide and produce uneven results reducing effectiveness over dipping techniques.[1, 2] Regardless of the technique used in re-treating nets, there are safety concerns associated with the handling of concentrated insecticide during net re-treatment[1, 2].

Traditionally, ITM's are made by either incorporating the insecticide into the spinning dope or by dipping or spraying the net with insecticide [2, 28]. Both processes require re-treatment of nets. In response to the considerable challenges caused by the need to re-treat ITM's new techniques are being developed to produce long lasting, wash resistant bednets and include incorporating insecticides into materials through the use of micro-encapsulation techniques[29, 30], dyeing and padding techniques[28] and the use of coatings using polymer bindings, thickening agents[31, 32] and particle films[23].

Microcapsules, cyclic structures having a hydrophobic interior and a cellulosic exterior, are being used in novel textile applications to impart special qualities to textiles including durable fragrances, skin softeners, flame retardants, anti-microbial properties, drug delivery, and

insecticidal properties[29, 30]. Microcapsules release their embedded substance when ruptured by gentle abrasive forces such as those exerted on a fabric during normal use. Microcapsules are embedded in the fiber using extrusion processes and/or applied to the surface of the fabric using standard finishing techniques such as padding (a process where the fabric is passed through in a coating solution and then dried), dye processes and polymer binders. [29, 30]

Cyclodextrins, cyclical sugars, have been used successfully to create insecticidal textiles using permethrin and DEET, due to their high heat tolerance. Insecticides were protected from degradation from the sun by UV additives. The insecticide/repellant embedded cyclodxtrins were grafted onto cotton fabric and proved to be effective against *Aedes aegypti*[29].

Yeast-based microcapsules have been developed to bind long lasting finishes (insecticides, fragrances, phase changing materials, etc.) to textiles and other products. Yeast cells have carbohydrate cell walls and a lipid bilayer membrane that protect volatile active compounds from evaporation. Textiles treated with these microcapsules, which range in size from 1 to 20 microns with the smaller sizes resulting in longer lasting efficacy and the larger size resulting in a greater release of encapsulated material, retain effectiveness for between 8 to 20 wash cycles.[30]

Researchers are working on improving the washfastness of ITM's. The addition of retentive additives such as polyvinyl acetate, dye agents and mild acids has been shown to improve washfastness. Polymeric binders can also be added to permethrin [32].

In addition, researchers are exploring the use of hollow fibers as insecticide reservoirs for sustained release.

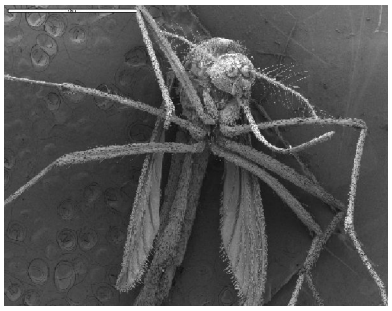

Figure 1: Scanning electrol microscope image of an *Aedes aegyti* mosquito

2.3 Anatomy of a Mosquito

The life cycle of a mosquito commences when its egg hatches, followed by four molting stages (instars) as a larva, the last molt producing a pupa, and finally emerges as an adult. Larvae feed on nutrients and micro-organisms in the water, while pupae do not feed. When adult mosquitoes first emerge, both males and females feed on nectar. The acquisition of a blood meal is necessary for oviposition (egg laying) and if mating has occurred the female mosquito becomes host-seeking. A female can oviposit two or three times in her adult lifespan and becomes gravid (egg carrying) shortly after blood feeding. The life span of a mosquito depends on the species and environmental conditions such as temperature.

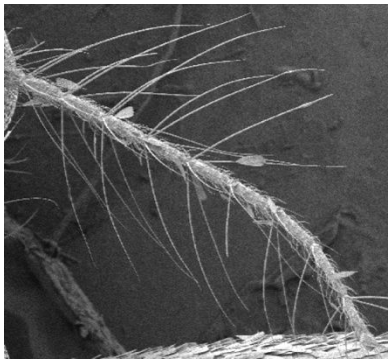

Figure 2: SEM image of an antenna from a female *Aedes agytie* mosquito

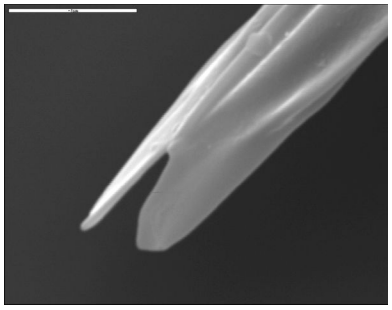

Figure 3: SEM image of a proboscis from a male mosquito[34]

Female mosquitoes find hosts by a complex interaction of cues including olfactory, visual, gastronomical and physical[11]. While significant differences exist between the antennae of the male and female mosquito, each sex being optimized for either finding nectar or hosts, olfactory cues are of primary importance in finding hosts and are detected by the antennae[11].

Although the function and composition of the antennae is not completely understood, it is known that CO_2 is detected from long distances and scents from sweat and body odors are detected at closer range by host-seeking female mosquitoes[33,] .

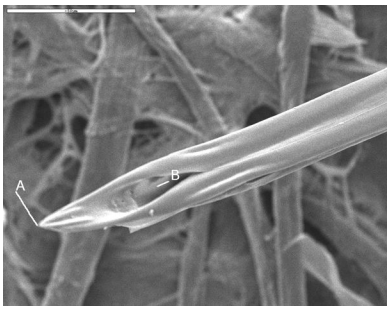

Figure 4: SEM image of a proboscis from a female mosquito where A shows the salivary channel and B shows the blood channel[34]

The proboscis (mouthparts) consists of an outer sheath called the labium and the inner needle-like fascicle. The tip of the male fascicle resembles a fork, and the tip of the female fascicle resembles a hypodermic needle[34]. In the female fascicle, the salivary channel is extremely small while the blood channel is relatively large.

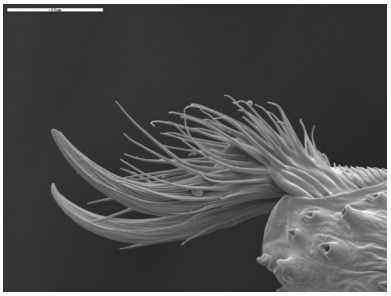

Figure 5: SEM image of the tarsus of Aedes aegypti

The female mosquito is assisted in obtaining a blood meal by microscopic hairs on the tarsus (feet) that cling to the host by Van Der Waals forces and by claws on the end of the tarsus[33, 35]. To obtain a blood meal, mosquitoes must penetrate the skin to a depth of 100-400 μm to reach the blood carrying arterioles and venules[34]. Initially, the mosquito pokes or probes the skin using just the tip of the fascicle, kicking up its back legs to generate force. Swaminathan observed that the proboscis is under considerable force during blood feeding, especially at the point that the fascicle first enters the host's skin[34]. It is believed that the probing behavior is to find soft spots in the skin to facilitate blood feeding.

2.3.1 Cuticle

The insect cuticle, which covers the outer surface of an insect including the tracheae (air passages), consists of four noncellular layers excreted by the epidermis. The cuticle layers are:

- the outermost thin hydrophobic layer called the epicuticle,
- the hard, rigid exocuticle layer containing melanin pigment,
- the endocuticle, which can be subdivided into two layers, consisting of a thick, pliable transparent material[34, 35]

The cuticle of most insects forms an effective moisture barrier due to lipids or waxes present in the epicuticle[22, 26]. The disruption of the epicuticular lipid layer results in dehydration of the insect[22, 26, 34].

3 Experimental Materials and Methods

Due to the groundbreaking nature of the task of developing a non-chemical method of insect control, the research was divided into two phases. The preliminary phase was exploratory in nature and focused on finding effective ways to make surfaces that harmed, entrapped, or otherwise incapacitated mosquitoes that interacted with the surface. The second phase focused on the optimization of the surface and the characterization of both its makeup and its effects.

3.1 Preliminary Research - Materials and Methods

Drawing on both available literature and first hand observation, multiple techniques were used to explore and quantify the vulnerabilities of mosquitoes to their environment, and to develop and test non-chemical insecticidal textiles.

3.1.1 Tarsus

In response to literature (Swaminathan) that described the considerable force required for the mosquito proboscis to penetrate human skin, it was hypothesized that if the tarsus could be clogged or otherwise hindered, the mosquito would be unable to generate sufficient force to push the proboscis through skin. To test the hypothesis, mosquitoes were exposed to various nanofibers, micro-fibers and fabrics, including a hydroentangled fabric made from islands in the sea fibers with 75% PLA and 25% nylon which had the PLA dissolved with a 3% Sodium Hydroxide (NaOH) solution. The loose, grabby fabric successfully entangled the hairs of the tarsus by adhesion due to Van Der Waal forces. To further test the hypothesis, legs were amputated in different combinations and at different lengths using the amputation method described below. (see **Amputations**) Combinations of amputations included:

- both front legs;
- both back legs;
- both middle legs;
- one front and one back leg;

Legs were amputated in whole and at ½ the length of the leg. Modified mosquitoes were subjected to bite tests.

3.1.2 Proboscis

Characterization of the ability of mosquitoes to bite through pores of a range of diameters was performed. Woven and nonwoven filter fabrics with well defined porosity were obtained. Bite tests were performed as described below (**Bite Test**) using the various porous filter fabrics as the barrier. Both histamine responses and blood meals were recorded.

Previous work done by Swaminathan suggested that the proboscis could be dulled, clogged or made rough enough to prevent the penetration of human skin. The hypothesis was tested by sandwiching abrasive dust (DE) between fine filter fabrics that had fine pores (20 μm). The outer sheath of the proboscis is larger than 30 μm, forcing the mosquito to expose the more delicate and vulnerable fascicle (inner mouth parts) to the abrasive dust. The sandwich was placed over the open end of a vial containing five host seeking mosquitoes and the apparatus was placed near human skin for 15 to 30 minutes.

3.1.3 Antennae

The antennae have been reported to be the sensing organs that guided the mosquito to its host[11, 33]. To explore the possibility of preventing host-seeking behavior, antennae from two groups of mosquitoes were amputated to different degrees including both antennae totally amputated (two replicates) and both antennae half amputated (one replicate). Four to five modified host-seeking mosquitoes of each amputation group were subjected to land (five replicates for each group) and bite tests both immediately after amputations (following the recovery period as described in **Amputation**) and again after 48 hours.

3.1.4 Desiccation

Unexpected mortality among test mosquitoes was observed while testing DE sandwiched between fine filter fabrics for the purpose of abrading the proboscis (as described in **Proboscis**). It was determined that the cause of death was the minute presence of DE that sifted through the filter fabrics and fell onto the test subjects. It was later observed that

mosquitoes could walk through drifts of DE without observable harm. It was hypothesized that mosquitoes could be killed by desiccation from DE, but that good body contact was required for mortality. The hypothesis was tested by making prototype fabrics (see **Creation of prototype fabrics**) using various fabric structures that were designed to reach past the mosquito's legs and reach its body. The fabrics were tested by covering the clear plastic cups containing five host-seeking mosquitoes. The cups were placed against human skin for 20 minutes during which time mosquitoes were observed crawling into and under the fabric structures in the same way as they would crawl under animal fur to obtain blood meals. The cup was removed from the host and histamine responses in the host were recorded. Test fabric was replaced with untreated fabric and the mosquitoes remained in the test apparatus for 24 hours, at which time mortality was recorded.

3.1.5 Amputations

In initial experiments, various body parts of mosquitoes were amputated to determine the impact of missing or harmed body parts on the mosquito's ability to find a host and obtain a blood meal. Prior to amputations, the mosquito was knocked down by chilling, using an ice bath for 20 min. Amputations were performed in a chilled environment, such as in a walk in refrigerator or over an ice bath, using either an X-acto knife or dissection scissors. Test subjects were allowed to recover for between 20 and 50 minutes on a moist paper towel at room temperature before the experiment was continued.

3.1.6 Bite Test

To determine whether a mosquito had the ability to bite through a test material or after being exposed to a procedure, bite tests were performed. Test and control fabrics were placed over the opening of a polypropylene vial containing five host seeking mosquitoes and placed on the bare skin of a host for 15 minutes. The mosquitoes were killed by freezing and crushed against clean white paper. Blood stains on the paper were counted and recorded as blood meals. Histamine responses in the host were also recorded.

3.1.7 Land Tests

To quantify the mosquito's ability to find a host, land tests were performed. Depending on the purpose of the test, either five or 20 mosquitoes were placed in a Plexiglas cube with one side covered with a surgical sock. A host inserted one hand into the test apparatus through the surgical sock, and for the duration of one minute, mosquitoes were allowed to land on the host and were shaken off before a blood meal could be obtained. The procedure was repeated between three and five times, and the number of lands that occurred was recorded.

3.1.8 Creation and Test of Prototype Fabrics

Prototype fabrics were created by spraying a 10% solution of an acrylic binder (Bayderm finish 91UD) in water onto a variety of fabric, then dredging the fabric in DE (Safer brand crawling insect killer) and drying at 60˚ C. Excess DE was shaken from the fabric before testing. Clear plastic cups containing five host-seeking mosquitoes were covered with the fabric. The fabric side was placed against human skin for 20 minutes. The cup was removed from the host and

histamine responses were recorded. Test fabrics were then replaced with untreated fabric. The mosquitoes remained in the container for 24 hours, when mortality was recorded.

3.1.9 Characterization of Pore Size

It was found that, although the proboscis is 30 μm in diameter[34], a woven fabric with a pore size of ≤11 μm was required to provide a barrier to mosquito bites, while a nonwoven with a 47 μm pore size was sufficient to provide a barrier due to its three dimensional structure.

3.2 Desiccating Fabric - Materials and Methods

After identifying a specific area of interest, fabric was made by controlled and repeatable processes and detailed and repeatable experiments were designed and implemented to verify early findings.

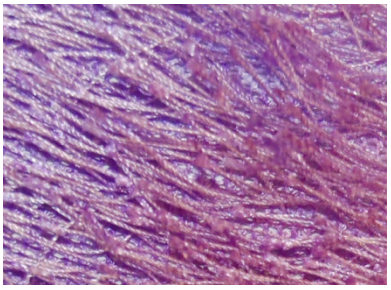

Figure 6: Texture of knit fabric

Food grade diatomaceous earth (DE) dust (Garden Harvest Supply) sold as fossil shell flour and insecticidal DE (Safer brand) was bound to three different types of fabrics chosen for their unique qualities:

- 100% polypropylene 30 filament 125 denier yarn, 200 holes per inch warp knitted mosquito net with a basis weight of 50 grams per square meter (gsm) was chosen for its wide use in bed nets and for the potential of interaction with the mosquito. Mosquitoes have been observed putting their head and thorax into the opening of mosquito netting[1].

- 100% cotton three pick terry cloth made from singles yarn with the weft and loop yarns having a cotton count of 25 and the warp yarns having a cotton count of 15 and the fabric having a basis weight of 300 gsm was chosen for it standard construction and availability and for the potential for interaction with the mosquito body.
- A 14 cut single knit jersey with a pile backing made with a 2 ply, 40 filament, 400 denier polyester yarn was chosen for its success in preliminary experiments and for the propensity of mosquitoes to treat the fabric structure as natural animal fur. The knit had a basis weight of 320 gsm and was purchased from JoAnn fabrics.

Table 2: Auto Jet atomizer settings

Auto Jet Technologies Settings	**Pressure**
Liquid pressure	5 PSI
Atomizer pressure	8 PSI
Fan air pressure	0

A uniform and consistent fine spray of 80% acrylic binder in water (Robond PS-2000 acrylic adhesive manufactured by Rohm Haas) was applied to each of the fabrics using an atomizer sprayer (Auto Jet Technologies) with the settings shown in table 2. Five spray nozzles were fixed 12 inches above the fabric, and the fabric was passed under the nozzles at a rate of four yards per minute. (Figure 7) The fabric samples were air dried overnight at room temperature in an area with good air circulation. Fabric samples were weighed and then placed in large plastic bags with an abundance of diatomaceous earth dusts. The bag was closed tightly and shaken to coat the fabric evenly until the fabric was totally saturated. The fabric was then removed from the bag and shaken until minimal visible dust fell from it. Samples were weighed to determine the amount of DE on the fabric. The fabric samples from all fabric types were heated to the film formation temperature of 121˚ C for two minutes. Samples were cut and the remainder of

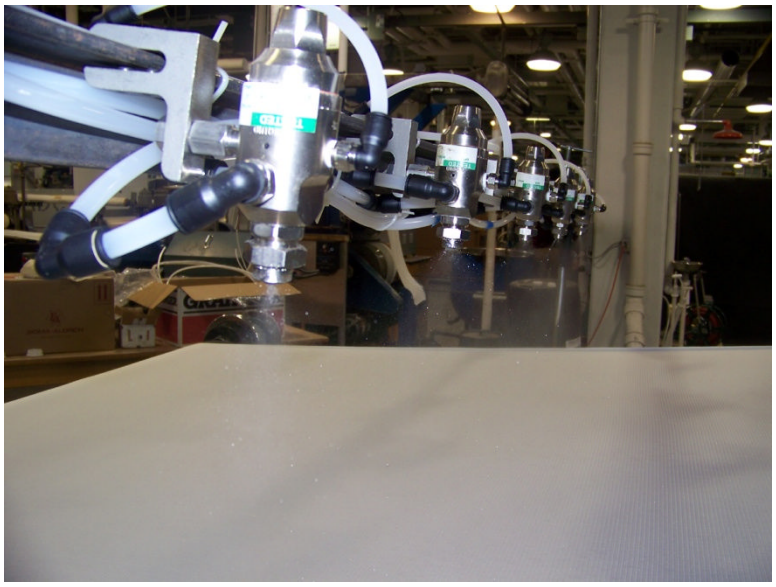

Figure 7: Set up for spraying adhesive onto test fabric. Photo courtesy of Jeff Kraus.

the fabric was cured at 148˚ C in a convection oven for two minutes (Warner Mathis AG Textile Machines). In the case of the Safer DE, fabric was stored undisturbed in zip lock plastic bags before heating to the curing temperature. Different loadings were obtained for the knit fabric treated with FSF DE by vigorously shaking separate samples until no visible dust fell from them. All samples were weighed throughout the process to calculate the DE loading. (Table 3)

Table 3: Fabric loading

Fabric type	Fossil shell flour	Safer DE
Net	0.40 mg/cm² 98.5 mg DE/g fabric	0.24 mg/cm² 40.8 mg DE/g fabric
Terry Cloth	1.9 mg/cm² 57.5 mg DE/g fabric	0.21 mg/cm² 7.1 mg DE/g fabric
Knit	2.4 mg/cm² 66.1 mg DE/g fabric	
Knit – well shaken	1.7 mg/cm² 41.5 mg DE/g fabric	1.3 mg/cm² 40.0 mg DE/g fabric

3.2.1 AATCC Wash

Two samples of the knit fabric were hand washed after the application of DE according to the American Association of Textile Colorist and Chemists (AATCC) Test Method 124-2001

section 8.2.1 (Hand Wash). Standard detergent (20g ± 0.1g) was added to two gallons of water at 41˚C ± 3˚C in a 10 quart pail. The fabric was gently agitated for two minutes by holding a corner and lowering and raising the fabric into and out of the pail at a rate of approximately 30 cycles per minute. The fabric was rinsed in clean water for two minutes and dried in a convection oven at 100˚C for 20 minutes.

3.2.2 Rearing Mosquitoes

An *Aedes aegypti* colony was established from eggs that were field collected from New Orleans, LA in 2003. Adults were added at six and eight months intervals to sustain diversity. The colony was maintained as described by Trexler et al (2003) [36] where mosquitoes were kept at 28º C and 97% relative humidity (RH). A photoperiod of 14 hours light and 10 hours of dark with 2 - one hour periods of twilight was used. Larvae were fed a mixture of liver and yeast in a ratio of 2 to 1. Adults were fed on a 10% sucrose solution.

3.2.3 Exposing Mosquitoes

Mosquitoes were exposed to each fabric in a test cell (Figure 8) made from a one pint round clear polypropylene container with a lid diameter of 4.5 inches. A round hole just large enough to accommodate a mosquito aspirator was cut from the lid and covered with layers of clear film adhesive to create an access port for the insertion of the aspirator.

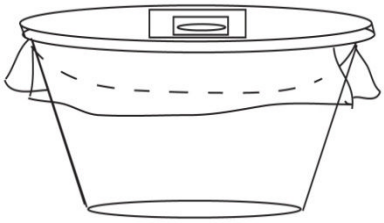

Figure 8: Schematic of Test Apparatus

Fabric samples measuring 8.5 x 7 inches ±1/2 inch (38 x 25 cm ± 1 cm) were cut, and positioned within the container with a minimum of .5 inch overlapped on all sides. The lid was then clamped into place. Five female *Aedes aegypti* mosquitoes, between two and five days old, were aspirated into two test apparati (apparatus A and apparatus B). The mosquitoes were exposed to the fabric for a total of 15 minutes by alternately agitating the mosquitoes by placing a rotating rigid rod aspirator tip through the lid opening following the procedure in table 4. Mosquitoes from both apparati were release into a 9 inch Plexiglas cube after exposure by placing the deli container inside the Plexiglas cube and opening the lid which removed any possibility of harm caused by unnecessary aspirations. The procedure was repeated, releasing the mosquitoes again into the same Plexiglas cube. Unless otherwise stated, mosquitoes had no access to food or water after exposure. Three replicates of each experiment were performed. Mortality was recorded at 1, 3, 24 and 48 hours. For the purpose of testing different conditions, variations were made in the test procedure (Table 5), including the addition of nectar to the test apparatus. Also, to determine the difference in mortality between test fabric and DE alone, the experiment was repeated using filter fabric taped to the bottom of the deli container and adding 0.15 ± .05 g of Safer brand DE.

Fabrics and mosquitoes were examined with a Hitachi S-3200N and the ultra high resolution Hitachi S-5500 Scanning Electron Microscope (SEM). Samples for the Hitachi S-3200N were prepared for viewing with a sputter coating of gold and palladium.

Table 3: Method of agitation

Step Number	Container	Time
1	A	3 minutes
2	B	3 minutes
3	A	3 minutes
4	B	3 minutes
5	A	1.5 minutes
6	B	1.5 minutes
7	Release mosquitoes into Plexiglas cube	

3.2.4 Statistical Analysis

Percentage mortality data were analyzed using a repeated measures ANOVA with a compound symmetry covariance structure. To determine if statistically significant differences in percentage mortality (P ≤ 0.05) existed between fabrics treated with different forms of DE (fossil shell flour or Safer brand DE) and untreated fabrics across all time points and at each time point, probability of differences values were calculated in least significant difference tests for least squares mean (LSM) mortalities under the hypothesis Ho: LSM(i) = LSM(j) (SAS Institute 2003) [37].

4 Results

4.1 Preliminary Research - Results and Discussion

After exploring several possible approaches to creating an insecticidal textile that used mechanical methods of killing insects, two points of vulnerability were identified. Damaging the antennae and the use of desiccating particle bound to a fabric showed promise, while approaches that focused on inhibiting skin penetration were less successful.

4.1.1 Inhibiting Skin Penetration

Multiple methods were found to inhibit the acquisition of blood meals including harming or clogging the tarsus and abrading the proboscis. Mosquitoes with one front leg and one back leg amputated on opposite sides of the body and mosquitoes with both front legs amputated were all inhibited from taking blood meals. Test results showed five bites (histamine responses) with no blood meals and one bite with no blood meals respectively. Although the number of blood meals was reduced or eliminated, the number of bites, which indicates saliva transfer, increased over the number of bites inflicted by mosquitoes which were not harmed (three control mosquitoes produce three bites and three blood meals). Mosquitoes who had been enticed to probe into abrasive particles (DE) were also less likely to obtain a blood meal but produced more histamine responses than mosquitoes that were unharmed. Although preventing the acquisition of blood meals prevent mosquitoes from becoming infected by malaria, the transmission of saliva is sufficient to transmit malaria from a mosquito to a human[38].

A number of ways have been identified to prevent a mosquito from successfully penetrating skin sufficiently to obtain a blood meal (clogging or damaging the tarsus and clogging or dulling the proboscis). Penetration of any depth transmits saliva. A close examination of the proboscis reveals that the salivary channel is extremely small compared to the blood channel and it is at the very end of the needle like proboscis[33] making it the first point of contact (see Figure 4). The increased probing, and subsequent transmission of saliva, makes the damaged mosquito more likely to transmit malaria.[38]

Table 4: Design of experiments: The table reads vertically so the columns show the variables tested in each experiment.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Knit	X			X	X			X	X		X		X		X	X
Netting		X				X						X				
Terry cloth			X				X							X		
No Fabric										X						
Binder + DE heated to 121˚ C								X					X	X		
Binder + DE cured at 148˚ C	X	X	X	X	X	X	X		X		X	X			X	X
7.1 mg DE/g fabric							X							X		
98.5 mg DE/g fabric		X										X				
57.5 mg DE/g fabric			X													
66.1 mg DE/g fabric				X											X	X
41.5 to 40.0 mg DE/g fabric	X				X	X		X	X		X		X			
Washed															X	
Agitated with a rod	X	X	X	X	X	X	X	X	X	X				X	X	X
Host enticed											X	X				
No agitation													X			
Fossil shell flour	X	X	X	X							X	X			X	X
Safer brand DE					X	X	X	X	X	X			X	X		
Access to sugar water																X

4.1.2 Antennae

Total and partial amputations of both antennae, the sensing organ primarily responsible for a mosquito's ability to find a host, were performed on *Aedes aegypti* and were followed by land tests. The results were dramatic, showing an average of 30 ± 6 lands per minute for the control group and zero lands for amputee groups regardless of whether the antennae were amputated in whole or part. After 48 hours the average number of lands by the amputee groups rose to 2 ± 2 lands per minute showing that the effects of the physical damage are long lasting.

In early experiments done to clog the tarsus of the mosquito, fibers were observed entangled in the tarsus after exposing the mosquito to fabrics containing loose, grabby nano fibers. It is hypothesized that a similar fabric could be made to entangle the antennae of the mosquito. It is further hypothesized that if a mosquito net were made with such a fabric, the mosquito would interact with the structure in such a way as to provide interaction of the fabric and the antennae. Early experiments that amputated antennae show that the antennae are places of vulnerability and harming them would incapacitate the mosquito.

4.1.3 Desiccation

It appeared that the unexpected mortality in mosquitoes observed while performing tests designed to abrade the proboscis using sharp abrasive powder (DE) sandwiched between filter fabrics, was caused by DE that had sifted through the filter fabrics onto the mosquitoes. In order to test the hypothesis, the conditions were repeated using filter fabric to allow DE to sift onto mosquitoes. The experiment caused death in the test subjects within one to two hours.

Later experiments found 100% mortality after 24 hours in mosquitoes that were exposed for 20 minutes to the knit prototype fabric that was treated with fossil shell flour. The discrepancy in time required to cause mortality between the loose DE and the fabric is curious and is not currently understood. It may be caused by any combination of variables discussed by Jones, including surface area of contact, particle size and quantity[23]. The loose DE may have been more plentiful. The particles may have been smaller because they had passed through a filter fabric and the smaller particles may have made better contact with the insects body because they were dropped directly onto the body of the insect. In addition to the variables identified by Jones, it was observed that static electricity was present in the polypropylene test containers and that mortality was high and unexpectedly sudden in test subjects where both DE and static electricity were present.

Previous studies (Wigglesworth and Jones) indicate that alumina and activated charcoal are more effective than DE[22, 26]. However, results from early experiments performed in this study showed higher mortality for DE than for alumina. This supports Jones' findings that abrasion is not as important as absorption[26]. The alumina acquired for this study was sold as abrasive powder and was optimized for abrasion with no claim for absorption. Due to the poor mortality in the alumina and the extreme safety of DE, the use of alumina was discontinued in this study.

The results of these early experiments warranted further exploration. Furthermore, additional experimentation could be successfully performed with available resources. The remainder of the research was focused on desiccating fabrics.

4.2 Desiccating Fabric - Results and Discussion

Non-chemical insecticidal fabrics were successfully created by binding diatomaceous earth (DE) to various fabrics causing significant mortality of mosquitoes within 24 hours of an initial exposure of 15 minutes. A statistical analysis was performed on the data using repeated measures ANOVA analysis. It was determined that fabric structure has no significant effect on mortality of mosquitoes exposed to fabrics treated with DE, while the presence of DE on the fabric is effective in producing mortality with a confidence level of greater than 99.99. It was also observed that mosquitoes recover completely with access to nectar, which was also observed by other researchers working with DE. [22, 26]

A method of comparing the quantity of DE on different fabrics is needed to compare experimental results. The quantity of DE per area of fabric is important to manufacturing costs, an important consideration in the task of controlling malaria. For evaluating experimental results, however, comparison of the quantity of DE deposited as it relates to initial fabric weight is more appropriate.

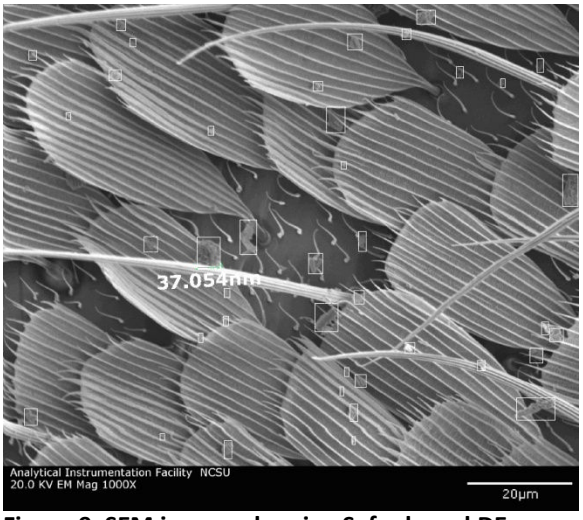

Figure 9: SEM images showing Safer brand DE on mosquito carcass

4.2.1 Comparing Types of DE

When comparing Safer brand insecticidal DE (Safer DE) and fossil shell flour (FSF DE), it was found that the knit fabrics treated with FSF caused a higher mortality than the same fabric treated with similar weights of Safer DE per gram of fabric where each was cured at 148º C. This was unexpected given that the Safer DE was presumably optimized as an insecticide since it was sold specifically for that purpose. The results could be explained by the fact that the optimizations commonly done to DE for insecticidal purposes (the addition of baits and binders[21, 27]) would not be beneficial in this case given that mosquitoes are not drawn to sugar or starch baits and a binder was added to the fabrics. Jones also observed that DE particles less than 5 μm were less effective than larger particles because they are more likely to be caught in the protective hair and scales of the insect[26]. Close examination of SEM images (Figure 9) of Safer DE on mosquitoes post-exposure show that the particles are sub-micron in size and usually rest on scales and hair.

4.2.2 Fabric Structure

Early experiments showed that mosquitoes could walk through drifts of DE without observable harm while mortality was observed when DE was sifted onto the insects. It was hypothesized that the long legs of the mosquito were adapted to prevent body contact from harmful environmental surfaces and that the fabric structure would be important to high mortality. Experimental data shows that there is no statistically significant difference (p value >.05 at 24 hours in all cases) in mortality between the fabrics tested that were treated with FSF cured at

148 º C despite the different fabric structures such as mosquito netting, terry cloth and the jersey knit with a pile backing. A significant difference in mortality was observed between the knit fabric and the terry cloth treated with Safer DE cured at 148º C. It is known that the terry cloth had a much lighter loading of Safer DE than the knit (7.1 mg DE/g and 40 mg DE/g fabric respectively) which is likely to be the cause of decreased mortality. Exposing the mosquitoes to DE particles on filter paper produced low mortality, showing that the presence of fabric increases the effectiveness of DE against mosquitoes.

4.2.3 Fabric Loading

The knit fabric with a loading of 66.1 mg FSF DE/g fabric performed better than the same fabric with a loading of 41.5 mg FSF DE/g fabric where both fabrics were cured at 148º C ($t = 6.17$; $df = 12, 63.3$; $p = <.0001$). It is also shown that there is no significant difference between the knit fabric with a loading of 66.1 mg FSF DE/g fabric and the net fabric with a loading of 98.5 mg DE/g fabric ($t = 0.48$; $df = 12, 63.3$; $p = .6310$). These results show that loading is a significant variable in mortality but not the most significant in all cases. Given that the latter comparison is between two different fabrics, no clear conclusion can be made about what other variable may be present.

4.2.4 Access to Nectar Post Exposure

Researchers (Wigglesworth and Jones) working with DE found that insects completely recovered when given access to water or nectar[22, 26]. Experiments were done to verify that mosquitoes behave similarly by allowing them access to nectar post-exposure. Statistical

analysis reveals no significant difference between the control group and groups given access to nectar after exposure ($t = .52$; $df = 13, 102$; $p = .5978$). These findings support work done by Wigglesworth and by Jones and further indicate that dehydration is the mechanism of death.

4.2.5 Film Formation Temperature vs. Curing Temperature

Binding DE to textiles poses challenges due to the need to preserve its porous nature. For this reason, the addition of DE to the binder prior to the application was avoided. Instead a tacky binder was sprayed onto the fabric, DE was applied and it was heated to 121 º C, the film formation temperature, causing the binder to flow evenly across the fabric and into the DE without filling the structures. The binder was then cured at 148º C. Fabric samples were tested for both temperatures producing data that shows a decrease in mortality for knit fabric treated with a loading of 40.0 mg Safer DE/g fabric when cured to 148º C over the same fabric that as heated to the film formation temperature only.

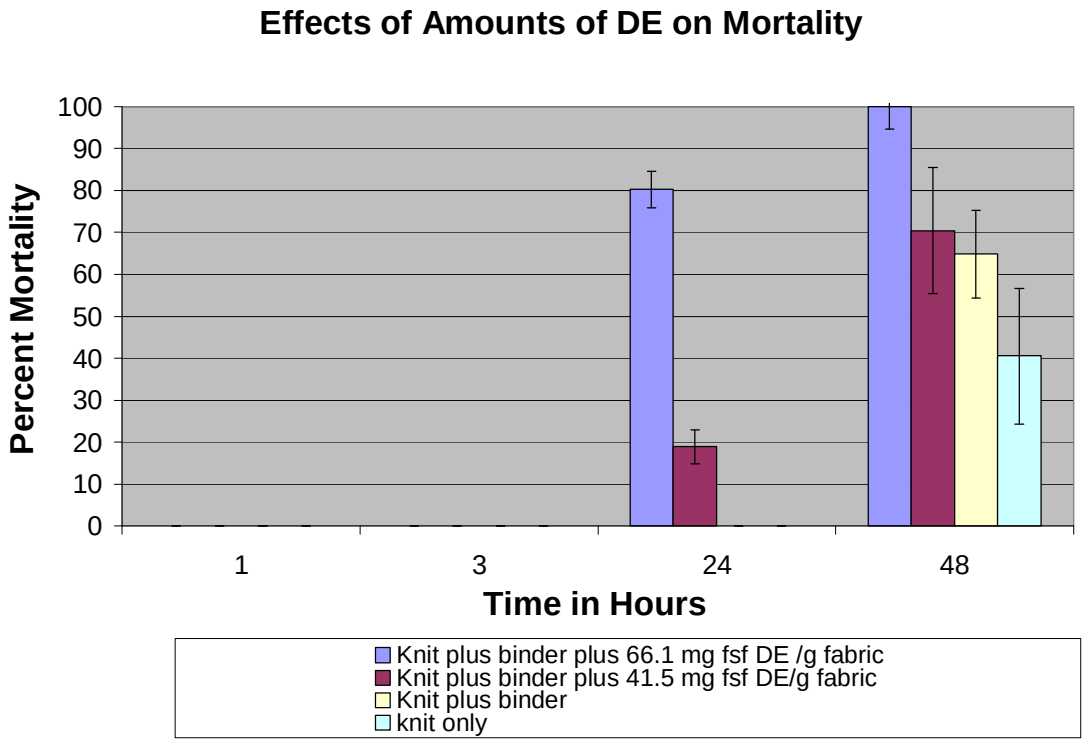

Figure 10: Effects of loading for FSF DE

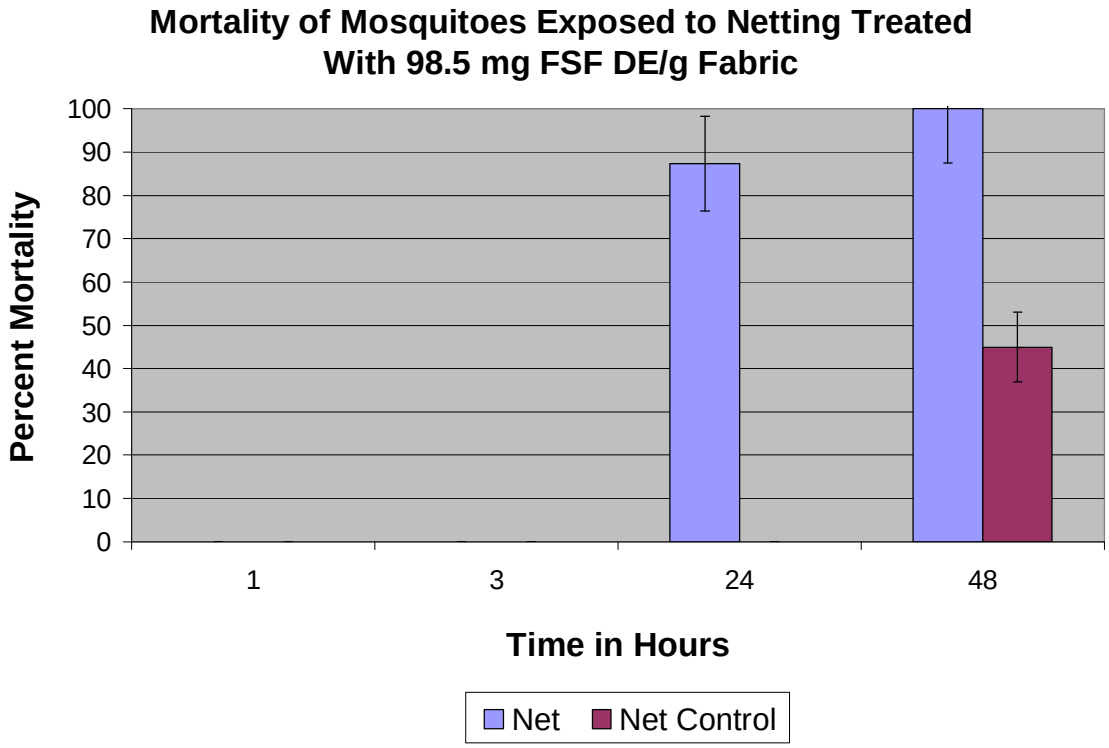

Figure 11: Mortality caused by nets treated with FSF DE

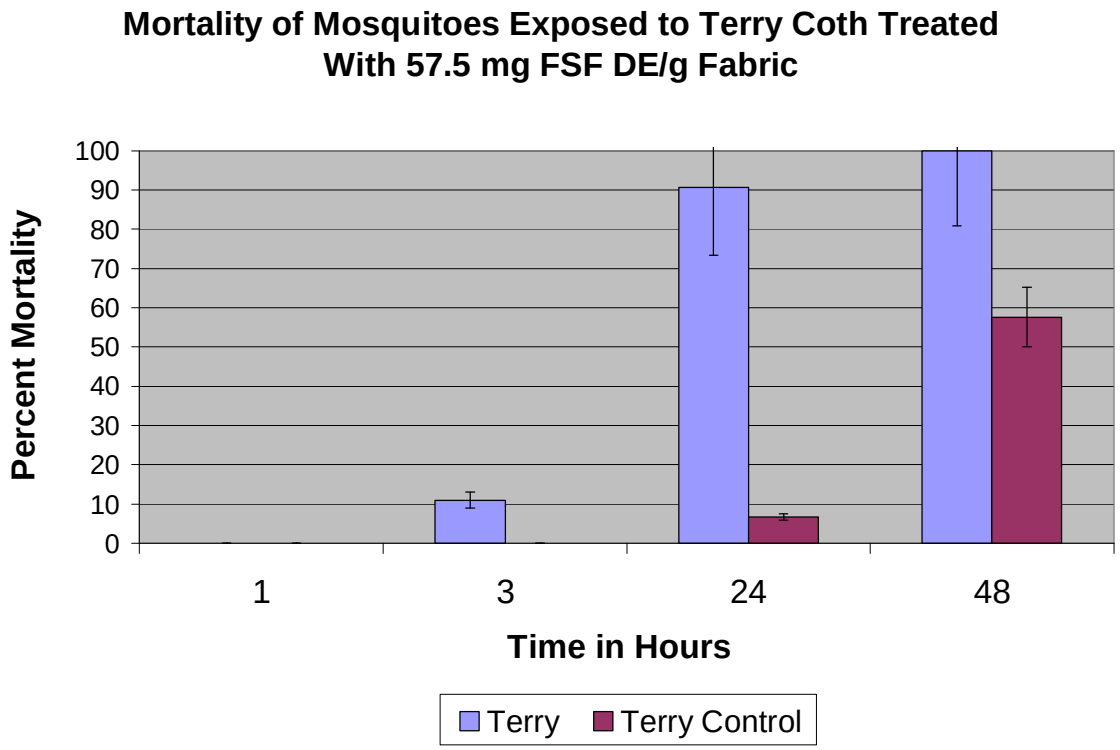

Figure 12: mortality caused by terry cloth treated with FSF DE

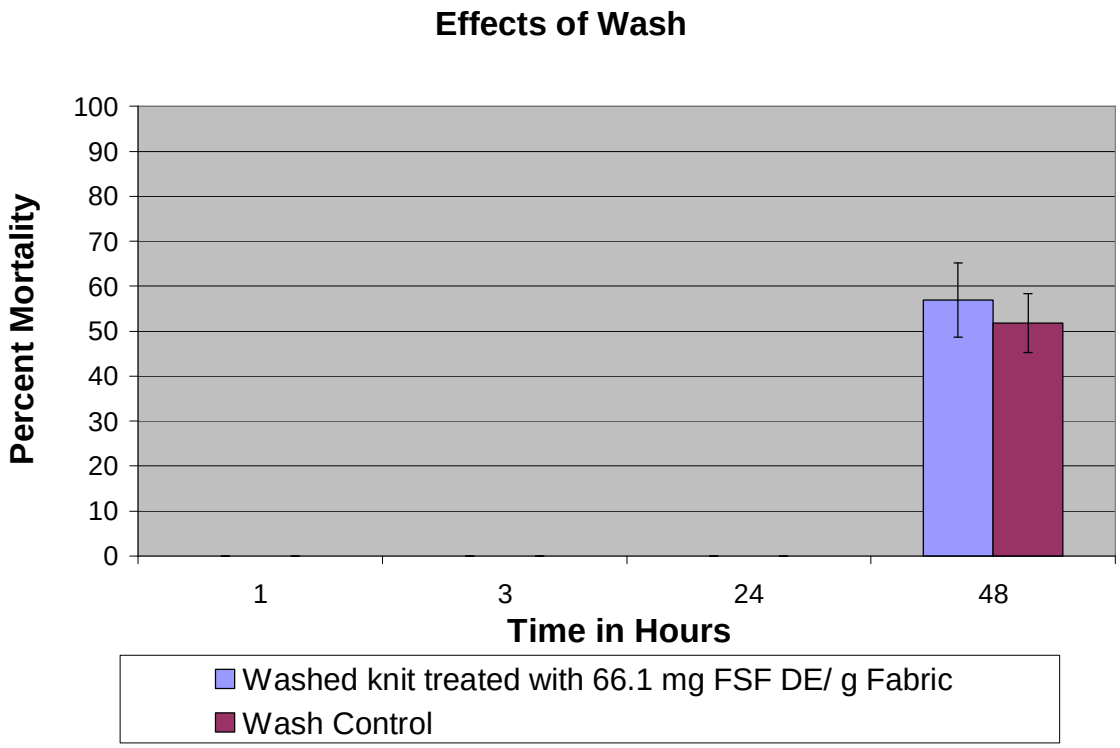

Figure 13: Effects of wash on mortality

4.2.6 Effects of Wash on Mortality

Statistical analysis showed that washed knit fabric treated with 66.1 mg FSF DE/g fabric performed identically to the washed control fabric at 24 hours ($t = 0$; $df = 12, 63.3$; $p = 1$) indicating that washing the fabric treated with DE nullified any effects. Examination of SEM images of washed and unwashed knit fibers reveal that nearly all DE was removed from all but recessed and protected areas of the fiber during wash (Figure 14). The presence of embedded DE particles, broken DE particles and imprints of missing DE particles (Figures 16, 17 and 18) in the binder were all evident in SEM images of the washed fibers. By comparing SEM images it was determined that the DE particles remaining on washed fibers were largely filled with binder while no such coating was found on DE remaining on mosquito carcasses (Figure 19).

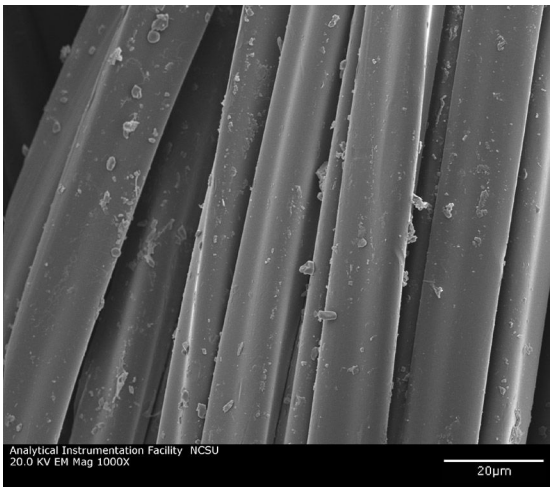

Figure 14: SEM image of untreated knit fiber at 500X magnification

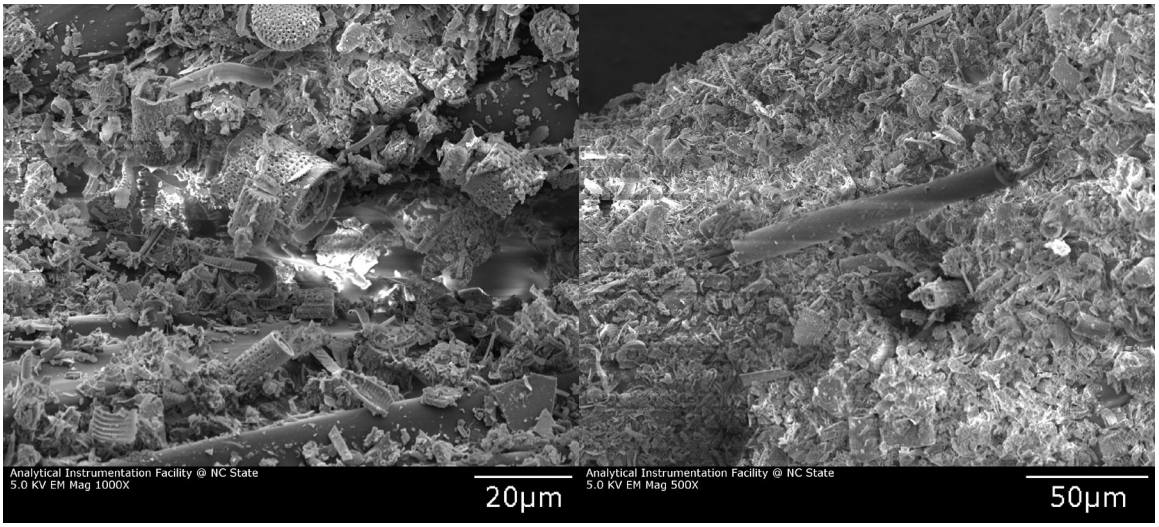

Figure 15: SEM images of knit fiber treated with 66.1mg FSF DE/g fabric. Magnifications from left to right are 1000X and 500X

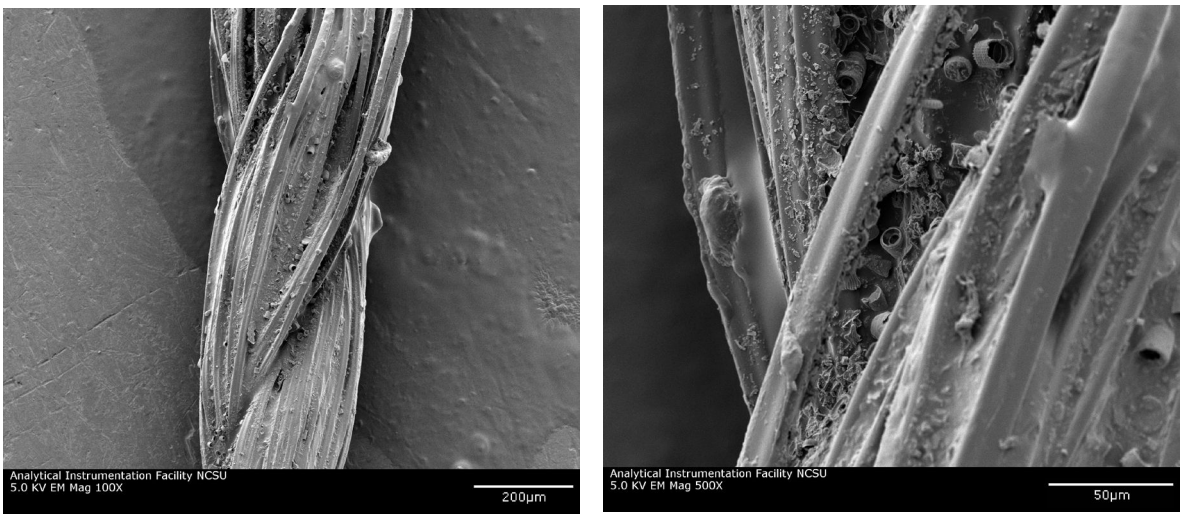

Figure 16: SEM images of washed knit fibers treated with 66.1 mg FSF DE/g fabric showing the loss of DE particles during wash. From left to right magnifications are 100X and 500X

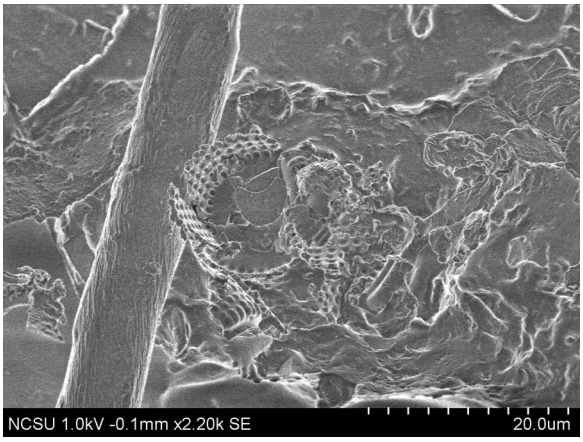

Figure 17: SEM image of pieces of DE left in the binder after washing of the knit fabric

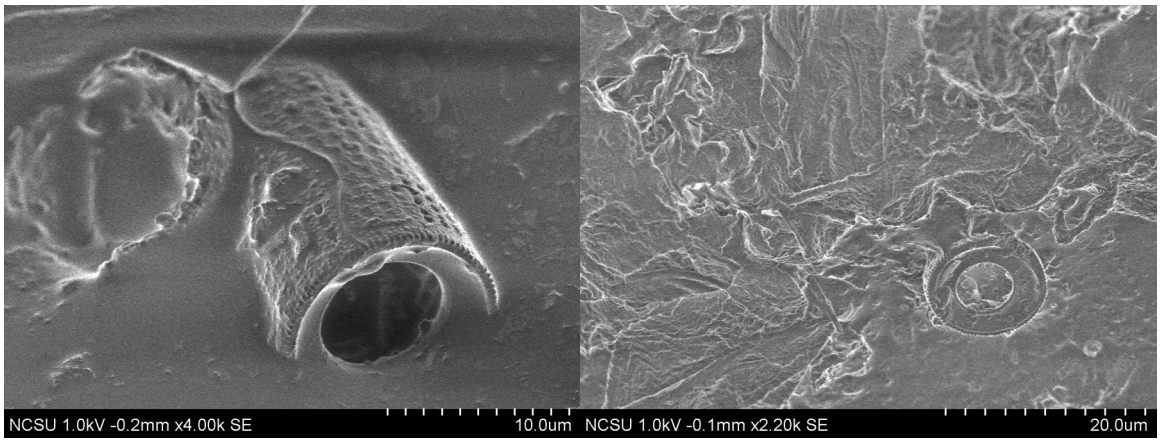

Figure 18: SEM images of embedded DE and imprints of DE after washing of the knit fabric

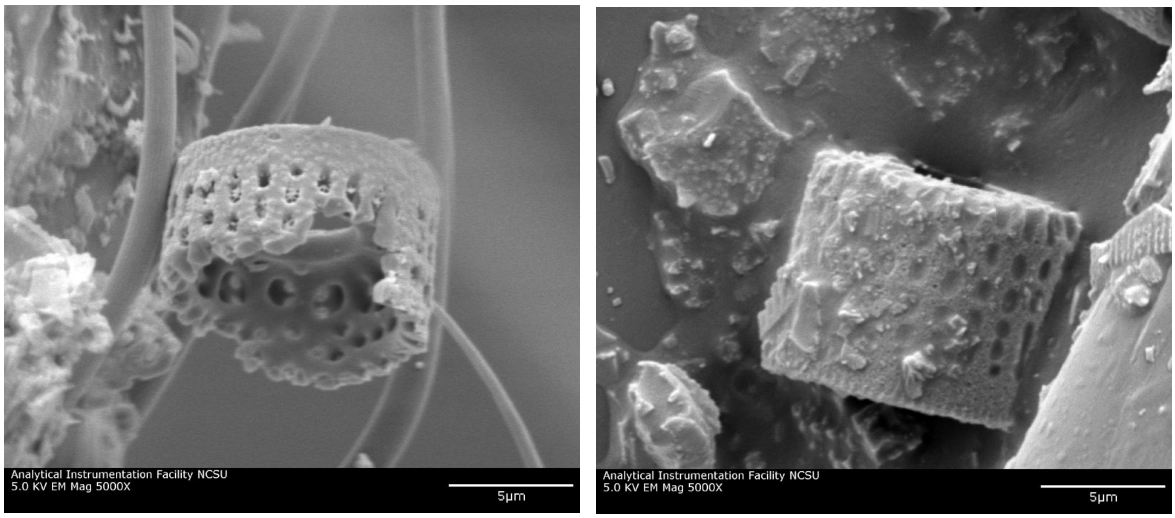

Figure 19: Left: SEM images of a DE piece shown on the antennae of an *Aedes aegypti* mosquito post-exposure. Right: a DE piece on the knit fabric after wash. The images show the flow of binder into the structure of pieces of DE left on the washed fiber.

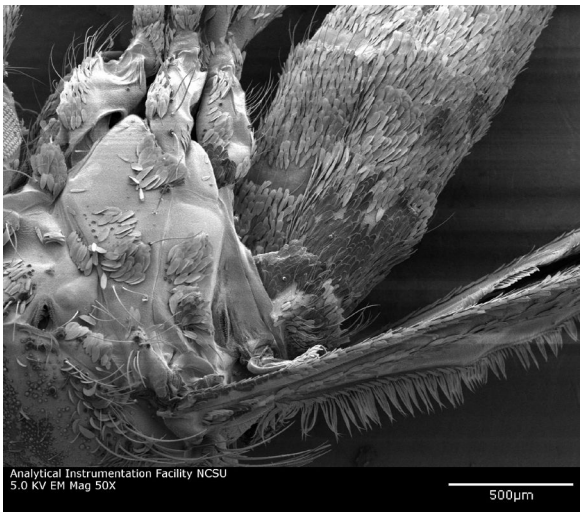

Figure 20: SEM image of the thorax and abdomen of an *Aedes aegypti* mosquito. Magnification is 50X

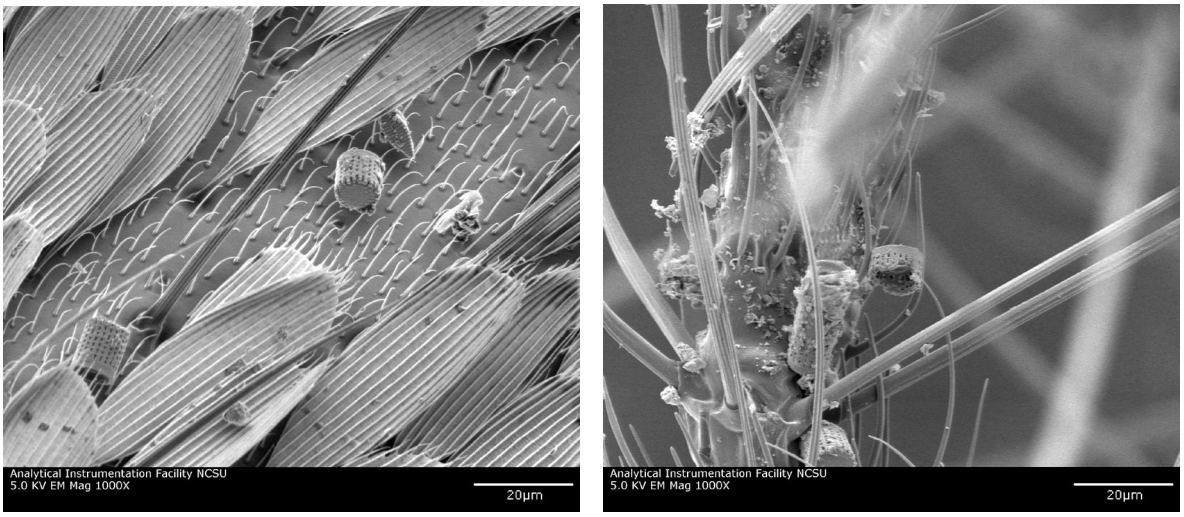

Figure 21: SEM images of DE particles which were transferred to the *Aedes aegypti* mosquito during exposure to the knit fabric treated with 66.1 mg FSF DE/ g fabric. Magnification is 1000X

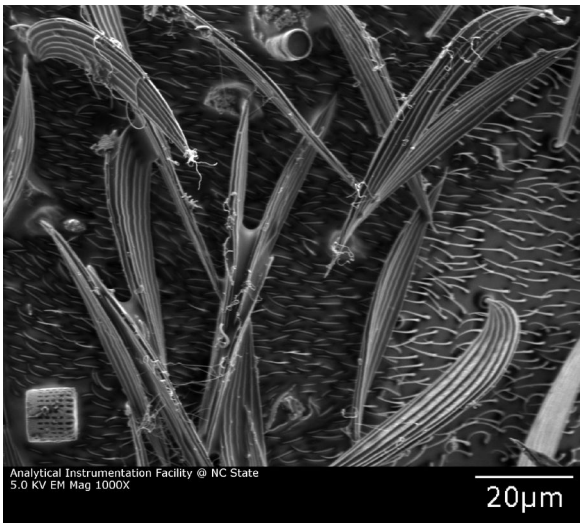

Figure 22: SEM images of DE particles which were transferred to the *Aedes aegypti* mosquito during exposure to the knit fabric treated with 1.7 mg/cm^2 of DE. Magnification is 1000X

5. Conclusion and Recommendations

Novel insecticidal textiles were created by binding safe, food-grade diatomaceous earth to several different fabrics. The physical nature of the killing mechanism should make DE treated fabrics effective against mosquitoes that are resistant to chemical insecticides and would make it less likely to develop cross resistance. Interaction of a mosquito with any of these fabrics resulted in a transfer of diatomaceous earth to the insect body and subsequent death by dehydration/desiccation, as indicated by the apparent recovery of insects that were permitted to imbibe nectar post-exposure. Fabric structure appears to have little effect on mortality.

5.1 Future Explorations

5.1.1 Antennae

Preliminary experiments showed a dramatic and immediate knock down of mosquitoes whose antennae were amputated even in part, showing that the antennae are both vulnerable and essential to host seeking behavior. SEM imagery shows significant accumulations of DE occur on the antennae of mosquitoes, showing a method of accessing the antennae.

Preliminary observations also showed that nano fibers successfully clung to and clogged the tarsus of a mosquito which has a construction similar to the antennae in that both consist of fine hairs. It may be possible to engineer fabrics and/or particles that may harm the antennae causing immediate and permanent knock down of mosquitoes.

5.1.2 Transfer of DE to Mosquitoes

SEM images reveal DE on the carcass of a mosquito exposed for 15 minutes to knit fabric treated with all loadings of DE (figures 21 and 22). Similar quantities of DE were seen on various body parts of this and other mosquitoes post-exposure (figure 21). The feather-like

structures in the image are scales that normally cover a mosquito's body.

Given that Jones determined that an increase in surface area of cuticular contact with DE increased mortality in honeybees[26], it is hypothesized that the transfer of DE to the mosquito is important in obtaining good mortality. The hypothesis is supported by, but not proven by, data

showing a decrease in mortality for fabrics made where binders were cured as opposed to those brought only to the film formation temperature, presuming that DE was held more tightly by the cured binder. It is also possible that the decrease in mortality in knit fabrics with a loading of 40 to 42 mg DE/g fabric compared to those with a loading of 66 mg DE/g fabric is caused by the absence of DE that is readily available to transfer to the mosquito. Further experimentation is required to prove or disprove the importance of the transfer of DE.

5.2 Applications

While the insect of focus in this research was the *Aedes aegypti* mosquito, it is believed that similar results would be obtained with Anopheline mosquitoes, the mosquito most commonly associated with the transmission of malaria to humans. It is also expected that this newly developed insecticidal fabric will be effective against other insect species as DE is currently used against many species of insects[21, 22, 26, 27]. Such fabrics might be useful in home textiles such as cupboard linings, curtains, bed skirts and the like to control fleas, bed bugs, ticks and roaches etc. Further research is necessary to verify the fabrics effectiveness on other insects.

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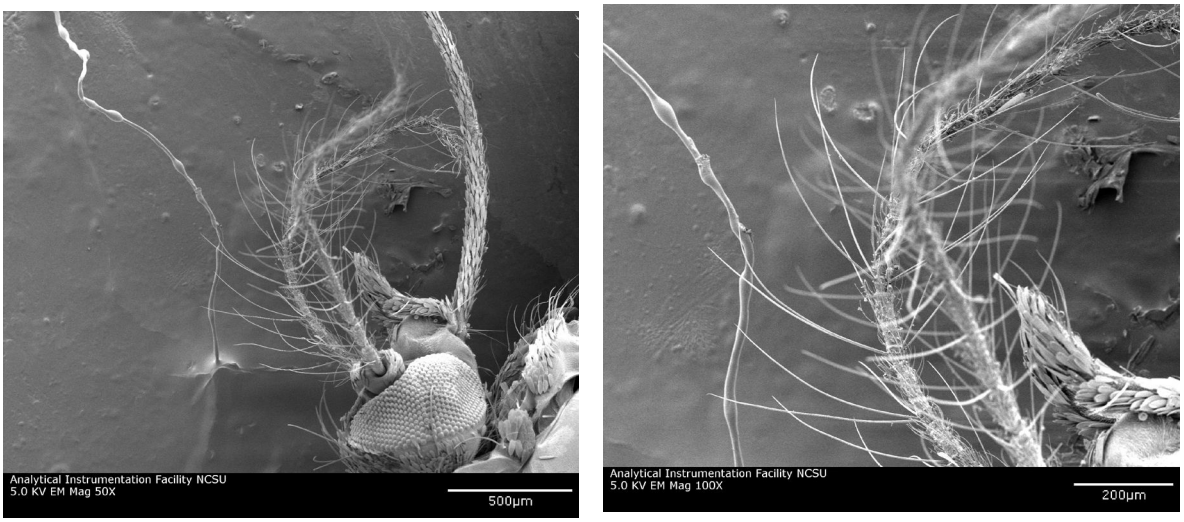
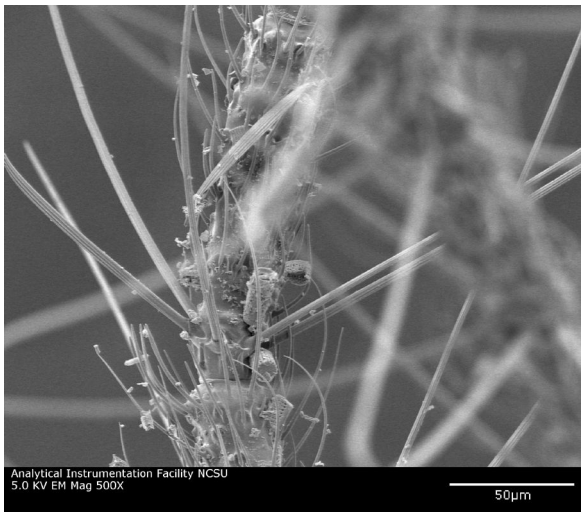
Appendix

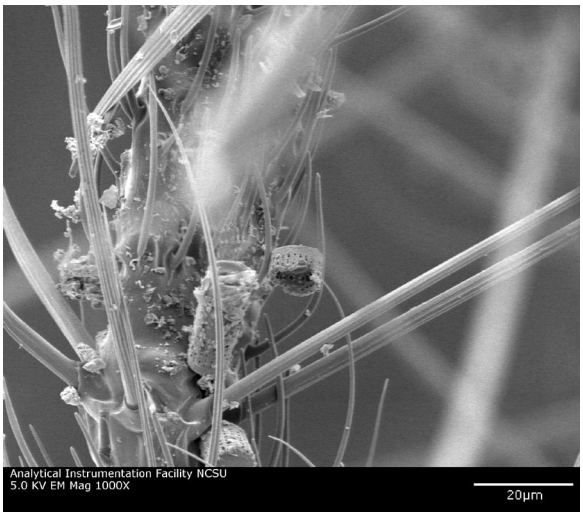
Appendix I

FSF DE on Antennae:

Scanning electron microscope images of the antennae of a female *Aedes agytie* mosquito show the proportionately large amount of DE accumulated on the antennae. Magnifications are from top left to bottom right, 50X, 100X, 500X, and 1000X. Preliminary studies showed that amputating the antennae incapacitated mosquitoes immediately. Future research may target the antennae.




Analytical Instrumentation Facility NCSU
5.0 KV EM Mag 500X
50µm


Analytical Instrumentation Facility NCSU
5.0 KV EM Mag 1000X
20µm

Appendix II

Data from experiments using fossil shell flour. High refers to 66.1 mg DE/g fabric and low refers to 41.5 mg DE/g fabric. Fabrics were cured at 148º C.

Treatment	Rep	Time	Percent Mortality
Knit High	1	1	0
Knit High	1	3	0
Knit High	1	24	85.7
Knit High	1	48	100
Knit High	2	1	0
Knit High	2	3	0
Knit High	2	24	75
Knit High	2	48	100
Knit High	3	1	0
Knit High	3	3	0
Knit High	3	24	80
Knit High	3	48	100
Knit Control	1	1	0
Knit Control	1	3	0
Knit Control	1	24	0
Knit Control	1	48	18.2
Knit Control	2	1	0
Knit Control	2	3	0
Knit Control	2	24	0
Knit Control	2	48	16.7
Knit Control	3	1	0
Knit Control	3	3	0
Knit Control	3	24	0
Knit Control	3	48	86.7

Knit Low	1	1	0
Knit Low	1	3	0
Knit Low	1	24	16.7
Knit Low	1	48	58.3
Knit Low	2	1	0
Knit Low	2	3	0
Knit Low	2	24	40
Knit Low	2	48	95
Knit Low	3	1	0
Knit Low	3	3	0
Knit Low	3	24	0
Knit Low	3	48	57.9
Knit Binder	1	1	0
Knit Binder	1	3	0
Knit Binder	1	24	0
Knit Binder	1	48	53.3
Knit Binder	2	1	0
Knit Binder	2	3	0
Knit Binder	2	24	0
Knit Binder	2	48	83.3
Knit Binder	3	1	0
Knit Binder	3	3	0
Knit Binder	3	24	0
Knit Binder	3	48	57.9
Knit Wash	1	1	0
Knit Wash	1	3	0
Knit Wash	1	24	0
Knit Wash	1	48	61.1

Knit Wash	2	1	0
Knit Wash	2	3	0
Knit Wash	2	24	0
Knit Wash	2	48	40.7
Knit Wash	3	1	0
Knit Wash	3	3	0
Knit Wash	3	24	0
Knit Wash	3	48	68.8
Knit Wash Control	1	1	0
Knit Wash Control	1	3	0
Knit Wash Control	1	24	0
Knit Wash Control	1	48	44.4
Knit Wash Control	2	1	0
Knit Wash Control	2	3	0
Knit Wash Control	2	24	0
Knit Wash Control	2	48	44.4
Knit Wash Control	3	1	0
Knit Wash Control	3	3	0
Knit Wash Control	3	24	0
Knit Wash Control	3	48	66.7
Knit High w/ nector	1	1	0
Knit High w/ nector	1	3	.
Knit High w/ nector	1	24	5
Knit High w/ nector	1	48	10
Knit High w/ nector	2	1	0
Knit High w/ nector	2	3	0
Knit High w/ nector	2	24	6.7
Knit High w/ nector	2	48	6.7

Knit High w/ nector	3	1	0
Knit High w/ nector	3	3	0
Knit High w/ nector	3	24	0
Knit High w/ nector	3	48	11.1
Knit Low w/ nector	1	1	0
Knit Low w/ nector	1	3	0
Knit Low w/ nector	1	24	0
Knit Low w/ nector	1	48	0
Knit Low w/ nector	2	1	0
Knit Low w/ nector	2	3	0
Knit Low w/ nector	2	24	0
Knit Low w/ nector	2	48	0
Knit Low w/ nector	3	1	0
Knit Low w/ nector	3	3	0
Knit Low w/ nector	3	24	0
Knit Low w/ nector	3	48	0
Knit Nector Control	1	1	0
Knit Nector Control	1	3	0
Knit Nector Control	1	24	0
Knit Nector Control	1	48	0
Knit Nector Control	2	1	0
Knit Nector Control	2	3	0
Knit Nector Control	2	24	0
Knit Nector Control	2	48	0
Knit Nector Control	3	1	0
Knit Nector Control	3	3	0
Knit Nector Control	3	24	0
Knit Nector Control	3	48	0
Net	1	1	0

Net	1	3	0
Net	1	24	75
Net	1	48	100
Net	2	1	0
Net	2	3	0
Net	2	24	100
Net	2	48	100
Net	3	1	0
Net	3	3	0
Net	3	24	87
Net	3	48	100
Net Control	1	1	0
Net Control	1	3	0
Net Control	1	24	0
Net Control	1	48	25
Net Control	2	1	0
Net Control	2	3	0
Net Control	2	24	0
Net Control	2	48	50
Net Control	3	1	0
Net Control	3	3	0
Net Control	3	24	0
Net Control	3	48	60
Terry	1	1	0
Terry	1	3	0
Terry	1	24	92.3
Terry	1	48	100
Terry	2	1	0

Terry	2	3	33.3
Terry	2	24	88.9
Terry	2	48	100
Terry	3	1	0
Terry	3	3	0
Terry	3	24	90.9
Terry	3	48	100
Terry Control	1	1	0
Terry Control	1	3	0
Terry Control	1	24	0
Terry Control	1	48	50
Terry Control	2	1	0
Terry Control	2	3	0
Terry Control	2	24	20
Terry Control	2	48	50
Terry Control	3	1	0
Terry Control	3	3	0
Terry Control	3	24	0
Terry Control	3	48	72.7

Second Data Set:

"a" refers to alumina. "k" refers to the knit fabric. Particle type and curing temperature are as noted in the table.

Treatment	Rep	Time	Percent Mortality
alumina 121 k	1	1	0
alumina 121 k	1	3	5
alumina 121 k	1	24	36.8
alumina 121 k	2	1	0

alumina 121 k	2	3	0
alumina 121 k	2	24	21.1
control 121 a	1	1	0
control 121 a	1	3	0
control 121 a	1	24	16.7
control 121 a	2	1	0
control 121 a	2	3	0
control 121 a	2	24	21.6
safer 121˚C knit	1	1	0
safer 121˚C knit	1	3	0
safer 121˚C knit	1	24	68.4
safer 121˚C knit	2	1	0
safer 121˚C knit	2	3	5.9
safer 121˚C knit	2	24	52.9
safer 148˚C knit	1	1	0
safer 148˚C knit	1	3	0
safer 148˚C knit	1	24	22.2
safer 148˚C knit	1	48	95.5
safer 148˚C knit	2	1	0
safer 148˚C knit	2	3	0
safer 148˚C knit	2	24	26.3
safer 148˚C knit	2	48	89.5
fsf 148 knit	1	1	0
fsf 148 knit	1	3	0
fsf 148 knit	1	24	85.7
fsf 148 knit	1	48	100
fsf 148 knit	2	1	0
fsf 148 knit	2	3	0
fsf 148 knit	2	24	75
fsf 148 knit	2	48	100
fsf 148 knit	3	1	0
fsf 148 knit	3	3	0

fsf 148 knit	3	24	80
fsf 148 knit	3	48	100
control safer 121 k	1	1	0
control safer 121 k	1	3	0
control safer 121 k	1	24	16.7
control safer 121 k	2	1	0
control safer 121 k	2	3	0
control safer 121 k	2	24	22.2
control safer 148 k	1	1	0
control safer 148 k	1	3	0
control safer 148 k	1	24	0
control safer 148 k	1	48	18.2
control safer 148 k	2	1	0
control safer 148 k	2	3	0
control safer 148 k	2	24	0
control safer 148 k	2	48	16.7
control safer 148 k	3	1	0
control safer 148 k	3	3	0
control safer 148 k	3	24	0
control safer 148 k	3	48	86.7
safer 121˚C terry	1	1	0
safer 121˚C terry	1	3	0
safer 121˚C terry	1	24	10
safer 121˚C terry	1	48	65
safer 121˚C terry	2	1	0
safer 121˚C terry	2	3	0
safer 121˚C terry	2	24	15
safer 121˚C terry	2	48	90
safer 148 terry	1	1	0
safer 148 terry	1	3	0
safer 148 terry	1	24	0
safer 148 terry	1	48	76.5

safer 148 terry	2	1	0
safer 148 terry	2	3	0
safer 148 terry	2	24	0
safer 148 terry	2	48	38.9
fsf 148 Terry	1	1	0
fsf 148 Terry	1	3	0
fsf 148 Terry	1	24	92.3
fsf 148 Terry	1	48	100
fsf 148 Terry	2	1	0
fsf 148 Terry	2	3	33.3
fsf 148 Terry	2	24	88.9
fsf 148 Terry	2	48	100
fsf 148 Terry	3	1	0
fsf 148 Terry	3	3	0
fsf 148 Terry	3	24	90.9
fsf 148 Terry	3	48	100
control terry	1	1	0
control terry	1	3	0
control terry	1	24	0
control terry	1	48	10.5
control terry	2	1	0
control terry	2	3	0
control terry	2	24	0
control terry	2	48	70
control terry	3	1	0
control terry	3	3	0
control terry	3	24	18.2
control terry	3	48	50
safer 148 net	1	1	0
safer 148 net	1	3	0
safer 148 net	1	24	15.8
safer 148 net	1	48	68.4
safer 148 net	2	1	0
safer 148 net	2	3	0

safer 148 net	2	24	35.7
safer 148 net	2	48	92.9
control net	1	1	0
control net	1	3	0
control net	1	24	0
control net	1	48	50
control net	2	1	0
control net	2	3	0
control net	2	24	0
control net	2	48	25
control net	3	1	0
control net	3	3	0
control net	3	24	0
control net	3	48	60
fsf 148 Net	1	1	0
fsf 148 Net	1	3	0
fsf 148 Net	1	24	75
fsf 148 Net	1	48	100
fsf 148 Net	2	1	0
fsf 148 Net	2	3	0
fsf 148 Net	2	24	100
fsf 148 Net	2	48	100
fsf 148 Net	3	1	0
fsf 148 Net	3	3	0
fsf 148 Net	3	24	87
fsf 148 Net	3	48	100

Appendix III

Agitation Data:

The degree of interaction between the mosquito and the test fabrics is an important variable. All of the experiments reported in this study used a forced interaction of 15 minutes by inserting and rotating a rigid rod into the test chamber. Other methods of getting the mosquitoes to interact with the fabric were explored including using a host as bait and allowing the mosquitoes to interact at will with no external enticement. Data from these experiments was not included in the main body of the study because of high mortality in the control groups and because of un-repeatable results. It is believed that the experiment did not sufficiently isolate the desired variables. Histamine responses were noticed in the host indicating that mosquitoes were able to interact with the host sufficiently to transmit saliva to the host which could have introduced new variables and invalidated the experiment. The data is included here. Mosquitoes were exposed to the host for 30 minutes.

Treatment	Rep	Time	Percent Mortality
FSF Net Host Enticed	1	1	0
FSF Net Host Enticed	1	3	0
FSF Net Host Enticed	1	24	10.3
FSF Net Host Enticed	1	48	92
FSF Net Host Enticed	2	1	0
FSF Net Host Enticed	2	3	0
FSF Net Host Enticed	2	24	24
FSF Net Host Enticed	2	48	69
FSF Net Host Enticed	3	1	0
FSF Net Host Enticed	3	3	
FSF Net Host Enticed	3	24	8.7

FSF Net Host Enticed	3	48	56.5
FSF Net Host Enticed	4	1	0
FSF Net Host Enticed	4	3	
FSF Net Host Enticed	4	24	7.1
FSF Net Host Enticed	4	48	42.9
Net Host Enticed Control	1	1	0
Net Host Enticed Control	1	3	0
Net Host Enticed Control	1	24	4
Net Host Enticed Control	1	48	87.5
Net Host Enticed Control	2	1	0
Net Host Enticed Control	2	3	0
Net Host Enticed Control	2	24	0
Net Host Enticed Control	2	48	35.3
Safer Knit No Agitation	1	1	0
Safer Knit No Agitation	1	3	0
Safer Knit No Agitation	1	24	18.8
Safer Knit No Agitation	1	48	50
Safer Knit No Agitation	2	1	0
Safer Knit No Agitation	2	3	0
Safer Knit No Agitation	2	24	15
Safer Knit No Agitation	2	48	75
Knit No Agitation Control	1	1	0
Knit No Agitation Control	1	3	0
Knit No Agitation Control	1	24	11
Knit No Agitation Control	1	48	66.7
Knit No Agitation Control	2	1	0
Knit No Agitation Control	2	3	0
Knit No Agitation Control	2	24	0
Knit No Agitation Control	2	48	66.7

Appendix IV

Statistical Analysis Output:
First Data Set Output:

The SAS System 17:28 Tuesday, November 20, 2007 9

The GLM Procedure

Level of Treatment	Level of Time	N	Mortality Mean	Mortality Std Dev
KNecCon	1	3	0.000000	0.0000000
KNecCon	3	3	0.000000	0.0000000
KNecCon	24	3	0.000000	0.0000000
KNecCon	48	3	0.000000	0.0000000
Kbind	1	3	0.000000	0.0000000
Kbind	3	3	0.000000	0.0000000
Kbind	24	3	0.000000	0.0000000
Kbind	48	3	64.833333	16.1571450
Khi	1	3	0.000000	0.0000000
Khi	3	3	0.000000	0.0000000
Khi	24	3	80.233333	5.3538148
Khi	48	3	100.000000	0.0000000
KhiNec	1	3	0.000000	0.0000000
KhiNec	3	3	0.000000	0.0000000
KhiNec	24	3	3.900000	3.4828150
KhiNec	48	3	9.266667	2.2898326
Klo	1	3	0.000000	0.0000000
Klo	3	3	0.000000	0.0000000
Klo	24	3	18.900000	20.0905450
Klo	48	3	70.400000	21.3051637
KloNec	1	3	0.000000	0.0000000
KloNec	3	3	0.000000	0.0000000
KloNec	24	3	0.000000	0.0000000
KloNec	48	3	0.000000	0.0000000
Knit	1	3	0.000000	0.0000000
Knit	3	3	0.000000	0.0000000
Knit	24	3	0.000000	0.0000000
Knit	48	3	40.533333	39.9885400
Kwash	1	3	0.000000	0.0000000
Kwash	3	3	0.000000	0.0000000
Kwash	24	3	0.000000	0.0000000
Kwash	48	3	56.866667	14.5204454
KwashCon	1	3	0.000000	0.0000000
KwashCon	3	3	0.000000	0.0000000
KwashCon	24	3	0.000000	0.0000000
KwashCon	48	3	51.833333	12.8749110
NBindDE	1	3	0.000000	0.0000000
NBindDE	3	3	0.000000	0.0000000
NBindDE	24	3	87.333333	12.5033329
NBindDE	48	3	100.000000	0.0000000
Net	1	3	0.000000	0.0000000
Net	3	3	0.000000	0.0000000

Net	24	3	0.000000	0.0000000
Net	48	3	45.000000	18.0277564
TBindDE	1	3	0.000000	0.0000000

The GLM Procedure

Level of Treatment	Level of Time	N	Mortality Mean	Mortality Std Dev
TBindDE	3	3	11.100000	19.2257640
TBindDE	24	3	90.700000	1.7088007
TBindDE	48	3	100.000000	0.0000000
Terry	1	3	0.000000	0.0000000
Terry	3	3	0.000000	0.0000000
Terry	24	3	6.666667	11.5470054
Terry	48	3	57.566667	13.1058511

The Mixed Procedure

Model Information

Data Set	WORK.A
Dependent Variable	Mortality
Covariance Structure	Compound Symmetry
Subject Effect	Rep
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Treatment	13	KNecCon Kbind Khi KhiNec Klo KloNec Knit Kwash KwashCon NBindDE Net TBindDE Terry
Time	4	1 3 24 48
Rep	3	1 2 3

Dimensions

Covariance Parameters	2
Columns in X	66
Columns in Z	0
Subjects	3
Max Obs Per Subject	52

Number of Observations

Number of Observations Read	156
Number of Observations Used	156
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	811.42328695	
1	1	811.02590180	0.00000000

The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	Rep	1.2259
Residual		81.4530

Fit Statistics

-2 Res Log Likelihood	811.0
AIC (smaller is better)	815.0
AICC (smaller is better)	815.1
BIC (smaller is better)	813.2

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	0.40	0.5284

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	12	102	44.65	<.0001
Treatment*Time	39	102	39.09	<.0001

Least Squares Means

Effect	Treatment	Time	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	KNecCon		-155E-16	2.6826	63.3	-0.00	1.0000
Treatment	Kbind		16.2083	2.6826	63.3	6.04	<.0001
Treatment	Khi		45.0583	2.6826	63.3	16.80	<.0001
Treatment	KhiNec		3.2917	2.6826	63.3	1.23	0.2244
Treatment	Klo		22.3250	2.6826	63.3	8.32	<.0001
Treatment	KloNec		-112E-16	2.6826	63.3	-0.00	1.0000
Treatment	Knit		10.1333	2.6826	63.3	3.78	0.0004
Treatment	Kwash		14.2167	2.6826	63.3	5.30	<.0001
Treatment	KwashCon		12.9583	2.6826	63.3	4.83	<.0001

The Mixed Procedure

Least Squares Means

Effect	Treatment	Time	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	NBindDE		46.8333	2.6826	63.3	17.46	<.0001
Treatment	Net		11.2500	2.6826	63.3	4.19	<.0001
Treatment	TBindDE		50.4500	2.6826	63.3	18.81	<.0001
Treatment	Terry		16.0583	2.6826	63.3	5.99	<.0001

Differences of Least Squares Means

Effect	Treatment	Time	_Treatment	_Time	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	KNecCon		Kbind		-16.2083	3.6845	102	-4.40	<.0001
Treatment	KNecCon		Khi		-45.0583	3.6845	102	-12.23	<.0001
Treatment	KNecCon		KhiNec		-3.2917	3.6845	102	-0.89	0.3738
Treatment	KNecCon		Klo		-22.3250	3.6845	102	-6.06	<.0001
Treatment	KNecCon		KloNec		-427E-17	3.6845	102	-0.00	1.0000
Treatment	KNecCon		Knit		-10.1333	3.6845	102	-2.75	0.0070
Treatment	KNecCon		Kwash		-14.2167	3.6845	102	-3.86	0.0002
Treatment	KNecCon		KwashCon		-12.9583	3.6845	102	-3.52	0.0007
Treatment	KNecCon		NBindDE		-46.8333	3.6845	102	-12.71	<.0001
Treatment	KNecCon		Net		-11.2500	3.6845	102	-3.05	0.0029
Treatment	KNecCon		TBindDE		-50.4500	3.6845	102	-13.69	<.0001
Treatment	KNecCon		Terry		-16.0583	3.6845	102	-4.36	<.0001
Treatment	Kbind		Khi		-28.8500	3.6845	102	-7.83	<.0001
Treatment	Kbind		KhiNec		12.9167	3.6845	102	3.51	0.0007
Treatment	Kbind		Klo		-6.1167	3.6845	102	-1.66	0.1000
Treatment	Kbind		KloNec		16.2083	3.6845	102	4.40	<.0001
Treatment	Kbind		Knit		6.0750	3.6845	102	1.65	0.1023
Treatment	Kbind		Kwash		1.9917	3.6845	102	0.54	0.5900
Treatment	Kbind		KwashCon		3.2500	3.6845	102	0.88	0.3798
Treatment	Kbind		NBindDE		-30.6250	3.6845	102	-8.31	<.0001
Treatment	Kbind		Net		4.9583	3.6845	102	1.35	0.1814
Treatment	Kbind		TBindDE		-34.2417	3.6845	102	-9.29	<.0001
Treatment	Kbind		Terry		0.1500	3.6845	102	0.04	0.9676
Treatment	Khi		KhiNec		41.7667	3.6845	102	11.34	<.0001
Treatment	Khi		Klo		22.7333	3.6845	102	6.17	<.0001
Treatment	Khi		KloNec		45.0583	3.6845	102	12.23	<.0001
Treatment	Khi		Knit		34.9250	3.6845	102	9.48	<.0001
Treatment	Khi		Kwash		30.8417	3.6845	102	8.37	<.0001
Treatment	Khi		KwashCon		32.1000	3.6845	102	8.71	<.0001
Treatment	Khi		NBindDE		-1.7750	3.6845	102	-0.48	0.6310
Treatment	Khi		Net		33.8083	3.6845	102	9.18	<.0001
Treatment	Khi		TBindDE		-5.3917	3.6845	102	-1.46	0.1464
Treatment	Khi		Terry		29.0000	3.6845	102	7.87	<.0001
Treatment	KhiNec		Klo		-19.0333	3.6845	102	-5.17	<.0001
Treatment	KhiNec		KloNec		3.2917	3.6845	102	0.89	0.3738
Treatment	KhiNec		Knit		-6.8417	3.6845	102	-1.86	0.0662
Treatment	KhiNec		Kwash		-10.9250	3.6845	102	-2.97	0.0038
Treatment	KhiNec		KwashCon		-9.6667	3.6845	102	-2.62	0.0100
Treatment	KhiNec		NBindDE		-43.5417	3.6845	102	-11.82	<.0001
Treatment	KhiNec		Net		-7.9583	3.6845	102	-2.16	0.0331
Treatment	KhiNec		TBindDE		-47.1583	3.6845	102	-12.80	<.0001
Treatment	KhiNec		Terry		-12.7667	3.6845	102	-3.46	0.0008
Treatment	Klo		KloNec		22.3250	3.6845	102	6.06	<.0001
Treatment	Klo		Knit		12.1917	3.6845	102	3.31	0.0013
Treatment	Klo		Kwash		8.1083	3.6845	102	2.20	0.0300
Treatment	Klo		KwashCon		9.3667	3.6845	102	2.54	0.0125

Treatment	Klo		NBindDE		-24.5083	3.6845	102	-6.65	<.0001
Treatment	Klo		Net		11.0750	3.6845	102	3.01	0.0033
Treatment	Klo		TBindDE		-28.1250	3.6845	102	-7.63	<.0001
Treatment	Klo		Terry		6.2667	3.6845	102	1.70	0.0920
Treatment	KloNec		Knit		-10.1333	3.6845	102	-2.75	0.0070
Treatment	KloNec		Kwash		-14.2167	3.6845	102	-3.86	0.0002
Treatment	KloNec		KwashCon		-12.9583	3.6845	102	-3.52	0.0007
Treatment	KloNec		NBindDE		-46.8333	3.6845	102	-12.71	<.0001
Treatment	KloNec		Net		-11.2500	3.6845	102	-3.05	0.0029
Treatment	KloNec		TBindDE		-50.4500	3.6845	102	-13.69	<.0001
Treatment	KloNec		Terry		-16.0583	3.6845	102	-4.36	<.0001
Treatment	Knit		Kwash		-4.0833	3.6845	102	-1.11	0.2704
Treatment	Knit		KwashCon		-2.8250	3.6845	102	-0.77	0.4450
Treatment	Knit		NBindDE		-36.7000	3.6845	102	-9.96	<.0001
Treatment	Knit		Net		-1.1167	3.6845	102	-0.30	0.7625
Treatment	Knit		TBindDE		-40.3167	3.6845	102	-10.94	<.0001
Treatment	Knit		Terry		-5.9250	3.6845	102	-1.61	0.1109
Treatment	Kwash		KwashCon		1.2583	3.6845	102	0.34	0.7334
Treatment	Kwash		NBindDE		-32.6167	3.6845	102	-8.85	<.0001
Treatment	Kwash		Net		2.9667	3.6845	102	0.81	0.4226
Treatment	Kwash		TBindDE		-36.2333	3.6845	102	-9.83	<.0001
Treatment	Kwash		Terry		-1.8417	3.6845	102	-0.50	0.6183
Treatment	KwashCon		NBindDE		-33.8750	3.6845	102	-9.19	<.0001
Treatment	KwashCon		Net		1.7083	3.6845	102	0.46	0.6439
Treatment	KwashCon		TBindDE		-37.4917	3.6845	102	-10.18	<.0001
Treatment	KwashCon		Terry		-3.1000	3.6845	102	-0.84	0.4021
Treatment	NBindDE		Net		35.5833	3.6845	102	9.66	<.0001
Treatment	NBindDE		TBindDE		-3.6167	3.6845	102	-0.98	0.3286
Treatment	NBindDE		Terry		30.7750	3.6845	102	8.35	<.0001
Treatment	Net		TBindDE		-39.2000	3.6845	102	-10.64	<.0001
Treatment	Net		Terry		-4.8083	3.6845	102	-1.31	0.1948
Treatment	TBindDE		Terry		34.3917	3.6845	102	9.33	<.0001
Treatment*Time	KNecCon	1	Kbind	1	7.15E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	Khi	1	1.34E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	KhiNec	1	1.34E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	Klo	1	4.49E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	KloNec	1	7.29E-16	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	Knit	1	1.34E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	Kwash	1	3.25E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	KwashCon	1	1.16E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	NBindDE	1	-84E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	1	Net	1	2.71E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	1	Terry	1	-151E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	3	Kbind	3	-141E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	3	Khi	3	4.81E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	3	KhiNec	3	-23E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	3	Klo	3	-407E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	3	KloNec	3	-112E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	3	Knit	3	4.81E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	3	Kwash	3	2.39E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	3	KwashCon	3	-407E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	3	NBindDE	3	4.81E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	3	Net	3	8.36E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	KNecCon	3	Terry	3	-94E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	24	Kbind	24	1.3E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	24	Khi	24	-80.2333	7.3690	102	-10.89	<.0001
Treatment*Time	KNecCon	24	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	KNecCon	24	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	KNecCon	24	KloNec	24	7.59E-15	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	24	Knit	24	1.92E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	24	Kwash	24	2.41E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	24	KwashCon	24	2.46E-14	7.3690	102	0.00	1.0000
Treatment*Time	KNecCon	24	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	KNecCon	24	Net	24	2.28E-14	7.3690	102	0.00	1.0000

Treatment*Time	KNecCon	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	KNecCon	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KNecCon	48	Kbind	48	-64.8333	7.3690	102	-8.80	<.0001
Treatment*Time	KNecCon	48	Khi	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KNecCon	48	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	KNecCon	48	Klo	48	-70.4000	7.3690	102	-9.55	<.0001
Treatment*Time	KNecCon	48	KloNec	48	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KNecCon	48	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	KNecCon	48	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	KNecCon	48	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	KNecCon	48	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KNecCon	48	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	KNecCon	48	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KNecCon	48	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Kbind	1	Khi	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Khi	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Khi	24	-80.2333	7.3690	102	-10.89	<.0001
Treatment*Time	Kbind	1	Khi	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	1	KhiNec	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	KhiNec	3	-888E-18	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	Kbind	1	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	Kbind	1	Klo	1	-266E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	Klo	3	-266E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	KloNec	1	-642E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	KloNec	3	-979E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	KloNec	24	-542E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	KloNec	48	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Knit	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Knit	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Knit	24	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	Kbind	1	Kwash	1	2.53E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Kwash	3	2.53E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Kwash	24	1.11E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	Kbind	1	KwashCon	1	4.44E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	KwashCon	3	-266E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	KwashCon	24	1.15E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	Kbind	1	NBindDE	1	-799E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	NBindDE	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Kbind	1	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	1	Net	1	-444E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	Net	3	9.77E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Net	24	9.77E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Kbind	1	TBindDE	1	2.75E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	1	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Kbind	1	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Kbind	1	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	1	Terry	1	-222E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	Terry	3	-799E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	1	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Kbind	1	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Kbind	3	Kbind	24	0	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Kbind	48	-64.8333	7.3690	102	-8.80	<.0001
Treatment*Time	Kbind	3	Khi	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Khi	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Khi	24	-80.2333	7.3690	102	-10.89	<.0001
Treatment*Time	Kbind	3	Khi	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	3	KhiNec	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	KhiNec	3	-888E-18	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978

Treatment*Time	Kbind	3	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	Kbind	3	Klo	3	-266E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	Kbind	3	Klo	48	-70.4000	7.3690	102	-9.55	<.0001
Treatment*Time	Kbind	3	KloNec	1	-642E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	KloNec	3	-979E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	KloNec	24	-542E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	KloNec	48	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Knit	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Knit	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Knit	24	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	Kbind	3	Kwash	1	2.53E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Kwash	3	2.53E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Kwash	24	1.11E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	Kbind	3	KwashCon	1	4.44E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	KwashCon	3	-266E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	KwashCon	24	1.15E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	Kbind	3	NBindDE	1	-799E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	NBindDE	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Kbind	3	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	3	Net	1	-444E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	Net	3	9.77E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Net	24	9.77E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Kbind	3	TBindDE	1	2.75E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Kbind	3	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Kbind	3	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	3	Terry	1	-222E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	Terry	3	-799E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	3	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Kbind	3	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Kbind	24	Kbind	48	-64.8333	7.3690	102	-8.80	<.0001
Treatment*Time	Kbind	24	Khi	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Khi	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Khi	24	-80.2333	7.3690	102	-10.89	<.0001
Treatment*Time	Kbind	24	Khi	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	24	KhiNec	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	KhiNec	3	-888E-18	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	Kbind	24	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	Kbind	24	Klo	48	-70.4000	7.3690	102	-9.55	<.0001
Treatment*Time	Kbind	24	KloNec	1	-642E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	KloNec	3	-979E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	KloNec	24	-542E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	KloNec	48	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Knit	1	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Knit	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Knit	24	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	Kbind	24	Kwash	1	2.53E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Kwash	3	2.53E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Kwash	24	1.11E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	Kbind	24	KwashCon	1	4.44E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	KwashCon	3	-266E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	KwashCon	24	1.15E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	Kbind	24	NBindDE	1	-799E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	NBindDE	3	6.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001

Treatment*Time	Kbind	24	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	24	Net	1	-444E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	Net	3	9.77E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Net	24	9.77E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Kbind	24	TBindDE	1	2.75E-14	7.3690	102	0.00	1.0000
Treatment*Time	Kbind	24	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Kbind	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Kbind	24	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Kbind	24	Terry	1	-222E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	Terry	3	-799E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kbind	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Kbind	24	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Kbind	48	Khi	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Khi	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Khi	24	-15.4000	7.3690	102	-2.09	0.0391
Treatment*Time	Kbind	48	Khi	48	-35.1667	7.3690	102	-4.77	<.0001
Treatment*Time	Kbind	48	KhiNec	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KhiNec	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KhiNec	24	60.9333	7.3690	102	8.27	<.0001
Treatment*Time	Kbind	48	KhiNec	48	55.5667	7.3690	102	7.54	<.0001
Treatment*Time	Kbind	48	Klo	48	-5.5667	7.3690	102	-0.76	0.4517
Treatment*Time	Kbind	48	KloNec	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KloNec	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KloNec	24	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KloNec	48	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Knit	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Knit	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Knit	24	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Knit	48	24.3000	7.3690	102	3.30	0.0013
Treatment*Time	Kbind	48	Kwash	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Kwash	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Kwash	24	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Kwash	48	7.9667	7.3690	102	1.08	0.2822
Treatment*Time	Kbind	48	KwashCon	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KwashCon	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KwashCon	24	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	KwashCon	48	13.0000	7.3690	102	1.76	0.0807
Treatment*Time	Kbind	48	NBindDE	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	NBindDE	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	NBindDE	24	-22.5000	7.3690	102	-3.05	0.0029
Treatment*Time	Kbind	48	NBindDE	48	-35.1667	7.3690	102	-4.77	<.0001
Treatment*Time	Kbind	48	Net	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Net	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Net	24	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Net	48	19.8333	7.3690	102	2.69	0.0083
Treatment*Time	Kbind	48	TBindDE	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	TBindDE	3	53.7333	7.3690	102	7.29	<.0001
Treatment*Time	Kbind	48	TBindDE	24	-25.8667	7.3690	102	-3.51	0.0007
Treatment*Time	Kbind	48	TBindDE	48	-35.1667	7.3690	102	-4.77	<.0001
Treatment*Time	Kbind	48	Terry	1	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Terry	3	64.8333	7.3690	102	8.80	<.0001
Treatment*Time	Kbind	48	Terry	24	58.1667	7.3690	102	7.89	<.0001
Treatment*Time	Kbind	48	Terry	48	7.2667	7.3690	102	0.99	0.3264
Treatment*Time	Khi	1	Khi	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Khi	24	-80.2333	7.3690	102	-10.89	<.0001
Treatment*Time	Khi	1	Khi	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Khi	1	KhiNec	1	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	KhiNec	3	-711E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	Khi	1	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	Khi	1	Klo	1	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	Klo	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	Khi	1	Klo	48	-70.4000	7.3690	102	-9.55	<.0001

Treatment*Time	Khi	1	KloNec	1	-126E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	KloNec	3	-16E-15	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	KloNec	24	-116E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	KloNec	48	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Knit	1	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Knit	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Knit	24	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	Khi	1	Kwash	1	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Kwash	3	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Kwash	24	4.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	Khi	1	KwashCon	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	KwashCon	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	KwashCon	24	5.33E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	Khi	1	NBindDE	1	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	NBindDE	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Khi	1	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Khi	1	Net	1	-107E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	Net	3	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Net	24	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Khi	1	TBindDE	1	2.13E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	1	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Khi	1	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Khi	1	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Khi	1	Terry	1	-284E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	Terry	3	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	1	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Khi	1	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Khi	3	Khi	24	-80.2333	7.3690	102	-10.89	<.0001
Treatment*Time	Khi	3	Khi	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Khi	3	KhiNec	1	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	KhiNec	3	-711E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	Khi	3	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	Khi	3	Klo	1	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	Klo	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	KloNec	3	-16E-15	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	KloNec	24	-116E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	KloNec	48	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Knit	1	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Knit	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Knit	24	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	Khi	3	Kwash	1	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Kwash	3	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Kwash	24	4.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	Khi	3	KwashCon	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	KwashCon	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	KwashCon	24	5.33E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	Khi	3	NBindDE	1	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	NBindDE	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Khi	3	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Khi	3	Net	1	-107E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	Net	3	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Net	24	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Khi	3	TBindDE	1	2.13E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351

Treatment*Time	Khi	3	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Khi	3	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Khi	3	Terry	1	-284E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	Terry	3	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	3	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Khi	3	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Khi	24	Khi	48	-19.7667	7.3690	102	-2.68	0.0085
Treatment*Time	Khi	24	KhiNec	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KhiNec	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KhiNec	24	76.3333	7.3690	102	10.36	<.0001
Treatment*Time	Khi	24	KhiNec	48	70.9667	7.3690	102	9.63	<.0001
Treatment*Time	Khi	24	Klo	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Klo	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Klo	24	61.3333	7.3690	102	8.32	<.0001
Treatment*Time	Khi	24	Klo	48	9.8333	7.3690	102	1.33	0.1850
Treatment*Time	Khi	24	KloNec	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KloNec	24	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KloNec	48	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Knit	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Knit	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Knit	24	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Knit	48	39.7000	7.3690	102	5.39	<.0001
Treatment*Time	Khi	24	Kwash	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Kwash	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Kwash	24	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Kwash	48	23.3667	7.3690	102	3.17	0.0020
Treatment*Time	Khi	24	KwashCon	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KwashCon	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KwashCon	24	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	KwashCon	48	28.4000	7.3690	102	3.85	0.0002
Treatment*Time	Khi	24	NBindDE	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	NBindDE	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	NBindDE	24	-7.1000	7.3690	102	-0.96	0.3376
Treatment*Time	Khi	24	NBindDE	48	-19.7667	7.3690	102	-2.68	0.0085
Treatment*Time	Khi	24	Net	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Net	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Net	24	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Net	48	35.2333	7.3690	102	4.78	<.0001
Treatment*Time	Khi	24	TBindDE	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	TBindDE	3	69.1333	7.3690	102	9.38	<.0001
Treatment*Time	Khi	24	TBindDE	24	-10.4667	7.3690	102	-1.42	0.1586
Treatment*Time	Khi	24	TBindDE	48	-19.7667	7.3690	102	-2.68	0.0085
Treatment*Time	Khi	24	Terry	1	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Terry	3	80.2333	7.3690	102	10.89	<.0001
Treatment*Time	Khi	24	Terry	24	73.5667	7.3690	102	9.98	<.0001
Treatment*Time	Khi	24	Terry	48	22.6667	7.3690	102	3.08	0.0027
Treatment*Time	Khi	48	KhiNec	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KhiNec	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KhiNec	24	96.1000	7.3690	102	13.04	<.0001
Treatment*Time	Khi	48	KhiNec	48	90.7333	7.3690	102	12.31	<.0001
Treatment*Time	Khi	48	Klo	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Klo	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Klo	24	81.1000	7.3690	102	11.01	<.0001
Treatment*Time	Khi	48	Klo	48	29.6000	7.3690	102	4.02	0.0001
Treatment*Time	Khi	48	KloNec	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KloNec	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KloNec	24	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KloNec	48	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Knit	48	59.4667	7.3690	102	8.07	<.0001
Treatment*Time	Khi	48	Kwash	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Kwash	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Kwash	24	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Kwash	48	43.1333	7.3690	102	5.85	<.0001
Treatment*Time	Khi	48	KwashCon	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KwashCon	3	100.00	7.3690	102	13.57	<.0001

Treatment*Time	Khi	48	KwashCon	24	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	KwashCon	48	48.1667	7.3690	102	6.54	<.0001
Treatment*Time	Khi	48	NBindDE	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	NBindDE	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	NBindDE	24	12.6667	7.3690	102	1.72	0.0887
Treatment*Time	Khi	48	NBindDE	48	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Khi	48	Net	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Net	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Net	24	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Net	48	55.0000	7.3690	102	7.46	<.0001
Treatment*Time	Khi	48	TBindDE	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	TBindDE	3	88.9000	7.3690	102	12.06	<.0001
Treatment*Time	Khi	48	TBindDE	24	9.3000	7.3690	102	1.26	0.2098
Treatment*Time	Khi	48	TBindDE	48	2.13E-14	7.3690	102	0.00	1.0000
Treatment*Time	Khi	48	Terry	1	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Terry	3	100.00	7.3690	102	13.57	<.0001
Treatment*Time	Khi	48	Terry	24	93.3333	7.3690	102	12.67	<.0001
Treatment*Time	Khi	48	Terry	48	42.4333	7.3690	102	5.76	<.0001
Treatment*Time	KhiNec	1	KhiNec	3	-711E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	KhiNec	1	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	KhiNec	1	Klo	1	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	Klo	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	KhiNec	1	Klo	48	-70.4000	7.3690	102	-9.55	<.0001
Treatment*Time	KhiNec	1	KloNec	1	-126E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	KloNec	3	-16E-15	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	KloNec	24	-116E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	KloNec	48	0	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Knit	1	0	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Knit	3	0	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Knit	24	0	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	KhiNec	1	Kwash	1	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Kwash	3	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	KwashCon	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	KwashCon	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	KwashCon	24	5.33E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	KhiNec	1	NBindDE	1	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	NBindDE	3	0	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	KhiNec	1	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KhiNec	1	Net	1	-107E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	Net	3	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Net	24	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	KhiNec	1	TBindDE	1	2.13E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	1	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	KhiNec	1	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	KhiNec	1	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KhiNec	1	Terry	1	-284E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	Terry	3	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	1	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KhiNec	1	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	KhiNec	3	KhiNec	24	-3.9000	7.3690	102	-0.53	0.5978
Treatment*Time	KhiNec	3	KhiNec	48	-9.2667	7.3690	102	-1.26	0.2114
Treatment*Time	KhiNec	3	Klo	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	Klo	3	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	KhiNec	3	Klo	48	-70.4000	7.3690	102	-9.55	<.0001
Treatment*Time	KhiNec	3	KloNec	1	-554E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	KloNec	3	-89E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	KloNec	24	-453E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	KloNec	48	7.11E-15	7.3690	102	0.00	1.0000

Treatment*Time	KhiNec	3	Knit	1	7.11E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Knit	3	7.11E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Knit	24	7.11E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	KhiNec	3	Kwash	1	2.62E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Kwash	3	2.62E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Kwash	24	1.2E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	KhiNec	3	KwashCon	1	5.33E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	KwashCon	3	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	KwashCon	24	1.24E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	KhiNec	3	NBindDE	3	7.11E-15	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	KhiNec	3	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KhiNec	3	Net	1	-355E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	Net	3	1.07E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Net	24	1.07E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	KhiNec	3	TBindDE	1	2.84E-14	7.3690	102	0.00	1.0000
Treatment*Time	KhiNec	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	KhiNec	3	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	KhiNec	3	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KhiNec	3	Terry	1	-213E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	Terry	3	-711E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KhiNec	3	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KhiNec	3	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	KhiNec	24	KhiNec	48	-5.3667	7.3690	102	-0.73	0.4681
Treatment*Time	KhiNec	24	Klo	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Klo	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Klo	24	-15.0000	7.3690	102	-2.04	0.0444
Treatment*Time	KhiNec	24	Klo	48	-66.5000	7.3690	102	-9.02	<.0001
Treatment*Time	KhiNec	24	KloNec	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	KloNec	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	KloNec	24	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	KloNec	48	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Knit	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Knit	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Knit	24	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Knit	48	-36.6333	7.3690	102	-4.97	<.0001
Treatment*Time	KhiNec	24	Kwash	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Kwash	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Kwash	24	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Kwash	48	-52.9667	7.3690	102	-7.19	<.0001
Treatment*Time	KhiNec	24	KwashCon	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	KwashCon	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	KwashCon	24	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	KwashCon	48	-47.9333	7.3690	102	-6.50	<.0001
Treatment*Time	KhiNec	24	NBindDE	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	NBindDE	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	NBindDE	24	-83.4333	7.3690	102	-11.32	<.0001
Treatment*Time	KhiNec	24	NBindDE	48	-96.1000	7.3690	102	-13.04	<.0001
Treatment*Time	KhiNec	24	Net	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Net	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Net	24	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	TBindDE	24	-86.8000	7.3690	102	-11.78	<.0001
Treatment*Time	KhiNec	24	TBindDE	48	-96.1000	7.3690	102	-13.04	<.0001
Treatment*Time	KhiNec	24	Terry	1	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Terry	3	3.9000	7.3690	102	0.53	0.5978
Treatment*Time	KhiNec	24	Terry	24	-2.7667	7.3690	102	-0.38	0.7081
Treatment*Time	KhiNec	24	Terry	48	-53.6667	7.3690	102	-7.28	<.0001
Treatment*Time	KhiNec	48	Klo	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Klo	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Klo	24	-9.6333	7.3690	102	-1.31	0.1941
Treatment*Time	KhiNec	48	Klo	48	-61.1333	7.3690	102	-8.30	<.0001

Treatment*Time	KhiNec	48	KloNec	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	KloNec	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	KloNec	24	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	KloNec	48	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Knit	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Knit	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Knit	24	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Knit	48	-31.2667	7.3690	102	-4.24	<.0001
Treatment*Time	KhiNec	48	Kwash	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Kwash	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Kwash	24	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Kwash	48	-47.6000	7.3690	102	-6.46	<.0001
Treatment*Time	KhiNec	48	KwashCon	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	KwashCon	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	KwashCon	24	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	KwashCon	48	-42.5667	7.3690	102	-5.78	<.0001
Treatment*Time	KhiNec	48	NBindDE	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	NBindDE	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	NBindDE	24	-78.0667	7.3690	102	-10.59	<.0001
Treatment*Time	KhiNec	48	NBindDE	48	-90.7333	7.3690	102	-12.31	<.0001
Treatment*Time	KhiNec	48	Net	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Net	3	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Net	24	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	Net	48	-35.7333	7.3690	102	-4.85	<.0001
Treatment*Time	KhiNec	48	TBindDE	1	9.2667	7.3690	102	1.26	0.2114
Treatment*Time	KhiNec	48	TBindDE	3	-1.8333	7.3690	102	-0.25	0.8040
Treatment*Time	KhiNec	48	TBindDE	24	-81.4333	7.3690	102	-11.05	<.0001
Treatment*Time	KhiNec	48	TBindDE	48	-90.7333	7.3690	102	-12.31	<.0001
Treatment*Time	KhiNec	48	Terry	48	-48.3000	7.3690	102	-6.55	<.0001
Treatment*Time	Klo	1	KloNec	1	-376E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	1	Knit	1	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	Kwash	1	2.8E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	KwashCon	1	7.11E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	NBindDE	1	-533E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	1	NBindDE	3	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Klo	1	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Klo	1	Net	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	1	Net	3	1.24E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	Net	24	1.24E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Klo	1	TBindDE	1	3.02E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	1	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Klo	1	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Klo	1	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Klo	1	Terry	1	-195E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	1	Terry	3	-533E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	1	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Klo	1	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Klo	3	Klo	24	-18.9000	7.3690	102	-2.56	0.0118
Treatment*Time	Klo	3	Klo	48	-70.4000	7.3690	102	-9.55	<.0001
Treatment*Time	Klo	3	KloNec	1	-376E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	3	KloNec	3	-712E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	3	KloNec	24	-275E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	3	KloNec	48	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Knit	1	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Knit	3	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Kwash	3	2.8E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Kwash	24	1.38E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	Klo	3	KwashCon	1	7.11E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	KwashCon	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	KwashCon	24	1.42E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	Klo	3	NBindDE	1	-533E-17	7.3690	102	-0.00	1.0000

Treatment*Time	Klo	3	NBindDE	3	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Klo	3	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Klo	3	Net	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	3	Net	3	1.24E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Net	24	1.24E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	Klo	3	TBindDE	1	3.02E-14	7.3690	102	0.00	1.0000
Treatment*Time	Klo	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Klo	3	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Klo	3	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	Klo	3	Terry	1	-195E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	3	Terry	3	-533E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Klo	3	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Klo	3	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Klo	24	Klo	48	-51.5000	7.3690	102	-6.99	<.0001
Treatment*Time	Klo	24	KloNec	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	KloNec	3	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	KloNec	24	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	KloNec	48	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Knit	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Knit	3	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Knit	24	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Knit	48	-21.6333	7.3690	102	-2.94	0.0041
Treatment*Time	Klo	24	Kwash	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Kwash	3	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Kwash	24	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Kwash	48	-37.9667	7.3690	102	-5.15	<.0001
Treatment*Time	Klo	24	KwashCon	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	KwashCon	3	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	KwashCon	24	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	KwashCon	48	-32.9333	7.3690	102	-4.47	<.0001
Treatment*Time	Klo	24	NBindDE	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	NBindDE	24	-68.4333	7.3690	102	-9.29	<.0001
Treatment*Time	Klo	24	NBindDE	48	-81.1000	7.3690	102	-11.01	<.0001
Treatment*Time	Klo	24	Net	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Net	3	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Net	24	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Net	48	-26.1000	7.3690	102	-3.54	0.0006
Treatment*Time	Klo	24	TBindDE	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	TBindDE	3	7.8000	7.3690	102	1.06	0.2923
Treatment*Time	Klo	24	TBindDE	24	-71.8000	7.3690	102	-9.74	<.0001
Treatment*Time	Klo	24	TBindDE	48	-81.1000	7.3690	102	-11.01	<.0001
Treatment*Time	Klo	24	Terry	1	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Terry	3	18.9000	7.3690	102	2.56	0.0118
Treatment*Time	Klo	24	Terry	24	12.2333	7.3690	102	1.66	0.1000
Treatment*Time	Klo	24	Terry	48	-38.6667	7.3690	102	-5.25	<.0001
Treatment*Time	Klo	48	KloNec	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	KloNec	3	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	KloNec	24	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	KloNec	48	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Knit	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Knit	3	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Knit	24	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Knit	48	29.8667	7.3690	102	4.05	<.0001
Treatment*Time	Klo	48	Kwash	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Kwash	3	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Kwash	24	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Kwash	48	13.5333	7.3690	102	1.84	0.0692
Treatment*Time	Klo	48	KwashCon	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	KwashCon	3	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	KwashCon	24	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	KwashCon	48	18.5667	7.3690	102	2.52	0.0133
Treatment*Time	Klo	48	NBindDE	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	NBindDE	3	70.4000	7.3690	102	9.55	<.0001

Treatment*Time	Klo	48	NBindDE	24	-16.9333	7.3690	102	-2.30	0.0236
Treatment*Time	Klo	48	NBindDE	48	-29.6000	7.3690	102	-4.02	0.0001
Treatment*Time	Klo	48	Net	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Net	3	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Net	24	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Net	48	25.4000	7.3690	102	3.45	0.0008
Treatment*Time	Klo	48	TBindDE	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	TBindDE	3	59.3000	7.3690	102	8.05	<.0001
Treatment*Time	Klo	48	TBindDE	24	-20.3000	7.3690	102	-2.75	0.0070
Treatment*Time	Klo	48	TBindDE	48	-29.6000	7.3690	102	-4.02	0.0001
Treatment*Time	Klo	48	Terry	1	70.4000	7.3690	102	9.55	<.0001
Treatment*Time	Klo	48	Terry	48	12.8333	7.3690	102	1.74	0.0846
Treatment*Time	KloNec	1	KloNec	3	-336E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KloNec	1	KloNec	24	1.01E-15	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	KloNec	48	1.26E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Knit	1	1.26E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Knit	3	1.26E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Knit	24	1.26E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	KloNec	1	Kwash	1	3.17E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Kwash	3	3.17E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Kwash	24	1.75E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001
Treatment*Time	KloNec	1	KwashCon	1	1.09E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	KwashCon	3	3.76E-15	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	KwashCon	24	1.8E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	KloNec	1	NBindDE	1	-157E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KloNec	1	NBindDE	3	1.26E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	KloNec	1	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KloNec	1	Net	1	1.98E-15	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Net	3	1.62E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Net	24	1.62E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	KloNec	1	TBindDE	1	3.4E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	1	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	KloNec	1	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	KloNec	1	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KloNec	1	Terry	1	-158E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KloNec	1	Terry	3	-157E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KloNec	1	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KloNec	1	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	KloNec	3	KloNec	24	4.37E-15	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	KloNec	48	1.6E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	Knit	1	1.6E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	Knit	3	1.6E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	Knit	24	1.6E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	KloNec	3	Kwash	1	3.51E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	Kwash	3	3.51E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	KwashCon	3	7.12E-15	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	NBindDE	3	1.6E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	Net	3	1.96E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	KloNec	3	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KloNec	24	Knit	24	1.16E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	24	Kwash	24	1.65E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	24	KwashCon	24	1.7E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	24	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	KloNec	24	Net	24	1.52E-14	7.3690	102	0.00	1.0000
Treatment*Time	KloNec	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	KloNec	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KloNec	48	Knit	48	-40.5333	7.3690	102	-5.50	<.0001
Treatment*Time	KloNec	48	Kwash	48	-56.8667	7.3690	102	-7.72	<.0001

Treatment*Time	KloNec	48	KwashCon	48	-51.8333	7.3690	102	-7.03	<.0001
Treatment*Time	KloNec	48	NBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KloNec	48	Net	48	-45.0000	7.3690	102	-6.11	<.0001
Treatment*Time	KloNec	48	TBindDE	48	-100.00	7.3690	102	-13.57	<.0001
Treatment*Time	KloNec	48	Terry	48	-57.5667	7.3690	102	-7.81	<.0001
Treatment*Time	Knit	1	Kwash	1	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	Knit	1	KwashCon	1	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Knit	1	NBindDE	1	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Knit	1	Net	1	-107E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Knit	1	TBindDE	1	2.13E-14	7.3690	102	0.00	1.0000
Treatment*Time	Knit	1	Terry	1	-284E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Knit	3	Kwash	3	1.91E-14	7.3690	102	0.00	1.0000
Treatment*Time	Knit	3	KwashCon	3	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Knit	3	NBindDE	3	0	7.3690	102	0.00	1.0000
Treatment*Time	Knit	3	Net	3	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	Knit	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Knit	3	Terry	3	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Knit	24	Kwash	24	4.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	Knit	24	KwashCon	24	5.33E-15	7.3690	102	0.00	1.0000
Treatment*Time	Knit	24	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Knit	24	Net	24	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	Knit	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Knit	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Knit	48	Kwash	48	-16.3333	7.3690	102	-2.22	0.0289
Treatment*Time	Knit	48	KwashCon	48	-11.3000	7.3690	102	-1.53	0.1283
Treatment*Time	Knit	48	NBindDE	48	-59.4667	7.3690	102	-8.07	<.0001
Treatment*Time	Knit	48	Net	48	-4.4667	7.3690	102	-0.61	0.5458
Treatment*Time	Knit	48	TBindDE	48	-59.4667	7.3690	102	-8.07	<.0001
Treatment*Time	Knit	48	Terry	48	-17.0333	7.3690	102	-2.31	0.0228
Treatment*Time	Kwash	1	KwashCon	1	-209E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	1	NBindDE	1	-333E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	1	Net	1	-298E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	1	TBindDE	1	2.22E-15	7.3690	102	0.00	1.0000
Treatment*Time	Kwash	1	Terry	1	-475E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	3	KwashCon	3	-28E-15	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	3	NBindDE	3	-191E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	3	Net	3	-155E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Kwash	3	Terry	3	-333E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	24	KwashCon	24	4.44E-16	7.3690	102	0.00	1.0000
Treatment*Time	Kwash	24	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	Kwash	24	Net	24	-133E-17	7.3690	102	-0.00	1.0000
Treatment*Time	Kwash	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Kwash	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Kwash	48	KwashCon	48	5.0333	7.3690	102	0.68	0.4961
Treatment*Time	Kwash	48	NBindDE	48	-43.1333	7.3690	102	-5.85	<.0001
Treatment*Time	Kwash	48	Net	48	11.8667	7.3690	102	1.61	0.1104
Treatment*Time	Kwash	48	TBindDE	48	-43.1333	7.3690	102	-5.85	<.0001
Treatment*Time	Kwash	48	Terry	48	-0.7000	7.3690	102	-0.09	0.9245
Treatment*Time	KwashCon	1	NBindDE	1	-124E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KwashCon	1	Net	1	-888E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KwashCon	1	TBindDE	1	2.31E-14	7.3690	102	0.00	1.0000
Treatment*Time	KwashCon	1	Terry	1	-266E-16	7.3690	102	-0.00	1.0000
Treatment*Time	KwashCon	3	NBindDE	3	8.88E-15	7.3690	102	0.00	1.0000
Treatment*Time	KwashCon	3	Net	3	1.24E-14	7.3690	102	0.00	1.0000
Treatment*Time	KwashCon	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	KwashCon	3	Terry	3	-533E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KwashCon	24	NBindDE	24	-87.3333	7.3690	102	-11.85	<.0001
Treatment*Time	KwashCon	24	Net	24	-178E-17	7.3690	102	-0.00	1.0000
Treatment*Time	KwashCon	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	KwashCon	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	KwashCon	48	NBindDE	48	-48.1667	7.3690	102	-6.54	<.0001
Treatment*Time	KwashCon	48	Net	48	6.8333	7.3690	102	0.93	0.3560
Treatment*Time	KwashCon	48	TBindDE	48	-48.1667	7.3690	102	-6.54	<.0001
Treatment*Time	KwashCon	48	Terry	48	-5.7333	7.3690	102	-0.78	0.4383

Treatment*Time	NBindDE	1	Net	1	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	NBindDE	1	TBindDE	1	3.55E-14	7.3690	102	0.00	1.0000
Treatment*Time	NBindDE	1	Terry	1	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	NBindDE	3	Net	3	3.55E-15	7.3690	102	0.00	1.0000
Treatment*Time	NBindDE	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	NBindDE	3	Terry	3	-142E-16	7.3690	102	-0.00	1.0000
Treatment*Time	NBindDE	24	Net	24	87.3333	7.3690	102	11.85	<.0001
Treatment*Time	NBindDE	24	TBindDE	24	-3.3667	7.3690	102	-0.46	0.6487
Treatment*Time	NBindDE	24	Terry	24	80.6667	7.3690	102	10.95	<.0001
Treatment*Time	NBindDE	48	Net	48	55.0000	7.3690	102	7.46	<.0001
Treatment*Time	NBindDE	48	TBindDE	48	3.55E-14	7.3690	102	0.00	1.0000
Treatment*Time	NBindDE	48	Terry	48	42.4333	7.3690	102	5.76	<.0001
Treatment*Time	Net	1	TBindDE	1	3.2E-14	7.3690	102	0.00	1.0000
Treatment*Time	Net	1	Terry	1	-178E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Net	3	TBindDE	3	-11.1000	7.3690	102	-1.51	0.1351
Treatment*Time	Net	3	Terry	3	-178E-16	7.3690	102	-0.00	1.0000
Treatment*Time	Net	24	TBindDE	24	-90.7000	7.3690	102	-12.31	<.0001
Treatment*Time	Net	24	Terry	24	-6.6667	7.3690	102	-0.90	0.3678
Treatment*Time	Net	48	TBindDE	48	-55.0000	7.3690	102	-7.46	<.0001
Treatment*Time	Net	48	Terry	48	-12.5667	7.3690	102	-1.71	0.0912
Treatment*Time	TBindDE	1	Terry	1	-497E-16	7.3690	102	-0.00	1.0000
Treatment*Time	TBindDE	3	Terry	3	11.1000	7.3690	102	1.51	0.1351
Treatment*Time	TBindDE	24	Terry	24	84.0333	7.3690	102	11.40	<.0001
Treatment*Time	TBindDE	48	Terry	48	42.4333	7.3690	102	5.76	<.0001

Second Data Set Output:

Obs	Treatment	Rep	Time	Mortality
1	fsf148kn	1	1	0.0
2	fsf148kn	1	3	0.0
3	fsf148kn	1	24	85.7
4	fsf148kn	1	48	100.0
5	fsf148kn	2	1	0.0
6	fsf148kn	2	3	0.0
7	fsf148kn	2	24	75.0
8	fsf148kn	2	48	100.0
9	fsf148kn	3	1	0.0
10	fsf148kn	3	3	0.0
11	fsf148kn	3	24	80.0
12	fsf148kn	3	48	100.0
13	Saf148kn	1	1	0.0
14	Saf148kn	1	3	0.0
15	Saf148kn	1	24	22.2
16	Saf148kn	1	48	95.5
17	Saf148kn	2	1	0.0
18	Saf148kn	2	3	0.0
19	Saf148kn	2	24	26.3
20	Saf148kn	2	48	89.5
21	Consaf14	1	1	0.0
22	Consaf14	1	3	0.0
23	Consaf14	1	24	0.0
24	Consaf14	1	48	18.2
25	Consaf14	2	1	0.0
26	Consaf14	2	3	0.0
27	Consaf14	2	24	0.0
28	Consaf14	2	48	16.7
29	Consaf14	3	1	0.0
30	Consaf14	3	3	0.0
31	Consaf14	3	24	0.0
32	Consaf14	3	48	86.7

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	3	Consaf14 Saf148kn fsf148kn
Time	4	1 3 24 48
Rep	3	1 2 3

Number of Observations Read 32
Number of Observations Used 32

The GLM Procedure

Dependent Variable: Mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	47452.84042	4313.89458	26.29	<.0001
Error	20	3281.89833	164.09492		
Corrected Total	31	50734.73875			

R-Square	Coeff Var	Root MSE	Mortality Mean
0.935313	45.76005	12.80995	27.99375

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	2	7333.73417	3666.86708	22.35	<.0001
Time	3	31373.22375	10457.74125	63.73	<.0001
Treatment*Time	6	8745.88250	1457.64708	8.88	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	7333.73417	3666.86708	22.35	<.0001
Time	3	31493.07970	10497.69323	63.97	<.0001
Treatment*Time	6	8745.88250	1457.64708	8.88	<.0001

The GLM Procedure

t Tests (LSD) for Mortality

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	164.0949
Critical Value of t	2.08596
Least Significant Difference	11.783
Harmonic Mean of Cell Sizes	10.28571

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	45.058	12	fsf148kn
B	29.188	8	Saf148kn
C	10.133	12	Consaf14

The GLM Procedure

t Tests (LSD) for Mortality

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	164.0949
Critical Value of t	2.08596
Least Significant Difference	13.361

Means with the same letter are not significantly different.

t Grouping	Mean	N	Time
A	75.825	8	48
B	36.150	8	24
C	0.000	8	1
C			
C	0.000	8	3

The GLM Procedure

Level of Treatment	Level of Time	N	Mortality Mean	Mortality Std Dev
Consaf14	1	3	0.000000	0.0000000
Consaf14	3	3	0.000000	0.0000000
Consaf14	24	3	0.000000	0.0000000
Consaf14	48	3	40.533333	39.9885400
Saf148kn	1	2	0.000000	0.0000000
Saf148kn	3	2	0.000000	0.0000000
Saf148kn	24	2	24.250000	2.8991378
Saf148kn	48	2	92.500000	4.2426407
fsf148kn	1	3	0.000000	0.0000000
fsf148kn	3	3	0.000000	0.0000000
fsf148kn	24	3	80.233333	5.3538148
fsf148kn	48	3	100.000000	0.0000000

The Mixed Procedure

Model Information

Data Set	WORK.FOSSIL
Dependent Variable	Mortality
Covariance Structure	Compound Symmetry
Subject Effect	Rep
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information

Class	Levels	Values
Treatment	3	Consaf14 Saf148kn fsf148kn
Time	4	1 3 24 48
Rep	3	1 2 3

Dimensions

Covariance Parameters	2
Columns in X	16
Columns in Z	0
Subjects	3
Max Obs Per Subject	12

Number of Observations

Number of Observations Read	32
Number of Observations Used	32
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	170.32792877	
1	2	170.32786844	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Subject	Estimate
CS	Rep	-0.1836
Residual		164.28

Fit Statistics

-2 Res Log Likelihood	170.3
AIC (smaller is better)	174.3
AICC (smaller is better)	175.0
BIC (smaller is better)	172.5

Null Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
1	0.00	0.9938

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	18.5	22.25	<.0001
Treatment*Time	9	16.5	27.13	<.0001

Differences of Least Squares Means

Effect	Treatment	Time	_Treatment	_Time	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	Consaf14		Saf148kn		-19.0283	6.5848	19.8	-2.89	0.0091
Treatment	Consaf14		fsf148kn		-34.9250	5.2326	16.5	-6.67	<.0001
Treatment	Saf148kn		fsf148kn		-15.8967	6.5848	19.8	-2.41	0.0256
Treatment*Time	Consaf14	1	Saf148kn	1	0.02590	12.0844	17.7	0.00	0.9983
Treatment*Time	Consaf14	1	fsf148kn	1	4.97E-14	10.4651	16.5	0.00	1.0000
Treatment*Time	Consaf14	3	Saf148kn	3	0.02590	12.0844	17.7	0.00	0.9983
Treatment*Time	Consaf14	3	fsf148kn	3	3.55E-14	10.4651	16.5	0.00	1.0000
Treatment*Time	Consaf14	24	Saf148kn	24	-24.2241	12.0844	17.7	-2.00	0.0606
Treatment*Time	Consaf14	24	fsf148kn	24	-80.2333	10.4651	16.5	-7.67	<.0001
Treatment*Time	Consaf14	48	Saf148kn	48	-51.9408	12.0844	17.7	-4.30	0.0005
Treatment*Time	Consaf14	48	fsf148kn	48	-59.4667	10.4651	16.5	-5.68	<.0001
Treatment*Time	Saf148kn	1	fsf148kn	1	-0.02590	12.0844	17.7	-0.00	0.9983
Treatment*Time	Saf148kn	3	fsf148kn	3	-0.02590	12.0844	17.7	-0.00	0.9983
Treatment*Time	Saf148kn	24	fsf148kn	24	-56.0092	12.0844	17.7	-4.63	0.0002
Treatment*Time	Saf148kn	48	fsf148kn	48	-7.5259	12.0844	17.7	-0.62	0.5414

Appendix V

Data from Undergraduate's experimentation:

Experiment 1 Antennas

(1/23/07) Hand Test

Control (9 mosquitoes)

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Back	13	19	23	21	15	18.2
Palm/Front	10	9	17	11	14	12.2
Total	23	28	40	32	29	30.4

Test group put in fridge at 7C/38F then taken outside to cut off antennae
Unsuccessful, therefore aspirated into ice bath, used vacuum needle to hold while cut with dissection scissors, then defrosted in a test tube with a moist paper towel. Waited 50 minutes, if did not revive, considered dead. 2 males discovered – unsure of whether from original test or xtras.

Test Group

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Back	0	0	0	0	0	0
Palm/Front	0	0	0	0	0	0
Total	0	0	0	0	0	0

Continued (1/25/07) Hand Test

Previously mutilated mosquitoes were tested again (5 survived)

Test Group (Again)

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Back	6	1	1	0	0	1.6
Palm/Front	0	0	0	0	0	0
Total	6	1	1	0	0	1.6

(1/25/07) Bite Test

Bite test on remaining mosquitoes

Tester	# Mosquitoes	# Histamine Rxns	# Blood Meals
Linda	2	7	2
Ben W	1	0	0

Experiment 2 Antennas

(1/25/07) Hand Test

- Collect host seeking mosquitoes
- Aspirate and separate into 3 test tubes (4 mosquitoes in each + one tube with xtas)
- Put all groups in ice bath for 22 minutes
- During ice bath
    - Group 2 – antennas were partially removed
    - Group 3 – antennas were almost entirely removed
    - One leg was lost – mosquito was replaced by a spare from the xtras tube
- All groups defrosted for 20 minutes
- Hand test performed

Group 1: Control

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Total	16	36	11	7	4	

One of the mosquitoes died (left 3 to test)

Group 2: Partially Removed

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Total	0	0	0	0	0	0

Group 3: Mostly Removed

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
Total	1	0	0	0	0	0.2

(1/36/07) Bite Test

Group	Tester	# Mosquitoes	# Histamine Rxns	# Blood Meals
1	Ben W	3	3	1
2	Bonnie	4	2	2 *
3	Ben Rice	4	2	1

*1 flew away while removing from skin