

Polyolefin copolymers as controlled release devices for  
insecticides and repellents

by

**Mthokozisi Mayibongwe Sibanda**

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## Declaration

I, Mthokozisi Sibanda, the undersigned, declare that the thesis that I hereby submit for the degree PhD in Chemical Engineering at the University of Pretoria is my own work, and has not previously been submitted by me for degree purposes or examination at this or any other University.

Pretoria, July 2015

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Mthokozisi Sibanda

# **POLYOLEFIN COPOLYMERS AS CONTROLLED RELEASE DEVICES FOR INSECTICIDES AND REPELLENTS**

Student: Mthokozisi M. Sibanda

Supervisor: Prof Walter W. Focke

Degree: PhD (Chemical Engineering)

Department: Chemical Engineering

## **Synopsis**

Malaria is a parasitic disease confined mostly to tropical areas and transmitted by female anopheles mosquitoes. It results in approximately 250 million clinical cases and nearly a million deaths annually. Malaria is particularly prevalent in sub-Saharan Africa where it affects mostly pregnant women and children less than five years of age. The World Health Organisation (WHO) mainly recommends the use of long lasting insecticide treated nets (LLIN) and indoor residual spray (IRS) to control mosquitoes. These interventions have been very effective for the most part however they leave gaps that threaten the goal of eliminating malaria: LLIN are only effective when a person is sleeping under the net whilst a mosquito can still bite and infect people outdoors. LLIN also relies on pyrethroid insecticides and mosquitoes are steadily becoming resistant to this class of insecticide. IRS is only effective when using DDT, a persistent organic pollutant whose use in public health is very contentious. Unfortunately insecticide alternatives to DDT, e.g. pyrethroids, carbamates and organophosphates fail prematurely due to alkaline hydrolysis in the environment. Using these alternatives will require repeated applications throughout the malaria transmission season making IRS unaffordable to relatively poor African countries. Lastly, both IRS and LLIN target the malaria vector indoors whilst infection can actually happen outdoors.

The work presented here sought to develop two cost effective and innovative ideas that may bridge the gap left by implementing current recommended vector control interventions. These ideas relied on the use of polymer matrices to stabilise and slowly release active agents used in malaria vector control thereby increasing their residual effectiveness.

The first idea pursued the development of an insecticide treated wall lining (ITWL). It was envisaged that the lining may substitute the use of IRS and also complement the use of LLIN. This lining was produced using simple extrusion of 10wt.% and 18wt.% (alphacypermethrin and deltamethrin loading respectively) polyethylene masterbatches with a 1:1 polymer blend of high density polyethylene (HDPE) and low density polyethylene (LDPE) to produce a Netlon® mesh. This mesh contained alphacypermethrin and deltamethrin in concentrations ranging from 0.29wt.% to 0.85wt.%. The mesh linings were evaluated for acceptability, durability and perceived effectiveness in field trials carried out in the Vhembe district of Limpopo province, north of South Africa. In these trials it was established that majority of the field trial participants perceived the Netlon® lining to be effective and user friendly. The linings were stable to environmental elements that persisted inside the dwellings where they were installed however there was some rodent damage observed. Standard bioassay tube tests indicated that these nets remained effective for at least 24 months in the field and 36 months after manufacture.

The second idea sought to address the need for protection against mosquito bites outdoors. It entailed the use of polymer matrices to trap large amounts of a repellent and to release it slowly over an extended period of time. This could increase the residual effectiveness of volatile repellents. Possible future product concepts based on this idea include long-life mosquito repellent bracelets and low cost slip slops.

The repellent N,N-Diethyl-meta-toluamide (DEET) was incorporated into the polymer via the technique of spinodal decomposition. It was found possible to trap up to 50wt.% in poly(ethylene-co-vinyl acetate) (EVA). Thermogravimetric analysis and oven mass loss studies showed that the filled EVA polymer matrix reduced the rate of release of the repellent. Laboratory repellency tests suggested that these bracelets may be effective in repelling mosquito bites for at least one month. This suggests that, in theory at least, low cost bracelets and slip slops can be designed that would last that long. Future products based on this idea could help reduce infections due to ankle biting by *An. gambiae* mosquitoes which are responsible for a significant number of malaria infections in Africa.

**Keywords: malaria, mosquito, controlled release, pyrethroids, repellent, DEET, polymer**

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*If I have seen further it is by standing on the shoulders of giants...*

## Preface

This thesis examines the weaknesses in current malaria vector control interventions and presents work done to formulate innovative interventions to address these weaknesses. The first part of this thesis examines all malaria vector control interventions ever implemented to control mosquitoes. This review critically compares these methods in respect to controlling mosquitoes in present day. This thesis goes on to review literature that reports on the mosquito's ability to adapt to current vector control interventions and avoid being killed.

The second part of this thesis describes the development of an Insecticide Treated Wall Lining (ITWL) or Netlon® produced by a simple process of compounding and extruding insecticide impregnated polyethylene directly into a net format. The advantages of producing the net in this manner is that the method is a departure from traditional fabric weaving processes that add to the cost of production. Use of alternative insecticides and polymer materials in the manufacture of ITWL were also examined in this section. These alternatives were not successful however insights developed will inform on how to improve their performance in future. This thesis further describes field trials of Netlon® undertaken in the Vhembe district of Limpopo and the results thereof.

The third part of this thesis describes the work done to develop mosquito repellent bracelets. It is envisaged that these bracelets will protect from ankle biting *An. gambiae* mosquitos which are responsible for a significant proportion of infections in Africa. These bracelets were produced by using the technique of spinodal decomposition to increase the amount of repellent loading in the polymer matrix by forming a bi-continuous phase between polymer and repellent (micro-porous polymer). To reduce the rate of loss of the repellent in the polymer matrix a polyamide coating was used to act as barrier to slow down the migration of repellent out of the bracelet polymer matrix. The loss of active from the polymer was studied by exposing prototype bracelets to accelerated temperatures in a convection oven. The change in mass of the bracelets was observed over a period of time. The mass loss kinetics of these micro-porous bracelets was compared to those of solid polymer bracelets produced and tested for efficacy in earlier studies.

The penultimate section gives an overall discussion on the two innovative techniques developed and their impact on current recommended vector control interventions. The last

section presents appendices of complementary data that could not to be included in the main text.

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## Nomenclature

Symbol	Property	Unit
$\mathfrak{RH}$	Relative humidity	[-]
$\Phi$	Dimensionless residual mass	[-]
$D$	Diffusion coefficient	[m <sup>2</sup> .s <sup>-1</sup> ]
$k_c$	Mass transfer coefficient	[m.s <sup>-1</sup> ]
$D$	Characteristic diameter	[m]
$P_A$	Vapour pressure of substance A	[kg.m <sup>-1</sup> .s <sup>-2</sup> ]
$P_{ow}$	Octanol water partition constant	[-]
$T_{UC}$	Upper critical solution temperature	[°C]
$\Delta G_{mix}$	Gibbs free energy of mixing	[J.mol <sup>-1</sup> ]
$\Delta H_{mix}$	Enthalpy of mixing	[J.mol <sup>-1</sup> ]
$\Delta S_{mix}$	Entropy of mixing	[J.mol <sup>-1</sup> .K <sup>-1</sup> ]
$\varphi_p$	Polymer volume fraction	[-]
$C$	Initial insecticide loading at time $t$	[wt.%]
$C_i$	Initial insecticide loading	[wt.%]
$C_{eq}$	Long term insecticide loading at equilibrium	[wt.%]
$w(t)$	Repellent loading at time $t$	[wt.%]
$w_i$	Initial repellent loading	[wt.%]
$w_\infty$	Long term equilibrium loading of repellent	[wt.%]
$\tau$	first order time constant	[days]

## List of acronyms and abbreviations

<b>CFM</b>	confocal fluorescence microscopy
<b>DDT</b>	dichlorodiphenyltrichloroethane
<b>DEET</b>	N,N-Diethyl-meta-toluamide
<b>DOP</b>	dioctylphthalate
<b>DSC</b>	differential scanning calorimetry
<b>EVA</b>	poly(ethylene-co-vinyl acetate)
<b>GC</b>	gas chromatography
<b>GMAP</b>	global malaria action plan
<b>HDPE</b>	high density polyethylene
<b>HPLC</b>	high pressure liquid chromatography
<b>IGR</b>	insect growth regulators
<b>IR</b>	infra-red spectroscopy
<b>IRS</b>	indoor residual spray
<b>ITN</b>	insecticide treated nets
<b>ITWL</b>	insecticide treated wall lining
<b>IUPAC</b>	International Union of Pure and Applied Chemistry
<b>KD</b>	knockdown
<b>LDPE</b>	low density polyethylene
<b>LLIN</b>	long lasting insecticide treated nets
<b>MB</b>	masterbatch
<b>MP</b>	melting point
<b>MS</b>	mass spectroscopy
<b>NMR</b>	nuclear magnetic resonance spectroscopy
<b>PCL</b>	polycaprolactone
<b>SD</b>	standard deviation
<b>SEM</b>	scanning electron microscope/microscopy
<b>SIT</b>	sterile insect technique
<b>TEM</b>	transmission electron microscopy
<b>TGA</b>	thermo gravimetric analysis
<b>TLC</b>	thin layer chromatography
<b>UV/VIS</b>	ultra-violet/visible light spectroscopy
<b>WHO</b>	World Health Organization

**wt.%**

weight of component as a percentage of total weight

## 1. Introduction

Malaria is a parasitic disease confined mostly to tropical areas and transmitted by female anopheles mosquitoes. It results in approximately 250 million clinical cases and nearly a million deaths annually. Malaria is particularly prevalent in sub-Saharan Africa where it affects mostly pregnant women and children less than five years of age (WHO, 2014). On the African continent this debilitating disease poses a major economic burden. It is estimated that malaria cost the African economy more than \$12 billion annually in lost economic output (WHO, 2014). Consequently the World Health Organisation (WHO), African governments and donor agencies at large, have mobilised resources to fight the scourge of malaria. The stated ultimate goal is malaria elimination. Since mosquitoes are the vectors in malaria transmission, decreasing their population will lead to a reduction and eventually elimination in malaria morbidity and mortality. The WHO recommends an integrated approach that combines clinical control of the malaria parasite along with the use of insecticide-treated nets (ITN), Long Lasting Insecticide Treated Nets (LLIN) and the use of Indoor Residual Spray (IRS).

In many instances interventions such as IRS and ITN (and LLIN) proved effective in dealing with the anopheles mosquito vectors. In sub-Saharan Africa, entomological inoculation rates (number of bites by infectious mosquitoes per person per unit time) can reach levels of more than a thousand infective bites per person per year (Kelly-Hope and McKenzie, 2009). With such high levels of entomological inoculation rates, it is estimated that existing interventions can only reduce the annual inoculation rate by an order of magnitude (Control, 2011).

Resurgence of malaria has been recorded in several countries, underscoring the limitations of IRS and LLIN to effectively eliminate malaria (WHO, 2014). In areas where malaria transmission has been brought under control such as South Africa, residual transmission remains stubborn prompting continued vigilance in maintaining large scale vector control interventions and entomological surveillance activities. Subsequently the conclusion is that current WHO recommended interventions cannot suffice for achieving malaria elimination in Africa. There is therefore a need for effective additional vector control interventions that can deal a “knockout blow” to malaria vectors.

It is important that before an attempt to address some of the problems with current vector interventions to critically appraise all vector control interventions (physical, chemical and

biological) that have been used to control mosquitoes. These ideas have been grouped into vector elimination; prevention of mosquito bite and killing of mosquito after they have bitten. It is also intended to highlight why current vector control interventions recommended by the WHO are inadequate for the elimination of malaria in Africa.

## **2. A review of physical, chemical and biological malaria vector control interventions**

### **2.1 Vector elimination**

#### **2.1.1 Aerial spraying**

Aerial spraying is a process of spraying the minimum effective volume of an undiluted formulation of insecticide. Ultra low volume spraying is the favoured aerial spray method used worldwide for mosquito adulticide control. Compared to other methods it features a high effective insecticide payload, lower pumping costs, less time used on formulation, reduced handling of the insecticide, etc. It is used in areas where the mosquitoes are predominantly exophilic. It is very expensive to operate and the insecticides currently in use do not have a long residual period.

Mount *et al.* (1996) have reviewed the use of aerial spraying for mosquito control. Small scale tests conducted using small fixed wing single-engine aircraft equipped with high-volume spray systems modified to allow aerial spraying applications showed satisfactorily mortality rates of various mosquito species (89-100%) with the following dosages; 91-95% malathion, 0.224-226 L/ha; 50% emulsifiable concentrate (EC) malathion, 0.952 L/ha; 93% fenthion, 0.03 L/ha; permethrin 0.042-0.063 L/ha.

#### **2.1.2 Larviciding**

The mosquito life cycle has four stages of which three are spent in a still aquatic environment. Destroying the larvae chemically or biologically whilst it is still developing into a mosquito is a viable option for eliminating mosquito vector populations. The earliest chemical larvicide to be used was Paris green (cupric acetoarsenite) (Gladwell, 2002). It proved to be more effective than the traditionally used oil. It was successfully used to eradicate *anopheles gambiae* in Brazil before World War 2. Arsenic compounds are toxic chemicals and are now banned for use in public health. Temephos, an organophosphate has been used as a larvicide. It is much safer to use than arsenic but also kills mosquito predators (WHO, 1995).

Recently insect growth regulators (IGRs) have been synthesized for use as larvicides (Mulla, 1995). IGRs work by mimicking insect infantile growth hormones. They bind onto larvae hormone receptors preventing attainment of the next stage of development. A significant

advantage of IGRs is that they are target specific and have shown a good margin of safety on non-target organisms i.e. invertebrates, fish and birds. However they may be toxic to immature stages of aquatic insects.

Plant extract have also shown to be very good in controlling mosquito larvicidal activity. The use of plant based products compared to synthetic products have shown that plant based products are much more environmentally friendly and often do not have significant effect on other organisms other than the targeted organism. Many of these compounds are easily biodegraded and the target range of organisms is limited thus making plant-based product an attractive alternative to synthetically produced products. An extract made from the fresh green berries of *Solanum villosum* has shown to have significant larvicidal activity against the tested mosquito vector larvae (Chowdhury *et al.*, 2008). The extract of *S. villosum* showed a diverse composition of many functional compounds like saponins, terpenoids, phenolics, essential oils, etc. thus emphasizing the complex nature of plant extract could be used to target vector larvae and especially those that are showing signs of resistance against current synthetic product. Kalu *et al.* (2010) studied the larvicidal effect of garlic and it was shown that an ethanol extract of the bulb was very effective as a larvicidal product and it was concluded that the crude or isolated phytochemicals from the bulbs could be used directly in stagnant water bodies known to be breeding grounds of mosquitoes. Several species of indigenous plant species found in Nigeria such as *Adenia cissampelli* showed potential as a larvicidal treatment (Ajayi, 2008).

### **2.1.3 Biological control of mosquito ovum, larvae and adults**

Biocontrol is the use of natural enemies to manage mosquito populations and includes the direct introduction of parasites, pathogens and predators that target various life stages of the mosquito. Particularly, biological agents including bacteria, fungi, viruses, nematodes, copepods and fish have been employed to decrease the mosquito larvae populations (Walker *et al.*, 1996). Fish feeding on mosquito larvae such as *Gambus affinis* and *G. holbrooki* have been used for many generations in malaria-endemic regions, with the added advantage that the fish can be harvested for food at a later stage thus providing sustenance or income for users (Blaustein, 1992). The use of larvivorous fish is sustainable as mosquitoes cannot develop physical resistance against this approach. However, most mosquito fish are generalist predators. They may feed on mosquito larvae only if there is a significant shortage of

alternative prey. Nevertheless this technique is of value in more permanent habitats where a balanced ecosystem can be established that favours the fish feeding on mosquito larvae (Murdoch *et al.*, 1985).

Bio-larvicides have been extensively developed as a means of reducing dependence on chemical larvicides (Karunamoorthi, 2011). They are inexpensive to implement, safe to humans and non-target organisms and pose a potentially environmentally friendly option (Mittal, 2003). Pathogenic bacteria such as *Bacillus thuringiensis var. israelensis* (Bti) and *Bacillus sphaericus* (Bs) have been shown to be highly effective in controlling mosquito larvae at very low doses (Mittal, 2003). The bacterium has the ability to produce pro-toxins (parasporal crystals in the bacterial spore coat) with an insecticidal action, due to their ability to act as stomach poisons after being solubilised in the alkaline pH of the mosquito larval mid-gut (Roh *et al.*, 2007, Prabakaran and Hoti, 2008). The active toxin subsequently binds to insect-specific receptors on the surface of the plasma membrane of mid-gut epithelial cells, inserts into the membrane to create trans-membrane pores that cause cell swelling and lyses, and eventually death of the insect. Although safe to use, the large-scale application of Bti is still questionable, and its efficacy dependent on formulations best suited to the biology of the specific mosquito species targeted. Bs spores have been reported to be preferred to Bti spores as it can self-recycle in the environment without negative environmental impacts, although it showed some preference to mosquito hosts, and is more effective against *Culex pipiens* compared to *An. stephensi* (Becker *et al.*, 1995). Biotechnological adaptation of both Bti and Bs strains to produce recombinants that are at least 10-times as potent have been reported, based on synergistic action of toxins in these recombinants (Becker *et al.*, 1995). However, the possibility of resistance development against both Bti and Bs should not be excluded. Besides *Bacillus*-based larvicides, obligate endosymbiotic bacteria such as *Wolbachia pipiens* causes male-female incompatibility resulting in death of uninfected eggs fertilized by infected sperm (Walker *et al.*, 1996). *Wolbachia* infections additionally induce immunity in the mosquitoes, causing significant *P. berghei* density reductions in *Aedes aegypti* (Kambris *et al.*, 2010) as well as *P. falciparum* infections in *An. Gambiae* (Hughes *et al.*, 2011). Unfortunately, wild *Anopheles* mosquitoes are not readily infected by *Wolbachia*, necessitating the development of a stable *Wolbachia*-infected *Anopheles* line that could be released into wild populations (Walker and Moreira, 2011).



Some fungi have been used to control mosquito larvae and adults (Farenhorst *et al.*, 2010). Such entomopathogenic fungi (e.g. the aquatic *Coelomomyces* sp and soil fungi *Beauveria bassiana*) can kill *An. gambiae* through tarsal contact. Unlike other mosquitocidal biocontrol agents such as bacteria, microsporidia and viruses, the fungi can infect and kill without being ingested by germination of their spores on the insect cuticle, followed by penetration and growth in the mosquito hemolymph within 1-2 weeks, requiring very low concentrations of spores (Mnyone *et al.*, 2009). Spores can survive for months after being sprayed and are therefore considered applicable to domestic use. They have been shown to reduce malaria transmission potential by up 90% as soon as the mosquitoes become infected with the fungus after ingesting Plasmodia (Blanford *et al.*, 2005). Transgenic fungi that can produce antimalarial peptides in the mid gut of the mosquito have also been reported (Fang *et al.*, 2011). The use of fungi to augment chemical insecticides showed synergistic interaction with permethrin, supporting the notion of using fungi in IVM programmes (Farenhorst *et al.*, 2010).

As with many other vector control strategies, there simply is no single, one-size-fits-all application of bacterial or fungal larvicides, and situation specific variances should be taken into account to enable efficient application thereof. Elango *et al.* (2010) showed the potential of several non-polar plant extracts (hexane and chloroform) as an ovicidal as well as an oviposition deterrent. In the study *Aegle lineata*, *Andrographis paniculata* and *Tagetes erecta* showed to be the best ovicidal extracts with very low egg hatchability (31 – 45%) at concentrations as low as 125 ppm, with 100% morality achieved at 1000 ppm against *Anopheles subpictus*. As an oviposition deterrent *Aegle lineata*, *Cocculus hirsutus* and *Eclipta prostrate* hexane and chloroform extract showed very promising results with effective repellency at very low concentration (31.25 ppm) of 73.47%, 62.22% and 75.48% respectively. The study also emphasized that the plant material of all these plants was easily obtainable and affordable and thus the use of such biological prevention methods should be encouraged to local residence in rural communities in order to reduce the man – vector contact as well as vector borne disease in general.

#### **2.1.4 Environmental control**

The WHO defines environmental management as “the planning, organisation, carrying out and monitoring of activities for the modification and/or manipulation of environmental

factors or their interaction with man with a view to preventing or minimising vector propagation and reducing man-vector pathogen contact.” The basic rationale of environmental management is to eliminate all possible mosquito breeding sites.

Environmental management was used extensively before the advent of DDT which offered a single effective way of fighting malaria. Environmental management has been applied in four distinct epidemiological settings namely malaria in forest areas; rural malaria attributable to water resources development and management; rural malaria attributable to wetlands, rivers, coasts and non-agricultural man-made habitats; and urban and peri-urban malaria (Keiser *et al.*, 2005). With rising health concerns related to the use of DDT and the build-up of multiple resistance mechanisms to insecticides by mosquitoes, environmental management offers a cost effective, sustainable way of controlling mosquitoes. Environmental control can reduce the risk malaria transmission by up to 88% (Keiser *et al.*, 2005).

### **2.1.5 Sterile insect technique (SIT)**

There is renewed interest in sterile insect techniques (SIT) for the control of mosquitoes (Helinski *et al.*, 2009). This technique relies on the mass release of sterile male mosquitoes. Sterilisation may be done by exposing pupae stage or adult mosquitoes to high energy ionising radiation. Highly penetrative gamma rays produced by  $^{60}\text{Co}$  or  $^{137}\text{Cs}$  are commonly used. When biological material is irradiated, molecular bonds are broken and ions and free radical are formed. This causes damage to DNA which may lead to the formation of lethal mutations in the germ cells and damage to the somatic cells undergoing mitosis. In order to achieve maximum damage to germ cells and minimise damage to somatic cells, radiation should be done at or near to the completion of development i.e. late pupal and adult stages.

If a female mosquito mates with an infertile male, she becomes infertile for the rest of her lifespan. In order for this technique to be effective, a sustained release of indigenous sterile male mosquitoes among the target population is necessary. Continually releasing sterile males in large quantities to outnumber fertile native males (“overflooding”) and over a time period that is sufficient to cover several generations of the target population, will lead to a gradual decrease in the productive capacity of the mosquitoes. Eventually, so few fertile insects remain that fertile matings do not occur and the population is eliminated. SIT is initiated when the number of mosquitoes reaches a seasonal minimum e.g. at the end of

winter. SIT has been applied with success to eliminate screw worms in the USA (Backri, 2012).

### **2.1.6 Biological control of *plasmodium* parasite in mosquitoes**

The successful transmission of malaria relies on the development of the *Plasmodium* parasite in the lumen of the mid-gut of the mosquito host. During this development, the *Plasmodium* parasite suffers huge losses due to the hostile environment of the mid-gut (Vaughan *et al.*, 1994). However, survival of the few ensures onward transmission of the parasite. It has been shown that mid-gut microbiota have the ability to inhibit growth of the different parasite growth stages (ookinetes, oocysts and sporozites) (Pumpuni *et al.*, 1996). Gram negative (G-) bacterium isolated from wild *An. arabiensis* mosquitoes in southern Zambia has been shown to retard anopheles *Plasmodium* growth in *An. gambiae* and *An. stephensi* mosquitoes whilst gram positive bacteria isolated does not. The level of inhibition has also been shown to be bacteria dependant. The *Enterobacter* sp. (*Esp\_Z*) bacterium retarded ookinete, oocyst, and sporozoite development of a virulent laboratory *Plasmodium* strain by 98%, 99%, and 99% respectively.

Novel approaches to controlling the mosquito vector is the development of transgenic mosquitoes that are resistant to diseases and have the ability to survive and out compete the wild type population of mosquitoes. Transgenic mosquitoes carry specific genes that make them resistant to *Plasmodium* or inhibit the vector specific stages of the parasite's lifecycle (Amenya *et al.*, 2010). As these "disease resistant" genes are passed on to following generations of mosquitoes the disease will consequently fade away after some time. Since there will be interactions between transgenic and wild type mosquito populations once the transgenic mosquitoes have been released, it must be ensured that the disease resistant genes are more dominant than the wild type genes to ensure that the disease resistant phenotype is not diluted, and eventually lost in later generations (Rafikov *et al.*, 2009). However, clear evidence of the efficacy of these parasites in field studies are not available as yet.

## **2.2 Preventing mosquito bites**

### **2.2.1 Insecticide Treated Nets (ITN) and Long life insecticide treated nets (LLIN)**

There are two types of LLIN that have been approved by WHO for use in mosquito control (Table 1). Promising results have been achieved with these polymer nets either coated with or

incorporating pyrethroid insecticides (Graham *et al.*, 2005, Focke and Van Preen, 2011). PermaNet<sup>®</sup> produced by Vestergaard Frandsen in Thailand and Vietnam, was found to retain an effective efficacy of about 97% mortality of anopheles mosquitoes after 3 min contact exposure even after 21 washes (Graham *et al.*, 2005). PermaNet is composed of a polyester net coated with polymer resin containing pyrethroid insecticide deltamethrin 55mg a.i./m<sup>2</sup>. Bayer vector control has recently reported a deltamethrin impregnated polypropylene net under the trade name of Lifenet<sup>®</sup>. Over time deltamethrin migrates to the surface of the fibre to replenish residues removed by washing. It is claimed that it can withstand more than 35 washes and can last for five years (Bayer., 2011). The Olyset<sup>®</sup> net is a product developed by Sumitomo Chemical Company of Japan. In Africa, Olyset<sup>®</sup> nets are manufactured by A to Z Textile Mills in Tanzania, under a technology transfer agreement with Sumitomo. This polyethylene netting has permethrin incorporated in the fibres during the net manufacture. The permethrin is slowly released onto the surface of the fibre over time until the insecticide concentration on the surface reaches equilibrium. When the insecticide is removed from the fibre by washing, the surface concentration is replenished from the reservoir within. Through this slow release mechanism the concentration is maintained at a level sufficient to provide sufficient activity against mosquitoes (Sumitomo, 2012).

### **2.2.2 Repellents and attractants**

Before the advent of DDT and pyrethroids in the 1950s and 1970s respectively, researchers were working on diverting the female mosquitoes away from human beings by either attractants or repellents. With the initial perceived strength of DDT and pyrethroid insecticides, research in this research field became subdued. However recently, with the controversy arising from the use of DDT and the emergence of resistance to pyrethroids, there is renewed interest in the alternative control methods.

#### **2.2.2.1 Repellents**

Repellents are chemical products that give off an offensive smell or taste to mosquitoes. The mechanism of function of most repellents is not yet understood. It has been suggested however that if a repellent incorporates a ring structure there is usually a carbonyl group immediately above the ring (Schreck *et al.*, 1986). In the past, most non-US produced repellent formulations in the United States contain essential oils and DEET (Schreck *et al.*, 1986).

**Table 1: WHO approved LLIN**

<b>Product name</b>	<b>Product type</b>	<b>WHO recommendation</b>
DawaPlus®	Deltamethrin coated on polyester	Interim
Duranet®	Alphacypermethrin incorporated into polyethylene	Interim
Interceptor®	Alphacypermethrin coated on polyester	Full
LifeNet®	Deltamethrin incorporated into polypropylene	Interim
MAGNet®	Alpha-cypermethrin incorporated into polyethylene	Interim
Netprotect®	Deltamethrin incorporated into polyethylene	Interim
Olyset®	Permethrin incorporated into polyethylene	Full
Olyset® Plus	Permethrin and PBO incorporated into polyethylene	Interim
PermaNet® 2.0	Deltamethrin coated on polyester	Full
PermaNet® 2.5	Deltamethrin coated on polyester with strengthened border	Interim
PermaNet® 3.0	Combination of deltamethrin coated on polyester with strengthened border (side panels) and deltamethrin and PBO incorporated into polyethylene (roof)	Interim
Royal Sentry®	Alphacypermethrin incorporated into polyethylene	Interim
Yorkool LN®	Deltamethrin coated on polyester	Full

The compounds that have been identified in plants to have repellent properties are most likely chemical compounds produced by the plant to defend itself against insects that pose a threat to them. These chemicals can be grouped into different classes based on their functional chemical composition i.e. nitrogen containing compounds, terpenoids, phenolics, proteinase inhibitors and growth regulators. These compounds are in general designed to fight off a broad spectrum of insects and thus have shown in many cases to be very effective against mosquitos. The plants with the best plant based repellent properties researched fall into distinct families of which the *Poaceae* family (Citronella based) is the best known. Other families are *Lamiaceae*, *Fabaceae* and *Asteraceae* (Maia and Moore, 2011). The future potential is thus very clear as these families cover large number of plants of which many have never been researched.

**Essential oils.** Prior to the extensive use of synthetically produced repellents, aromatic/essential oils were mostly used. A large consumer of these oils was the military. Soldiers were issued with creams containing citronella, camphor and paraffin (Covell, 1945). Numerous essential oil producing plants from the *Lamiaceae*, *Poaceae*, and *Myrtaceae* families have very well-known repellent activity. The best candidates against *Aedes aegypti* were *Pogostemon cablin* (Patchouli), *Cymbopogon nardus* (Citronella), *Zanthoxylum limonella* and *Syzygium aromaticum* (Clove) feature repellent activity of up to 120 min (Trongtokit *et al.*, 2005).

Neem oil produced from *Azadirachta indica* has shown, on top of being larvicidal, that it also offers protection against mosquito bites for up to 12 hours when 2wt.% is mixed with coconut oil (Sharma *et al.*, 1993). A eucalyptus based repellent, which contains *p*-methane-3, 8-diol (PMD) as the principal ingredient, apparently offers repellent protection similar to DEET in efficacy and duration against *An. gambiae* and *An. funestus* mosquitoes (Trigg, 1996). There are several other commercial plant based repellents that are used widely around the world. These repellents mainly comprise of citronella oil, but many make use of other essential oils. The essential oil based repellents perform significantly worse than DEET with repellence times of a few minutes in some cases. This makes essential oils on their own not very promising for disease transmission prevention, but it does indicate the future potential of essential oils with other product to produce superior repellent products (Barnard and Xue, 2004).

**Permethrin.** Permethrin has been used as a 1wt.% suspension in oil. In a field study trial, persons were positioned inside and outside a tent treated with permethrin. An untreated tent was used as control. Those inside the treated tent experienced up to 94% reduction in mosquito bites ( $p \leq 0.5$ ) for 42 days. Subjects outside the treated tent experienced 42% to 82% fewer mosquito bites than those outside the untreated tent (Schreck *et al.*, 1986).

Since permethrin combines very good insecticidal properties with low mammalian toxicities it is ideal for application in agriculture and public health systems. Permethrin is a neurotoxin which works by modifying the sodium channel from very brief opening to a prolonged opening causing hyper muscular activity (Narahashi, 2001, Costa *et al.*, 2008). Permethrin is susceptible to oxidation by mono-oxygenase enzymes. The use of synergists such as

piperonylbutoxide, sesamex, etc., that inhibit this enzyme, markedly increase the toxicity of permethrin.

**N,N-Diethyl-meta-toluamide (DEET).** DEET is the most widely used insect repellent today. The repellence of DEET is thought to be due to the blockage of lactic acid receptors, resulting in the insect losing the trail leading to the host (Davis and Sokolove, 1976). It was discovered and developed by the US army for use on military personnel in overseas missions. DEET has the ability to keep mosquitoes and a wide range of insects repelled. It was commercially marketed first in 1956 and has been the best performing insect repellent. DEET can be used safely on cotton wool and nylon fabrics but it may cause damage to a wide range of polymer derived material such as spandex.

Several cases of dermatitis, allergic reactions, neurologic and cardiovascular toxicities have been reported due to the use of DEET (Couch and Johnson, 1992, Tenenbein, 1987). In most of these cases it was concluded that the side effects were due to the misuse of DEET. However, if properly used the risk of serious adverse effects is slight. To reduce the likelihood of side effects, the lowest effective concentration of DEET for a given application should be used.

Initially the repellence effect of DEET against *Aedes aegypti* and *Culex pipiens* increases with concentration but it reaches a plateau value above a concentration of about 50% (Buescher *et al.*, 1984). Formulations containing high amounts of DEET tend to be oily, greasy or sticky and generally unpleasant to use. Current DEET formulations have been improved by use of slow release technologies that increased the protection time of DEET by up to 300% (Andrews *et al.*, 1992). They feature less odour, reduced skin absorption and therefore enhanced comfort of use.

**Indalone (butyl-3, 4-dihydro-2, 2-dimethyl-4-oxo-2H-pyran-6-carboxylate).** It is a slightly volatile repellent. The insect has to come into contact with indalone before being repelled (Garson and Winnike, 1968). It is safe to use on clothing or direct skin application.

**Dimethylphthalate (dimethyl 1, 2-benzenedicarboxylate).** Dimethyl phthalate is a synthetic repellent. It was initially used as a solvent in trial solid insect repellents. The minimum

dosage of dimethyl phthalate necessary to prevent mosquito bites is about 12.4 g/m<sup>2</sup> while the required minimum dosage for DEET is much lower at about 0.558 to 0.775 g/m<sup>2</sup> (Maibach *et al.*, 1974).

**Rutgers 612 (2-ethyl-1, 3-hexanediol).** Rutgers 612 became commercially available in 1939 (Peterson and Coats, 2001). It was used in combination with other commercially available repellents at the time to form a mixture called “6-2-2” (6 parts dimethyl phthalate, 2 parts indalone<sup>®</sup> and 2 parts Rutgers 612) in military personnel during World War 2. 2-ethyl-1, 3-hexanediol, the active ingredient in Rutgers has been shown to repel mosquitoes (Quintana *et al.*, 1972). Its effectiveness is limited by the high volatilisation and its ease of absorption through the skin. However its volatility was reduced by reacting it with acidic amino groups which acted as anchors on the epidermis of the skin.

#### 2.2.2.2 Attractants

Attractants present a possibility of controlling mosquito vector populations by using a mosquito trap. Host seeking female mosquitoes are guided by attractant odours released by their target. *An. gambiae* is attracted to ammonia, lactic acid and carboxylic acids (Okumu *et al.*, 2010, Knols, 2012). These are produced naturally by warm blooded animal body odour and sweat (Bernier, Kline *et al.* 2000). These compounds attract more mosquitoes as blends than when applied in isolation (Smallegange *et al.*, 2005, Geier *et al.*, 1996) suggesting a synergistic relationship. Blends of lactic acid, carboxylic acids and ammonia have been shown to be attractive in laboratory tests and field tests (Okumu *et al.*, 2010, Smallegange *et al.*, 2005). However, relative to human sweat, these blends are still less attractive (Okumu *et al.*, 2010). This implies that there are additional other compounds that are yet to be discovered which are synergistic in the attraction of mosquitoes. Another chemical odour that plays an important role in mosquito host seeking behaviour is CO<sub>2</sub> (Mboera *et al.*, 2000). In field tests, mosquito trap catches increased with the addition of CO<sub>2</sub> to odour blend. *An. gambiae* has been shown to attack its victims on the feet and ankles. It is attracted to this particular area because of odour emitted by the feet. This odour is produced by *Brevibacterium linens* that survive in humid and warm clefts between toes. These odours resemble those of limburger cheese. Knols (2012) confirmed the attractiveness of limburger cheese to *An. gambiae*.



Brown (1966) showed that female mosquitoes are attracted to human steel dummies set at 37°C, releasing CO<sub>2</sub> and with a wet surface especially if this is human sweat. It can be concluded therefore that, CO<sub>2</sub>, lactic acid, carboxylic acids, heat and moisture are the cues that direct female mosquitoes to their blood hosts. However, it is likely that there are many unknown compounds that work in synergy with these chemicals to produce an effective cue that attracts mosquitoes.

An alternative technique to inhibit a mosquito in locating its blood host is to disrupt its odour receptors. Stopfer (2011) described three classes of receptor disruptors. Inhibitors such as hexanol and butanol have the ability to inhibit CO<sub>2</sub> receptors in mosquitoes and flies in general. Secondly, irritators such as 2-butanone can be used to simulate CO<sub>2</sub> and thus has the ability to attract mosquitoes away from human beings. Lastly, blinders such as 2, 3-butanedione have the ability to cause prolonged activation of CO<sub>2</sub> sensory neurons and thus disabling the ability of the mosquito to sense CO<sub>2</sub> coming from human beings.

It has been reported that sugar is an attractant for mosquitoes (Müller *et al.*, 2010). In general, a sugar meal is the main source of nutrition for mosquitoes. Female mosquitoes additionally require a blood meal in order to grow their eggs. The sugar meal is usually obtained from plant nectar. This sugar meal can be mixed with additives that can kill a mosquito. Sprayed fermented plant juice mixed with boric acid reduced the population of *An. gambiae* by 90% (Müller *et al.*, 2010).

### **2.2.3 House design**

Careful design of human settlements effectively reduces the spread of malaria. Pioneering work was done by Angelo Celli at the end of the 19<sup>th</sup> century (Lindsay *et al.*, 2002). In malaria endemic areas, transmission was reduced by up to 96% by covering windows and doors of human dwellings with thin muslin. Due to these excellent results, the practice of house proofing spread across Europe, America and to European settlers in tropical regions. House proofing was used extensively during the construction of the Panama Canal. The decrease in malaria in England in early 19<sup>th</sup> century and in the USA was attributed to improved housing (Boyd, 1926, Byrd, 1914). Another study reports that the British army mosquito-proofed their barracks in Lahore which resulted in the reduction of mosquito

incidence by 68% (Rutherford, 1928). These results clearly point out that mosquito proofing significantly reduces malaria incidence.

Most African mosquitoes are adapted to entering a human dwelling in search of a blood meal. The most efficient vectors in Africa are excellently adapted to feeding on human beings. *An. gambiae* for example is attracted to human odour coming out of a dwelling. It flies upwards as soon as it meets an obstacle such as the wall of a dwelling (Snow, 1987). If there is an opening on the roof, such as open eaves, the vector will use this as an entry point. Fewer mosquitoes were experienced in indoors in experiments where the eaves were closed (Lindsay *et al.*, 2002). In most studies fewer malaria cases were recorded in such dwellings. However, closure of eaves or the presence of a ceiling in a dwelling may point out why affluence seemingly provides protection against malaria.

Installing a ceiling or a net screen is beneficial as it reduces house entry of mosquitoes (Lindsay *et al.*, 2003). In this study house entry was reduced by 59% for a ceiling, 79% when using synthetic netting, 78% when using insecticide treated synthetic netting, 80% when using plastic synthetic netting and 37% when eaves were closed. The high percentage of reduction for nets was attributed to the creation of a decoy that attracts mosquitoes to the roof space. Incorporating an insecticide in the plastic screens did not enhance the reduction in house entry. Generally installing ceilings and closing eaves reduces mosquito entry into houses. However installing ceilings and closing eaves presents a problem of their own. They restrict ventilation and make the rooms warmer. This may reduce the acceptability of such interventions in generally warmer climates where mosquitoes are prevalent. Insecticide netting seems to be a reasonable entry level protection compared to closing eaves.

Materials of construction were shown to affect the rate of house entry by mosquitoes. In many rural African dwellings, mud is used as the main material of construction. Overtime the mud walls develop cracks which provide access points into the house and environments conducive for mosquito survival. Mud walls are usually associated with thatched roofing with open eaves. Thatch requires regular maintenance and the eaves provide easy access for mosquitoes. However, modern western style housing is built using bricks and mortar and typically utilizes galvanized iron roofing or ceramic tiling. The eaves are usually closed. It is no surprise that studies show that typical traditional African dwellings experience high

mosquito entry rates compared to western styled houses and have been positively linked to high malaria infection rates (Sintasath *et al.*, 2005).

## **2.3 Killing mosquitoes after they have bitten**

### **2.3.1 Indoor residual spray (IRS)**

The World Health Organization (WHO) Global Malaria Action Plan (GMAP) promotes indoor residual spraying (IRS) in addition to long-life insecticide impregnated nets (LLIN) as a primary operational vector control intervention to reduce and eliminate malaria transmission. IRS is deemed particularly effective and widely applied in Southern Africa. IRS is an annual activity and WHO has approved twelve insecticides for use in IRS. Six of the insecticides are classified as pyrethroids (alphacypermethrin, betacyfluthrin, bifenthrin, deltamethrin, etofenprox, and lamdacyhalothrin), three as organophosphates (malathion, fenitrothion, pirimiphos-methyl), two as carbamates (propoxur, bendiocarb) and one as an organochlorine (DDT). DDT is the most preferred for IRS because it has an efficacy of up to 12 months or more depending on the application surface, while the rest of the insecticides have an efficacy of up to 6 months. The longer lasting efficacy of DDT provides a low cost option as one spray cycle is required in a year as compared with two or more spray cycles for the alternative insecticides. Unfortunately, the use of DDT has health implications for both applicators and residents (Eskenazi *et al.*, 2009). In birds, DDT through its metabolite DDE has been well documented to cause the thinning of eggshells which causes breakage of eggs and embryo deaths (Bitman *et al.*, 1970, Blus, 1971).

Other WHO approved pyrethroid, organophosphate and carbamate insecticides are limited in effective IRS residual life. Furthermore, repeated application of these alternatives is required in order to provide year round protection significantly increases the costs of IRS (Sadasivaiah *et al.*, 2007). Formulations based on micro-encapsulated insecticides have been tested with great success (Amelotti *et al.*, 2009). These results show that shielding the insecticides from the outside environment stabilizes them against premature degradation. However, the higher costs associated with such formulations may limit their widespread implementation as replacements for DDT in IRS. Recent work suggests that precipitation of insecticides on phosphogypsum (Sibanda *et al.*, 2011) and co-intercalation of the insecticides in organo-clays (Merckel *et al.*, 2012) can provide significantly improved stabilization of selected WHO approved insecticides.

### 2.3.2 Insecticide treated wall linings (ITWL)

IRS dust residues may play a crucial role in both the loss of active insecticide from the sprayed walls and the accumulation of DDT in humans (Van Dyk *et al.*, 2010, Aneck-Hahn *et al.*, 2007, De Jager *et al.*, 2009). This means that future efforts should also consider ways to reduce dust formation. One possible way could be to use netting with embedded insecticide as a wall covering. Thus it could well be that insecticides incorporated into a polymeric netting or a fine mesh affixed to indoor walls may provide an even safer and longer lasting alternative to wall spraying. These polymers have the ability to store the insecticide and slowly release them onto the surface of the polymer (Messenger *et al.*, 2012b).

IRS was developed over 60 years ago, following the invention of DDT, the first insecticide with a sufficient residual activity to be used for this purpose (Hays, 2000). Since then, hardly anything has changed in the way IRS is implemented. The only major change has been that there is now a greater range of insecticides (twelve different products in four chemical classes) with some of the newer products being longer lasting formulations (up to 12 months residual effect). On the same model of how conventional ITN with an insecticidal effect of maximum 12 months moved on to become LLIN that do not need to be re-treated for 3-5 years, there is a great rationale for making IRS also much longer lasting. This is especially important in areas that are difficult to reach, or where the weakness of the health system is such that repeated applications are difficult.

Insecticide treated wall linings (ITWL) represents a new vector control technology that could alleviate the problem of insecticide dusting and also the premature failure of alternative insecticides used in IRS. So far, the most advanced product is based on woven shade cloth made of polyethylene with 50% shading and treated with deltamethrin 4.4 g a.i./kg material (Messenger *et al.*, 2012a, Messenger *et al.*, 2012b). The type of treatment is similar to Type 2 LLIN (the insecticide being included in the fibre itself). This product was developed and manufactured by Durable Activated Residual Textiles S.A. (DART), a consortium of three partners: Vestergaard Frandsen®, Acumen Fund and Richard Allan. Similar products are currently in the pipeline from a number of manufacturers.

### 2.3.3 Natural insecticides for IRS

Natural insecticides present immense possibilities as alternative insecticides to synthetics. This includes different modes of action and rapid degradation in the environment. Research into natural insecticides subsided during the early 1970s with the successful synthesis of synthetic pyrethroids from pyrethrum. However there are renewed interests in natural insecticides as green alternatives. Sukumar *et al.* (1991) reviewed the available botanical derivatives for malaria control. Research on the efficacy of natural insecticides has mainly focused on larviciding and growth inhibition of insects. Essential oils, turpenoids, lactones, alkaloids and phenolic botanical derivatives were found to be effective repellents and growth inhibitors of adult mosquitoes (Makhaik *et al.*, 2005). Some natural insecticides derive from bacterial processes. Spinosad, a mixture of spinosyn A and spinosyn D is a stomach poison that is an effective mosquito larvicide (Duchet *et al.*, 2008).

Research into mosquito adulticides has been very minimal (Shalaan *et al.*, 2005) and up to now commercial natural insecticides are rare in the market. In recent years some essential oils were tested for their effectiveness as mosquito adulticides. These are thymoquinone, nootkatone and carvacol, components of Alaska yellow cedar *chamaecypris nootkatensis* (D. Don) *spach* and incense cedar, *calocedrus decurrens* (Torr) (Dolan *et al.*, 2009, McAllister and Adams, 2010). Their mode of action has shown to act through epithelium of the midgut, thereby presenting a different mechanism to that of synthetic insecticides. However the residual effect of these botanicals is short (azadirachtin is 4-8 days in the field) (Schmutterer, 1988).

A suitable natural insecticide that may be used for mosquito control is pyrethrum (Duchon *et al.*, 2009). Pyrethrum is a natural pyrethroid insecticide of low mammalian toxicity derived from plants of the *chrysanthemum* genus. This insecticide has been used for centuries (Casida, 2010). Its main limitation is its instability towards ultraviolet light and heat. This prompted the development of more robust synthetic pyrethroids (Casida, 2010). It is possible that pyrethrum may be stabilised by a protective polymer matrix making it a potential natural insecticide that can be used in malaria control.

Chaiyasit *et al.* (2006) investigated the effectiveness of the essential oils of five plant species, caraway (*Carum carvi*), celery (*Apium graveolens*), Chinese star anise (*Illicium verum*), long

pepper (*Piper longum*) and zedo-ary (*Curcuma zedoaria*). All the essential oils investigated in this particular study showed significant effectiveness against both the pyrethroid resistant as well as the sensitive *A. aegypti* strains. The activity ( $\mu\text{g}/\text{mg}$  female) of these oils was calculated to be 5.44, 5.96, 8.52, 6.21 and 5.94 respectively. These promising results were found to be consistent with previous studies (Amonkar and Reeves, 1970, Thomas and Callaghan, 1999) thus emphasizing the effectiveness of essential oils as possible adulticidal agents. The effectiveness of these oils against the pyrethroid resistant strains was also a major find, as this highlighted the potential use of essential oils in resistant prone areas especially when used in combination with current synthetical products.

The field of natural insecticides is still extremely underdeveloped and there are no promising “silver bullets” for replacing very stable and very effective synthetics. However it might be interesting to evaluate the performance of pyrethrum in a polymer net.

#### **2.4 Future developments in vector control**

During a recent poster competition run by the Global Alliance for developing and deploying alternatives to DDT for disease vector control, very interesting innovations were proposed. Plants with repellent properties were shown to build mosquito repelling gaseous envelopes around the area where they grow. Plant species such as *Azadirachta indica*, *Ocimum suave*, *Cymbopogon citrates*, *Lantana camara* and *Cymbopogonnardus* contain essential oils that act as repellents. Sowing such plants in homesteads coupled with sleeping under ITN has the potential to increasing protection from mosquito bites.

Plant based insecticides have received very little attention in past few decades and very little is still known and understood how these bio-insecticides actually work. The notion, that due to the unique nature of bio-insecticides resulting from their complex mixture of compounds, insect resistance to them has never been reported. This complex mixture of compounds potentially represents many different modes of actions that take place with one application of a bio-insecticide. A second factor that should not be ignored is that the half-life, low frequency of application and small scale of applications are also possible contributors to the lack of reported resistant cases (Chaiyasit *et al.*, 2006). It is thus important that research in plant based insecticides receives a new lease on life to find new candidate like the very successful pyrethrum compound discovered from nature in *Chrysanthemum cinerariaefolium*.

It has also been proposed to use the LLIN concepts in the form of artefacts such as dummy flowers, etc. that can be used interior decorations. The LLIN can also be applied as curtains, window screens etc. The rationale of this method is to extend the use of mosquito control interventions to areas outside the bedroom and also to increase the acceptability of such interventions.

Recently at a talk organised by TEDx (Knols, 2012), Dr Bart Knols presented novel ideas on fighting malaria. Mosquito larvae produce odour. Dr Knols demonstrated that dogs can be used to detect areas with mosquito larvae through the odour they produce. This will allow larvicides to be applied directly in areas with larvae. A more exciting demonstration was the use of oral medication to kill mosquitoes after biting. In this technique, a pill which imparts toxic properties to blood is ingested beforehand and can kill mosquitoes hours after biting.

Effective vector control methods are those that suppress vector populations to a bare minimum. This reduces the capacity of the mosquito to transmit the malaria parasite. DDT has been shown to be an effective chemical in achieving this. The goal is to find an alternative technology that is both as effective as DDT and cost effective or better. This paper has reviewed the various possible techniques that can be used to achieve more effective control of malaria. These techniques are summarised in a cartoon sketch shown in Figure 1. The suitability of these techniques to achieve the desirable malaria vector control has been summarised in Table 2.

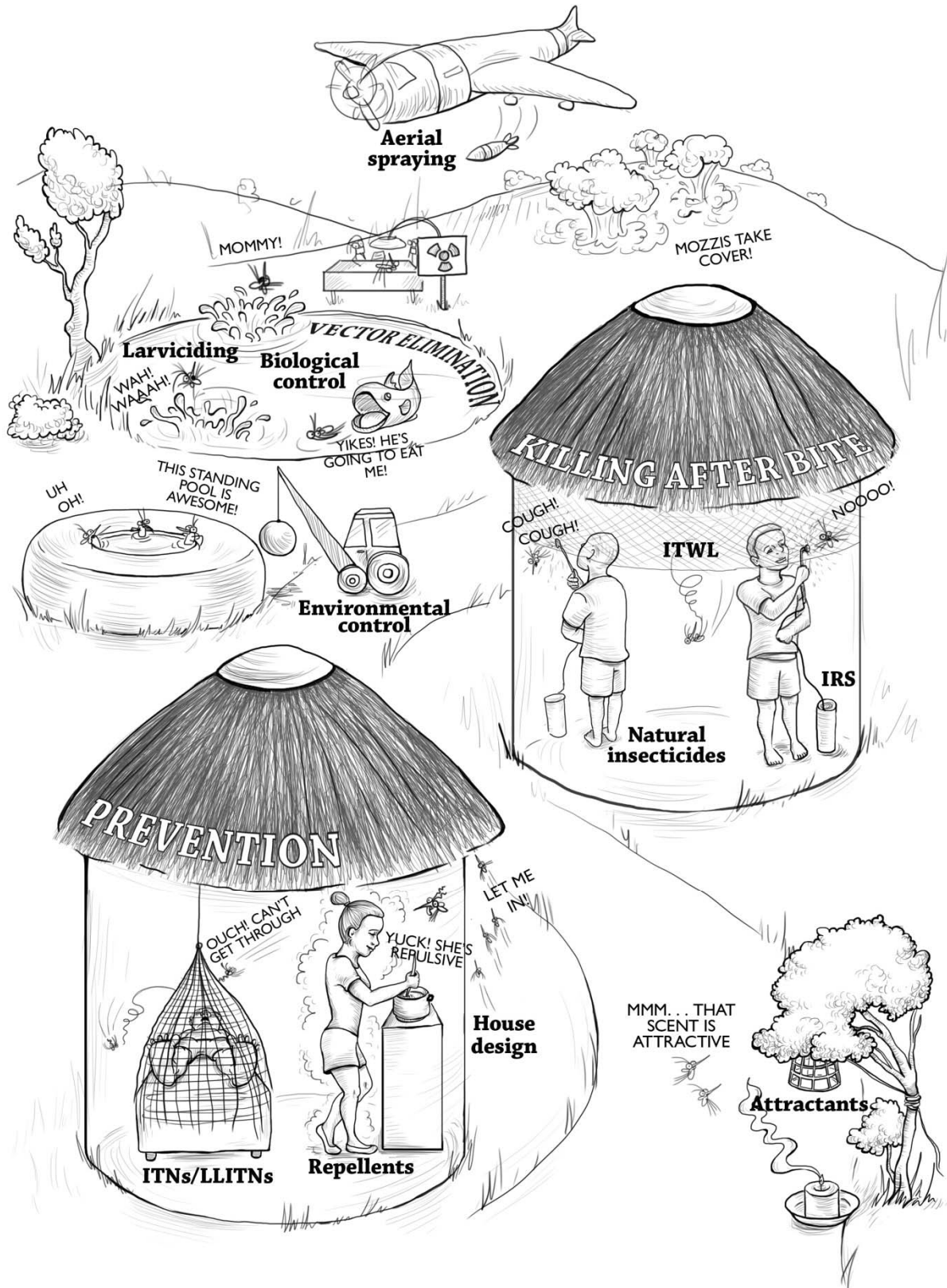


Figure 1: Physical, chemical and biological vector control methods



**Table 2: Summary of physical, chemical and biological vector control strategies**

<b>Group</b>	<b>Subgroup</b>	<b>Advantages</b>	<b>Disadvantages</b>	<b>Comments</b>
<b>Vector elimination</b>	Aerial spraying	Cheap effective and simple to implement.	Only effective on controlling exophilic mosquitoes	It may fail as it does not target indoor feeding mosquitoes, which are responsible for the bulk of malaria transmission
	Larviciding	It can eliminate source of mosquitoes	There is need to develop a cheap and effective larvicide. IGRs are expensive and limited in availability.	Anopheles gambiae was eliminated in Brazil using arsenic larviciding.
	Environmental control	It can lead to vector elimination	Environmental modification is expensive and may be possible in more advanced economies	Potential to bring about vector elimination
	Sterile insect techniques	Effective if implemented correctly	Cost is prohibitive to poorer countries	Potential to bring about vector elimination
	Biological	Effective if implemented correctly	Expensive and technically challenging for poorer countries	Potential to bring about vector elimination
<b>Preventing mosquito bites</b>	ITNs/LLIN	Cheap and easy to implement	Only offers protection during bed time; it does not kill vector	Mosquitoes can still transmit malaria before bedtime.
	Repellents	Effective in preventing bites	Short residual efficacy, strong smell, irritating to the skin	Does not reduce vector populations and they will simply migrate to areas where repellents are not used
	Attractants House design	Safe to humans and environment, cheap Very effective and cheap	Chemicals that attract not fully isolated Closing eaves increases indoor temperatures	Very promising technique! Does not reduce vector populations however worked well for Europe and America
<b>Killing</b>	IRS	Breaking transmission cycle	Too much reliance on DDT; dusting of sprayed insecticides a problem, labour intensive	Residual efficacy limited to at most one season
	ITWL	Similar to IRS but eliminates dusting and short residual efficacy of insecticides	User acceptability may be a challenge	Emerging polymer technology which will eliminate the need to spray chemicals
	Natural insecticides	Low mammalian toxicity	Short residual efficacy	Pyrethrin is the most effective

## **2.5 Limitations (gaps) of current vector control interventions**

The main vector interventions recommended by the WHO are the use of Indoor Residual Spraying (IRS) and LLIN. However these interventions have limitations. These limitations are discussed in the following sections.

### **2.5.1 Indoor residual spray**

The limitations of IRS have been discussed in section 2.3.1. IRS is most effective when using DDT. The main reason for this is that insecticides approved for malaria vector control in IRS do not last long. The use of DDT has become contentious as it is a persistent organic pollutant and can last up to 12 years in the environment. DDT has been linked to adverse health effects on humans and animals because of its persistence in the environment.

### **2.5.2 Insecticide treated nets (ITN) and Long lasting insecticide treated nets (LLIN)**

Most African countries favour LLIN (and ITN) programmes because unlike IRS programmes they are inexpensive and easy to implement. The main disadvantage of LLIN is that protection is only offered during sleeping time. It is possible to get infected at dusk when the mosquitoes start to be active and occupants in the house are not subject to protection by LLIN. The effectiveness of LLIN is also limited by the need to wash the nets from time to time, gradually diminishing the insecticidal activity.

### **2.5.3 Pyrethroid resistance (biochemical and kdr)**

Mosquitoes biochemically resist the effect of an insecticide by producing enzymes that metabolise the insecticide before it can kill the mosquito (Georghiou, 1972). Mosquitoes can also adapt their physiology to prevent the insecticide from being effective e.g. a hairy cuticle or thicker skin to prevent the penetration of the insecticide. This is known as knockdown resistance (kdr). Malaria vector control programs in Africa are currently dependant on the pyrethroid class of insecticides. This group of insecticides is the only class approved by the WHO for use on ITN and LLIN (Zaim *et al.*, 2000). Pyrethroids are also being increasingly deployed for use in IRS programmes across sub-Saharan Africa. With increasing agricultural production levels in Africa there is widespread use of pyrethroids for pest control. This wide use of pyrethroids has led to a dramatic increase in reports of pyrethroid resistance in malaria vectors over the past decade across sub-Saharan Africa (Santolamazza *et al.*, 2008, Ranson *et*

*al.*, 2011, Chandre *et al.*, 1999). Table 3 shows a list of countries where resistance has been reported.

There are very few studies on the epidemiological implications of this widespread reported pyrethroid resistance by mosquitoes. In South Africa DDT was temporarily replaced with the pyrethroid deltamethrin between 1996 and 1999. This was part of efforts by the South African government to scale down the use of DDT. However, DDT had to be reintroduced in 2000 when malaria transmission reached epidemic proportions. The failure of the pyrethroid was attributed to the return of the major vector mosquito, *An. funestus* that was shown to be resistant to pyrethroids but fully susceptible to DDT (Hargreaves *et al.*, 2000). A decline of 91% in malaria cases was recorded when the IRS program reverted back to DDT (Maharaj *et al.*, 2005). In the Bioko Islands off the coast of Equatorial Guinea, IRS was undertaken using lambda-cyhalothrin. This campaign was unable to reduce the population density of *An. gambiae* resistant to pyrethroids. However when the IRS program switched to the carbamate Bendiocarb, a decline in mosquito populations was observed (Sharp *et al.*, 2007, Kleinschmidt *et al.*, 2006). In Côte d'Ivoire an experimental hut study between two adjacent areas one with pyrethroid resistant and other fully susceptible *An. gambiae* populations showed no differences on the efficacy of ITN (Hougard *et al.*, 2003). However in an area in Benin lambda-cyhalothrin used for IRS and net treatment showed a significant loss in effectiveness to pyrethroid resistant *An. gambiae* compared to the northern part of Benin where vector populations remained largely susceptible (N'Guessan *et al.*, 2007).

#### **2.5.4 Mosquito feeding and resting behaviour and behavioural avoidance**

WHO recommended flagship interventions for mosquito control i.e. IRS and LLIN target indoor feeding and/or resting mosquitoes (endophagic and/or endophilic). These interventions have dramatically reduced malaria mortality and morbidity. For example using DDT in IRS, South Africa has managed to reduce malaria transmission to the WHO defined pre-elimination stage, one step before total elimination (Coetzee *et al.*, 2013). Similar success was recorded in India where the occurrence of malaria was reduced from about 57 million cases a year in the 1930s to 110000 cases per year in the 1960s, a massive reduction 99.8% (Cutler *et al.*, 2010). The basis of recommending IRS and LLIN as the main control interventions is based on an out-dated characterization of the behavioural phenotypes of the main malaria vectors in Africa, *An. gambiae* and *An. funestus* (Gillies and Coetzee, 1987).

**Table 3: Reported pyrethroid resistance in sub-Saharan Africa**

Region	Country	Species	Type of resistance	Reference
West Africa	Benin	-	kdr	(Corbel <i>et al.</i> , 2007)
	Cameroon	<i>An. Gambiae</i>	kdr	(Etang <i>et al.</i> , 2006)
	Nigeria	<i>An. Gambiae</i>	kdr	(Awolola <i>et al.</i> , 2007)
Central Africa	Central Africa		kdr	(Santolamazza <i>et al.</i> , 2008)
	Equatorial Guinea	<i>An. Gambiae</i>	kdr	(Moreno <i>et al.</i> , 2008)
	Gabon	<i>An. Gambiae</i>	kdr	(Pinto <i>et al.</i> , 2007)
East Africa	Ethiopia	-	-	(Balkew <i>et al.</i> , 2003)
	Kenya	-	kdr	(Stump <i>et al.</i> , 2004)
	Uganda	<i>An. Gambiae</i>	kdr	(Verhaeghen <i>et al.</i> , 2006)
	Sudan		kdr	(Abdalla <i>et al.</i> , 2014)
Southern Africa	Malawi	<i>An. Gambiae</i>	kdr	(Pinto <i>et al.</i> , 2007); (Coleman <i>et al.</i> , 2008); (Chandre <i>et al.</i> , 1999)
	Mozambique	<i>An. Gambiae</i>	kdr	(Pinto <i>et al.</i> , 2007); (Coleman <i>et al.</i> , 2008); (Chandre <i>et al.</i> , 1999)
	Tanzania	-	kdr	(Stump <i>et al.</i> , 2004); (Kulkarni <i>et al.</i> , 2007)
	South Africa	<i>An. Arabiensis</i>	-	(Hargreaves <i>et al.</i> , 2003)
	Zimbabwe	-	-	(Munhenga <i>et al.</i> , 2008)

#### 2.5.4.1 Selective targeting

IRS and LLIN select for indoor feeding and/or resting mosquitoes. Outdoor feeding and resting mosquitoes (exophagic and exophilic) have not been targeted for vector control. An example is in Garki, Nigeria where an IRS campaign was undertaken to interrupt malaria transmission (WHO, 2007). The insecticide was only exposed to indoor resting mosquitoes resulting in the decimation of their populations however the outdoor resting mosquito populations were not affected. Malaria transmission was only marginally reduced. It is thus clear that elimination of malaria transmission may not be possible without targeting outdoor resting mosquitoes.

#### 2.5.4.2 Behavioural resistance

In recent years mosquito behavioural phenotypes that make them avoid coming into contact with vector control interventions have been reported due to prolonged and widespread scaleup of vector control programs (Takken, 2002, Durnez, 2013). This phenomenon is less

recognised than insecticide resistance in the vector control community. These phenotypes consist of feeding outdoors or in the early evening to avoid vector control interventions that target indoors (Pates and Curtis, 2005, Geissbuhler *et al.*, 2007, Braimah *et al.*, 2005). The behavioural change might have come about by the selection of genetically inherited characteristics or simply to behavioural plasticity to avoid ITN or IRS (Pates and Curtis, 2005, Takken, 2002). Two vectors genotypes have been reported to behave in this way i.e. *An. funestus* and *An. gambiae* complex (*An. gambiae s.s.* and *An. arabiensis*). *An. arabiensis* has been shown to feed early evenings when humans are outdoors to avoid contact with IRS and LLIN. However *An. gambiae s.s.* has been shown to be unable to alter its nocturnal feeding habits because it is unable to adapt to low humidity (Lindsay *et al.*, 2003) A study in Benin found that *An. funestus* changed its biting times following universal coverage of long lasting insecticide treated nets (Moiroux *et al.*, 2012). In the Punta Europa area of Bioko Islands (Equatorial Guinea) long term application of IRS led to behavioural shift in the host seeking behaviour of *An. gambiae (s.l.)*. A similar study in Benin showed a shift in biting behaviour of *An. gambiae (s.l.)* to outdoors after scaling up LLIN and IRS necessitating the need to explore alternative outdoor vector control interventions (Padonou *et al.*, 2012). In rural southern Tanzania the use of bed nets in the last decade has reduced the malaria transmission intensity by 94%. However there is a reported increase in outdoor feeding of mosquitoes after exposure to LLIN (Russell *et al.*, 2011). In Papua New Guinea a change to an earlier biting time in *An. farauti* populations was recorded when pyrethroid impregnated bed nets were introduced (Charlwood and Graves, 1987). This was attributed to the fact that when female mosquitoes returned to a netted village after laying eggs to feed they had difficulty finding a blood meal by dawn and remain hungry the rest of the day and attempt to feed as soon as dusk fell.

### **2.5.5 Repellent residual effectiveness**

Judicious use of repellents might prevent or at least reduce outdoor malaria transmission. Repellents are most often used as topical formulations (lotions, sprays, emulsions, etc.) applied to exposed skin. DEET is the most popular topical repellent in use. Mosquito repellents such as DEET provide outdoor personal protection against malaria (Walker *et al.*, 1996). DEET is the most widely used repellent because of its superior residual efficacy. The relatively high cost of DEET and the need for repeated application to the skin at high concentrations (10–70%) precludes its use in tropical countries (Turner *et al.*, 2011). Also

reliance on DEET for outdoor mosquito bite protection has led to observed resistance in *An. albimanus* (Klun *et al.*, 2004) and *An. aegypti* (Stanczyk *et al.*, 2010) mosquitoes. Potential repellent alternatives to DEET that are derived from plant extracts have been studied (Sharma *et al.*, 1993, Chokechaijaroenporn *et al.*, 1994, Barnard, 1999). While they have demonstrated good efficacy, their high volatility prevents their use in topical skin applications (Lalko and Api, 2006).

## **2.6 Conclusion**

It is speculated that the gaps in vector control presented above are responsible for the residual transmission in malaria in areas where the disease has been brought largely under control e.g. South Africa. It is also suggested that without closing these gaps, the goal of malaria elimination in Africa will remain elusive. The study presented here sought to close these gaps. It is envisaged that if successfully developed, additional vector control interventions to close these gaps will have a high impact.

### **3. Problem statement**

Current vector control interventions recommended by the WHO do not protect from all possible scenarios (indoors and outdoors) in which a mosquito can bite and consequently infect a person with malaria.

#### **3.1. Aim**

The aim of this research is to develop long lasting and cost effective alternative vector control interventions that can fill in the gaps left by implementing current interventions.

#### **3.2. Objectives**

The objectives of this study were:

1. Conduct exploratory studies into whether polymer matrices can be used for the controlled release of alternative insecticides and volatile repellent actives, and
2. To evaluate the residual effectiveness of these controlled release formulations.

The ultimate aim of malaria vector control interventions is malaria elimination. Malaria elimination is defined as the permanent reduction to zero incidence of locally contracted cases, although imported cases will continue to occur and continued interventions are required. Existing interventions have been met with limited success. Elimination of malaria is probably not possible using current interventions only, because over-reliance on pyrethroids leads to biochemical and physiological resistance. Vector control interventions that select for indoor resting and feeding targets and also leads to behavioural resistance. These gaps are exacerbated by the very high entomological inoculation rate.

It is apparent that in order to make the required “quantum” improvement in current vector control interventions there is a need for effective holistic indoor and outdoor protection i.e. constant protection from mosquito bites when indoors and outdoors. The main problem is lack of adequate residual effectiveness of the active ingredients used in mosquito vector control i.e. alternatives to DDT in IRS and repellents for outdoor protection. In this light slow release of active ingredients using polymer matrices was explored. Certain polymers can be used as slow release insecticide carriers for malaria vector control. These polymers have the ability to offer protective environments to keep the active ingredients stable. Depending on the solubility in the polymer matrix, the active ingredient is slowly released into the

surrounding ambience. It was intended to use this slow release phenomenon of polymer matrices to control the release rate of alternative insecticides and repellents. If engineered correctly the polymer matrix can slowly release the active ingredient into the surrounding ambience over a period of time thereby elongating the length of residual effectiveness of the active ingredient. To achieve the aim and objectives of this study two ideas were explored.

### **3.2.1. Idea 1: Insecticide treated wall lining**

To solve the problem of ITN/LLIN not providing all round protection in the house and lack of adequate stability of alternative insecticides used in IRS, the manufacture and use of novel slow release insecticide treated wall lining (ITWL) was explored. ITWL is a slow release technology that combines the advantages of LLIN and IRS. Current commercial wall linings are produced using labour intensive fabric weaving methods. This study explored the effectiveness of inexpensive monofilament mesh linings produced by extruding insecticide impregnated polyethylene and poly(ethylene-co-vinyl acetate) (EVA) directly into a net format. It was envisaged that if such wall linings had adequate residual effectiveness then they could be used instead of spraying DDT directly onto the wall and may complement the use of ITN/LLIN.

### **3.2.2. Idea 2: Mosquito repellent bracelets**

To solve the problem of the inadequate residual activity of topical repellents for outside protection we explored the use of polymer matrices to slowly release repellents at a constant rate over an extended period of time thereby increasing their residual effectiveness. In this study we incorporated DEET into polymer matrices that can be used to manufacture bracelets or parts of low cost slip slops and used to prevent infections due to ankle biting *An. gambiae* mosquito. In this way it may be possible to protect people against malaria infection during the time they spend outdoors.



## 4. Development of ITWL

### 4.1 Aim

The aim was to explore producing ITWL using simple and cost effective extrusion through a die to form a mesh compared to the current labour intensive method of weaving a fabric in commercially available nets.

### 4.2 Objectives

1. Blow films using melts containing insecticides to be stabilised at different concentrations to test for processing safety and effectiveness against laboratory reared mosquitoes.
2. Incorporate insecticides into polymer matrices and extrude through a die to form a net.
3. Test the effectiveness of these nets using standard WHO recommended bioassay tests.
4. Carryout field trials of the net lining to evaluate overall user acceptability, lining durability and perceived effectiveness.

### 4.3 Theory

ITWL has been reviewed in section 2.3.2. ITWL is a concept that may combine the advantages of LLIN and IRS i.e. a wall lining with long lasting residual efficacy and no insecticide dusting. The most advanced product is based on woven shade cloth made of polyethylene with 50% shading and treated with deltamethrin 4.4 g a.i./kg material (Messenger *et al.*, 2012a, Messenger *et al.*, 2012b). This type of wall lining covers virtually all of the surface area of the inner walls. This may present several problems. The wall lining is within reach of children and this may cause serious health implications. In many African households interior decoration of mud huts by drawing patterns and other associated art is very popular. Putting a net over the wall prevents this and may lead to user rejection.

After a blood meal feed, a female mosquito typically rests on the upper parts of the walls. This therefore means that targeting only the upper parts of the walls may still have a similar impact as covering the whole wall.

Insecticides used to control mosquitoes are contact poisons. They have to be available on surface in adequate concentration for mosquitoes to come into contact with them. The desired end result is the release of the insecticides from within the polymer to the surface of the polymer over an extended period of time. This suggests that the insecticide must be trapped in a super saturated state i.e. be available in a concentration that exceeds its solubility limit. The diffusion of the insecticide from within the matrix to the surface should be slow enough to achieve controlled release. However this may not be the case. Focke and Van Preen (2011) found that pyrethroid insecticides almost bloom immediately in polypropylene after processing because these insecticides stay amorphous for extended periods of time when cooled from melt. They then proposed an idealized additive blooming process for an amorphous additive present above its glass transition temperature and illustrated schematically in **Error! Reference source not found.**

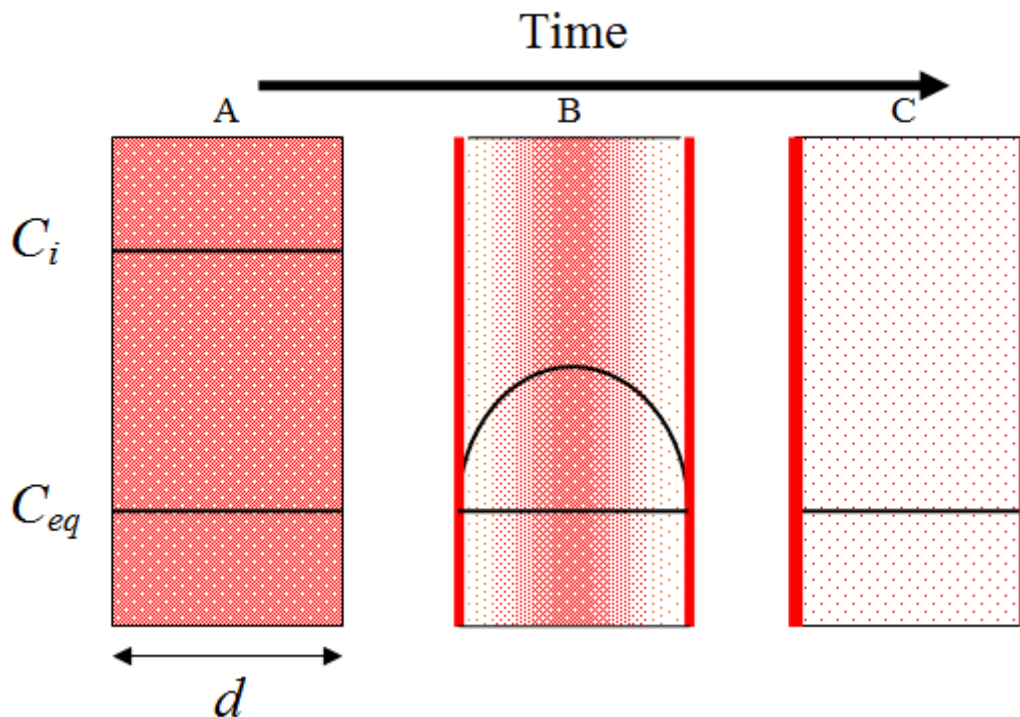


Figure 2: Schematic of the surface blooming mechanism in a fibre with diameter  $d$ . The intensity of the red colour scales with the insecticide concentration.

It was assumed that the fibre can be approximated by an infinite cylinder of diameter  $d$  with isotropic properties in the hoop and radial directions. It was also assumed that after compounding the melt, the active is homogeneously dispersed throughout the amorphous regions in the fibre (state A). The concentration  $C_i$  is determined by the dosage and exceeds

the equilibrium concentration  $C_{eq}$ . The additive diffuses to the surface setting up a concentration profile inside the fibre (state B). After a certain amount of time, the concentration in the fibre is reduced to a homogeneous concentration equal to the solubility limit  $C_{eq}$  (state C).

There exists very little further literature on the development and insecticide release mechanisms of various ITWL technologies (or LLINs). There are however a number of publications in the area of entomological testing of these products in the laboratory and field (Faulde et al., 2012, Messenger et al., 2012a, Messenger et al., 2012b). These products are by commercial companies who would not want to publish their intellectual property. It was not possible to perform a comprehensive literature review on the various ITWLs currently available.

Polyethylene has been widely used to carry pyrethroid insecticides especially for wall lining manufacture (Messenger *et al.*, 2012b). This is also confirmed in Table 1. Polyethylene is produced by Sasol in excess and therefore it is widely available and relatively cheap. This satisfies the condition of cost effectiveness. Polyethylene has low polarity and highly crystalline. Crystalline regions in polymer matrices reduce the solubility of additives and increase the chances of super saturation of an insecticide in a polymer matrix. The choice of polymer to stabilise the organophosphate insecticide was not very simple because no previous work has been done to stabilise organophosphates using polymer matrices. Organophosphate insecticides are liquid at room temperature. They are also relatively volatile compared to pyrethroids. They are unstable, releasing pungent sulphur-based compounds as temperature is increased. Ideally they must be compounded at the lowest possible temperature in order to avoid thermal degradation. Various polymers were considered for use with organophosphates. These are polycaprolactone (Capa 6500 and Capa 6800), dimer polyamide resins (1:1 blend of Euremelt 2130 with Euremelt 2140) and a 1:1 blend of EVA (Elvax 210) with polyamide (Euremelt 2130). These polymer matrices allowed for compounding and extrusion to occur at lower temperatures than polyethylene-pyrethroid compounding. Technical information about the polymers used in this study is contained in Table 4.

There are a number of wall lining products that have been tested in the field using carbamates as the active ingredient (Messenger *et al.*, 2012a, Messenger *et al.*, 2012b). Manufacture and

field tests of such products should be strongly discouraged. In preliminary studies it was discovered that when carbamates are exposed to high temperatures they degrade to release by-products that contain isocyanate functional groups. This is a very toxic functional group. It was responsible for the death of 3787 people in the Bhopal India in 1984.

**Table 4: Technical information of polymer matrices considered to stabilize insecticides**

Name	Grade	Melting point/ °C	MFI <sup>§</sup>	Supplier	
<b>LDPE</b>	LT 1050	120	20	Sasol	
	LT 033	120	0.33		
<b>HDPE</b>	F7650	130	0.33	Safripol	
<b>Polycaprolactone</b>	Capa 6500	58-60	6-8	Solvay Interlox	
	Capa 6800	58-60	2-4		
<b>Ethylene Vinyl Acetate (EVA)</b>	Elvax 210 (28% VA)	60	7	DuPont	
	Repsol PA440 (28% VA)	75	7		
	Seetec VS 430 (19% VA)	84	2.5		Honan
					Petrochemical
<b>Polyamide</b>	Euremelt 2130	125-135 <sup>#</sup>	3.4-4.6*	Huntsman	
	Euremelt 2140	135-145 <sup>#</sup>	7.6 – 10**		

<sup>§</sup>MFI units are g/10 min@ 190 °C; 2.16 kg except for poly(caprolactone) for which they are given at 160 °C using 2.16 kg for CAPA 6500 and 5.0 kg for CAPA6800; <sup>#</sup>Softening point; \* Viscosity at 200 °C in Pa s; \*\* Viscosity at 220 °C in Pa s.

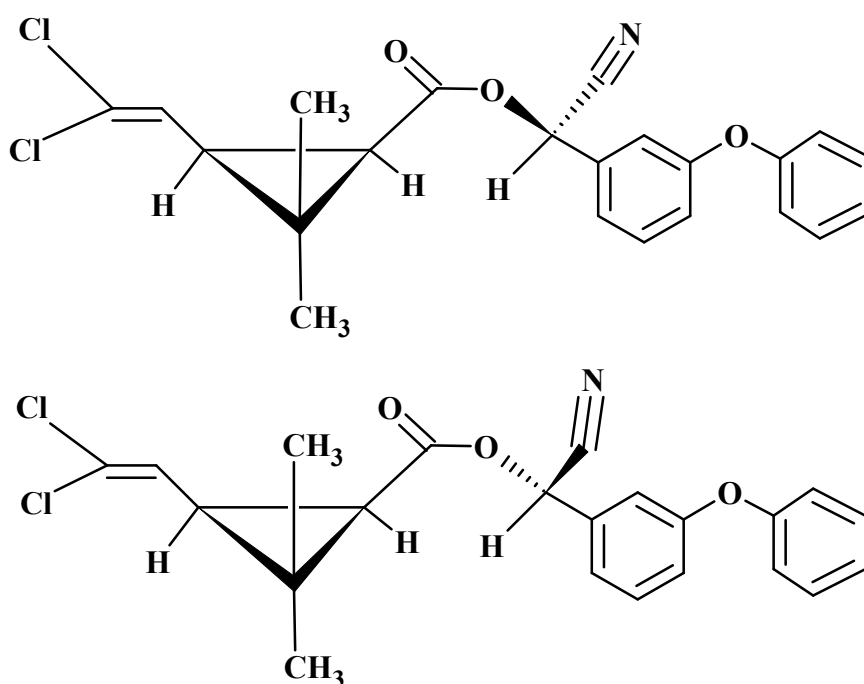
The methyl isocyanate gas was released from a fertilizer plant owned by Union Carbide after an industrial accident. It is highly possible that in rural areas where malaria is mostly prevalent people may use these wall linings as fuel to light fires. Subsequent thermal degradation of carbamates will likely release gases containing isocyanate functional groups leading to possible deaths. Carbamates were therefore not considered for stabilisation using polymer matrices.

Two types of pyrethroids and two types of organophosphate insecticides were considered for use in the manufacture of polymer wall linings. It is prudent to list the physical and chemical properties of these insecticides are described in the following section.

### 4.3.1 Physical properties of pyrethroids and organophosphates considered

#### 4.3.1.1 Alphacypermethrin

Technical alphacypermethrin is a white, water wetttable powder. Alphacypermethrin is described by the International Union of Pure And Applied Chemistry (IUPAC) as a racemic mixture of (S)- $\alpha$ -cyano-3-phenoxybenzyl-(1R, 3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (R)- $\alpha$ -cyano-3-phenoxy-benzyl-(1S,3S)-3(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate. Figure 3 and Table 5 show the molecular structure and the physical and chemical properties of alphacypermethrin respectively. Analysis of alphacypermethrin can be done by GC and IR spectroscopy.



**Figure 3: Molecular structure of alphacypermethrin**

**Table 5: Physical and chemical properties of alphacypermethrin (WHO, 2009)**

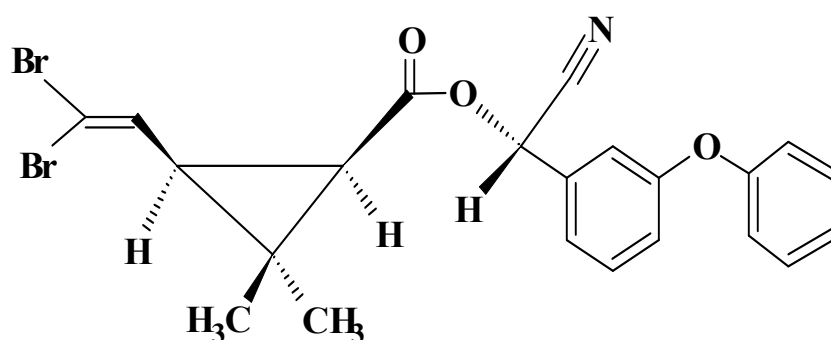
CAS registry number	67375-30-8
Molecular formula	C <sub>22</sub> H <sub>19</sub> Cl <sub>2</sub> NO <sub>3</sub>
Molar mass	416.3
Thermal properties	Melting point is 81–83°C at 95% purity
Density	1.329 ± 0.06 g.cm <sup>-3</sup> at 20°C
Vapour pressure	9.0 × 10 <sup>-6</sup> Pa at 25°C and 95% purity
Solubility in water	6 µg.l <sup>-1</sup> at 20 ± 0.5°C, pH ≈ 7 and 97.8% purity

Octanol water partition coefficient  $\text{Log } P_{\text{ow}}$  is 6.64 at 25°C and 95% purity

Toxicity,  $\text{LD}_{50}$  360mg.kg<sup>-1</sup> body weight (male rat)

#### 4.3.1.2 Deltamethrin

Technical deltamethrin is an off-white, crystalline, water wettable powder. Deltamethrin has eight stereoisomers and the isomers exhibit the structure in Figure 4. It is characterised by HPLC retention time, thin layer chromatography (TLC), IR, NMR and MS (WHO, 2010a). Table 6 shows the physical and chemical properties of deltamethrin.



**Figure 4: Molecular structure of deltamethrin**

**Table 6: Physical and chemical properties of deltamethrin (WHO, 2010a)**

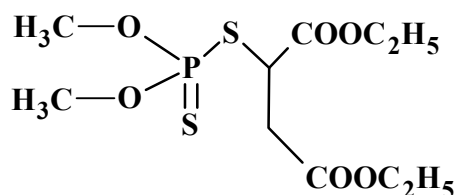
IUPAC name	(S)- $\alpha$ -cyano-3phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate
CAS registry number	52918-63-5
Molecular formula	C <sub>22</sub> H <sub>19</sub> Br <sub>2</sub> NO <sub>3</sub>
Molar mass	505.2g.mol <sup>-1</sup>
Thermal properties	Melting point is 99°C (decomposition temperature >300°C)
Density	1.595 ± 0.06g.cm <sup>-3</sup> at 20°C
Vapour pressure	1.24 × 10 <sup>-7</sup> Pa at 25°C and 98% purity
Solubility in water	0.0907mg.L <sup>-1</sup> at 25°C and 98% purity
Octanol water partition coefficient	Log $P_{\text{ow}}$ is 4.61 at 25°C and 98% purity
Toxicity, $\text{LD}_{50}$	87.4mg.kg <sup>-1</sup> body weight (male rat)

#### 4.3.1.3 Malathion

Technical malathion is a colourless, oily liquid with a characteristic pungent smell. Malathion is identified by GC retention time and IR spectroscopy (WHO, 2004). Table 7 and Figure 5 show the molecular structure and the physical and chemical properties of malathion respectively.

**Table 7: Physical and chemical properties of malathion (WHO, 2004)**

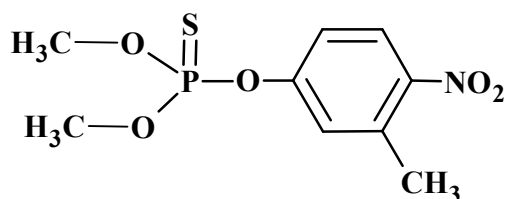
IUPAC name	<i>S</i> -1,2-bis(ethoxycarbonyl)ethyl <i>O,O</i> -dimethyl phosphorodithioate
CAS registry number	121-75-5
Molecular formula	C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub>
Molar mass	330.36g.mol <sup>-1</sup>
Thermal properties	Decomposition temperature > 174°C at 99.1% purity
Density	1.272 ± 0.06g.cm <sup>-3</sup> at 20°C
Vapour pressure	4.5 × 10 <sup>-4</sup> Pa at 25°C and 98.9% purity
Solubility in water	148mg.L <sup>-1</sup> at 25°C and 98.4% purity
Octanol water partition coefficient	log P <sub>ow</sub> is 2.7 at 25°C and 98% purity
Toxicity, LD <sub>50</sub>	1768mg.kg <sup>-1</sup> body weight (male rat)



**Figure 5: Molecular structure of malathion**

#### 4.3.1.4 Fenitrothion

Technical fenitrothion is a reddish-brown, oily liquid with a characteristic pungent smell. It can be identified by using HPLC retention time and IR spectroscopy. Table 8 and Figure 6 show its molecular structure, physical and chemical properties.



**Figure 6: Molecular structure of fenitrothion**

**Table 8: Physical and chemical properties of fenitrothion (WHO, 2010b)**

IUPAC name	<i>o,o</i> -dimethyl <i>o</i> -4-nitro- <i>m</i> -tolyl phosphorothioate
CAS registry number	112-14-5
Molecular formula	C <sub>9</sub> H <sub>12</sub> NO <sub>5</sub> PS
Molar mass	277.25g.mol <sup>-1</sup>
Thermal properties	Melting point is -1°C ± 1°C
Density	1.229 ± 0.06g.cm <sup>-3</sup> at 20°C
Vapour pressure	1.57 × 10 <sup>-3</sup> Pa at 25°C and 99.1% purity
Solubility in water	19.0mg.L <sup>-1</sup> at 20 ± 0.5°C and 99.1% purity
Octanol water partition coefficient	Log P <sub>ow</sub> is 3.319 ± 0.080 at 25°C and 99.3% purity
Toxicity, LD <sub>50</sub>	1 700mg.kg <sup>-1</sup> body weight (male rat)

#### 4.3.1.5 Pirimiphos-methyl

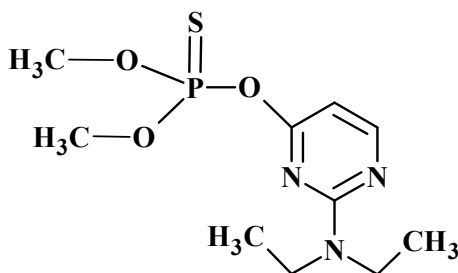
Technical pirimiphos-methyl is an orange, oily liquid with a characteristic pungent smell.

Pirimiphos-methyl is identified using ultraviolet/visible (UV/VIS) spectroscopy, IR spectroscopy, NMR and MS (FAO, 2007). Table 9 and Figure 7 show the molecular structure and the physical and chemical properties of pirimiphos-methyl respectively.

**Table 9: Physical and chemical properties of pirimiphos methyl (FAO, 2007)**

IUPAC name	<i>O</i> -2-diethylamino-6-methylpyrimidin-4-yl- <i>O,O</i> -dimethyl phosphorothioate
CAS registry number	29232-93-7
Molecular formula	C <sub>11</sub> H <sub>20</sub> N <sub>3</sub> O <sub>3</sub> PS <sub>2</sub>
Molar mass	305.3g.mol <sup>-1</sup>
Thermal properties	Melting point is -20°C at 99.1%
Density	1.229±0.06g.cm <sup>-3</sup> at 20°C
Vapour pressure	2.0×10 <sup>-6</sup> at 20°C and 99% purity
Solubility in water	10mg.L <sup>-1</sup> at pH 7, 20°C and 99% purity
Octanol water partition coefficient	Log P <sub>ow</sub> is 4.2 at pH 7, 25°C and 99% purity
Toxicity, LD <sub>50</sub>	1414mg.kg <sup>-1</sup> body weight (male rat)





**Figure 7: Molecular structure of pirimiphos-methyl**

#### 4.4 Work plan

This work considered the development of pyrethroid and organophosphate impregnated polymer wall linings. These linings were to be produced using simple extrusion through a die to form a net directly. It was envisaged that a successful product will be tested using standard WHO laboratory tests followed by standard WHO field tests. The linings to be produced were to be different compared to current commercial linings. They were intended to cover the upper parts of inner walls of a dwelling where blood fed mosquitoes are likely to rest.

##### 4.4.1 Pyrethroid mesh linings

Work was started with the most promising product development i.e. pyrethroids incorporated in polyethylene to form a mesh lining. Initial work comprised of the masterbatching of deltamethrin and alphacypermethrin in low density polyethylene followed by extrusion film blowing of the masterbatch with a 1:1 weight ratio blend of HDPE and LDPE at different pyrethroid concentrations to form a bilayer film. The reason for initially blowing a bilayer film is explained as follows. It was of paramount importance to ensure that the compounding of selected polymers and insecticides does not release any fumes. Such a scenario will be unsafe in a manufacturing plant set up. To test whether it is safe to compound selected polymers with insecticides, a bilayer film was blown using a polymer melt containing typical insecticide loadings under consideration. The outer layer consisted of a neat polymer film whilst the inner layer consisted of the polymer mixed with the insecticide. If any fumes were formed due to processing, such fumes will be visible to the naked eye and would be trapped inside the blown film bubble. Once it was established that it was safe to compound the polyethylene with pyrethroids at the different concentrations then single layer films containing the insecticides at different concentrations were blown. These single layer films were tested for efficacy using the WHO recommended bioassay tests. The reason for considering different insecticide concentrations was to estimate the minimum concentration

required to pass the standard WHO bioassay tests. Concentrations ranging from 0.27wt.% to 0.85wt.% were selected as initial estimates of the effective insecticide concentration. All the films easily passed the WHO bioassay tests. Lastly the polymer mesh was similarly manufactured by the process of masterbatching followed by a simple extrusion process with insecticide loadings considered above. To test whether a netlon mesh lining with a soft feel could be produced, EVA was also used as a base polymer to manufacture the Netlon lining. All manufactured linings underwent standard WHO recommended laboratory bioassay tests followed by field trials.

#### **4.4.2 Organophosphate mesh linings**

The development of this product was a very challenging as there is no other similar product that has ever been developed. The liquid insecticides were masterbatched into the selected polymers. Malathion and Fenitrothion in EVA were extruded through a die to form mesh linings. Fenitrothion and malathion in polycaprolactone, polyamide and EVA-polyamide blend were extruded into filaments that were manually meshed using a polyethylene template. Products developed were tested using the WHO standard bioassay tube tests.

### **4.5 Experimental**

#### **4.5.1 Materials**

LDPE powder (Sasol grade LT 1050) and pellets (Sasol grade LT 033) were sourced from Sasol. HDPE (Safripol grade F7650) was sourced from Safripol. Polycaprolactone (Capa 6500 and Capa 6800) were sourced from Solvay Interlox Ltd. EVA (Elvax 210) was sourced from Dupont. Polyamides Euremelt 2130 and Euremelt 2140 were sourced from Huntsman. Table 4 and Appendix 6 presents the specification sheets of polymers used in this study.

Alphacypermethrin (ca. 95% technical) was supplied by Bilag; deltamethrin (ca. 98% technical) was supplied by Tagros. Pirimiphos methyl (ca. 90% technical) was supplied by Avima, malathion (ca. 95% technical) and fenitrothion (ca. 90 % technical) were supplied by Agrochina.

## 4.5.2 Equipment and methods

### 4.5.2.1 Compounding

A 40 mm Berstorff Model EV 40 co-rotating twin screw machine with an L/D of 42 fitted with two kneader block mixer sections was used for the extrusion. Granulation was done using a LabTech Engineering model LSC 108 pelletizer. A Rapra CTM 25 mm laboratory extruder was used to extrude polymer filaments to mesh manually on a polyethylene template. Prepared mixtures were fed into the Berstorff compounder via a screw conveyor. The exiting polymer strands were then cooled using a water bath and then granulated using the LabTech pelletizer.

**Pyrethroid masterbatching.** For safety reasons, initial trials involved blowing bilayer polyethylene films with pyrethroids incorporated in the inner layer only. Once it was established that it is safe to compound pyrethroids in polyethylene further work was done by melt extruding low density polyethylene (LDPE) powder (Sasol grade LT 1050) and pellets (Sasol grade LT 033) as joint carrier polymers for the 10wt.% alphacypermethrin and 18wt.% deltamethrin masterbatch (Table 10).

**Organophosphate masterbatching.** These actives have a low thermal stability that limits the processing temperature that could be used. Thus polymers with low melting points were used as carriers i.e. polycaprolactone and EVA (Elvax 210). The active in liquid form was mixed with the polymer pellets and the precipitated silica to form a dry blend that was then compounded (Table 10). Flat temperature profiles were used: 90°C, 90°C and 140°C for the malathion and fenitrothion masterbatches respectively.

**Table 10: Insecticides compounded into masterbatches**

Insecticide masterbatch	Active (wt.%)	Carrier polymers	Composition (wt.%)
Alphacypermethrin	10	LT1050	45
		LT033	45
Deltamethrin	18	LT1050	82
Pirimiphos Methyl	10	Capa 6500	31.4
		Capa 6800	53.9
		Ultrasil VN3	4.6

Malathion	10	Elvax 210	84.9
		Ultrasil VN3	5.1
Fenitrothion	4.65	Elvax 210	46.5
		Euremelt 2140	46.5
		Ultrasil VN3	2.3

#### 4.5.2.2 Extrusion and meshing

**Netlon meshing.** The Netlon® mesh samples were produced on a commercial line at the Huhthamaki factory in Springs. The line comprised a 60 mm diameter Bandera extruder with L/D of 21. The extruder speed was varied between 38 rpm and 64 rpm. Standard colour masterbatches, used in the manufacture of Netlon, were used to tag the insecticide-containing samples for identification purposes.

**Alphacypermethrin and deltamethrin in polyethylene blend.** Monofilament woven grids and Netlon meshing containing pyrethroid insecticides in a 1:1 blend of LDPE (LT033) and HDPE (F7650) were produced. The insecticides were incorporated via masterbatches (Table 16). The meshing produced was 380 mm wide and had a mass of ca. 82 g/m<sup>2</sup>. The extrusion temperature profile is shown in Table 11.

**Table 11: Alphacypermethrin and deltamethrin in polyethylene blend extrusion temperature profile**

Feed	Zone1	Zone 2	Zone 3	Screen changer	Die body	Die lip
160-170 °C	180 °C	190-195 °C	220-225 °C	216 °C	215 °C	218 °C

**Alphacypermethrin in LDPE + EVA blend.** The purpose of this trial was to see if a Netlon with a softer feel could be made using a 1:1 blend of LDPE (LT 033 ex Sasol Polymers) and EVA (Seetec VS 430 ex Honan Petrochemical) (Table 16). The 10% alphacypermethrin masterbatch was dosed at the 5% add on level. 1% each of a yellow and a green masterbatch was added as well. The temperature profile along the extruder starting from the feed section, via three heating zones to the screen changer, die body and die lips as shown in Table 12.

**Table 12: Alphacypermethrin in LDPE/EVA blend extrusion temperature profile**

Feed	Zone1	Zone 2	Zone 3	Screen changer	Die body	Die lip
185 °C	180 °C	200 °C	225 °C	200 °C	215 °C	230 °C

**Alphacypermethrin in EVA.** The purpose of this trial was to see if a Netlon with a very soft feel could be made using EVA (Seetec VS 430 ex Honan Petrochemical) (Table 16). The 10% alphacypermethrin masterbatch was dosed at the 5% add on level. 1% each of a yellow and a red masterbatch was added as well. The temperature profile along the extruder starting from the feed section, via three heating zones to the screen changer, die body and die lips as shown Table 13.

**Table 13: Alphacypernethrin in EVA extrusion temperature profile**

Feed	Zone1	Zone 2	Zone 3	Screen changer	Die body	Die lip
125 °C	150 °C	165 °C	170 °C	155 °C	160 °C	175 °C

**Malathion in EVA.** The purpose of this trial was to see if a Netlon with a very soft feel could be made using EVA (Repsol PA440) (Table 16). The 10% malathion masterbatch was dosed at the 5.9% add on level. Orange masterbatch was added at 2% add on. The extruder speed was set at 23 rpm and temperature profile along the extruder starting from the feed section, via three heating zones to the screen changer, die body and die lips as shown in Table 14.

**Table 14: Malathion in EVA extrusion temperature profile**

Feed	Zone1	Zone 2	Zone 3	Screen changer	Die body	Die lip
125 °C	150 °C	165 °C	170 °C	155 °C	160 °C	175 °C

**Fenitrothion in EVA.** The purpose of this trial was to see if a Netlon® with a very soft feel could be made using EVA (Repsol PA440) (

Table 16). The 4.7% fenitrothion masterbatch was dosed at 11.8% add on level. Green masterbatch was added at 2% add on level. The extruder speed was set at 23 rpm and temperature profile along the extruder starting from the feed section, via three heating zones to the screen changer, die body and die lips was as shown in Table 15.

**Table 15: Fenitrothion in EVA extrusion temperature profile**

Feed	Zone1	Zone 2	Zone 3	Screen changer	Die body	Die lip
125°C	150°C	165°C	170°C	155°C	160°C	175°C

The Netlon mesh produced was ca. 320mm wide and had a mass of ca. 80 – 120g/m<sup>2</sup>.

**Manually woven mesh.** Thin polymer strands were extruded on a Rapra CTM 25mm laboratory extruder. The emerging filaments were pulled through a water bath and they were ca. 0.5mm thick. They were woven into a mesh using a polyethylene mesh as template. The masterbatches were added, at various let-down levels. Final active compositions and extrusion temperature profiles are shown in Table 17.

**Table 16: Extruded Netlon® mesh samples**

Insecticide	Base polymer	MB add on (wt.%)	Final active (wt.%)
Alphacypermethrin	LDPE (LT 033)	2.9	0.29
	HDPE (F7650)		
Alphacypermethrin	LDPE (LT 033)	4.7	0.47
	HDPE (F7650)		
Deltamethrin	LDPE (LT 033)	2.9	0.52
	HDPE (F7650)		
Deltamethrin	LDPE (LT 033)	4.7	0.85
	HDPE (F7650)		
Alphacypermethrin	LDPE (LT 033)	10	0.47
	HDPE (F7650)		

Alphacypermethrin	EVA (Seetec VS 430)	10	0.47
Malathion	EVA (Repsol PA440)	5.5	0.55
Fenitrothion	EVA (Repsol PA440)	10.53	0.49

**Table 17: Extruded and manually woven filament samples (10% masterbatch add-on)**

Insecticide	Base polymer	Active (wt.%)	Temperature profile (°C)
Fenitrothion	PCL (Capa 6500)	0.47	67/90/90/110
Pirimiphos methyl	PCL (Capa 6500)	1.0	80/150/160/170
Fenitrothion	Polyamide (Euremelt 2130)	0.47	67/90/90/110
Fenitrothion	Polyamide (Euremelt 2130)	0.47	80/150/160/170
	EVA (Elvax 210)		

#### 4.5.2.3 Microscopy

**Scanning electron microscope (SEM).** The surface morphology of neat insecticides and Netlon® filament samples were examined by low-resolution scanning electron microscopy (SEM) using a JEOL JSM-5800LV instrument.

A small quantity of neat insecticide powders and Netlon® filament samples were placed onto carbon tape on an aluminium sample holder. Excess powder was removed using a single compressed air blast. The samples were then coated two times with gold under vacuum at 0.1 mbar for four minutes respectively. The coating was done using an EMITECH K550X SEM auto-coating unit.

**TEM.** Samples were pre-treated by means of cryo-ultramicrotomy and sliced at -120°C using a diamond knife. Each section was then mounted on a 300 mesh copper grip and viewed. The cross-sectional analysis of Netlon® filaments was carried out on a JEOL 2100 microscope. The accelerating voltage was 200kV. Samples were prepared by means of cryo-ultramicrotomy in a Leica EM UC6 with a cryo-attachment.

**Confocal fluorescence microscopy (CFM).** The spatial distribution of alphacypermethrin and deltamethrin in the fibres was investigated by confocal laser scanning microscopy (CFM)

on a Zeiss 510 META instrument (Carl Zeiss, Jena, Germany). It employed a Plan Apochromat 20X objective and a laser with a wavelength of 405 nm. Small amounts of samples were mounted on a microscope cover slip. The slide was then inserted into the microscope for viewing.

#### 4.5.2.4 Thermal analysis

**Thermo gravimetric analysis (TGA).** TGA was performed on a Mettler Toledo STAR<sup>e</sup> System. Samples weighing ca. 30mg were placed in an open 70  $\mu$ L alumina pan. The temperature was scanned from 25 to 300°C at a scan rate of 2°C/min. Throughout the experiment the TG cell was purged with nitrogen flowing at a rate of 50 mL/min.

**Differential scan calorimetry (DSC).** Melting and crystallization behaviour of the insecticides and the Netlon<sup>®</sup> filament was studied by DSC on a Perkin-Elmer DSC 7 instrument. A small amount of sample (ca. 10mg) was placed in a 40 $\mu$ L aluminium sealed pan. The temperature was scanned at 20°C/min from 25°C to 200°C. The sample was then held isothermally for 1 minute at 200°C and then cooled to 20°C at 20°C/min.

#### 4.5.2.5 Bioassay testing

Standard WHO bioassays tube tests (WHO, 2006) were used to evaluate the residual efficacy against mosquitoes. In these tests, 25 non-blood-fed female *An. arabiensis* mosquitoes (KGB colony housed at the National Institute for Communicable Diseases, Johannesburg), 3-5 days old, were exposed for 30 minutes to Netlon<sup>®</sup> samples. Knockdown was recorded 30 minutes after end of exposure and mortality was recorded 24 hours after initial exposure. During the 24 hour waiting period, mosquitoes were given sugar solution as nourishment. For field trials samples of these nets were taken periodically over a period of 24 months for bioassay efficacy testing. A typical bioassay tube test set up for the Netlon<sup>®</sup> is shown in Figure 8.





**Figure 8: Typical WHO bioassay tube test set up**

## **4.6 Results**

### **4.6.1 Bioassay tests**

Table 18 lists the bioassay results for the Netlon® samples and the woven monofilaments respectively. The WHO effectiveness criterion for IRS is a mortality exceeding 80% after 24 hours following a 30 minute exposure. The knock down (KD) after 60 minutes only provides a secondary indication of effectiveness i.e. the bioavailability of the insecticide to the mosquitoes coming into contact with it. The data in Table 18 reveals that pyrethroid samples in polyethylene passed the WHO bioassay test. Samples of insecticides incorporated into EVA and polyamide failed the WHO effectiveness criterion. Raw bioassay results are presented in Appendix 1. The process of producing Netlon meshes containing pyrethroid insecticides via extrusion through a die was patented. These meshes were taken for further studies.

**Table 18: WHO tube bioassays on Netlon® formulations**

Sample name	Active (wt.%)	Knock down (%)	SD	Mortality (%)	SD
Plain Netlon®	0	0	0	0	0
Alphacypermethrin in polyethylene	0.29	100	0	100	0
Alphacypermethrin in polyethylene	0.47	100	0	100	0
Deltamethrin in polyethylene	0.52	100	0	100	0
Deltamethrin in polyethylene	0.85	100	0	100	0
Alphacypermethrin in 1:1 EVA-LDPE blend	0.47	99	2	59	15
Alphacypermethrin in EVA	0.47	92	8	50	26
Malathion in EVA	0.55	3	2	5	6
Fenitrothion in EVA	0.49	5	6	22	15
Fenitrothion in PCL (woven)	0.47	0	<sup>1</sup> n/a	12	n/a
Pirimiphos methyl in PCL (manually meshed)	1.0	0	<sup>1</sup> n/a	60	n/a
Fenitrothion in EVA-Polyamide blend (1:1) (manually meshed)	0.47	0	<sup>1</sup> n/a	12	n/a
Fenitrothion in Polyamide (manually meshed)	0.47	0	<sup>1</sup> n/a	19	n/a

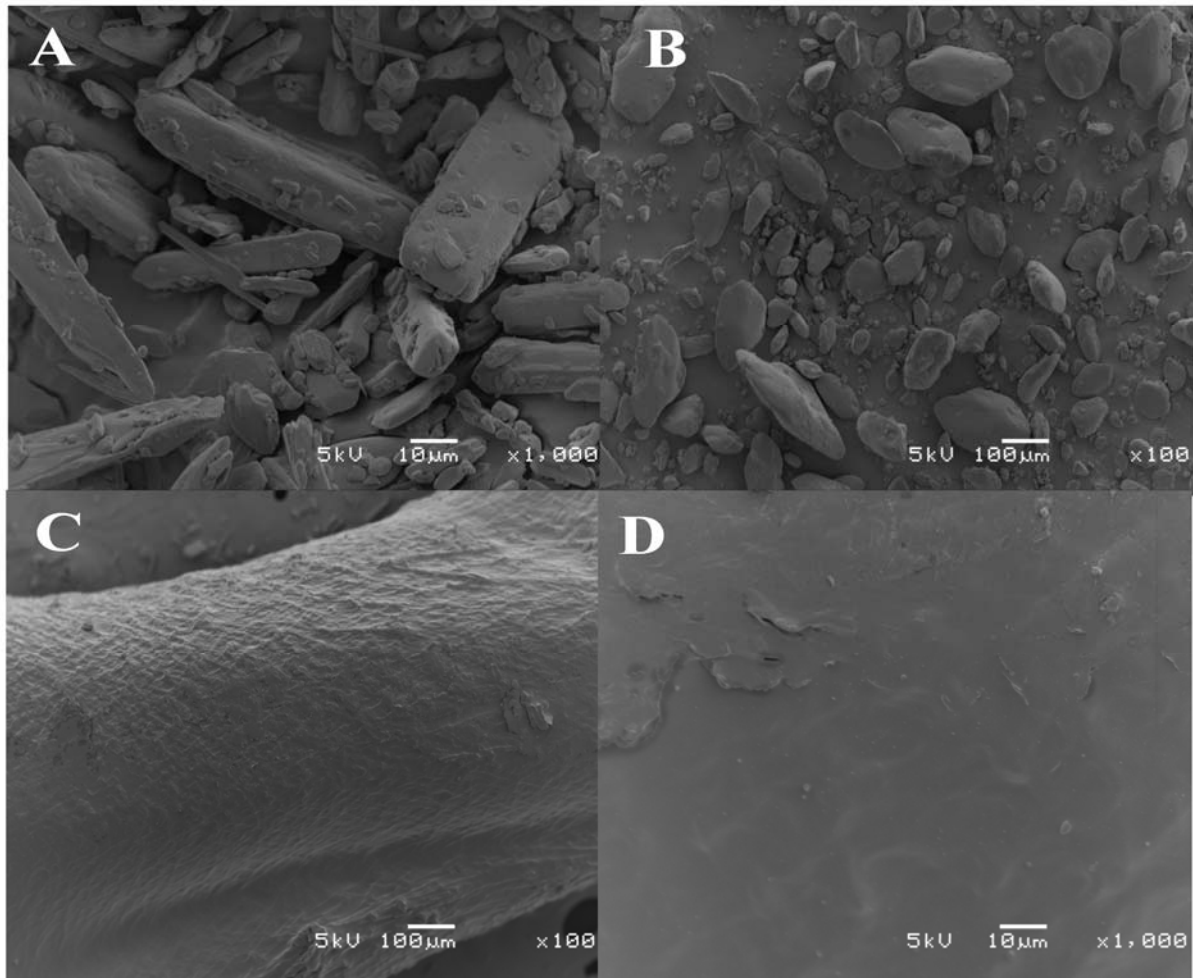
<sup>1</sup>These samples did not have replicates because they were produced using a laborious meshing process hence the standard deviation was not calculated.

## 4.6.2 Microscopy

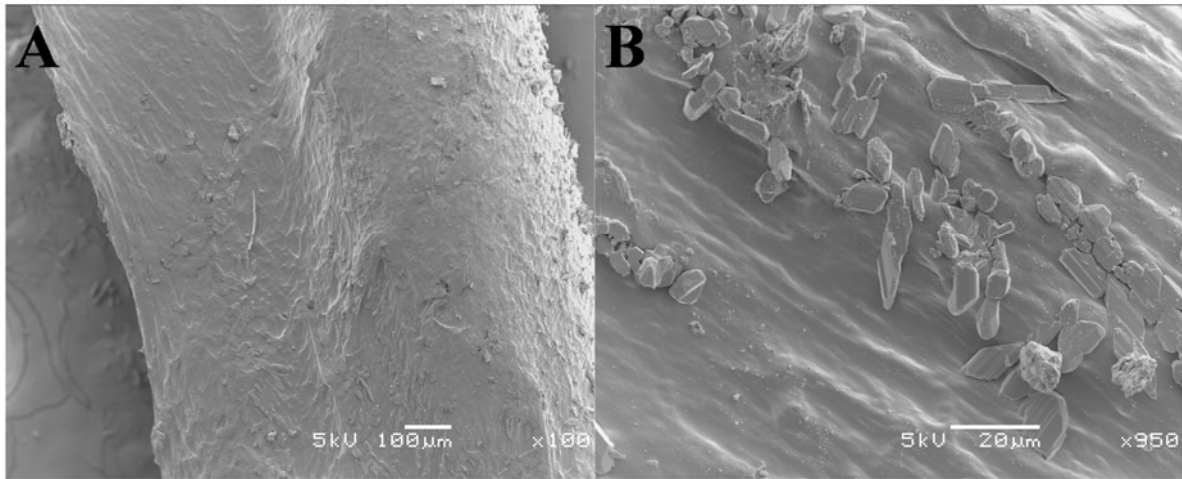
### 4.6.2.1 Scanning electron microscopy (SEM)

Figure 9 shows scanning electron micrographs of neat deltamethrin, neat alphacypermethrin and neat Netlon®. The deltamethrin particles have a needle like morphology whilst alphacypermethrin particles have larger and more spherical particle morphology. It can also be seen that deltamethrin particles are larger than alphacypermethrin particles. Figure 10; Figure 11, Figure 12 and Figure 13 shows SEM micrographs of Netlon® impregnated with

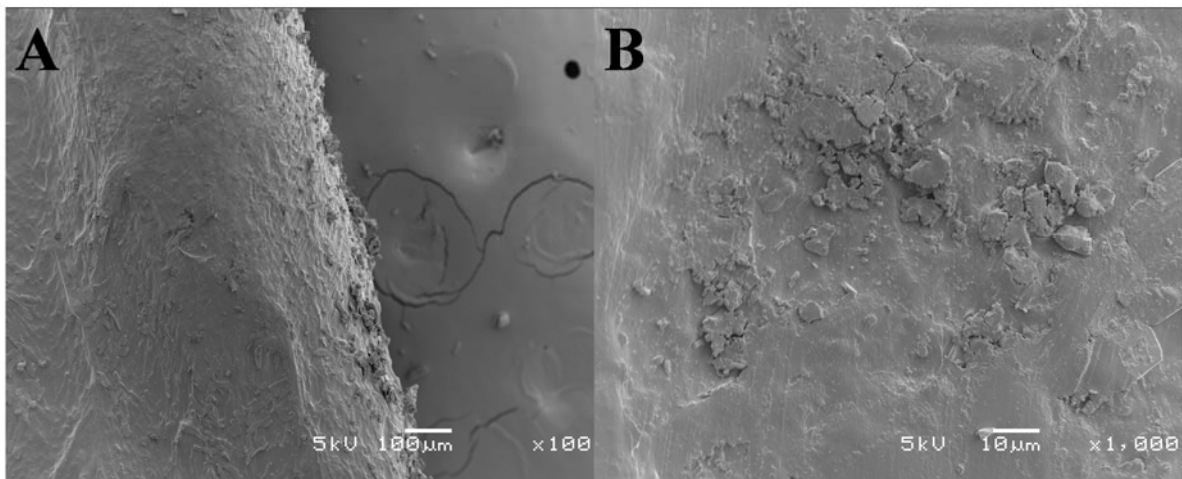
deltamethrin and alphacypermethrin. Insecticide particles are visible on the surface of the polymer filament. Additional results are presented in Appendix 2.



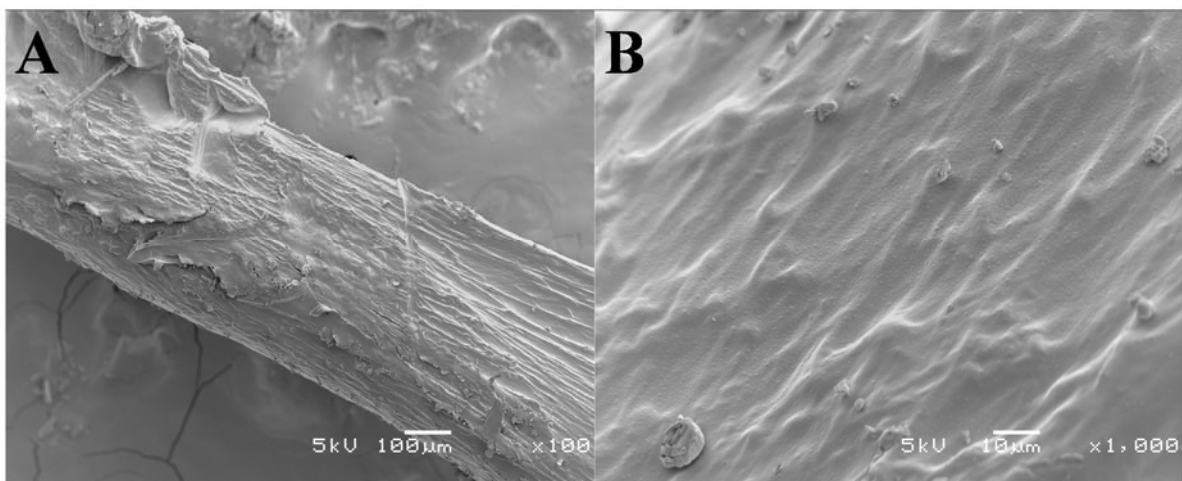
**Figure 9: SEM micrographs of (A) neat deltamethrin (B) neat alphacypermethrin and (C), (D) Neat Netlon® mesh filaments**



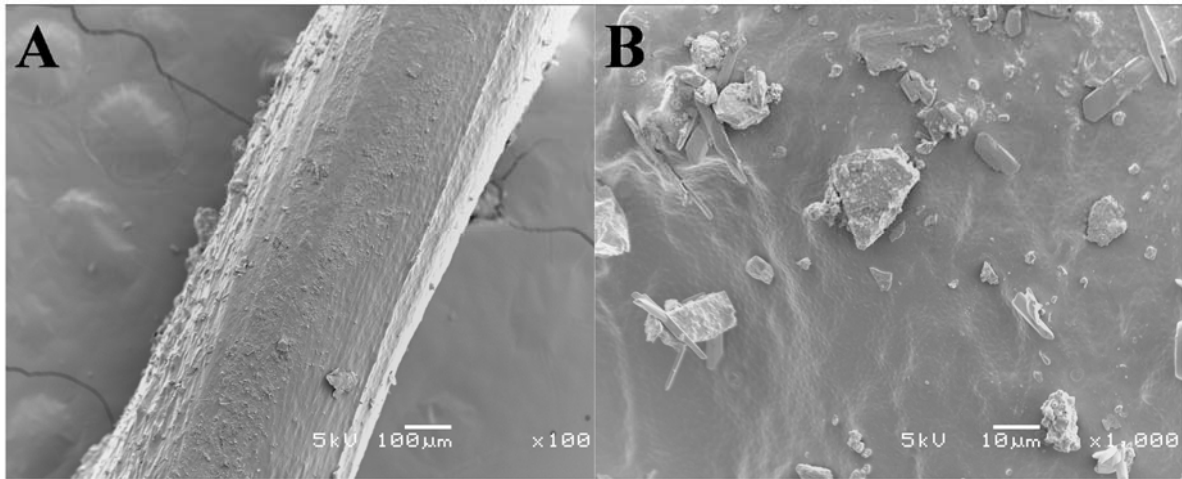
**Figure 10: SEM micrographs of Netlon® filament loaded with 0.27wt.% alphacypermethrin**



**Figure 11: SEM micrographs of Netlon® filament loaded with 0.47wt.% alphacypermethrin**



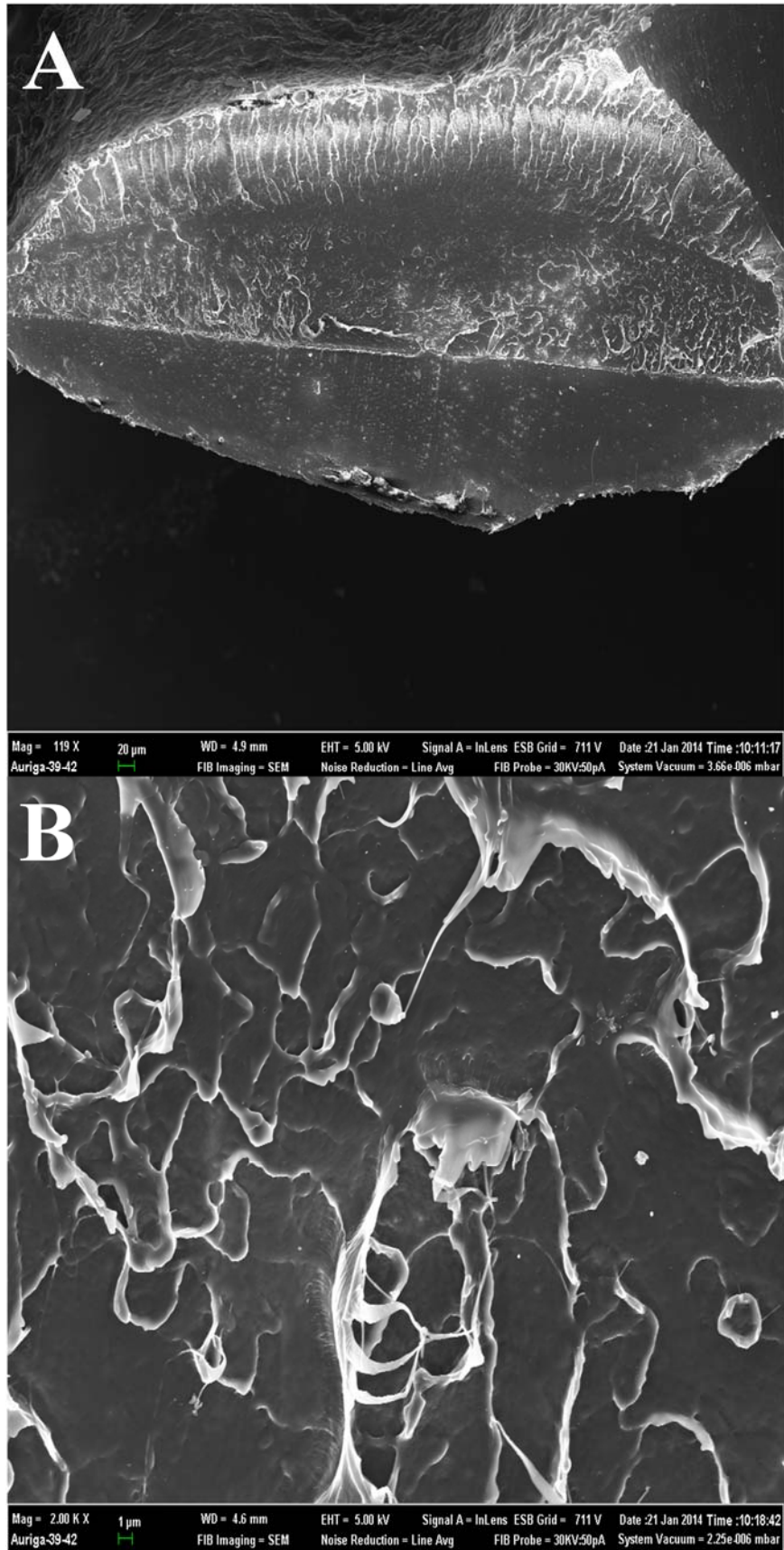
**Figure 12: SEM micrographs of Netlon® filament loaded with 0.52wt.% deltamethrin**



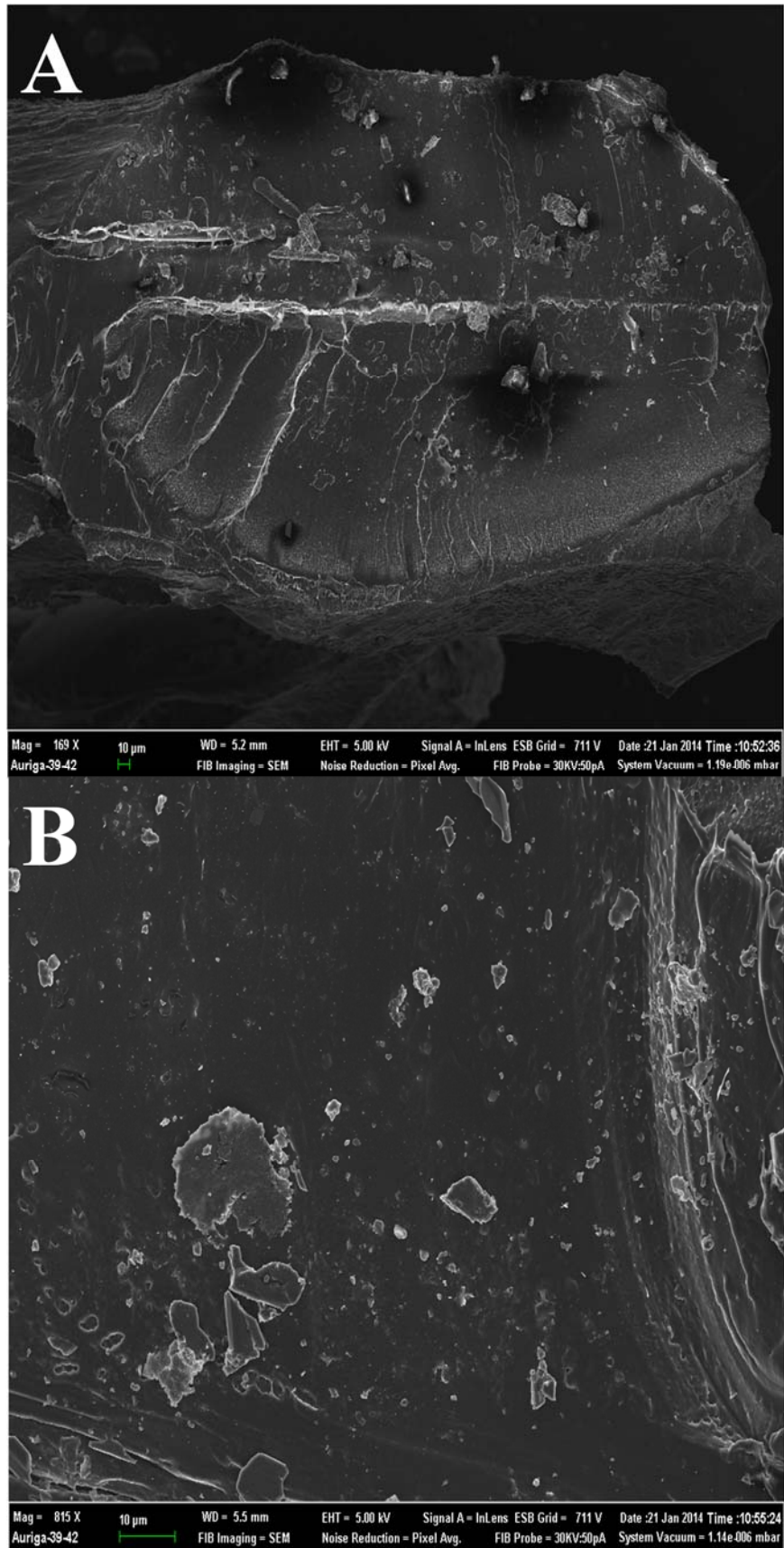
**Figure 13: SEM micrographs of Netlon® filament loaded with 0.85wt.% deltamethrin**

#### 4.6.2.2 Transmission electron microscopy (TEM)

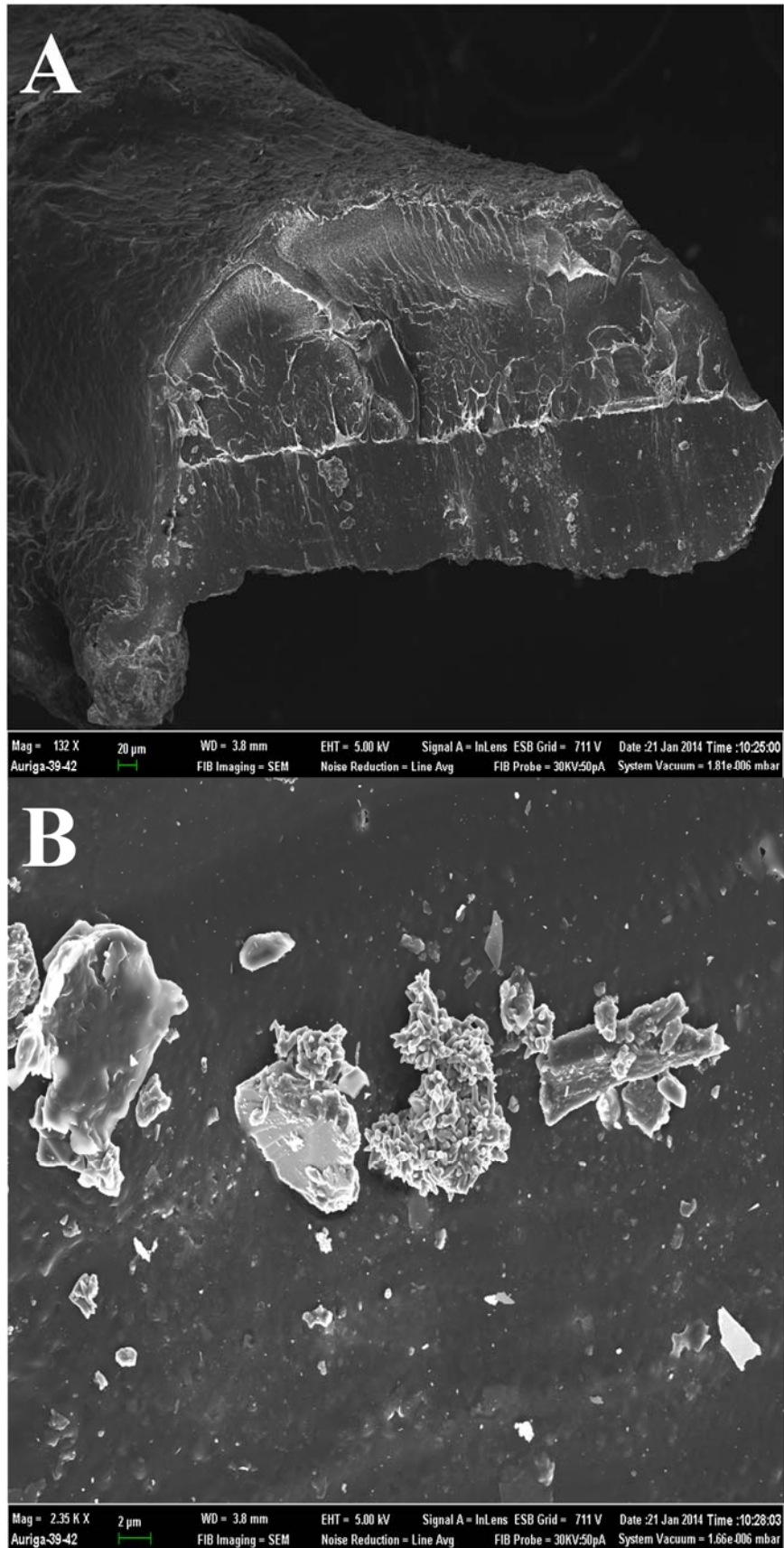
Figure 14, Figure 15, Figure 16, Figure 17 and Figure 18 show TEM micrographs of neat Netlon® linings and Netlon® containing deltamethrin and alphacypermethrin. The micrographs indicate that there is a presence of pyrethroid active within the polymer matrix. Additional results are presented in Appendix 3.



**Figure 14: Transmission electron micrographs of Neat Netlon® filament.** The green scale bar is 20μm in (A) and 1μm in (B).

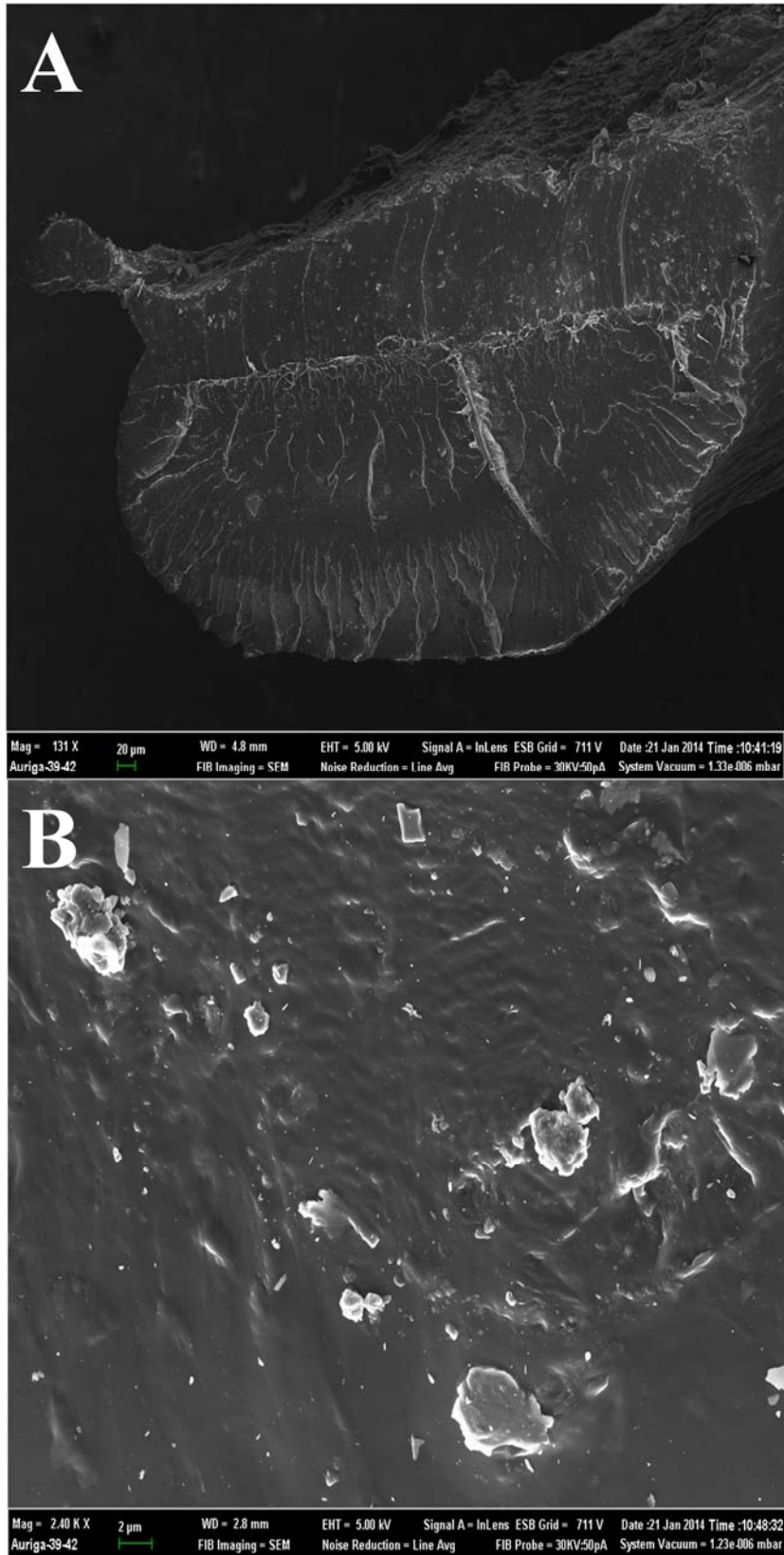


**Figure 15: Transmission electron micrographs Netlon® filament loaded with 0.29wt.% alphacypermethrin. The green scale bar is 10 $\mu$ m in (A) and (B).**

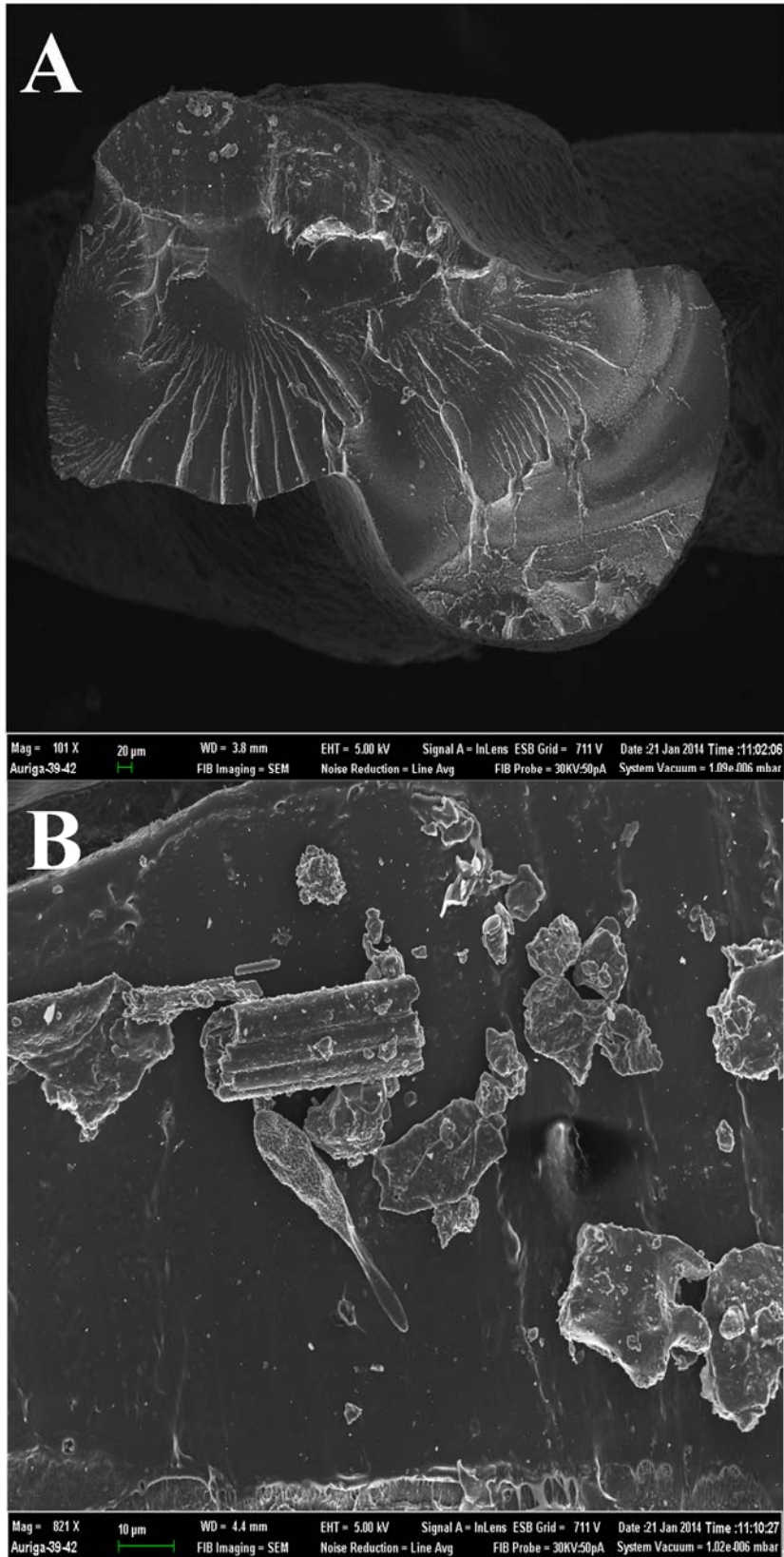


**Figure 16: TEM micrographs Netlon® filament loaded with 0.47wt.% alphacypermethrin. The green scale bar indicates 20 $\mu$ m in (A) and 2 $\mu$ m in (B).**





**Figure 17: TEM micrographs Netlon® filament loaded with 0.52wt.% deltamethrin.**  
The green scale bar indicates 20 $\mu$ m in (A) and 2 $\mu$ m in (B).

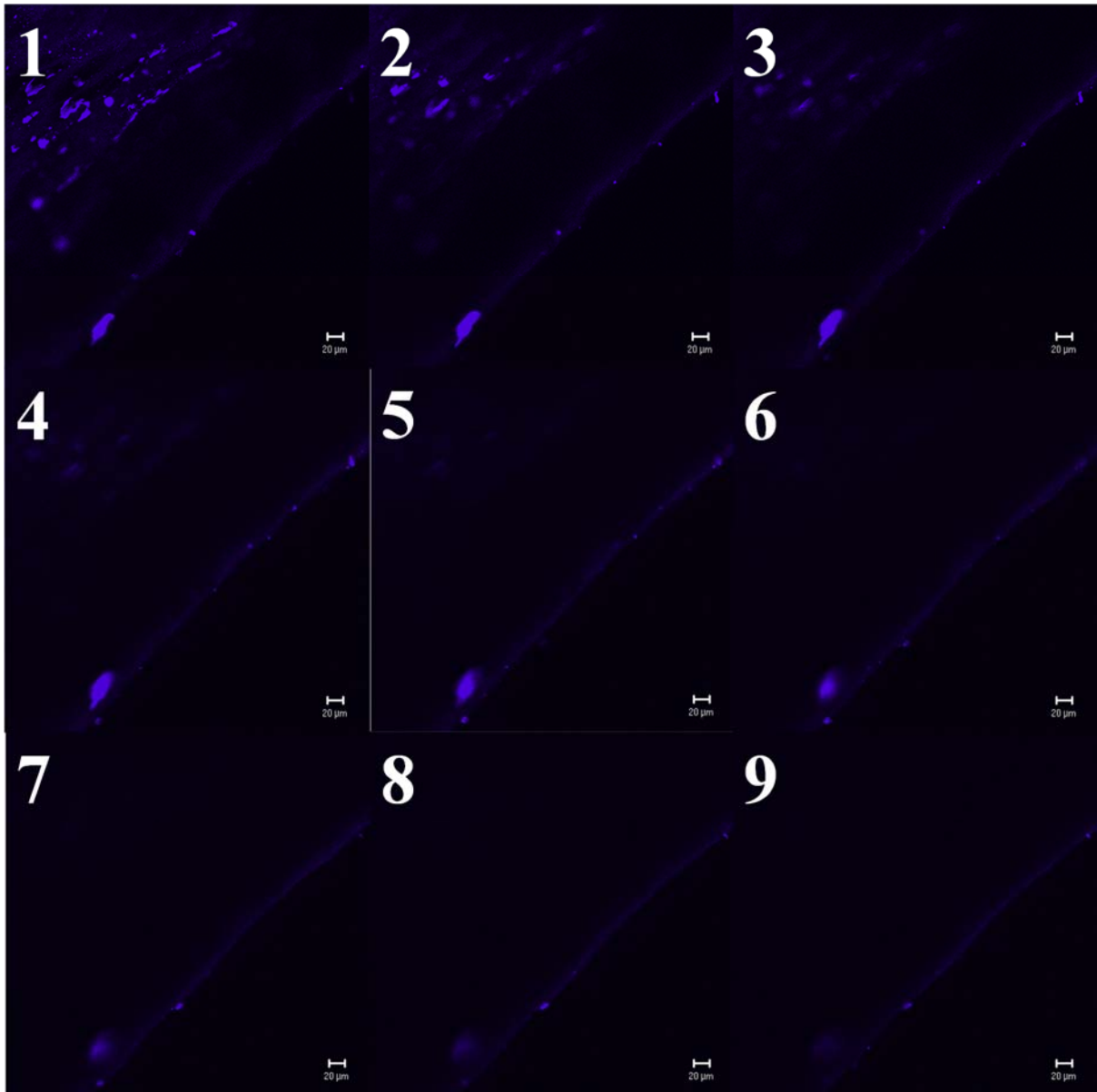


**Figure 18: TEM micrographs Netlon® filament loaded with 0.85wt.% deltamethrin. The green scale bar indicates 20μm in (A) and 10μm in (B).**

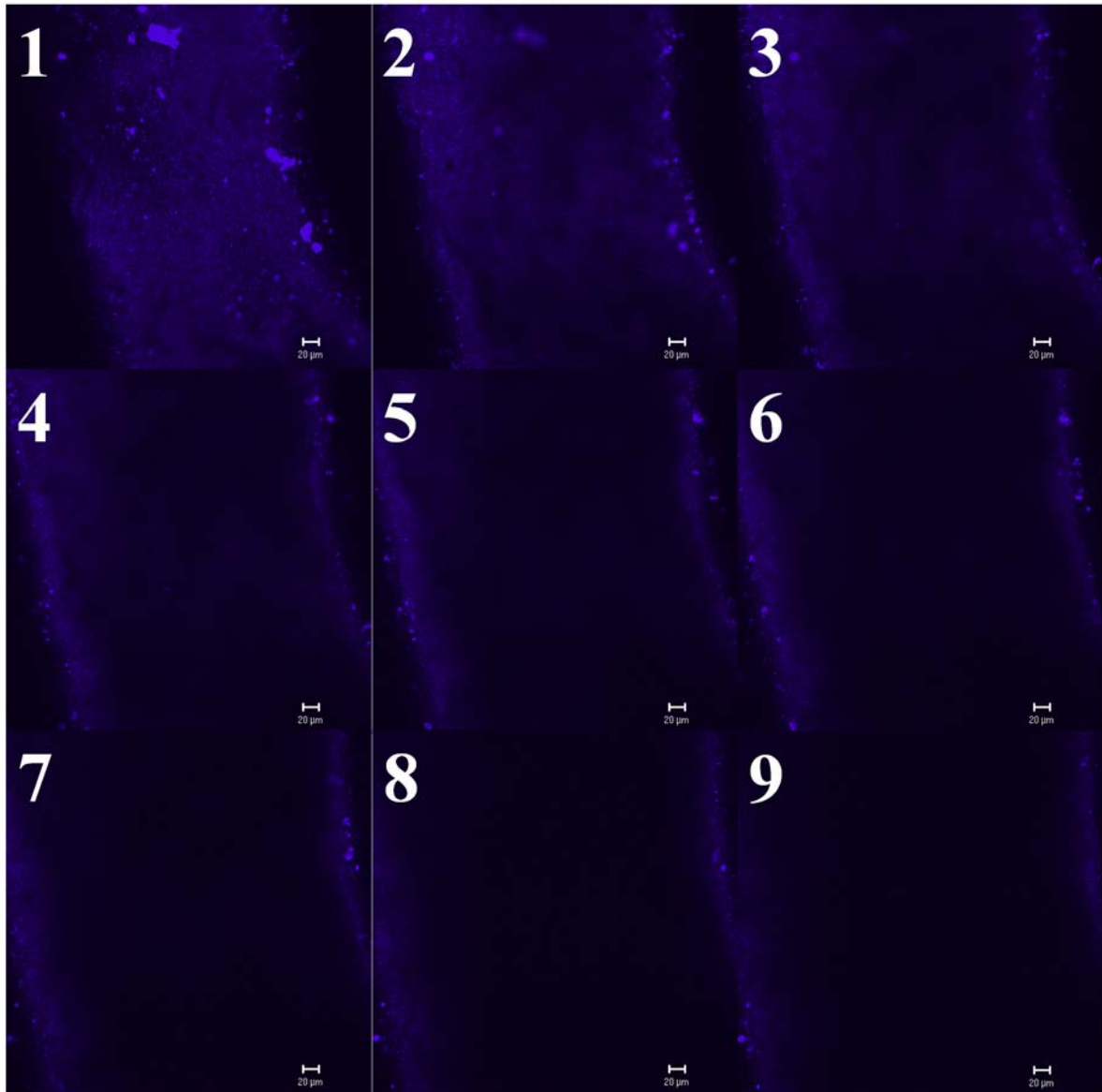
#### 4.6.2.3 CFM

Figure 19, Figure 20, Figure 21 and Figure 22 show images obtained using the CFM technique for Netlon® filament loaded with 0.85wt.% deltamethrin and 0.29wt.% alphacypermethrin. This technique allows for the optical depth profiling of a sample. The fluorescence observed in these micrographs is attributed to the presence of insecticides along a planar cross-section of the sample. The micrographs shown are surface slices of the filament in the XY plane as the laser light focused on fixed distance along the Z direction. Micrograph 1 (Figure 19, Figure 20, Figure 21 and Figure 22) can be attributed to the distribution of the insecticide on the top surface of the filament. There is evidence of agglomeration of actives in some areas. As the laser penetrated the sample (Micrographs 2-9) there is a decrease of insecticide the middle position of the image corresponding to the inside of the polymer filament. A distinct outline of fluorescence is visible on the sides of the image along the axial direction. This indicates that the insecticide is located near the surface of the filament and not in the internal regions of the filament.

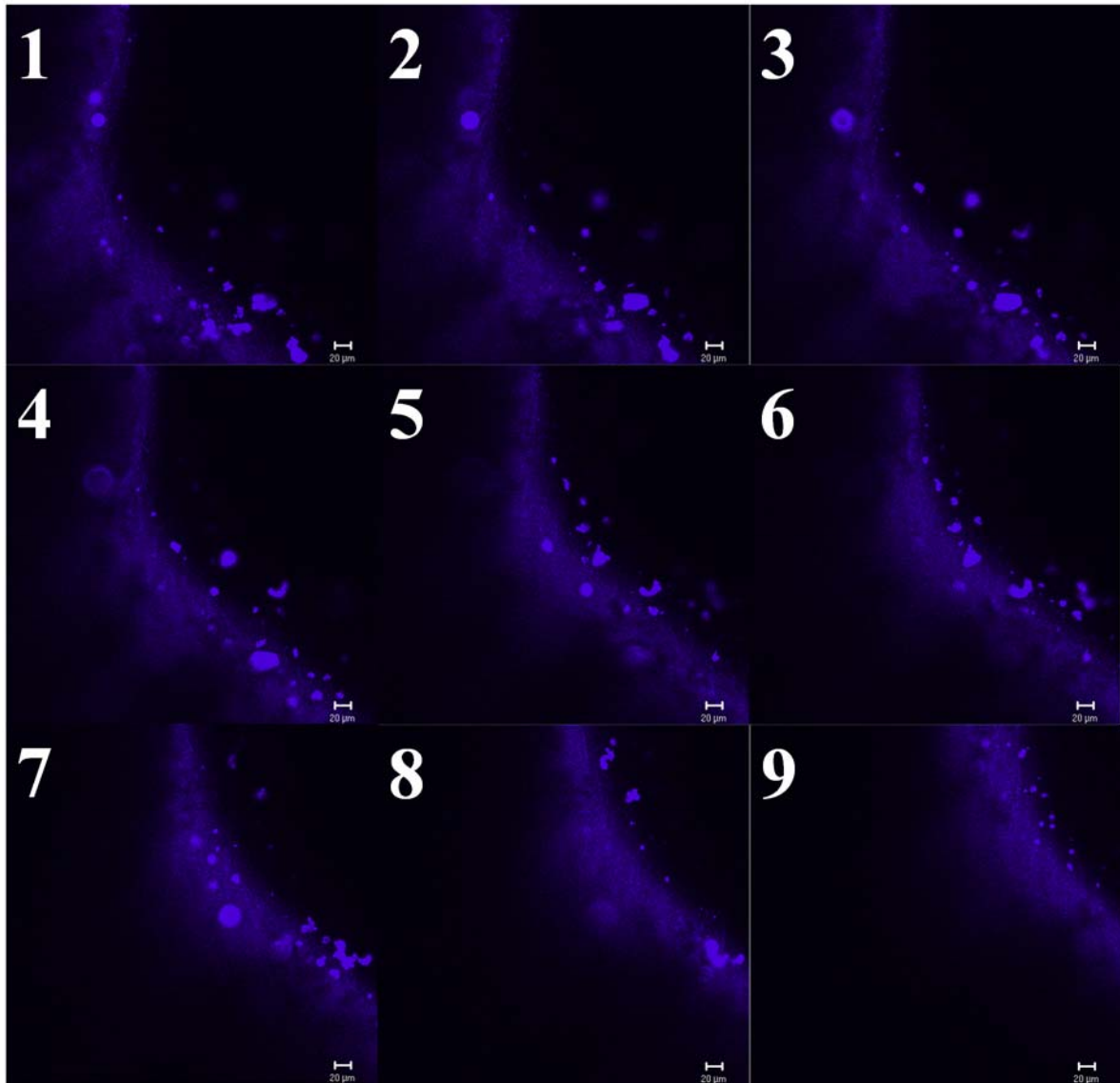
To view the cross-section of the Netlon® filaments, an attempt was made to mount the samples with cross-section facing upwards towards the incoming laser light. Generally there was no fluorescence observed except for one micrograph of Netlon® filament containing 0.29wt.% alphacypermethrin (Figure 23). In this micrograph presence of the insecticide at the edge of the filament is seen. These results clearly reveal that most the insecticide has migrated towards the surface of the Netlon® filament. This suggests that the additive has bloomed to the surface of the filament rather than being trapped inside.



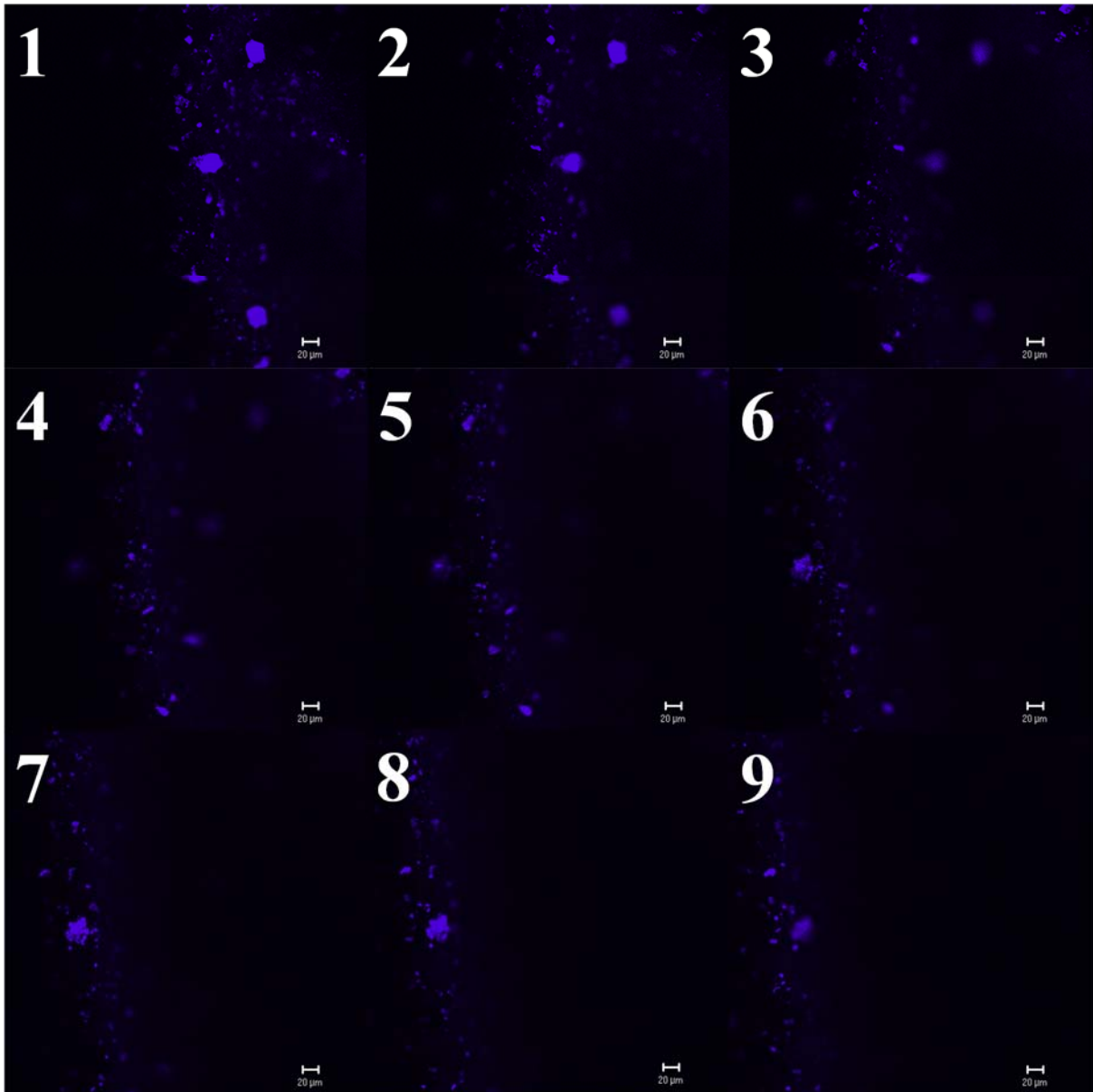
**Figure 19: CFM micrographs showing depth profiling of Netlon® filament loaded with 0.52wt.% deltamethrin. The micrographs are numbered in order of increasing depth into the sample.**



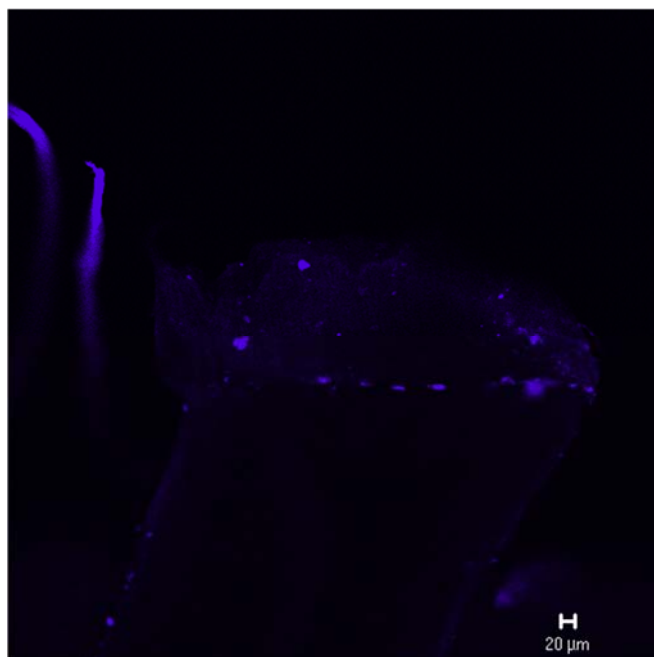
**Figure 20: CFM micrographs showing depth profiling of Netlon® filament loaded with 0.85wt.% deltamethrin. The micrographs are numbered in order of increasing depth into the sample.**



**Figure 21: CFM micrographs showing depth profiling of Netlon® filament loaded with 0.29wt.% alphacypermethrin.** The micrographs are numbered in order of increasing depth into the sample. In these micrographs we can only see one edge of the filament. This is due to the sectioned surface area of the filament being bigger than XY plane that the microscope could view.



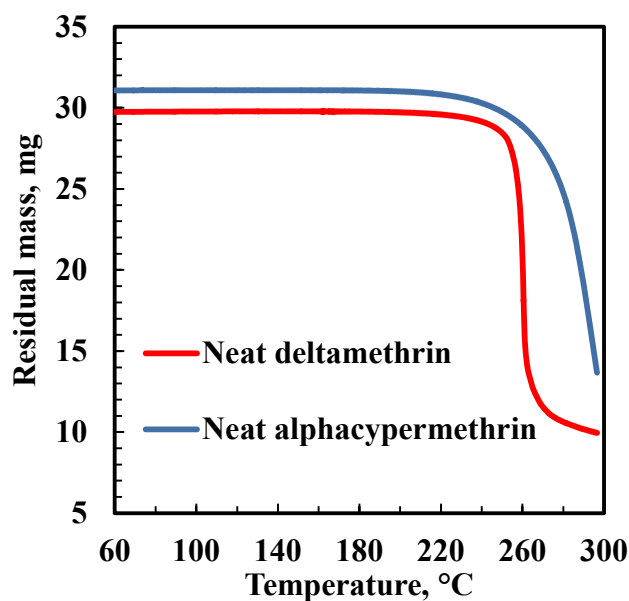
**Figure 22: CFM micrographs showing depth profiling of Netlon® filament loaded with 0.47wt.% alphacypermethrin.** The micrographs are numbered in order of increasing depth into the sample. Note that the left half of the filament is only visible.



**Figure 23: CFM cross-sectional micrograph of Netlon® 0.29wt.% alphacypermethrin**

#### 4.6.3 Thermal analysis

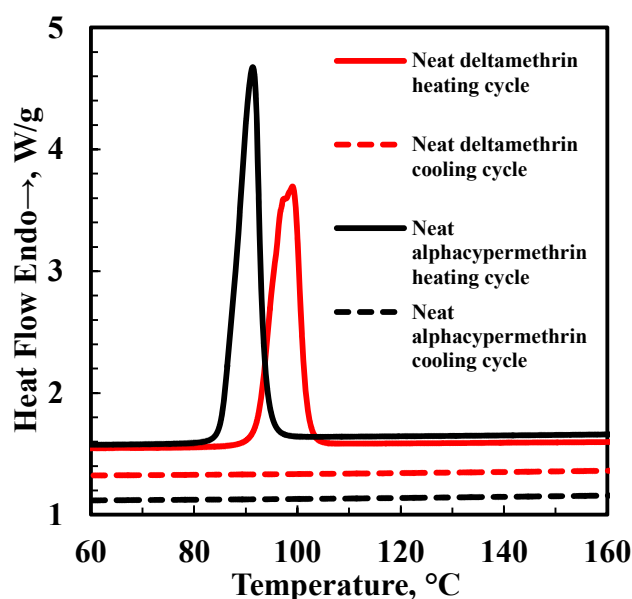
Figure 24 shows the TGA traces of neat alphacypermethrin and neat deltamethrin. The loss of mass indicates the evaporation of the active into the nitrogen atmosphere. TGA provides an indication of the volatility of the insecticides at elevated temperatures. The actives are stable to temperatures above the extrusion processing temperature of 220 °C for the Netlon® mesh. This suggests that very little active is lost during processing.



**Figure 24: TGA traces of neat deltamethrin and neat alphacypermethrin.** The samples were heated from 25°C to 300°C at 2°C/minute with a nitrogen purge of 50 mL/minute.



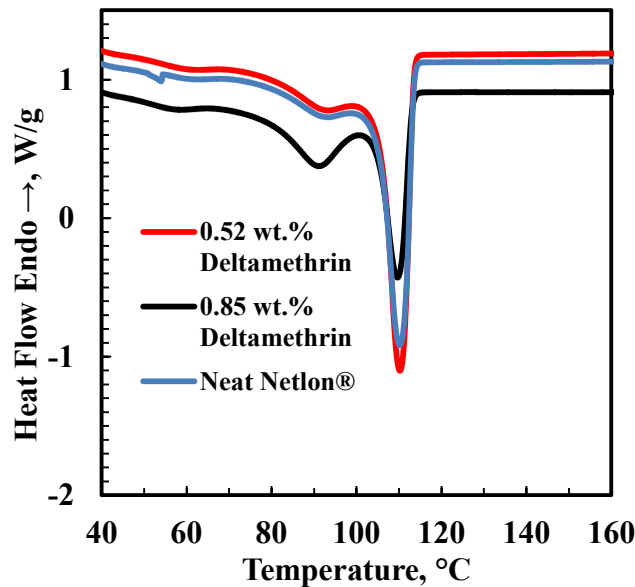
Figure 25 shows the DSC traces of neat deltamethrin and neat alphacypermethrin as the samples were heated to 200°C and then cooled. Onset of melting endotherms, ca. 102°C for deltamethrin and ca. 95°C for alphacypermethrin, are observed for both insecticides during the heating cycle. However on cooling there is a conspicuous absence of crystallisation exotherms. The reasons for this observation are not understood as yet. However this observation suggests that these insecticides require a relatively extended period of time to crystallize from their melt. They thus remain amorphous soon after a typical extrusion process. Their amorphous state will affect their rate of diffusion through the polymer matrix i.e. they will diffuse out of the matrix faster.



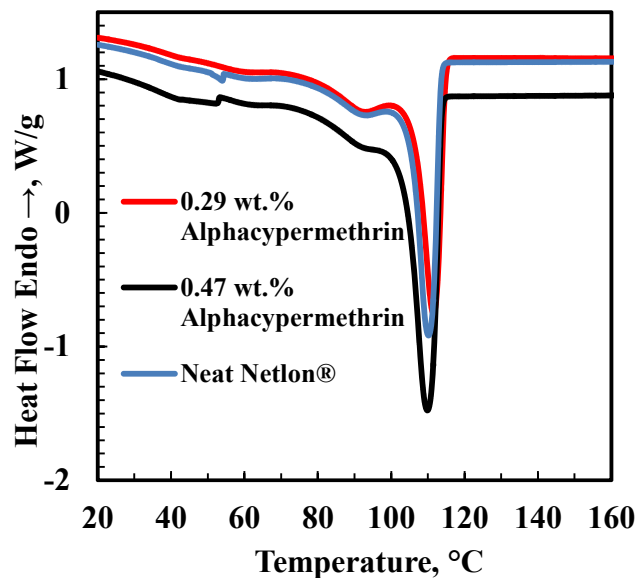
**Figure 25: DSC traces of neat alphacypermethrin and neat deltamethrin.** The temperature was scanned at 20°C/min from 25°C to 200°C. The sample was held isothermally for 1 minute at 200°C and then cooled to 20°C at 20°C/min.

Figure 26 and Figure 27 show the DSC traces of Netlon® containing different concentrations of deltamethrin and alphacypermethrin respectively. At a level of 0.52wt.%, deltamethrin does not seem to affect the crystallization kinetics of the LDPE/HDPE base polymer. However at a level of 0.85wt.% there is a decrease in the crystallization peak at ca. 110°C and an increase at ca. 95°C. This is attributed to deltamethrin interfering with the crystallization of components that make up the base polymer at higher concentration levels because it might have reached the solubility limit at this temperature in HDPE phase but not in LDPE phase. This effect is not observed in the lining samples containing

alphacypermethrin. It is tentatively speculated that a reason for this may be that alphacypermethrin is present concentrations lower than the solubility limit.



**Figure 26: DSC cooling traces of Netlon® incorporated with deltamethrin active.** The temperature was scanned at 20°C/min from 25°C to 200°C. The sample was held isothermally for 1 minute at 200°C and then cooled to 20°C at 20°C/min.



**Figure 27: DSC cooling traces of Netlon® incorporated with alphacypermethrin active.** The temperature was scanned at 20°C/min from 25°C to 200°C. The sample was held isothermally for 1 minute at 200°C and then cooled to 20°C at 20°C/min.

#### 4.7 Discussion

Insecticide solubility in polymers is dependent on the temperature of the polymer and the amount of amorphous phase available. Polymer additives are soluble in the amorphous parts of polymer matrices only but are excluded from (i.e. insoluble in) crystalline regions. Solubility generally increases with temperature. This implies that the insecticide will have a higher solubility in the fully molten polymer than in the Netlon® at ambient temperature. High dissolution of the insecticide in the melt is desirable as it aids homogeneous dispersion of the solute in the polymer matrix.

Preliminary bioassay results indicated that pyrethroid samples in polyethylene passed the WHO criterion easily whilst samples of insecticides incorporated in EVA and polyamide failed the test. A possible explanation is polyethylene is a nonpolar and highly crystalline polymer matrix. Both these features contribute to a low solubility of the polar insecticides in the solid polymer at room temperature. If the solubility limit is exceeded in the bulk polymer, an additive will migrate to the surface and deposit there. This phenomenon is called “blooming” and is exactly what is required to happen with the insecticide in the Netlon® mesh. These insecticides are contact poisons and there must be enough on the surface to ensure a fatal dose when the mosquito touches the mesh. EVA is more polar than polyethylene and also less crystalline. Small organic molecules are only solubilized in the amorphous regions of a polymer. Thus the apparent solubility in EVA is expected to be higher because the higher polarity of the matrix matches better with the polar nature of the insecticide, and in addition, the amorphous phase comprises a large fraction of the polymer host. The poor performance of alphacypermethrin in EVA and even in the 1:1 EVA/LDPE blend indicates that the dosage level was probably insufficient to guarantee adequate blooming to the surface. The dimer acid based polyamides are highly polar in nature and the poor performance of the organophosphate insecticides in this matrix is probably due to a high solubility of the insecticide in this polymer. Polycaprolactone can be processed at very low temperatures. It is also a semi-crystalline polymer and it was expected that it will perform similarly to the EVA. The main difference compared to EVA is that the esters groups in this polymer form part of the polymer backbone rather than being part of the side group.

During Netlon® extrusion, the mesh is rapidly cooled. While in some polymers rapid cooling may hinder the formation of crystalline regions within the polymer increasing the amorphous

phase, this is not expected for the polymers considered in the present study. Bioassay and SEM results indicate that the insecticides did in fact migrate from within the polyethylene polymer matrix to the surface. CFM results confirmed that the majority of the insecticides had accumulated at the filament surface. This suggests that during quenching of the melt a sufficient amount of the insecticide active existed in a super-saturated state and diffused to the surface.

DSC analysis presented above indicated that the pyrethroid additives stay amorphous when cooled slowly from their melt. It is therefore assumed that after quench-cooling the Netlon® mesh during processing, the insecticides within the lining persisted in an amorphous state above their glass transition temperature for a sufficiently long time to allow migration to the surface before crystallizing. Focke and Van Preen (2011) studied the idealized additive blooming process for an amorphous additive present above its glass transition temperature from within a polypropylene matrix for woven polypropylene LLIN applications. Their system is similar to the system currently being studied here with the difference that for Netlon® linings there is no need for periodic washings as in LLIN.

The idealized additive blooming process for an amorphous additive present above its glass transition temperature proposed by Focke and Van Preen (2011), illustrated schematically in **Error! Reference source not found.**, was applied to estimate the time it will take for 95 % of the additive to bloom out of polyethylene polymer. The Fickian diffusion equation can be applied to describe the diffusion of the insecticide from polymer matrix according to the above mentioned model i.e.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial r^2} \quad (1)$$

Initial condition:  $C = C_i$  at  $t = 0$  (2)

Boundary condition:  $C = C_{eq}$  at  $r = d/2$  (3)

Since heat and mass transport are analogous, solutions obtained for the equivalent heat transfer problem are applicable (Beek *et al.*, 1999). The analytical infinite series solution to equation (1) and its associated initial and boundary conditions is inconvenient as it converges

slowly. For “long times” (defined by  $Fo = Dt/d^2 > 0,1$ ) a useful approximate expression for the mean excess concentration in the fibre is (Beek *et al.*, 1999):

$$\frac{\langle C \rangle - C_{eq}}{C_i - C_{eq}} = 0,692 \exp\left(-23,13 \frac{Dt}{d^2}\right) \quad (4)$$

The diffusion coefficients of the pyrethroids in polyethylene are not known. However, a useful correlation between molecular weight and diffusion coefficient of an additive has been proposed by (Piringer, 1994).

$$D \text{ (cm}^2/\text{s)} = 10000 \exp\left(A_p - 0.01M_w - \frac{10450}{T}\right) \quad (5)$$

Where  $A_p$  is a coefficient dependant on the type of polymer;  $M_w$  is the molecular weight and  $T$  is the temperature. The polymer matrices used to manufacture the Netlon® linings are 1:1 blend of LDPE and HDPE. The coefficient  $A_p$  is 8 for HDPE and 9 for LDPE (Reynier *et al.*, 1999). Using these coefficients and the molecular weight of deltamethrin and alphacypermethrin, the diffusion co-efficients were estimated at 30°C. The Piringer model is used to estimate the worst case scenario of diffusion of food additives through a plastic film. Consequently the diffusion co-efficients are over estimated by an order of magnitude or two compared to empirical data (Reynier *et al.*, 1999). Therefore, in this study the estimate of diffusion of these pyrethroids has been reduced by three orders of magnitude lower than estimated by the Piringer model to obtain the lower range of the diffusivity coefficients. This gives a ball park range of upper and lower estimates of diffusion coefficients for alphacypermethrin and deltamethrin in polyethylene i.e.  $1 \times 10^{-12}$  to  $1 \times 10^{-9} \text{ cm}^2/\text{s}$ . The equilibrium solubility of deltamethrin and alphacypermethrin has been estimated to be 0.01 wt.%, a value similar to a phenol of almost similar molecular weight and diffusion coefficient (Roe *et al.*, 1974).

Using these parameters and Equation (4) the time to 95% depletion of the excess insecticide initially trapped in the fibre was estimated. The results are shown in Figure 28 and Figure 29. It shows two solid lines calculated using the limiting diffusion coefficients given above. It is clear from Figure 28 and Figure 29 that, for the current Netlon® diameter of  $d = 0.5 \text{ mm}$ , 95% of the pyrethroid will diffuse out of the polymer matrix within a few hours after

processing. This analysis implies that Netlon® does not slowly release the insecticides but rather releases all of the insecticides onto the polymer surface soon after processing. The insecticide that we see using TEM analysis is most likely the equilibrium amount of insecticide left within the polymer matrix after blooming.

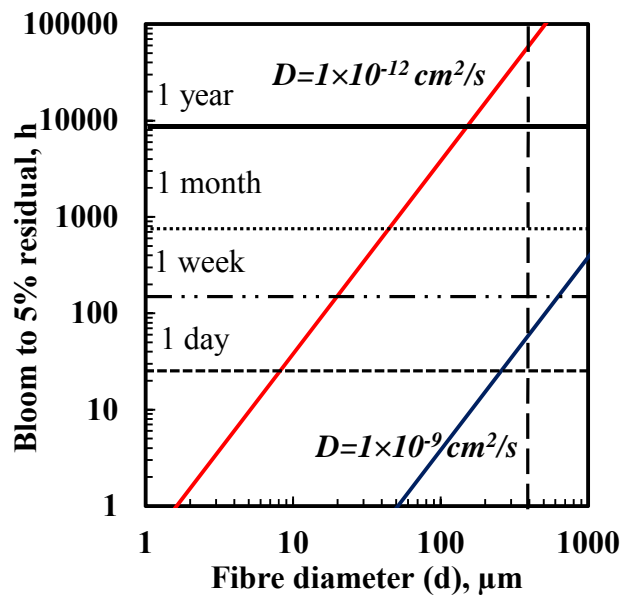


Figure 28: Effect of filament diameter on the time to bloom down to 5% residual of 0.47wt.% alphacypermethrin

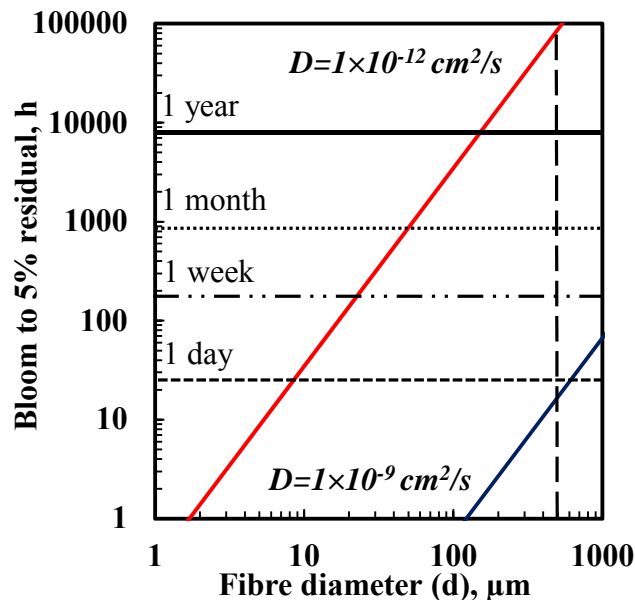


Figure 29: Effect of filament diameter on the time to bloom down to 5% residual of 0.85wt.% deltamethrin

In a previous study (Sibanda *et al.*, 2011) it was determined that alkaline hydrolysis was the cause of premature loss of activity for pyrethroids in field use. On Netlon® linings, the bulk of the insecticide is embedded on the surface of the polymer and the insecticide is not in contact with any alkaline material. In the absence of abrasion the pyrethroid insecticides will be active for a very long time (Sibanda *et al.*, 2011).

## **4.8 Field Trial of Netlon®**

### **4.8.1 Method**

#### 4.8.1.1 Trial area and trial village

Villages in the northern and eastern parts of the Vhembe district, Limpopo province, are situated in a medium to high risk malaria zone. Spraying for vector control does occur in many of the villages. The trial village is partly sprayed during the annual malaria spraying programme. In addition to mosquitoes, people living in this area are also exposed to annoying and biting insects. The housing in the village consists of traditional mud huts with thatched roofs and brick and cement houses with mostly metal or tiled roofs (western styled houses). The trial was performed on homes in the unsprayed section of the village.

#### 4.8.1.2 Enrolment

A total of 40 households (20 traditional mud huts and 20 western style houses) were included in the trial. The Netlon® lining was installed in the sleeping room of the household. Data was collected using a questionnaire and physical observation of the linings. A trained field worker fluent and familiar in the local language and culture conducted the interview. Participants were people who mostly slept in the sleeping area. Participants were enrolled prior to installation of the linings and an enrolment questionnaire was completed, which served as a baseline for future questionnaires.

#### 4.8.1.3 Lining installation

The Netlon® lining was installed on the upper parts of the wall near the roof. Wooden strips were first bolted onto the wall. The lining was then fixed on these wooden strips using Velcro® tape. One part of the Velcro® tape was attached onto the wooden strip using the adhesive side. The Netlon® lining was then fixed in between the two Velcro® tape parts (male and female). Each respective type of lining (Table 19) was installed in eight sleep areas (four huts and four houses).

**Table 19: Netlon® insecticide concentration and color coding<sup>#</sup>**

<b>Insecticide</b>	<b>Insecticide concentration (wt.%)</b>	<b>Colour</b>
Alphacypermethrin	0.29	Green
Alphacypermethrin	0.47	Orange
Deltamethrin	0.52	Brown
Deltamethrin	0.85	Purple
Negative control	None	White

<sup>#</sup>All nets were based on 1:1 blends of HDPE (Safripol F7650) and LDPE (Sasol LT033)

4.8.1.4 Overall user acceptability, lining durability and perceived effectiveness of linings. Face to face interviews to ascertain the overall user acceptability, lining durability and perceived effectiveness of linings were done monthly over a period of six months. Questions focused on the use of household pesticides and cleaning products within the sleeping area for that month; perceived activity of insects in the sleeping area since the lining was installed; physical change of the lining such as damage and discolouration, integrity of the installation and perceived adverse effects on the occupants and domesticated animals that might have had access to the sleeping area.

During face to face interviews conducted once every month, participants were asked if they had noticed a decrease in the number of mosquitoes and other annoying insects over the period between the last interview and the current one. This question did not distinguish between mosquitoes and other nuisance insects. The participants were given options to select from to indicate as to how they noticed a decrease over the time period. The options were:

- a) More dead mosquitoes and/or insects on the floor.
- b) More dead mosquitoes and/or insects on the furniture.
- c) More dead mosquitoes and/or insects when cleaning.
- d) Less bites noticed on people.
- e) Less irritation by mosquitoes and/or other insects.

Each month the participants were asked if they thought the decrease was due to the linings.



After the field trial period of six months was completed, participants were asked if they would like to keep the linings in their homes for longer. Permission was obtained to collect samples for further efficacy testing over the next three years. An ultimate interview was completed a few days after the final monthly interview on the sixth month. This interview focused on the participant's overall assessment of the linings, method of attachment, positioning of linings, impressions about the efficacy of linings in controlling nuisance insects and mosquitoes, colour preferences for future production and criticisms of the products they might have had.

## 4.8.2 Field trial results and discussion

### 4.8.2.1 Enrolment

Only one western style house had ever been sprayed by DDT spray workers to prevent malaria, whilst 35% of the huts had been sprayed. None of dwellings were sprayed less than six years ago based on what the participants could remember. Not all participants were head of the house or kept the spraying records, so they did not necessarily have accurate information on the spray history of the dwelling. In general, 30% of participants in western style houses and 45% in mud huts used insecticides to kill mosquitoes and other nuisance insects. Only two participants in western styled houses indicated that they had an untreated mosquito net to sleep under, which they have used before. In general 40% in western styled houses and 60% in huts had burnt mosquito coils before.

All participants except for one had an electricity connection to their home during the field trial. This made it possible to use electric drills to install wooden strips for attaching the wall linings. The general features of the households involved in the field trial are shown in Table 20.

**Table 20: Some features of the homes or huts where the linings were installed**

Houses (n=20)	Huts (n=20)
90% with corrugated zinc roofs and 10% tiled	All the huts had thatch roofs
15% with eaves	95% with eaves
All lining rooms had windows	55% had windows, all with glass, all but one could open

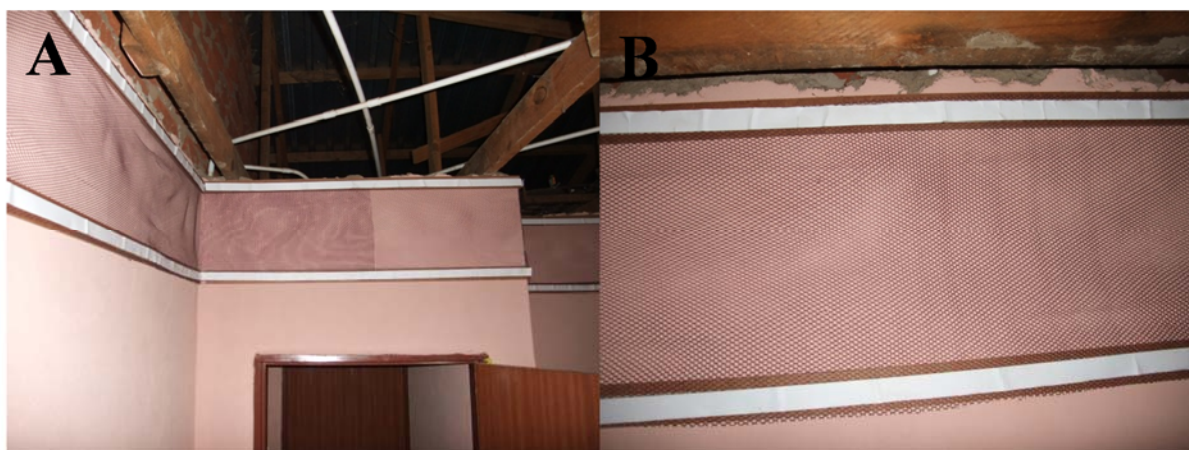
40% of inner walls painted and 60% plastered	60% of inner walls daub smeared, 25% lime white-washed, 10% plastered and 5% painted
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Prior to installation, 70% of the respondents indicated that they were irritated by mosquito bites while sleeping. After installation of linings, this was reduced to 40% of the participants. The change of this over the trial period (six months), along with insecticide and mosquito coil use is discussed in the following section.

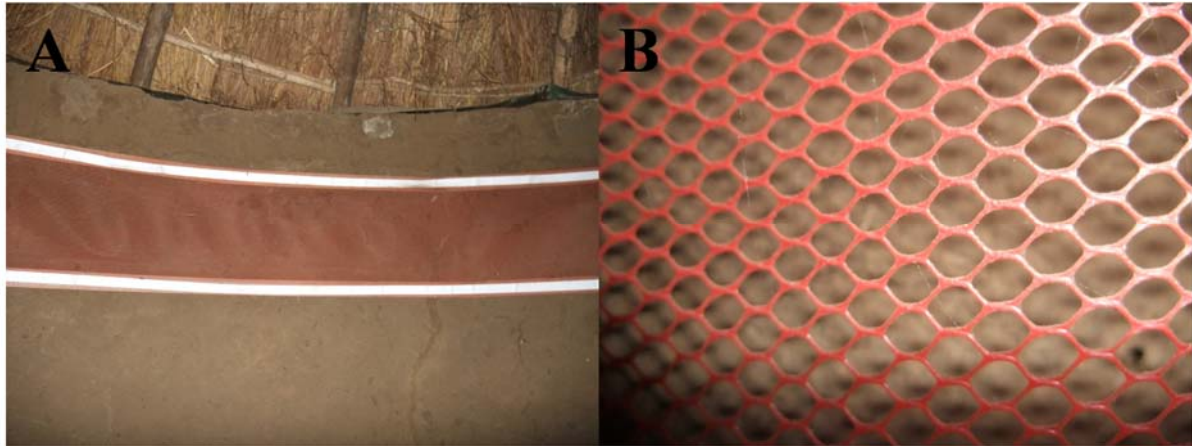
#### 4.8.2.2 Lining installation

Installation of the linings was expensive and labour intensive. For example, the wooden strips cost about R16000 for all the houses (or R400 per household per room). It also required the drilling of the inner walls. This caused some damage to the wall especially if the plastering (cement or mud) was not strong enough. This method required at least two people for the installation. Ideally manufacture and installation of the lining should be cost effective.

However once the lining has been installed it was easy to remove from the wooden strips. This is a very important feature of the net installation particularly in rural areas. It is practise to re-smear the inner walls with mud or cow dung or a mix of both. This is done to beautify the hut especially if visitors are expected or just before Christmas time when the husbands come home from the cities for holidays. Ability to remove the lining will also be convenient for western style type of houses if repainting the interior is necessary. All the participants appeared to be happy with the linings upon completion of installation. The attachment method in both a room in a western style house and in a hut is shown in Figure 30 and Figure 31 respectively. Further photographs of installed nets are shown in Appendix 4.



**Figure 30: Purple lining inside a room of a western style house.** (A) shows the wooden strip along the wall with the lining attached to the wooden strip by white strips of Velcro®. The section of overlap is where sampling of the lining occurred. (B) is a close up of the installed lining.



**Figure 31: Orange lining inside a mud hut.** (A) shows the wooden strip along the wall with the lining attached to the wooden strip by white strips of Velcro®. (B) is a close up of the installed lining.

#### 4.8.2.3 Overall user acceptability, lining durability and perceived effectiveness of linings

**Overall user acceptability.** On the first monthly visit one participant from a western style house complained that there was an unusual smell for a number of days after installation of the lining. No other adverse effects on the people sleeping in the room where the lining was installed were reported due to the smell. There was also no report of people or domesticated animals that came into contact with the lining. The obvious reason for this is that the lining is positioned on the upper parts of wall. The pyrethroid group of insecticides is used as the active ingredient to manufacture the lining. This group of insecticides is not toxic to human beings in small amounts such as those used to load Netlon® linings and they are quickly metabolised by enzymes in mammalian bodies (Sibanda *et al.*, 2011). There is no danger *per se* that might be involved in humans or animals coming into contact with the lining. However, the positioning of the lining may allow for the use of other contact poisons that may be regarded as relatively toxic to mammals e.g. DDT.

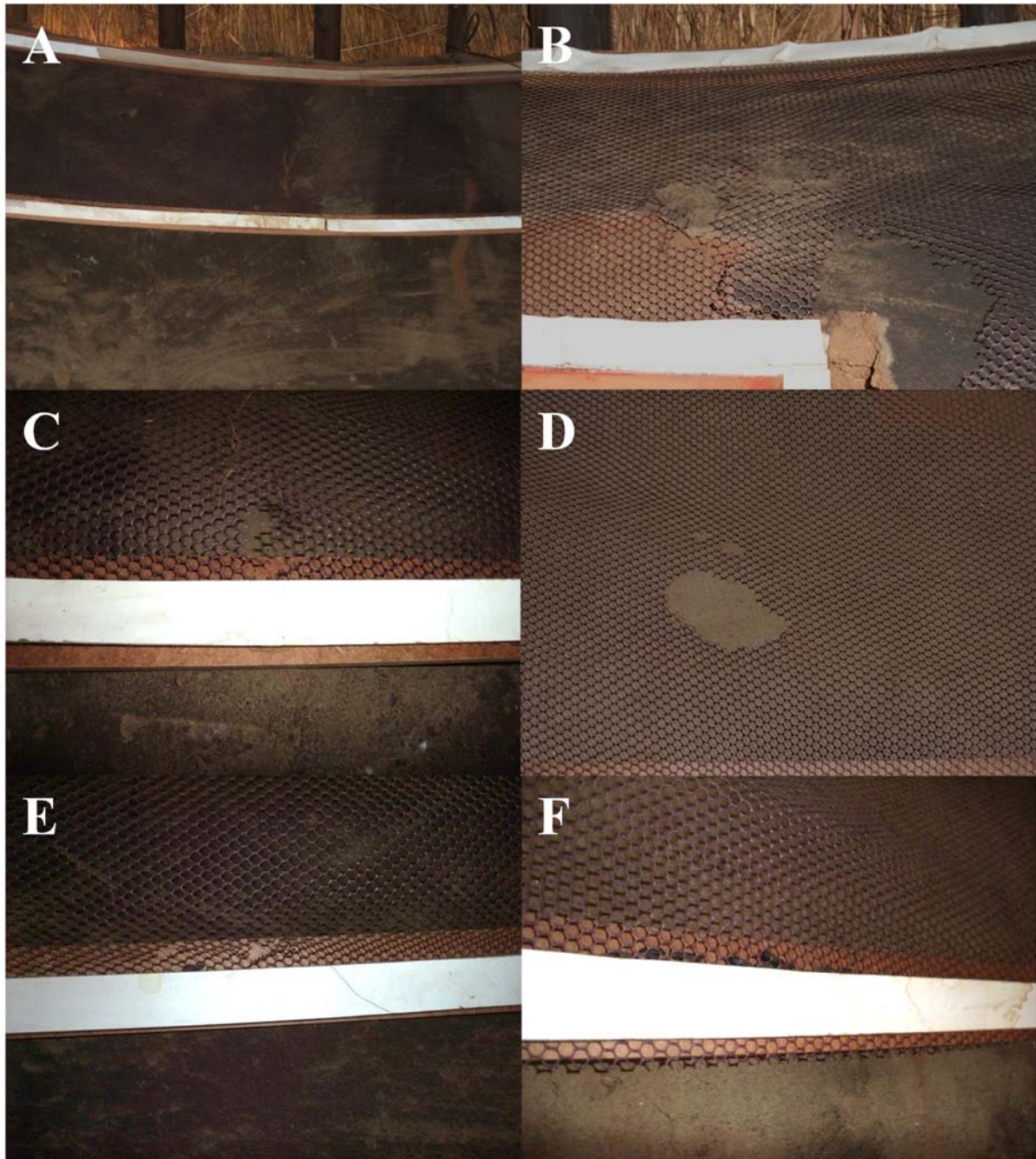
A sample of the comments made by participants during the final interview on what they liked about the lining included:

- The lining reduced mosquitoes and other insects
- Less biting by mosquitoes
- They could sleep peacefully
- That the colour was not too bright
- The lining matched the inside wall colour of the room
- The colour drew attention
- The colour does not show dirt too easily
- The lining decorates the wall

After the trial of six months was completed, participants were asked if they would like to keep the linings in their homes for longer. All 40 participants agreed and permission was obtained to collect samples for further efficacy testing over the next three years.

**Lining durability.** Participants were asked during each monthly visit, whether their lining underwent any colour change, if there was any damage noticed and did it come loose on its own or fall down totally. This was a very important question with regards to the stability of the plastic lining to ambient conditions persisting in the households. No discolouration was noticed in any of the linings over the six month trial in the huts or houses except for white linings which were reported to have turned to a brownish colour. This indicates that the linings did not suffer any form of degradation. The discolouration in white linings was due to dust accumulation on the linings that would expectedly contrast more with the white colour. One participant living in a hut reported damage on the lining. This damage was in the form of holes on the lining (Figure 32). This damage was attributed to rodents that may have come into contact with the linings. Rodent damage can potentially be a major problem for linings particularly in rural areas where rodent control may not exist. One participant reported that the lining had come loose and started falling down. This occurrence was attributed to major water damage in the hut due to heavy rains during the month of January (third monthly visit). The damage was especially visible on the white Velcro® strips in the form of brown water marks (Figure 32). Two other huts underwent slight water damage, but the lining installation was robust enough to hold its position. These occurrences bring into focus the fragility of mud walls characteristic of traditional huts.

**Perceived effectiveness of linings.** Over the course of the six months some of the participants decided to change their sleeping areas to cooking areas, and one changed it to a living area only. The latter, however, changed the living area back to a sleep area due to more mosquito bites in the room they slept in without the lining. This is an important piece of evidence that may indicate that the Netlon® does suppress mosquito activity. After the second monthly visit, one participant changed her sleeping hut into a kitchen and cooking occurred over an open fire for the remainder of the study. Table 21 and Figure 33 show the results of the perceived effectiveness by the respondents of the linings.



**Figure 32: Purple lining inside a hut with water and possible rodent damage.** (A) Water damage can be seen as a discolouration on the white parts on the Velcro®. (B), (C) and (D) lining showing possible rodent damage. (E) and (F) show possible rodent excrement.

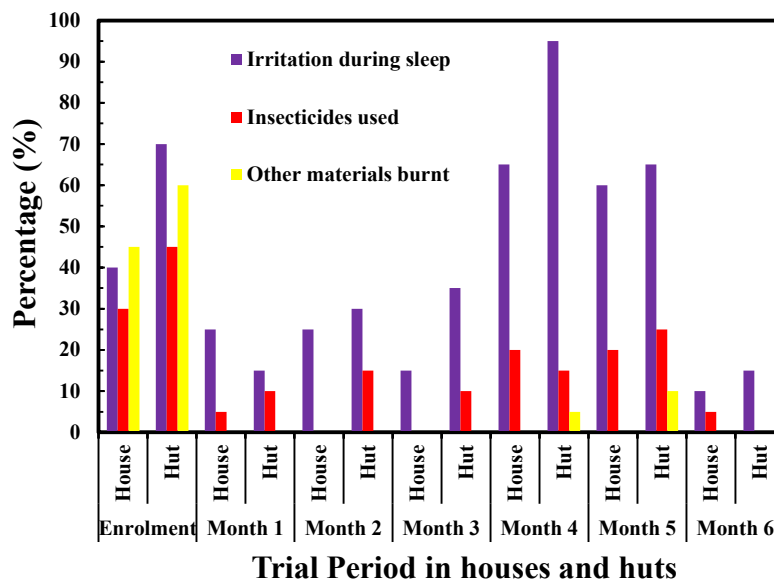
**Table 21: Decrease in mosquitoes and other biting and annoying insects noticed during periods between monthly interview visits**

<b>Time period</b>	<b>House (n = 20)</b>	<b>Hut (n = 20)</b>
First monthly visit	90% noticed a decrease since lining installation	100% noticed a decrease since lining installation
Second monthly visit	100% noticed a decrease since first monthly visit	95% noticed decrease since first monthly visit
Third monthly visit	100% noticed a decrease since second monthly visit	100% noticed a decrease since second monthly visit
Fourth monthly visit	85% noticed a decrease since third monthly visit	55% noticed a decrease since third monthly visit
Fifth monthly visit	90% noticed a decrease since fourth monthly visit	95% noticed a decrease since fourth monthly visit
Sixth monthly visit	100% noticed a decrease since fifth monthly visit	100% noticed a decrease since fifth monthly visit

The sum of those who responded to a question (positively or negatively) was divided by the sample population (n=20) for both the western type of houses and traditional huts respectively. On enrolment about 40% of the house respondents and 70% of the hut respondents reported being irritated by mosquitoes and other annoying insects. This number reduced during the first monthly interview and gradually increased up to month 4. The gradual increase in irritation is attributed to the general increase in the number of mosquitoes as the wet season progressed. During the same time there was a drastic decrease in the use of household insecticides and mosquito coils. This reinforces the view that respondents did find the linings to be effective in suppressing mosquitoes and other domestic insects. An important point to note is that irritation in huts was comparatively more than in western style houses. This is due to the differences in design of the household which affects the ability of the mosquito to enter the household. Traditional huts have an eave opening which allows mosquitoes to gain access into the hut whilst western style type of houses do not have this

feature. Therefore it is expected that traditional huts will have more mosquito populations than western style type of houses and hence more irritation. Perhaps the lining can be optimised to cover eaves in traditional huts. This clearly will be beneficial in reducing mosquito populations in the house.

Referring to Figure 33 a drastic increase in vector populations can be noted on month 4 of the interview. This increase in irritation coincided with the heavy rains that fell in the Vhembe district for more than two weeks, resulting in heavy floods all the way into Kruger National Park and Mozambique. Heavy rains will generally increase the number of suitable sites for female mosquitoes to lay their eggs and in turn lead to a drastic increase in mosquito populations. Towards the end of the study a large decrease in reported irritation occurred. This can be attributed to the decrease in the amount of rains a summer was coming to an end. It is important to note also that over study period the decrease in the use of household insecticides and mosquito coils was consistent indicating that indeed the effect the linings had was such that household occupants did not see the need to use insecticides and coils and at the same levels as before the installation of the lining.



**Figure 33: The trend, in percentages, in mosquito irritation whilst sleeping, and the use of insecticides and mosquito coils to lower mosquito burden over the trial period starting from enrolment to after six month in houses**



After six months of the same questionnaire being repeated, a final and shorter summary questionnaire was completed. All participants answered that they liked their lining and specifically the following criteria:

- Colour of the lining
- The decorative aspect
- Easy attachment of the lining
- Position of the lining
- Easy to re-attach when it came loose
- Easy to keep clean
- Less irritating insects in the hut/house
- Less mosquitoes in the hut/house

The participants were happy with the colour of the lining that was installed in their homes. However, they were given colour samples to select. Bright red followed by bright green was the favourite option in the houses, with bright orange followed by bright yellow was favoured in the huts.

All the participants were happy with the placement of the linings. They were given options of other positions to select from and replies varied. Options were:

- a) Against the wall
- b) Against the ceiling; from ceiling to the wall
- c) In front of the windows
- d) Entire inside of the room
- e) Under furniture

Two mentioned that they would prefer the lining to be lower down the wall, and one specifically mentioned that the lining should cover his/her bed. The participants were asked what other methods of attachment they would prefer. They could select from glue, tape, staples, capped nails, screws, rope or spring rod. Although participants did indicate what they deemed other possibilities, 35% with linings in houses and 30% with linings in huts commented they preferred the way it was attached. All the participants indicated that they

would recommend the linings for future use against mosquitoes and other insects to other members of their village or people from other villages.

Overall, the participants did notice a decrease in mosquitoes and other insects after installation of the linings. Participants were asked to describe what happened to mosquitoes and annoying insects over time as the study progressed. 15% of participants with linings in western style houses and 20% in huts replied that they noticed a decrease at first and then an increase. The respondents were asked what they thought was the cause of the gradual increase in mosquito irritation as the study continued. The respondents indicated that there were too many mosquitoes due to the heavy rains whilst some also indicated that there were usually more mosquitoes later on in the season. Other household pests that were reported to decrease were cockroaches, ants, flies and spiders.

**Bioassay efficacy testing.** The WHO effectiveness criterion is a mortality exceeding 80% after 24 hours following a 30 minute exposure of *An. arabiensis* mosquitoes to test samples. All the pyrethroid impregnated mesh samples achieved 100% knockdown and mortality even after more than 24 months after installation in trial houses (Figure 34). Raw bioassay results are presented in Appendix 1. This effectiveness is in addition to 12 months of storage after manufacture prior to installation. The linings have shown that they are at least effective for 36 months after manufacture. Knock down is sometimes used to indicate the bio-availability of the insecticide i.e. a change in the lethal concentration of insecticide present on the lining surface. There was no apparent change in the time it took to knockdown test mosquitoes. Some linings were extensively covered in dust and soot. These linings performed just as well as the linings that were not covered in dust and soot.

There was some knockdown and mortality observed in the white Netlon®. These samples were supposed to be the negative control and did not contain any insecticide. This result is attributed to contamination on white Netlon® samples caused by storage of all linings in one room. Placing the nets in polythene plastic bags did not prevent contamination of neat samples. An alternative control where bioassay tests were done on empty tubes was then used.

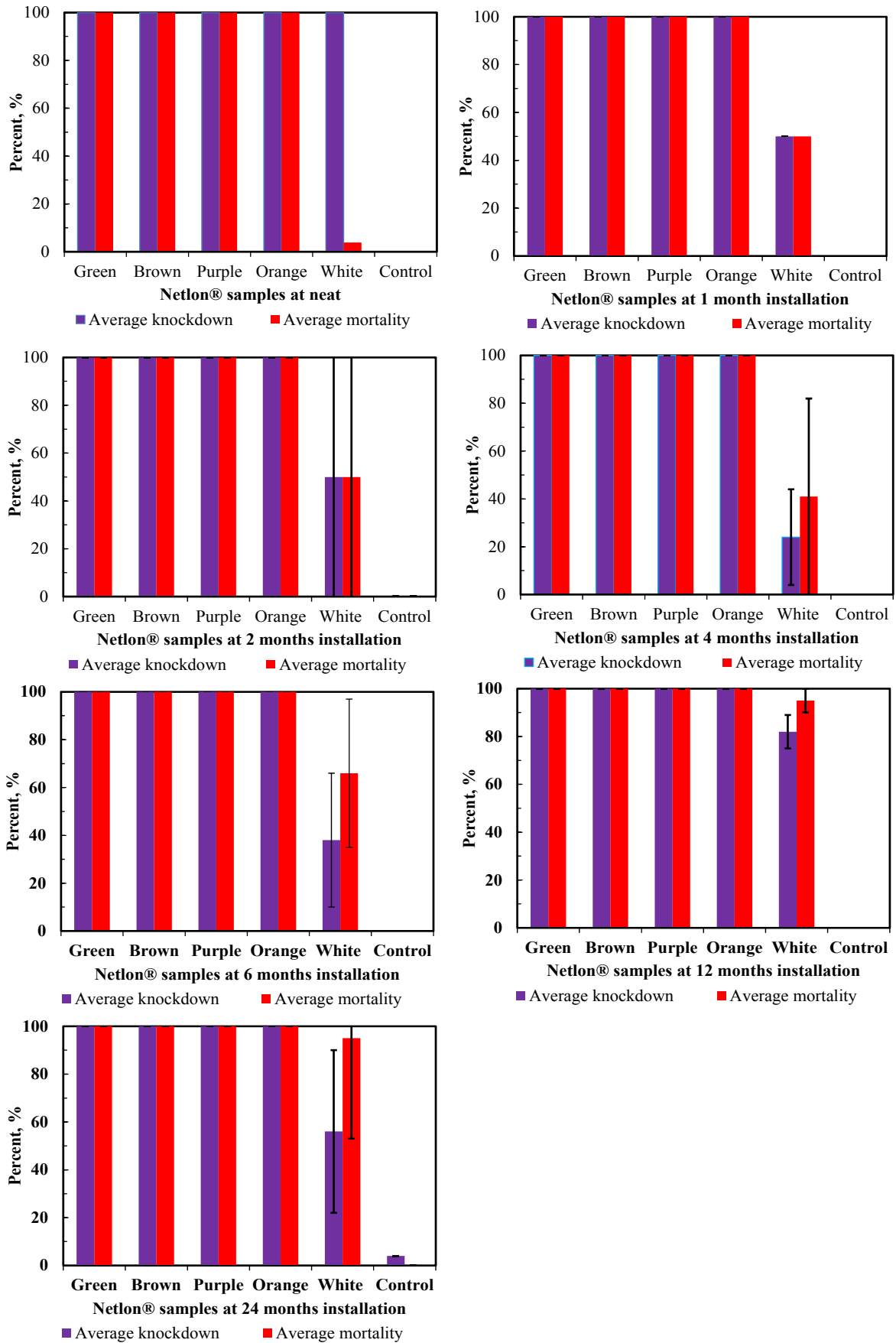


Figure 34: Plot of bioassay results for samples of installed Netlon®

We have established that these linings are not a slow release technology. The majority of insecticides incorporated into the polymer bloom to the surface of the polymer filament within a few hours. However the insecticides sit on a surface that is benign i.e. does not react with insecticide. Such a scenario still guarantees long lasting efficacy of the insecticides because they are not exposed to alkaline surfaces that have been shown to be the main mechanism of degradation of pyrethroid insecticides.

#### **4.9 Conclusions**

Monofilament polyethylene mesh, impregnated with pyrethroid insecticides, was successfully produced by a simple direct extrusion technique. It is an inexpensive method for the production of polymer based wall linings. Laboratory efficacy tests and field trials suggest that the Netlon® linings may be a potential substitute for IRS and it may also complement the use of LLIN. The results of previous work (Sumitomo, 2012, Focke and Van Preen, 2011) suggest that the residual effectiveness of Netlon® linings may be expected to last for an extended period of time. Results presented here show that the polyethylene provides a matrix suitable for production of effective and affordable wall linings. The superior performance of the polyethylene host is attributed to its low polarity and high crystallinity, attributes that reduce the solubility of the insecticide in the matrix and thereby favouring blooming of the contact poison to the polymer surface. This insight also suggests that addition of additives, e.g. crystalline waxes, could be beneficial to increase blooming of the insecticide. Such additives would have a preferential solubility in the amorphous regions of the polymer and their presence would reduce the volume fraction matrix available to solubilize the insecticides.

#### **4.10 Recommendations**

There is need to optimise the installation process of the Netlon® linings. The method has to be simple and cost effective for rural people to be able install the linings. It will be prudent to proceed to the WHO recommended small scale field trials followed by large scale field trials. These standardised tests enable the comparison of the performance of Netlon® with current products on the market.

## 5. Conceptual development of a mosquito repellent bracelet

### 5.1 Introduction

In Africa, a significant proportion of malaria infections may be due to exposure to vector mosquitoes during the early hours of the night when people are active outdoors, and not subject to protection by LLIN or IRS. In section 2.5, it was mentioned that mosquitoes are changing their biting behaviour from inside the house to outside in response to aggressive vector control inside human dwellings. Judicious use of repellents might prevent or at least reduce outdoor malaria transmission. Repellents are most often used as topical formulations (lotions, sprays, emulsions, etc.) applied to exposed skin. DEET is the most popular topical repellent in use and has a residual effectiveness that can last up to 4 hours (Ma *et al.*, 1999). The relatively high cost of DEET and the need for repeated application to the skin at high concentrations (10–70%) to maintain constant residual effectiveness precludes its use in tropical countries.

Outdoor malaria infection mostly occurs through bites on the ankle and feet and these bites decrease rapidly above ankle height (Braack *et al.*, 1994). The *An. gambiae* complex is one of the most efficient and most abundant malaria vectors in Africa. It is attracted by human foot odour, a smell similar to limburger cheese and tends to bite its victims in the ankle area (Dekker *et al.*, 1998, Knols and De Jong, 1996, Braack *et al.*, 1994). Wearing closed shoes or elevating feet of the ground may reduce mosquito bites and hence malaria infection. Closed shoes may be uncomfortable in areas where malaria is endemic because of the hot tropical weather. This may discourage people particularly in rural areas to wear closed shoes all the time. To solve this problem ankle bracelets or slippers made of polymer material impregnated with an effective repellent may be worn to prevent or drastically reduce mosquito bites on the ankle and feet area. The work in this study sought to develop an insecticide repellent bracelet that stores DEET within the polymer matrix and slowly releases it at effective levels over extended periods of time.

### 5.2 Problem statement

Current vector control interventions focus on minimising malaria infections indoors whilst infection can still occur outdoors. Current repellents that can be used to prevent outdoor infection have a short residual efficacy i.e. four hours at most.

### 5.3 Aim

The aim of this study was to increase the length of residual effectiveness of current commercial repellents for use in preventing outdoor malaria infection.

### 5.4 Objectives

Preliminary experiments were conducted to test the following hypotheses:

1. Polymers can be used as slow release agents of volatile repellents thereby increasing their residual effectiveness.
2. Use such polymers impregnated with volatile repellents to manufacture mosquito repellent bracelets.

### 5.5 Theory

In this study DEET, citronellal and dioctyl phthalate (DOP) were initially considered for incorporation into polymer matrices that can be used to manufacture bracelets or parts of low cost slip slops. The goal was to ensure long lasting efficacy, i.e. slow release of the active over an extended period of time. In this way it may be possible to protect people against malaria infection during the time they spend outdoors. The mechanism of slow release is important. The phenomenon of active blooming was again scrutinised in this study. However in this case the additive is a liquid. The liquid additives of this study diffused too quickly out of the polymer matrix. They were also more volatile than solid insecticides used in the manufacture of LLIN and ITWL. The combination of these two factors leads to a premature loss of repellent efficacy by the bracelets and slip slops. There is therefore a need to make the repellent additives more compatible with the polymer in order to make them last longer.

There are various long lasting mosquito repellent products that have been reported. Faulde et al. (2010) reported on a mosquito repellent fabric made by a polymer coating technique. In this technique a cotton fabric was immersed in a monomeric solution which contained DEET or IR3535 dissolved. The solution was made to polymerise at 110°C. The resultant polymer matrix which contained DEET or IR3535 entrapped in it formed a coating on the cotton fibres. Standard repellent efficacy tests revealed that this product had a residual efficacy of up to 61 weeks at 10.48 g/m<sup>2</sup> of DEET. Mokhtari et al. (2014) describes a chemical process of modifying a functional moiety in DEET in order to make it bind covalently onto Nylon 6. This was done by nitrating DEET at the para-position relative to the amide functional group.

This product was then reduced in the presence of HCl, SnCl<sub>2</sub> and C<sub>2</sub>H<sub>5</sub>OH to form an amine. This amine was condensed by reacting with 2, 4, 6-trichloro-1, 3, 5-triazine (cyanuric chloride) at below 5°C. The resultant product was reacted with the amino group in 7-Amino-4-hydroxy-naphthalenesulfonic acid producing 7-(4-chloro-6-(4-(diethylcarbamoyl)-2-methylphenylamino)-1,3,5-triazin-2-ylamino)-4-hydroxynaphthalene-2-sulfonic acid. The azo dye was manufactured by diazotizing sulfanilic acid using HCL and NaNO<sub>2</sub>. The resultant product of this reaction was combined with 7-(4-chloro-6-(4-(diethylcarbamoyl)-2-methylphenylamino)-1,3,5-triazin-2-ylamino)-4-hydroxynaphthalene-2-sulfonic acid. This azo dye could covalently bond with Nylon 6 and still show repellent activity.

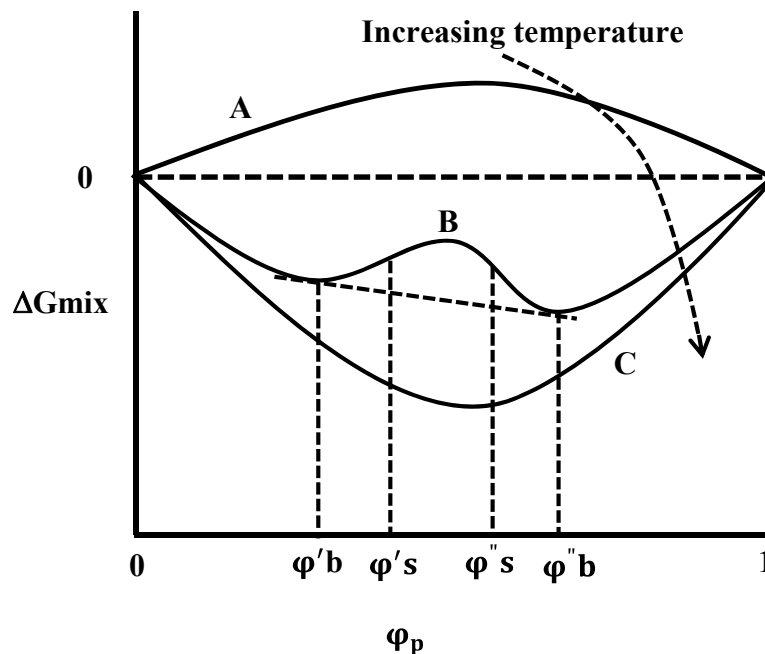
N'Guessan et al. (2008) has reported on a polyester LLIN that was treated with microencapsulated DEET supplied by Sumitomo. This product exhibited up to six months repellent residual activity.

In this study we proposed an alternative route to stabilise repellents and render them long lasting. The proposed route was through the formation of a bi-continuous phase between the polymer and repellent active through the phenomenon of spinodal decomposition of the polymer and repellent active. It is used mainly for the manufacture of porous membranes. This phenomenon has been well studied and is summarised below (Lloyd *et al.*, 1990, Lloyd *et al.*, 1991, Cahn, 1965).

Liquid-liquid phase separation has been shown to occur in crystalline or amorphous polymers. The miscibility between the polymer and liquid determines whether solid-liquid or liquid-liquid phase separation occurs. This miscibility is expressed by the Flory-Huggins interaction parameter of the system (Burghardt, 1989). If the interaction parameter is small then there is strong interaction and hence high miscibility between solid and solvent. The separation in this case is via solid-liquid separation when the polymer crystallizes. If the interaction parameter is large then there is weak interaction and hence less miscibility. Such a blend becomes easily unstable when cooled and undergoes liquid-liquid phase separation to show an upper critical solution temperature ( $T_{UC}$ ). The miscibility of a system can be described by the Gibbs free energy of mixing ( $\Delta G_{mix}$ ) and its second derivatives with respect to polymer volume fraction ( $\phi_p$ ) at constant temperature T and pressure P. The criterion for complete miscibility is:

1.  $\Delta G_{mix} < 0$
2.  $\left(\frac{\partial^2 \Delta G_{mix}}{\partial \phi_p^2}\right)_{T,P} > 0$

$\Delta G_{mix}$  is given by the equation  $\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix}$  where  $\Delta H_{mix}$  and  $\Delta S_{mix}$  represent the enthalpy and entropy of mixing respectively. If this criterion is not met then the solution may separate into two phases at equilibrium. Polymer-liquid mixtures may behave in three possible ways (Figure 35) i.e.



**Figure 35: Sketch of  $\Delta G_{mix}$  as a function of  $\phi_p$  and temperature for a fully miscible (A), partially miscible (B) and immiscible (C) system**

1. Immiscibility of the two phases throughout the whole composition range  $0 \leq \phi_p \leq 1$  (A);
2. Partial miscibility in the composition range where the second derivative of  $\Delta G_{mix}$  with respect to  $\phi_p$  is positive (B).
3. Miscibility across the entire composition range (C).

In general majority of the systems exhibit a combination of the three possible scenarios presented above i.e. above a certain  $T_{UC}$  the system is fully miscible, below the  $T_{UC}$  the

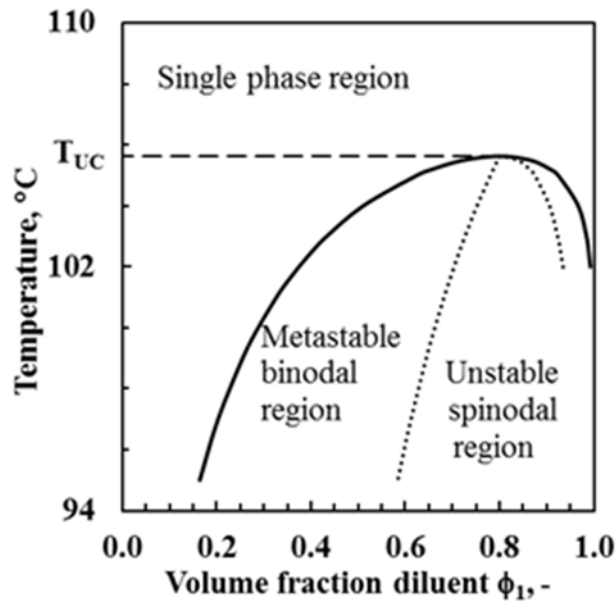


system is partially miscibility and at a temperature further below the  $T_{UC}$  the system is fully immiscible.

Considering partially miscible mixtures, a homogenous mixture is formed only under certain conditions of composition and temperature. Referring to curve B in Figure 35,  $\Delta G_{mix}$  is negative across the whole composition range suggesting miscibility. There is however an upward bend in the  $\Delta G_{mix}$  against composition curve between compositions  $\phi'_b$  and  $\phi''_b$  i.e. between the cotangential points. In this region the homogenous mixture can undergo phase separation to form compositions  $\phi'_b$  and  $\phi''_b$  to attain minimum energy. The mixtures are in equilibrium and have the same chemical potential i.e. the derivative of  $\Delta G_{mix}$  with respect to  $\phi_p$  indicated by the common tangent. The second derivative of  $\Delta G_{mix}$  is positive between compositions  $\phi'_b$  and  $\phi'_s$  and between compositions  $\phi''_b$  and  $\phi''_s$ . Although this region is not at equilibrium there is complete miscibility and stable to small concentration fluctuations between  $\phi_p \leq \phi'_s$  and  $\phi''_s \leq \phi_p$ . Concentration fluctuations between compositions  $\phi'_s \leq \phi_p \leq \phi''_s$  will lead to spontaneous phase separation. The system in this region is termed as *meta-stable*. The second derivative of  $\Delta G_{mix}$  is negative between compositions  $\phi'_s$  and  $\phi''_s$  i.e. the inflection points on the  $\Delta G_{mix}-\phi_p$  curve and this condition does not satisfy the conditions required for full mixing to occur i.e.

1.  $\Delta G_{mix} < 0$
2.  $(\frac{\partial^2 \Delta G_{mix}}{\partial \phi_p^2})_{T,P} > 0$

The system is therefore unstable in this composition range and spontaneous phase separation occurs. This region is termed as *unstable*. If the locus of co-tangential points (*binodal*) and the locus of inflection points (*spinodal*) are plotted on a temperature composition diagram then a phase diagram such as Figure 36 is obtained.



**Figure 36: Phase diagram of a typical partially miscible system exhibiting an upper critical temperature showing the stable single phase region, metastable and unstable regions**

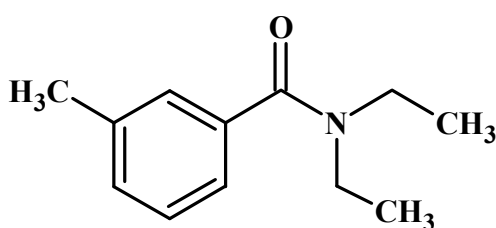
At temperatures above the  $T_{UC}$  the system is fully miscible for all mixture compositions. Below this temperature phase separation can occur. The compositions of the two phases in equilibrium at any temperature are defined by the binodal lines. In the meta-stable region indicated in the phase diagram, the phase separation will occur via a nucleation and growth mechanism. This is the usual scenario for liquid-liquid phase separation. If the polymer represents the minority phase, it initially leads to the undesirable formation of polymer particles that are suspended in the continuous liquid repellent phase.

In the unstable region, phase separation occurs spontaneously via spinodal decomposition. Phase separation by this mechanism leads to a finely dispersed microstructure via diffusion processes that amplify intrinsic thermodynamic spatial composition fluctuations (Cahn, 1965). Ultimately this co-continuous structure is fixed by the subsequent crystallization of the polymer phase. This means that the majority liquid phase is trapped inside a solid polymer structure. In practice such micro-porous microstructures can be achieved by rapid quenching of a homogeneous melt in a cold water bath.

From this theory we deduce that in order to form a co-continuous phase between the polymer and diluent, the polymer-diluent mixture should exhibit an upper critical temperature above

which polymer and diluent are fully miscible and below which they spontaneously separate. We also learn that if we rapidly cool the polymer-diluent mixture into the *spinodal* region we will get instantaneous formation of a micro-porous polymer structure. However if we do not have the correct polymer-diluent composition we may rapidly cool the mixture into the binodal region. It is necessary to develop phase diagrams of the polymer-diluent systems to inform on the conditions necessary for the formation of open cell microstructures especially when a particular pore size is required such as in membrane applications. McGuire *et al.* (1994) have proposed a simple method to determine such phase diagrams. In this study however, we simply used trial and error to rapidly cool fully miscible polymer-diluent mixtures at different compositions to obtain a micro-porous polymer structure.

It is possible that a micro-porous extrudate will form a denser skin layer on the outer surface. However, it is prudent to add membrane like coatings on the outside as they can be designed to reduce the rate of release even further. Hence, in this study a polyamide film coating was employed to act as a barrier for slowing down the loss of repellent from the polymer matrix. Polyamides are also thermoplastic and this makes it possible to add the outer layer by a co-extrusion process. However, in the present investigation the coatings were applied using a solvent technique. It relied on the fact that the polyamide used was soluble in acetic acid but not in water (Rulkens and Koning, 2012). The repellent used in this study is DEET. It is the most popular repellent and most effective repellent in use because of its relatively superior residual efficacy. It is slightly yellow in colour. The molecular structure of DEET and physical and chemical properties are shown in Figure 37 and Table 22.



**Figure 37: Molecular structure of DEET**

**Table 22: Physical and chemical properties of DEET**

CAS registry number	134-62-3
Molecular formula	C <sub>12</sub> H <sub>17</sub> NO
Molar mass	191.27 g/mol
Thermal properties	-45°C (MP), 288°C (BP)
Density	0.998 gm <sup>-3</sup>
Vapour pressure	0.75 Pa
Solubility in water	>1 g.dm <sup>-3</sup> at 25°C
Octanol water partition coefficient	2.02
Toxicity, LD <sub>50</sub>	2170 to 3664mg/kg in rats

## 5.6 Work plan

Initial experiments were conducted using DEET, citronellal and dioctyl phthalate. It was found that it was possible to produce microporous substrates using all three additives. See Appendix 5. However, of these compounds, DEET is by far the most effective insect repellent. Therefore it was decided to focus on the incorporation of DEET into polymer matrices that can be used to manufacture bracelets or parts of low-cost slip slops. For such an application it was required to select a polymer that would be most likely acceptable to the typical consumer. EVA was the most suitable candidate because of its soft rubbery feel. The initial objective was to determine the maximum amount of DEET that could be incorporated into the EVA matrix directly. The second stage was to explore whether we can increase the amount of repellent loading using the technique of spinodal decomposition. The release rate of DEET out of the polymer matrix was to be studied by exposing polymer strands impregnated with DEET to high temperatures in forced convection ovens to accelerate the release rate of the active whilst measuring the change in mass of the polymer strands periodically. Finally suitable formulations were to be tested for repellent effectiveness using arm-in-cage repellent tests. The ultimate goal was to prove the concept of controlled release of the active over an extended period of time using polymer matrices.

## **5.7 Equipment**

### **5.7.1 Compounding**

Compounding was done on a TX28P 28 mm co-rotating twin screw laboratory extruder with a screw diameter of 28 mm and an L/D ratio of 18. The screw design of this machine comprised intermeshing kneader blocks that also imparted a forward transport action.

### **5.7.2 Microscopy**

Processed polymer cross-section morphology was examined by low resolution SEM using a JEOL (Tokyo) 500 instrument. Sample coating was done using a SEM Polaron E5200 from Polaron Equipment Ltd.

A Carl Zeiss Axiovert 200 optical microscope was used to determine the diameter of the strands and the thickness of the coatings before ageing tests were conducted.

### **5.7.3 Thermal analysis**

The thermal behavior of DEET in its neat form and when incorporated in EVA was studied via TGA analysis in a TA instruments SDT Q600 simultaneous DSC/TGA and also in a PerkinElmer TGA 400 thermo gravimetric analyser respectively.

### **5.7.4 Accelerated mass loss studies**

The ovens used to study the loss of active at accelerated temperatures were a Labcon FSOH 16 and a Scientific Series 9000. These ovens are equipped with a digital temperature controller that could control temperature to within  $\pm 0.1^\circ\text{C}$ .

## **5.8 Materials**

DEET was obtained from Merck. This repellent has a density of  $0.998\text{ g cm}^{-3}$  and its normal boiling point is  $288^\circ\text{C}$ . EVA powder grade AMS 3042 was sourced from Affirm Marketing Services. The vinyl acetate content was 18%, the density was  $0.939\text{ g cm}^{-3}$  and melt flow index was  $1.7\text{ g}/10\text{ min}$  at  $190^\circ\text{C}$ . The copolyamide used for coating was Euremelt 2170 sourced from Huntsman. It had a softening point of ca.  $170^\circ\text{C}$  and a melt viscosity of  $5.8\text{ mPa}\cdot\text{s}$  at  $200^\circ\text{C}$ . Precipitated silica Ultrasil VN3 was supplied by Evonik. According to the manufacturer it had a surface area of  $180\text{ m}^2\text{ g}^{-1}$ . Properties of the repellent active and

polymers used are shown in **Error! Reference source not found.** and Table 23. Appendix 6 also presents the specification sheets of the polymers used in this study.

**Table 23: Polymer properties**

Name	Units	Ethylene Vinyl Acetate (EVA)	Polyamide
Grade		AMS 3042 Natural (18% VA)	Euremelt 2170
Softening point	°C	60	167-175
MFI at 190 °C/2.16 kg	g/10 min	1.7	-
Viscosity@ 200°C	Pa s	-	5.2-6.4
Supplier		Affirm Marketing Services	Huntsman

## 5.9 Methods

### 5.9.1 Free absorption of DEET by EVA studies

Five 5 cm lengths were cut from a neat EVA polymer strand. The initial mass and diameter were measured using a digital scale and an engineer's callipers respectively. These strands were submerged in DEET in a beaker. The change in mass and diameter were measured after 21 days.

### 5.9.2 Polymer repellent solubility studies

Theory dictates that the polymer and the diluent must be miscible above a certain  $T_{UC}$  in order to make open cell microstructures. To determine this temperature the polymer was mixed with the repellent in a 60:40 weight ratio in small glass bottles. These mixtures were heated up in an oven, with the oven temperature increased stepwise by 10°C every 10 minutes from 80°C. The temperature at which a single phase was formed was noted. Another similar mixture was made and exposed to the temperature where a single phase is formed, this time recording the amount of time that it took for the polymer and repellent to fully form one phase. This was taken as the maximum residence time required compounding the polymer and repellent. It was found that EVA-DEET formed a single phase if left at 170°C for about 55 minutes. To form micro-porous structures, the polymer and repellent will be mixed in a compounding extruder. Due to forced mixing the residence time required to form a single phase will be less. The results established here only served as a baseline to set compounding parameters in order to form a micro-porous polymer.

### 5.9.3 Compounding

#### 5.9.3.1 Solid strand compounding

The DEET liquid (50 parts by mass) was first mixed with 5 parts by mass precipitated silica and then mixed with 150 parts by mass of the polymer powder in a high speed mixer. This was necessary in order to facilitate feeding of the blend into the compounding extruder. The blend was fed into the extruder operated at a screw speed of 168 rpm. The extrusion parameters are shown in Table 24. The extruded strands were cooled in a water bath to yield the solid formulation.

Table 24: TX28P extrusion parameters for solid polymer compounding

Hopper	Zone 2	Zone 3	Die	Screw speed
85 °C	110 °C	120 °C	140°C	168 RPM

#### 5.9.3.2 Micro porous strand compounding

The DEET liquid (50 parts by mass) was first mixed with 5 parts by mass precipitated silica and then mixed with 50 parts by mass of the polymer powder in a high speed mixer. The blend was fed into the screw compounder operated at a screw speed of 105 rpm to increase the residence time compared to solid polymer formulations. The extrusion parameters are shown in Table 25. Higher compounding temperatures were employed to ensure that the DEET was fully dissolved in the EVA melt. The extruded strands were quenched in an ice bath, i.e. at a temperature of ca. 0°C to ensure that a micro-porous microstructure is generated by rapidly cooling to the spinodal region.

Table 25: TX28P extrusion parameters for micro-porous polymer compounding

Hopper	Zone 2	Zone 3	Die	Screw speed
90 °C	110 °C	170 °C	170°C	105 RPM

### 5.9.4 Microscopy

#### 5.9.4.1 SEM

The micro-porous material was leached free of DEET with dichloromethane. The samples were then frozen by submerging in liquid nitrogen and fractured. The fractured samples were coated four times with carbon.

#### 5.9.4.2 Light microscopy

The samples were mounted on a cover slip using adhesive tape and then viewed on the microscope.

#### 5.9.5 Thermal analysis

Samples weighing ca. 30mg were placed in open 70  $\mu$ L alumina pan. The temperature was scanned from 25 to 450°C at a scan rate of 10°C/min. Throughout the experiment the TG cell was purged with nitrogen flowing at a rate of 50 mL/min.

#### 5.9.6 Coating of strands

To slow down the initial rate of loss of repellent active, extruded micro-porous strands were cut to a length of 20 cm and dipped once and twice respectively in a 40wt.% solution of polyamide dissolved in acetic acid. The dipped strands were hanged on a line to allow the acetic acid to evaporate precipitating the polyamide on the strands. The polyamide solution was kept at a constant temperature by use of a hot plate with a temperature controller.

#### 5.9.7 Accelerated mass loss studies

##### 5.9.7.1 Solid polymers

Accelerated mass loss studies on solid strands were performed in forced convection ovens. The strands were cut to a length of 20 cm. The samples were studied in triplicates. Neat EVA was included in the study as a negative control. These samples were placed in the ovens at 60°C, 50°C and 40°C respectively. The loss of active was followed by weighing the samples at regular intervals using a four decimal output scale.

##### 5.9.7.2 Micro-porous polymers

Accelerated mass loss studies on micro-porous strands were performed in forced convection ovens on five 20 cm strands for each of the three formulations (not coated, single dip and double dip). Neat uncoated and coated samples were included in the study as negative controls. Uncoated, single dip and double dip strands were placed in the ovens at 60°C and 40°C respectively. The loss of active was followed by weighing the samples at regular intervals using a four decimal output scale.



### 5.9.8 Repellent bracelet testing

To test the concept of using repellent impregnated polymer bracelets in the early stages of the study, preliminary repellent studies were done on solid (non micro-porous) bracelets using arm-in-cage test. In this test a fore arm with a repellent bracelet placed on the wrist area was inserted in a cage with 40 female non blood fed susceptible *An. arabiensis* mosquitoes (3-5 days old) for 5 minutes at a time. This was done every hour for 4 hours. After 2 hours an extra 40 female mosquitoes were added into the cage. Similarly a forearm with a neat bracelet was also placed in a cage as negative control. The number of mosquitoes that landed on the forearm and the number of mosquitoes that landed on the bracelet area was recorded. This test was done once weekly over a 25 day period. In between testing, the bracelets were stored at 30°C and 90% RH.

## 5.10 Results

### 5.10.1 Free absorption of DEET by EVA matrix

Table 26 shows the results of the free absorption of DEET by EVA (18%) matrix. The results indicate that the EVA (18%) can freely absorb  $4.19 \pm 0.35$ wt.% DEET into its matrix at ambient conditions.

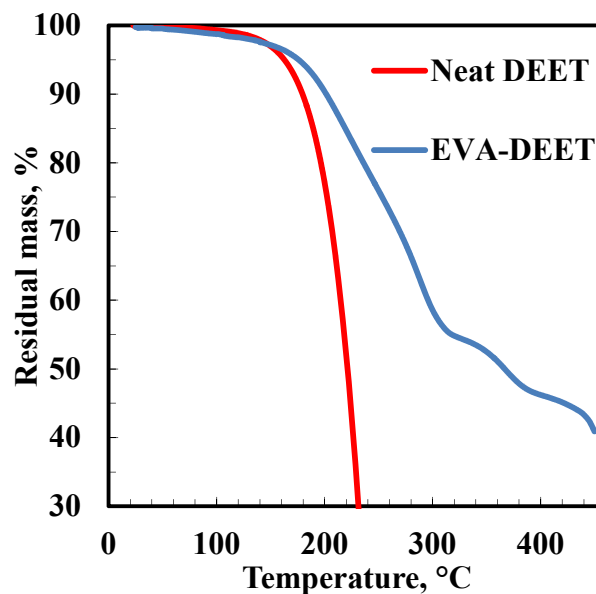
**Table 26: Absorption of DEET by EVA**

Sample	Initial mass (g)	Final mass (g)	Change (wt.%)	DEET (wt.%)
1	1.125	1.169	3.9	3.8
2	1.167	1.214	4.0	3.9
3	0.75	0.781	4.1	4.0
4	0.957	1.003	4.8	4.6
5	0.81	0.843	4.1	3.9

### 5.10.2 Thermal analysis

Figure 38 shows the TGA traces of neat DEET and micro-porous EVA (18%) formed by compounding 47wt.% DEET. The results show that DEET is volatile, immediately evaporating as the temperature is ramped up. It is reasonably stable up to ca. 160°C. This influenced the processing of the polymer strands to form a micro-porous polymer. The maximum compounding temperature was about 170°C above which visible fumes were formed during compounding. The EVA-DEET traces show three mass loss steps. During the

first step of mass loss, ca. 42wt.% DEET was freely held within the micro-porous structure. A further ca. 6wt.% DEET was lost during the second mass loss step. This was attributed to DEET bound by EVA polymer structure. This amount is about ca. 2wt.% more than found in the free absorption studies (Table 26). It is expected that more DEET dissolved in the polymer matrix at high processing temperatures of extrusion compared to the free absorption tests done at room temperature with no forced mixing. The third mass loss step is attributed to the degradation of the EVA polymer. The delayed onset of mass loss of the EVA-DEET sample compared to DEET is indicative of the stabilizing effect of the polymer to DEET volatility.

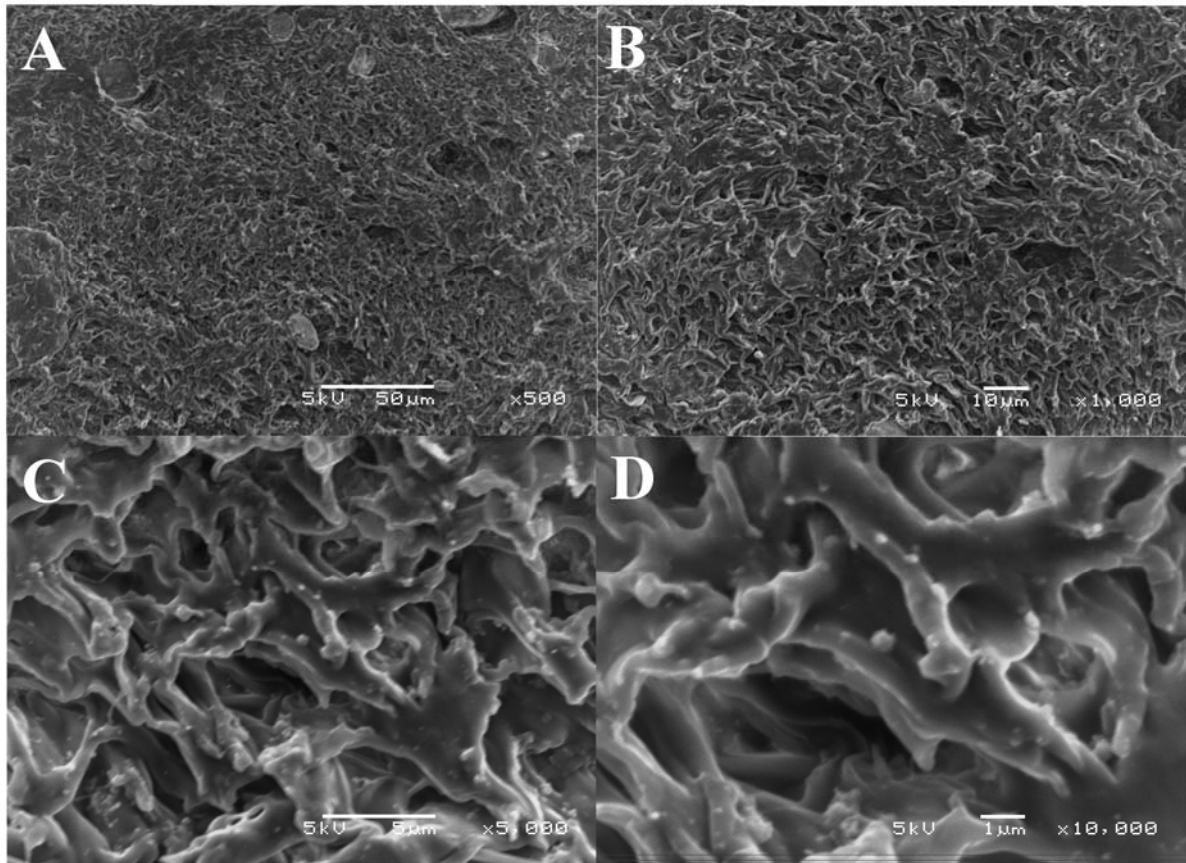


**Figure 38: TGA traces of Neat DEET and a micro-porous EVA (18%) formed by compounding with 47.6wt.% DEET.** The temperature was scanned from 20°C to 450°C at 10°C/ minute.

### 5.10.3 Microscopy

The nominal diameter of the solid EVA-DEET strands was  $4.18 \pm 0.18$  mm and that of the micro-porous DEET containing strands was  $2.91 \pm 0.06$  mm. The target was that both should contain the same nominal amount of DEET per unit length. However, in the end the micro-porous material contained about 6% less DEET than the solid strands when compared at equal lengths. The average thickness for the single coating was  $0.23 \pm 0.06$  mm while for the double coating the value was  $0.44 \pm 0.17$  mm. Figure 39 shows SEM micrographs of open cell micro-porous structure obtained with EVA-DEET. It reveals a complex co-continuous

polymer microstructure. For EVA-DEET micro-structure note that this structure represents a state where the repellent was removed and shrunk the polymer matrix. It is likely that in the loaded state the repellent actually swells the polymer and the pores would be much larger.



**Figure 39: SEM micrographs at four different magnifications of the open cell microstructure of an EVA-DEET strand initially containing 47.6wt.% DEET.** The structure appears less porous than expected owing to shrinkage of the polymer following the leaching of the DEET with dichloromethane. The length bar in (A) equals 50 µm, (B) corresponds to 10 µm, (C) corresponds to 5 µm and (D) corresponds to 1 µm.

Appendix 5 shows further examples of micro-porous polymer structures of EVA and linear low density polyethylene (LLDPE) produced using DEET and dioctylphthalate (DOP) as diluents. Appendix 5 also shows the results of unsuccessful compounding trials.

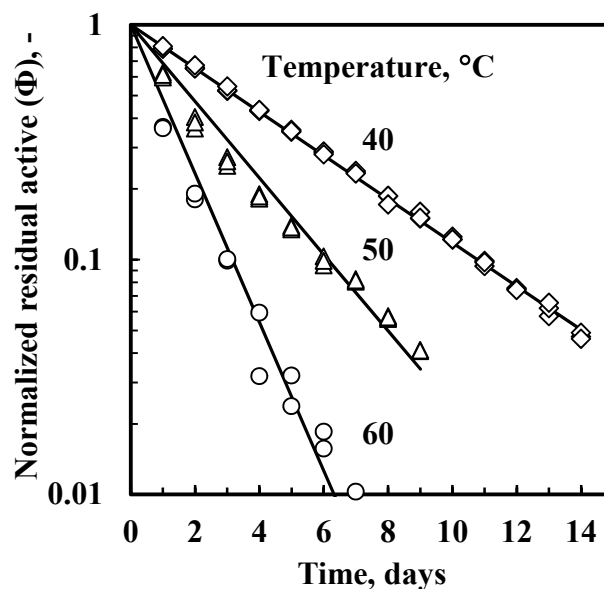
#### 5.10.4 Accelerated mass loss studies

Figure 40 and Figure 41 shows the change in the normalised repellent content as a function of time and temperature. Surprisingly all the mass loss data followed first order kinetics. This

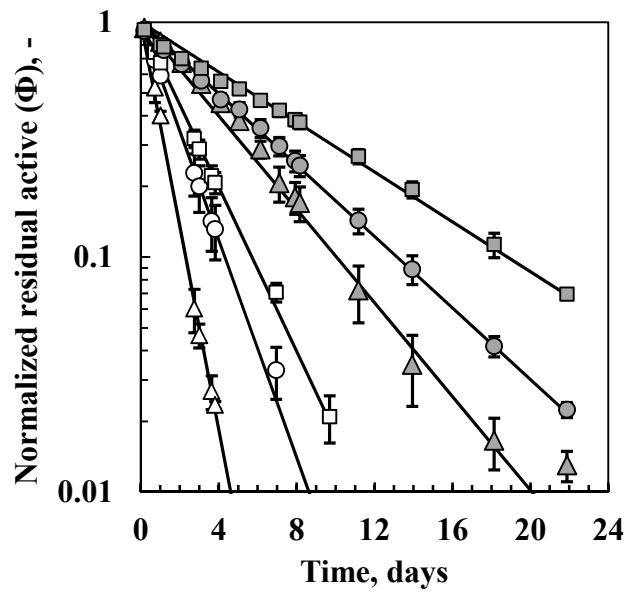
means that the time dependence of the normalised residual active content was described by the following expression:

$$\Phi = (w(t) - w_{\infty}) / (w_i - w_{\infty}) = e^{-t/\tau}$$

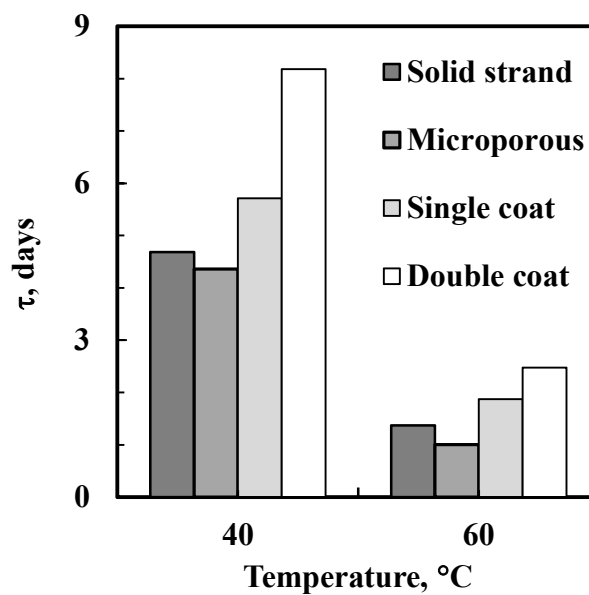
where  $\Phi$  is the normalized residual active content of the repellent;  $t$  is the time in days;  $w(t)$ ,  $w_i$  and  $w_{\infty}$  are the repellent loadings at time  $t$ , the initial loading and the long term (equilibrium) loading of the repellent in wt.% respectively, and  $\tau$  is the first order time constant in days. The time constant represented a measure of the rate at which the active is released. In any time interval of  $\Delta t = \tau$ , the residual amount of active in the system is reduced by a factor  $1/e \approx 0.368$ . If the sample had the same initial active content, then the effective life time should scale according to the corresponding  $\tau$  values. The time constant values evaluated for the different samples are indicated in Figure 42. For the solid EVA strand with a nominal DEET content of 24.4wt.%, the time constant was 4.7 days when aged at 40 °C. As expected,  $\tau$  values for the micro-porous strands were shorter because the DEET was partly available as a free liquid. Adding the polyamide coatings increased the time constant considerably. For a double coating the time constant was 8.2 days at 40 °C, i.e. almost twice the value found for the uncoated micro-porous strand (4.4 days).



**Figure 40: Effect of temperature on the normalised residual active content ( $\Phi$ ) for solid EVA strands with a nominal initial DEET content of 24.4wt.%**



**Figure 41: Variation of normalised residual active content ( $\Phi$ ) for microporous strands aged at 40°C (filled symbols) and 60 °C (open symbols). The initial nominal DEET content was 47.6wt.%. Key:  $\Delta$ ,  $\blacktriangle$  uncoated;  $\circ$ ,  $\bullet$  single coating; and  $\square$ ,  $\blacksquare$  double coating.**



**Figure 42: Variation of  $\tau$  the first order time constant with temperature and the microstructure of the strands**

### 5.10.5 Bracelet repellent tests

The general observation was that fewer mosquitos landed on the forearm with the treated bracelet compared to the non-treated bracelet. There was also no increase in the number of

mosquitos that landed on the forearm with the treated bracelet as testing time progressed. There was no mosquito that landed on or near the bracelet area (*ca.*  $\pm 4$  cm from the bracelet) throughout the whole testing period. Results are presented in Table 27, Table 28, Table 29 and Table 30.

**Table 27: Day 4 of arm-in-cage test**

Test	General forearm area with control bracelet	Forearm with treated bracelet	
		General forearm area	Bracelet area
1	11	0.4	0
2	12.2	1	0
3	8.4	5.4	0
4	9	1.4	0

**Table 28: Day 11 of arm-in-cage test**

Test	General forearm with control bracelet	Forearm with treated bracelet	
		Forearm	Bracelet area
1	7	9.4	0
2	2	3.2	0
3	3	3.2	0
4	5.8	7.4	0

**Table 29: Day 18 of arm-in-cage test**

Test	General forearm with control bracelet	Forearm with treated bracelet	
		Forearm	Bracelet area
1	4.2	0.8	0
2	4.2	1.4	0
3	5.8	2	0
4	5.4	1	0

**Table 30: Day 25 of arm-in-cage test**

Test	General forearm with control bracelet	Forearm with treated bracelet	
		Forearm	Bracelet area
1	4.4	1.2	0
2	3.6	1.4	0
3	6.2	2.2	0
4	6	1.8	0

These results may be expressed as the protective efficacy (Table 31) (Logan *et al.*, 2010). The protective efficacy may be expressed as:

$$\text{Protective efficacy} = (\text{Landings on control} - \text{landings on test specimen}) / \text{landings on control}$$

The protective efficacy measured after four days was actually negative. The difference in the mean landings for the control and the test was not statistically significant either. This unexpected result is not understood at present. However, for the other test days the differences in the means were statistically significant ( $p < 0.05$ ). If only these measurements are considered, the DEET bracelet did provide some protection against mosquito landings.

**Table 31: Protective efficacy (%) of over time in arm-in-cage experiments conducted with solid EVA strands**

Days	Test <sup>1</sup>	Control <sup>1</sup>	Protection efficacy <sup>2</sup>	<i>p</i> -value <sup>3</sup>
		10.15 ±		
0	2.05 ± 2.27	1.76	80 ± 28	0.017
4	5.8 ± 3.11	4.45 ± 2.34	-30 ± 22	0.609
12	1.30 ± 0.53	4.85 ± 0.89	73 ± 9	0.004
21	1.60 ± 0.43	5.05 ± 1.26	68 ± 6	0.015

<sup>1</sup>Average landings per test with a plain EVA strand was used as control.

<sup>2</sup>The protection efficacy is defined as (control mean-test mean)/control mean expressed as a percentage.

<sup>3</sup>The *p*-value represents the outcome of a *t*-test for equality of the test and control means under the assumption of equal variances. A significant difference is indicated in the event that  $p < 0.05$ .

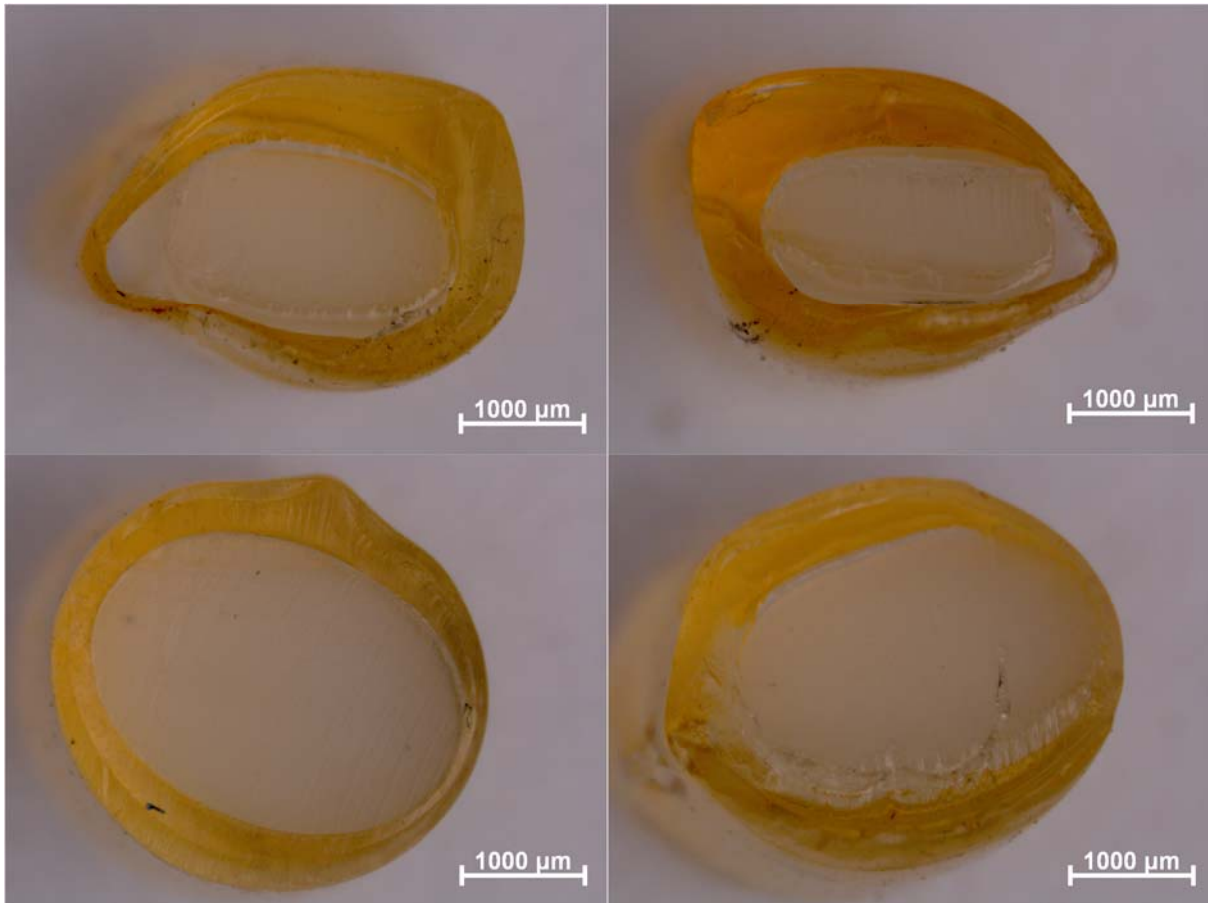
## 5.11 Discussion

Two objectives have to be met when developing a product to protect against mosquito bites in the ankle region. The product should be long lasting and affordable. Slow release of actives using polymer matrices can meet these objectives. In preliminary studies it was discovered that no more than 25wt.% DEET could be incorporated into EVA by simple direct extrusion without any additive bleeding. Repellent testing via arm in a cage tests has shown that an EVA bracelet impregnated with this amount of DEET remained active for at least a 21 days at 30 °C. Furthermore, using open cell micro-structures allowed for the incorporation of higher amounts of liquid active into the polymer matrix. For example in the case of EVA, by

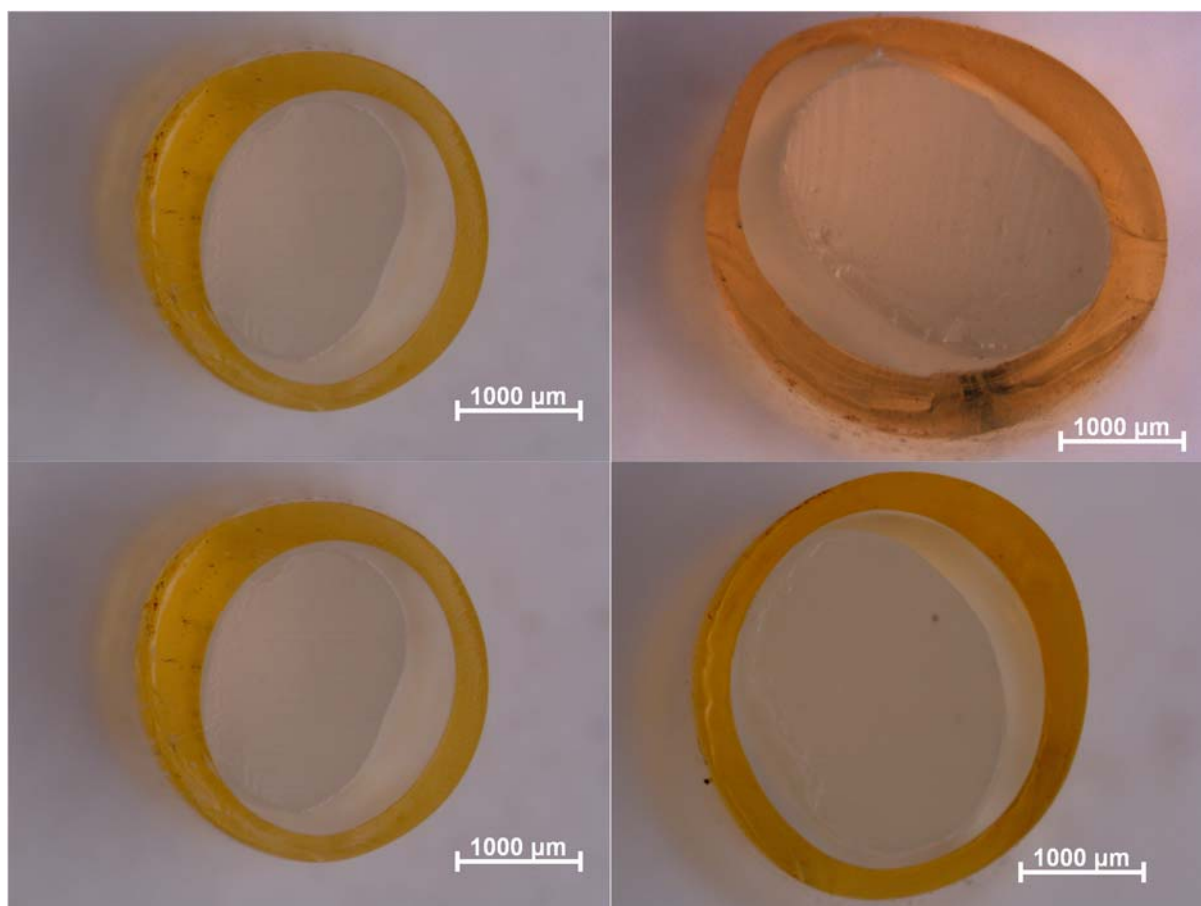
a simple compounding process it was not possible to incorporate more than 25wt.% DEET into the matrix without active blooming. However, using the technique of spinodal decomposition, it was possible to incorporate 47.6wt.% DEET without any blooming.

Such high amounts of active in the polymer will increase the length of residual effectiveness of the repellent polymer bracelet. EVA has a soft rubbery feel that makes it aesthetically desirable for the manufacturing of repellent bracelets. However, some DEET is retained within the EVA matrix. As the DEET active is transferred from the polymer matrix to the surrounding ambience the polymer matrix shrinks in volume. This means that during use the bracelet will become shorter. Figure 43 and Figure 44 shows optical micrographs of a cross section of a double coated micro-porous strand after the DEET was depleted during mass loss studies at 40°C and 60 °C respectively in a convection oven. It is clear that the outer membrane eventually became detached owing to differential shrinkage. The level of detachment increased with the temperature of ageing. The length of the micro-porous strands also decreased as the DEET was progressively depleted (Table 32). However, the presence of the coating did reduce the tendency to shrink to some extent. Lengthwise the uncoated micro-porous strand shrank by 13%, the single coat strand by ca. 11% and the double coated strand by ca. 10%.





**Figure 43: Optical microscope micrograph showing the uneven shrinkage of different EVA (18%)-DEET polymer strands double coated with polyamide and exposed to 40 °C**



**Figure 44: Optical microscope micrograph showing the uneven shrinkage of different EVA (18%)-DEET polymer strands double coated with polyamide and exposed to 60 °C**

**Table 32: Length change of strands after oven exposure**

Temperature	Coating	Initial length (cm)	Final length (cm)	Decrease (%)
40	None	20.00	17.39	13.05
	Single	20.00	17.62	11.90
	Double	20.00	18.29	8.55
60	None	20.00	17.32	13.40
	Single	20.00	17.74	11.30
	Double	20.00	17.90	10.50

Extrapolation of the time constant to 30 °C, on the assumption of Arrhenius temperature dependence, predicts a time constant of 9.0 days for the solid bracelet containing DEET. The corresponding value for the double coated micro-porous strand is 15.8 days. The former was still effective after 21 days. These results suggest that long life bracelets are at least a

theoretical possibility. More development and testing work is required before viable bracelets designs will be obtained.

### **5.12 Conclusion**

This study has shown that a bracelet made of EVA copolymer impregnated with DEET can be used to protect from *An. gambiae* mosquito bites. This bracelet can be produced by a simple extrusion process followed by quenching in an ice bath. Use of a barrier film may not slow down the loss of active from a shrinking polymer matrix. Repellent tests show that the EVA matrix slowly releases the active thereby increasing the length of the residual effectiveness. From these results it can be concluded that long life bracelets are a theoretical possibility. Shrinkage of the EVA polymer matrix as it loses active will cause a decrease in the size of the bracelet during use.

## 6. Overall discussion

It is possible to increase the residual effectiveness of alternative insecticides and repellents. Stabilising these actives are however different because of the different mechanisms that cause premature loss of insecticidal activity of these actives in the field. Pyrethroid insecticides are unstable in alkaline environments. These insecticides are quickly hydrolysed at high pHs (>7). At pH 7 the half-life of alphacypermethrin is about 100 days and at pH 9 it is just under 10 days (**Error! Reference source not found.**) (WHO, 2009). In the absence of pH hydrolysis, pyrethroid insecticides can last very long in the environment. It has also been established that Netlon® is not a slow release technology. However the polymer surface offers a benign environment that ensures that pyrethroids will be stable for a very long time. These conditions are good enough to ensure long lasting efficacy of the Netlon® wall lining.

The mechanism of premature loss of efficacy of DEET is due to the volatility of the active. DEET has several magnitudes higher vapour pressure than alphacypermethrin or deltamethrin. The experimental results in this study have shown that polymer matrices can reduce the volatility of volatile repellents. Adding an extra coating may also assist in reducing this volatility further thereby increasing the residual effectiveness of the repellents. Such an approach may be beneficial in stabilising liquid insecticides such as organophosphates.

The two techniques developed in this study can have a significant impact in reducing the prevalence of malaria. However, not all gaps in vector control have been covered. Insecticidal nets (LLIN and ITWL) still use pyrethroid insecticides only. Mosquitoes resistant to pyrethroids cannot be controlled using current LLIN and ITWL. New focus should be placed on developing insecticidal nets that use alternative insecticides. Carbamate insecticides must not be considered because they possibly release very toxic fumes upon thermal degradation. New focus should particularly be aimed at incorporating organophosphate insecticides into polymer matrices. Direct incorporation using simple compounding processes has not been successful. New techniques to incorporate organophosphate insecticides into polymers should be devised.

DEET has been a very effective repellent to use for topical applications because it has a relatively long residual efficacy compared to its alternatives. Efforts should be made to manufacture formulations that use alternative repellents such as citronellal and IR3535.

A very important part of product development for malaria vector control is efficacy testing. The tests performed for ITWL and mosquito repellent bracelets were very basic. The objective was to determine whether the product is achieving the intended objective i.e. long lasting effectiveness. Such a result is important in order to optimise the development of the product. Much more comprehensive bio-efficacy tests (laboratory and field) should be carried out in collaboration with experts in entomology.

The concept of mosquito repellent bracelets may be hard to sell to all stakeholders. A point that critics have raised time and again is that the area of repellent effectiveness for bracelets is very small to deter mosquitoes from obtaining a blood meal. The concept of protecting ankles only needs to be validated to warrant further developmental work of repellent bracelets. Another challenge is that there is no standard protocol from the WHO for testing mosquito repellent bracelets. New test protocols should then be developed with the assistance of entomologists.

The products developed in the studies presented in this thesis have a very high potential to be commercialised. It is therefore important to obtain high quality entomological results for the purposes of approval by the WHO and also funding to start-up production.

## 7. Overall conclusions and recommendations

This study provides, for the first time, detailed information about the location of pyrethroid insecticides in a polymer filament and this information is relevant to long life insecticide treated nets (LLIN) and insecticide treated wall linings (ITWL). The key finding is that the insecticides rapidly bloom to the surface owing to a low solubility and delayed crystallization. This conclusion is based on the confocal fluorescence microscopy and bioassay test results. Further development of ITWL or LLIN using other alternative insecticides should take this fact into account. It is recommended to consider ITWL based on the use of organophosphate insecticides as active ingredients are considered to control pyrethroid resistant mosquitoes.

The suppression of repellent volatility by polymer matrices has also been demonstrated via thermogravimetric analysis (TGA) studies. It was shown that up to 50wt.% liquid repellent can be incorporated into an EVA polymer matrix. It has also been shown that this polymer matrix can lengthen the residual effectiveness of a repellent such as DEET by up to about a month. More experimental and fundamental work needs to be done to improve the mosquito repellent bracelet concept in order to design a product far superior to any currently on the market. It will be prudent to develop mosquito repellent bracelets or slip-slop bands that use alternative repellents such as citronellal and IR3535.

Research into malaria vector control is a very broad based requiring close collaboration between product developers, public health officials and entomologists. The research presented in this thesis was done with minimal collaboration with public health officials and entomologists. Going forward it is recommended that strong collaborations be established with local and international public health and entomological experts for the purposes of testing products developed.

## 8. Publications and conference presentations

### Publications

1. Sibanda, MM. Focke, WW. Heyman, HM. and Birkholtz, L-M (2013). “Physical, chemical and biological methods of vector control” *Innovate*, 8, 40-44.
2. Sibanda, MM. Focke, WW, Smart, J. “Development of a mosquito repellent bracelet for malaria control.” (Submitted to the malaria journal for publication).
3. Sibanda, MM and Focke, WW. “Development of an insecticide impregnated wall lining for malaria vector control.” (Publication withheld pending licensing of the technology).
4. Kruger, T. Sibanda, MM. Focke, WW. de Jager, D. “Pilot evaluation on the user acceptability, durability, and perceived effectiveness and laboratory tested efficacy of wall linings for malaria vector control in Vhembe district, Limpopo province” (Submitted to the malaria journal for publication).

### Conference presentations

1. Sibanda M, and Focke W. Development of an insecticide impregnated polymer wall lining for malaria vector control. Challenges in malaria research: Core science and innovation, Oxford, United Kingdom, 22-24 September, 2014.  
<http://www.malariajournal.com/content/13/S1/P80>.
2. Kruger T, De Jager, C. Focke, W. Sibanda, M. and Bornman, R. Slow release pyrethroid-impregnated indoor linings as a new malaria vector control method. Environment and Health – Bridging South, North, East and West. Conference of ISEE, ISES and ISIAQ. Basel, Switzerland 19 – 23 August, 2013.  
<http://ehp.niehs.nih.gov/ehbasel13/p-3-23-24/>.
3. Sibanda M, Focke W, Kruger T. Development of an Insecticide Treated Wall Lining for malaria vector control. 6<sup>th</sup> MIM Pan-African Malaria, The International Convention Centre, Durban, South Africa, 6-11 October, 2013.
4. Kruger T, De Jager C, Focke W, Sibanda M, Bornman R. Slow release pyrethroid-impregnated indoor linings as a new malaria vector control method. 6<sup>th</sup> MIM Pan-African Malaria The International Convention Centre, Durban, South Africa, 6-11 October, 2013.

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## 10. Appendices

### 10.1 Appendix 1: Raw bioassay results

**Table 33: Bioassay tube test results for knock down at 60 min and mortality after 24 h for blown films in preliminary studies**

<b>Sample Code</b>	<b>Active (wt.%)</b>	<b>Knock down (%)</b>	<b>Mortality (%)</b>
Blank film A	None	0	0
Blank film B		0	0
P-EF-5A	etofenprox (0.48wt.%)	96	77
P-EF-5B		100	96
P-EF-10A	etofenprox (0.91wt.%)	92	13
P-EF-10B		79	0
P-EF-15A	Etofenprox (1.40wt.%)	93	100
P-EF-15B		100	92
P-AC-5A	alphacypermethrin (0.48wt.%)	100	100
P-AC-5B		96	100
P-AC-10A	alphacypermethrin (0.91wt.%)	96	100
P-AC-10B		100	100
P-AC-15A	alphacypermethrin (1.40wt.%)	100	100
P-AC-15B		100	100
P-BF-5A	bifenthrin (0.48wt.%)	100	100
P-BF-5B		100	100
P-BF-10A	bifenthrin (0.91wt.%)	100	100
P-BF-10B		100	100
P-BC-5A	betacyfluthrin (0.48wt.%)	100	100
P-BC-5B		100	100
P-BC-10A	betacyfluthrin (0.91wt.%)	100	100
P-BC-10B		100	100
P-LH-5A	lamdacyhalothrin (0.48wt.%)	100	100
P-LH-5B		100	100
P-LH-10A	lamdacyhalothrin (0.91wt.%)	100	100
P-LH-10B		100	100
P-DM-5A	deltamethrin (0.49wt.%)	100	100
P-DM-5B		100	100
P-DM-10A	deltamethrin (0.95wt.%)	100	100
P-DM-10B		100	100
P-DM-15A	deltamethrin (1.40wt.%)	89	93
P-DM-15B		100	100
OG-10A	DDT (0.91wt.%)	100	100
OG-10B		100	100
OG-15A	DDT (1.30wt.%)	100	96
OG-15B		100	100

**Table 34: Bioassay tube test results for knock down at 60 min and mortality after 24 h for preliminary Netlon® extrusion trials**

Code	Colour	Knock down (%)	SD	Mortality (%)	SD
Plain Netlon	No colour	1.9	2.6	0	0
alphacypermethrin 0.48wt.%	Yellow	100	0	100	0
alphacypermethrin 0.91wt.%	Blue	100	0	100	0
bifenthrin 0.48wt.%	Orange	100	0	100	0
betacyfluthrin 0.48wt.%	Green	100	0	100	0
deltamethrin 0.95wt.%	Red	100	0	100	0
deltamethrin 1.82wt.%	Brown	100	0	100	0
alphacypermethrin 0.91wt.%	Blue	100	0	100	0
deltamethrin 1.82wt.% (stretched)	Brown	100	0	100	0
DDT 0.91wt.%	Pink	72.6	15.5	15.4	4.4

**Table 35: Bioassay tube test results for knock down at 60 min and mortality at 24 h after one year of manufacture and before installation (Netlon® field trials)**

Sample	Total in tube	Knockdown (%)	Mortality (%)
White	26	100	4
Green	20	100	100
Brown	21	100	100
Purple	25	100	100
Orange	23	100	100



**Table 36: Bioassay tube test results 1 month after installation (Netlon® field trials)**

	Colour code	Insecticide	Total	Knockdown @ 1 h	Knockdown (%)	Mortality @ 24 h	Mortality (%)
A1	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
A2	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
A3	Orange	Cypermethrin (0.47wt.%)	25	22	88	25	100
A4	White	Neat	25	0	0	0	0
A5	Brown	Deltamethrin (0.52wt.%)	22	20	91	22	100
A6	Purple	Deltamethrin (0.85wt.%)	27	27	100	27	100
A7	Orange	Cypermethrin (0.47wt.%)	24	23	96	24	100
A8	White	Neat	25	0	0	0	0
A9	Brown	Deltamethrin (0.52wt.%)	26	26	100	26	100
A10	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A11	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A12	White	Neat	24	0	0	0	0
A13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A14	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A15	Green	Cypermethrin (0.29wt.%)	24	24	100	24	100
A16	White	Neat	25	0	0	0	0
A17	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A18	Purple	Deltamethrin (0.85wt.%)	26	26	100	26	100
A19	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
A20	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
B1	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
B2	White	Neat	24	0	0	0	0
B3	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B4	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B5	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B6	White	Neat	25	0	0	0	0
B7	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B8	Orange	Cypermethrin (0.47wt.%)	26	26	100	26	100
B9	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B10	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
B11	Green	Cypermethrin (0.29wt.%)	26	26	100	26	100
B12	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
B13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B14	White	Neat	24	18	75	0	0
B15	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B16	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B17	White	Neat	26	26	100	26	100
B18	Purple	Deltamethrin (0.85wt.%)	26	26	100	26	100
B19	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B20	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100

Please note that the codes given in the first column identify the dwelling where the corresponding net was installed. **A** represents a mud hut and **B** represents western style house.



**Table 37: Bioassay tube test results after 2 months of installation (Netlon® field trials)**

	Colour code	Insecticide	Total	Knockdown @ 1 h	Knockdown (%)	Mortality @ 24 h	Mortality (%)
A 1	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A 2	Purple	Deltamethrin (0.85wt.%)	26	26	100	26	100
A 3	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 4	White	Neat	25	5	20	25	100
A 5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 6	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 7	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 8	White	Neat	25	19	76	25	100
A 9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 10	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A 11	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 12	White	Neat	24	7	29	0	0
A 13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 14	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 15	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A 16	White	Neat	25	1	4	13	52
A 17	Brown	Deltamethrin (0.52wt.%)	27	27	100	27	100
A 18	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 19	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 20	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 1	Purple	Deltamethrin (0.85wt.%)	25	3	12	21	84
B 2	White	Neat	25	25	100	25	100
B 3	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 4	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B 5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B 6	White	Neat	25	0	0	8	32
B 7	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 8	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B 9	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B 10	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B 11	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 12	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B 13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B 14	White	Neat	24	0	0	5	21
B 15	Purple	Deltamethrin (0.85wt.%)	26	26	100	26	100
B 16	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B 17	White	Neat	25	0	0	6	24
B 18	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B 19	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 20	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
Control			25	0	0	0	0

Please note that the codes given in the first column identify the dwelling where the corresponding net was installed. **A** represents a mud hut and **B** represents western style house.



**Table 38: Bioassay tube test results after 4 months of installation (Netlon® field trials)**

Sample	Colour code	Insecticide	Total	Knockdown @ 1 h	Knockdown (%)	Mortality @ 24 h	Mortality (%)
A 1	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A 2	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 3	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 4	White	Neat	24	11	46	24	100
A 5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 6	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 7	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 8	White	Neat	25	8	32	6	24
A 9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 10	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A 11	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A 12	White	Neat	25	3	12	25	100
A 13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 14	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 15	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A 16	White	Neat	25	10	40	12	48
A 17	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A 18	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A 19	Orange	Cypermethrin (0.47wt.%)	26	26	100	26	100
A 20	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 1	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B 2	white	Neat	25	0	0	0	0
B 3	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 4	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B 5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B 6	White	Neat	24	12	50	12	50
B 7	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 8	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
B 9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B 10	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B 11	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 12	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B 13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B 14	White	Neat	23	0	0	0	0
B 15	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B 16	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B 17	White	Neat	25	3	12	2	8
B 18	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
B 19	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B 20	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
Control			27	0	0	0	0

Please note that the codes given in the first column identify the dwelling where the corresponding net was installed. **A** represents a mud hut and **B** represents western style house.



**Table 39: Bioassay tube test results after 6 months of installation (Netlon® field trials)**

	Colour code	Insecticide	Total	Knockdown @ 1 h	Knockdown (%)	Mortality @ 24 h	Mortality (%)
A1	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
A2	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A3	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A4	White	Neat	24	14	58	23	96
A5	Brown	Deltamethrin (0.52wt.%)	23	23	100	23	100
A6	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A7	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
A8	White	Neat	25	0	0	10	40
A9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A10	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
A11	Orange	Cypermethrin (0.47wt.%)	23	23	100	23	100
A12	White	Neat	25	9	36	17	68
A13	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
A14	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A15	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
A16	White	Neat	25	5	20	14	56
A17	Brown	Deltamethrin (0.52wt.%)	26	26	100	26	100
A18	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
A19	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
A20	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
B1	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B2	white	Neat	26	17	65	26	100
B3	Green	Cypermethrin (0.29wt.%)	26	26	100	26	100
B4	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B5	Brown	Deltamethrin (0.52wt.%)	23	23	100	23	100
B6	White	Neat	25	0	0	2	8
B7	Green	Cypermethrin (0.29wt.%)	24	24	100	24	100
B8	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
B9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B10	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B11	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
B12	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
B13	Brown	Deltamethrin (0.52wt.%)	22	22	100	22	100
B14	White	Neat	24	16	67	16	67
B15	Purple	Deltamethrin (0.85wt.%)	26	26	100	26	100
B16	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B17	White	Neat	26	15	58	24	92
B18	Purple	Deltamethrin (0.85wt.%)	23	23	100	23	100
B19	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B20	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
Control			25	0	0	0	0

Please note that the codes given in the first column identify the dwelling where the corresponding net was installed. **A** represents a mud hut and **B** represents western style house.





**Table 40: Bioassay tube test results after 12 months of installation (Netlon® field trials)**

	Colour code	Insecticide	Total	Knockdown @ 1 h	Knockdown (%)	Mortality @ 24 h	Mortality (%)
A1	Green	Cypermethrin (0.29wt.%)	22	22	100	22	100
A2	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
A3	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
A4	White	Neat	25	22	88	23	92
A5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A6	Purple	Deltamethrin (0.85wt.%)	23	23	100	23	100
A7	Orange	Cypermethrin (0.47wt.%)	24	24	100	24	100
A8	White	Neat	25	18	72	21	84
A9	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
A10	Green	Cypermethrin (0.29wt.%)	24	24	100	24	100
A11	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A12	White	Neat	25	21	84	24	96
A13	Brown	Deltamethrin (0.52wt.%)	26	26	100	26	100
A14	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A15	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A16	White	Neat	24	19	79	22	92
A17	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
A18	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
A19	Orange	Cypermethrin (0.47wt.%)	26	26	100	26	100
A20	Green	Cypermethrin (0.29wt.%)	23	23	100	23	100
B1	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
B2	white	Neat	25	19	76	25	100
B3	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B4	Orange	Cypermethrin (0.47wt.%)	23	23	100	23	100
B5	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B6	White	Neat	24	20	83	23	96
B7	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B8	Orange	Cypermethrin (0.47wt.%)	23	23	100	23	100
B9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B10	Purple	Deltamethrin (0.85wt.%)	22	22	100	22	100
B11	Green	Cypermethrin (0.29wt.%)	24	24	100	24	100
B12	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B13	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B14	White	Neat	25	24	96	24	96
B15	Purple	Deltamethrin (0.85wt.%)	24	24	100	24	100
B16	Brown	Deltamethrin (0.52wt.%)	24	24	100	24	100
B17	White	Neat	25	20	80	25	100
B18	Purple	Deltamethrin (0.85wt.%)	23	23	100	23	100
B19	Green	Cypermethrin (0.29wt.%)	26	26	100	26	100
B20	Orange	Cypermethrin (0.47wt.%)	26	26	100	26	100
Blank			24	0	0	4	17

Please note that the codes given in the first column identify the dwelling where the corresponding net was installed. **A** represents a mud hut and **B** represents western style house.



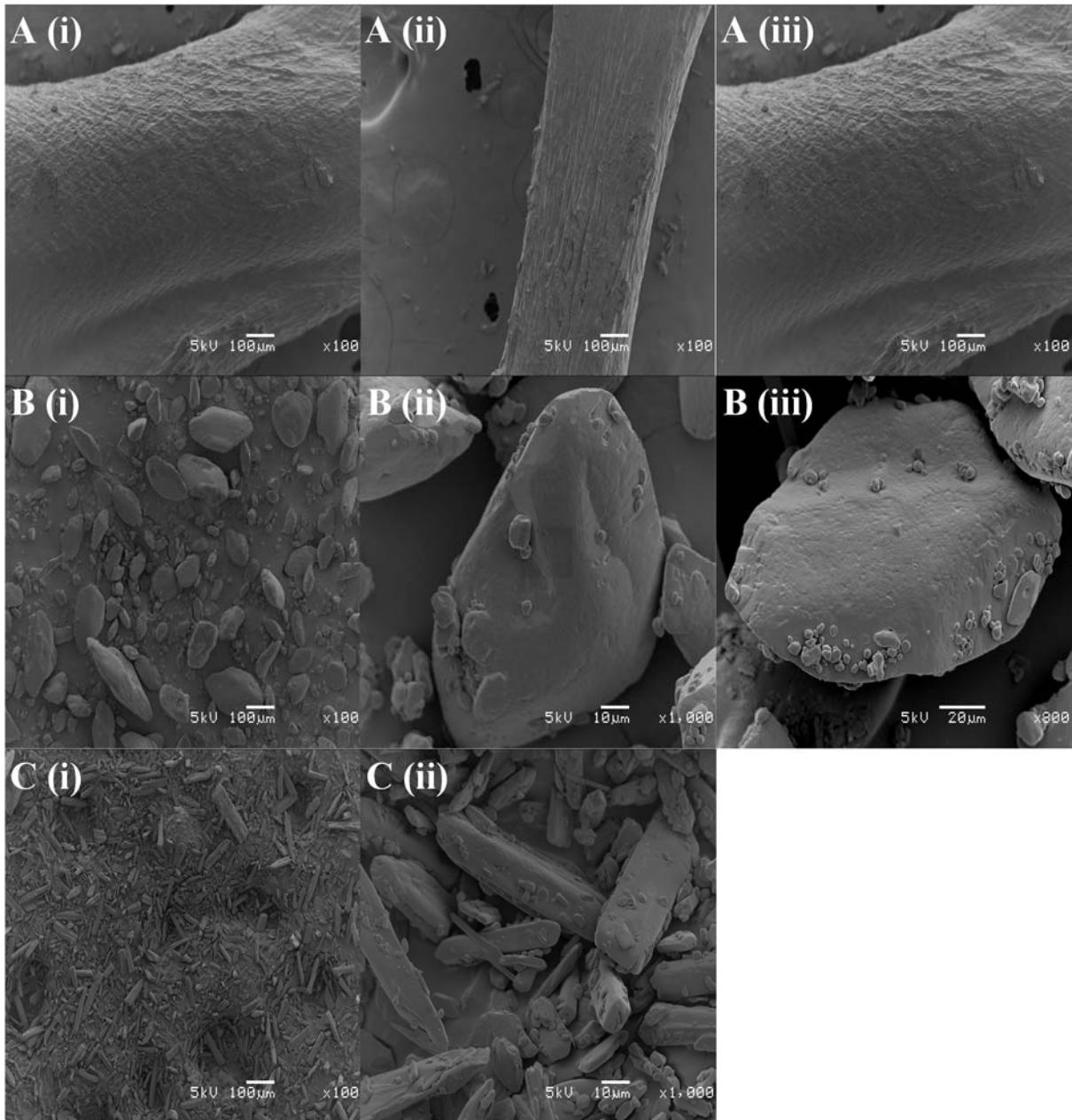
**Table 41: Bioassay tube test results 24 months after installation (Netlon® field trials)**

	Colour code	Insecticide	Total	Knockdown @ 1 h	Knockdown (%)	Mortality @ 24 h	Mortality (%)
A1	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A2	Purple	Deltamethrin (0.85wt.%)	N/A	N/A	N/A	N/A	N/A
A3	Orange	Cypermethrin (0.47wt.%)	25	22	88	25	100
A4	White	Neat	25	25	100	25	100
A5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A6	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A7	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A8	White	Neat	25	20	80	14	56
A9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A10	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A11	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A12	White	Neat	25	14	56	19	76
A13	Brown	Deltamethrin (0.52wt.%)	N/A	N/A	N/A	N/A	N/A
A14	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A15	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
A16	White	Neat	25	24	96	1	4
A17	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
A18	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
A19	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
A20	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B1	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B2	white	Neat	25	24	96	4	16
B3	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B4	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B5	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B6	White	Neat	25	0	0	1	4
B7	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B8	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B9	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B10	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B11	Green	Cypermethrin (0.29wt.%)	25	24	96	25	100
B12	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
B13	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B14	White	Neat	25	22	88	23	92
B15	Purple	Deltamethrin (0.85wt.%)	25	25	100	25	100
B16	Brown	Deltamethrin (0.52wt.%)	25	25	100	25	100
B17	White	Neat	25	25	100	24	96
B18	Purple	Deltamethrin (0.85wt.%)	N/A	N/A	N/A	N/A	N/A
B19	Green	Cypermethrin (0.29wt.%)	25	25	100	25	100
B20	Orange	Cypermethrin (0.47wt.%)	25	25	100	25	100
Blank			25	0	0	1	4

Please note that the codes given in the first column identify the dwelling where the corresponding net was installed. **A** represents a mud hut and **B** represents western style house.

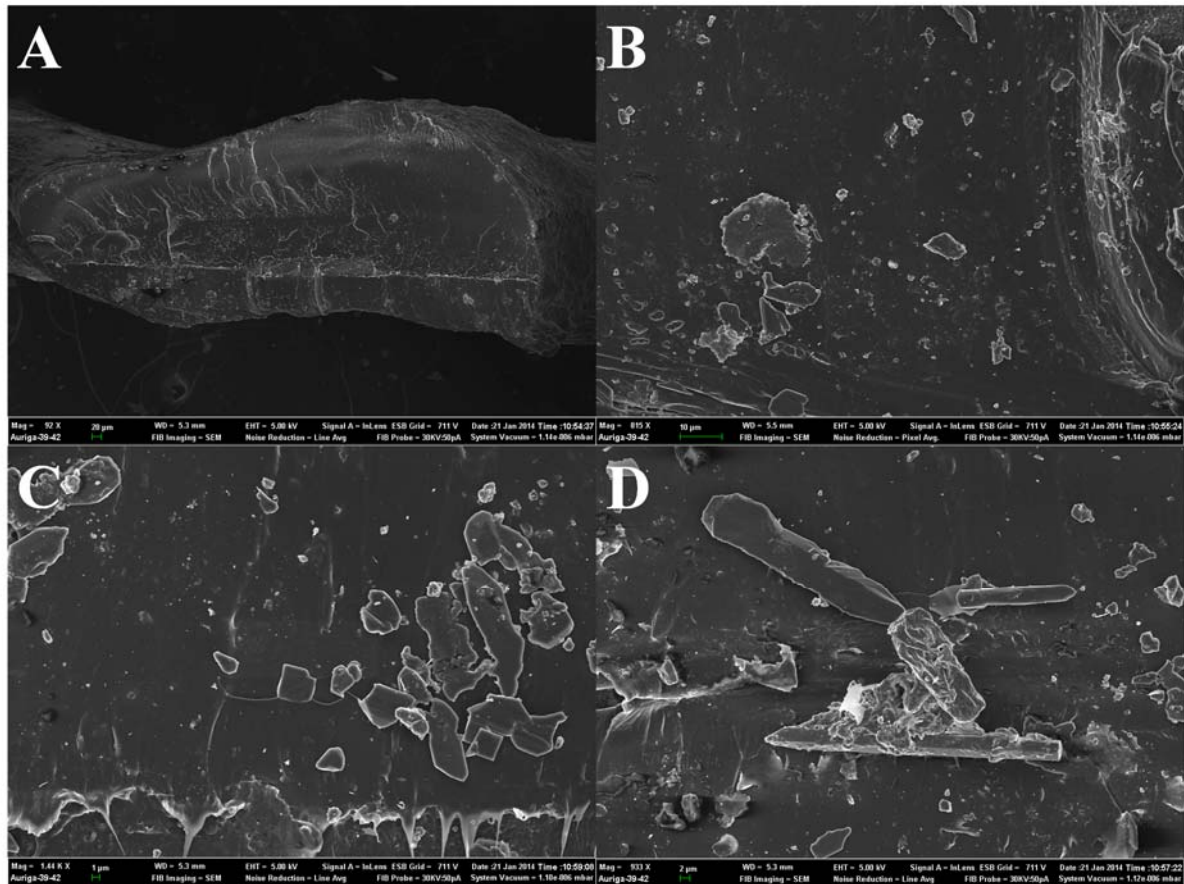
## 10.2 Appendix 2: SEM micrographs of Netlon® and neat insecticides

(A) Neat Netlon (B) Neat alphacypermethrin and (C) Neat deltamethrin

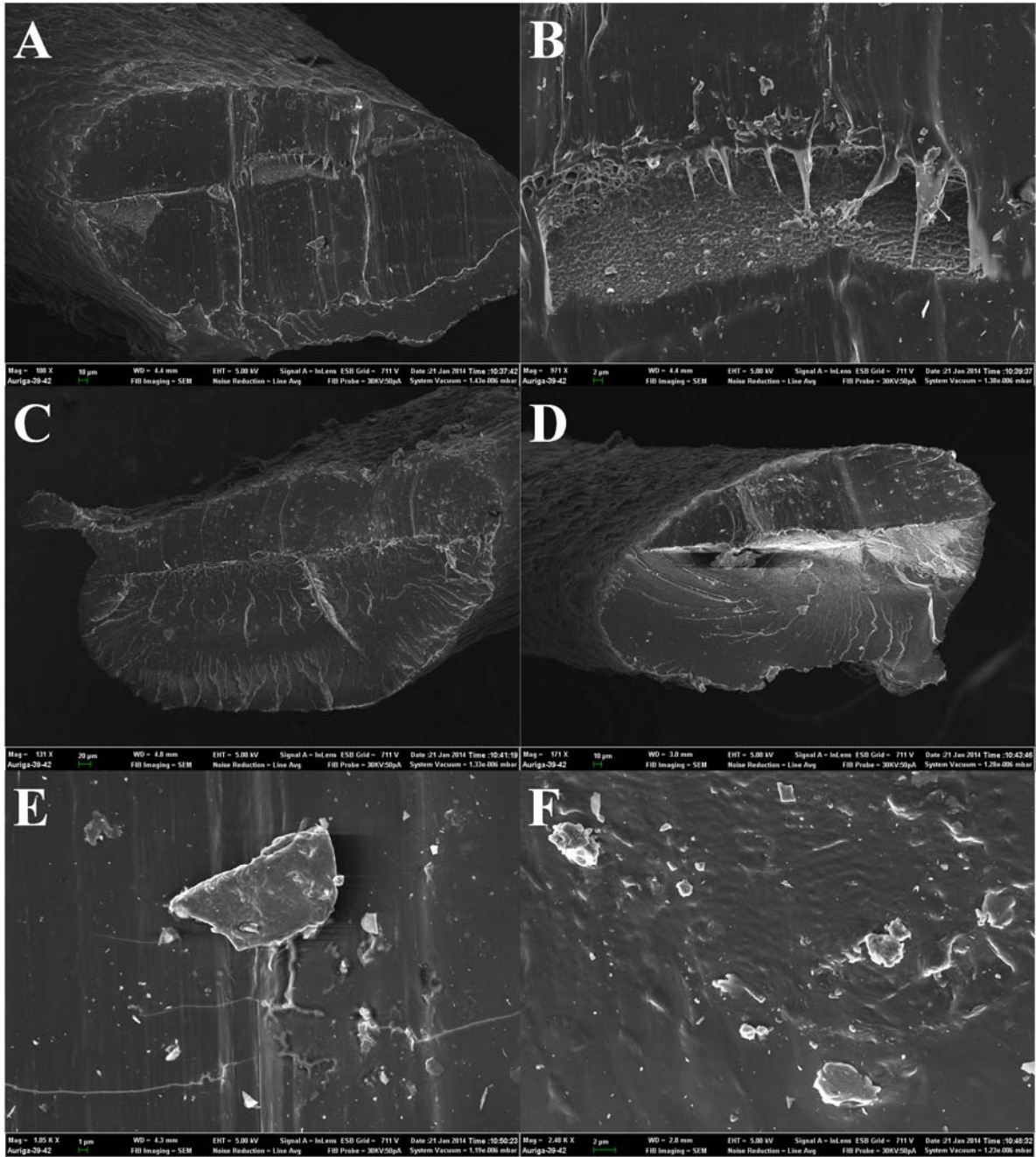


### 10.3 Appendix 3 TEM micrographs

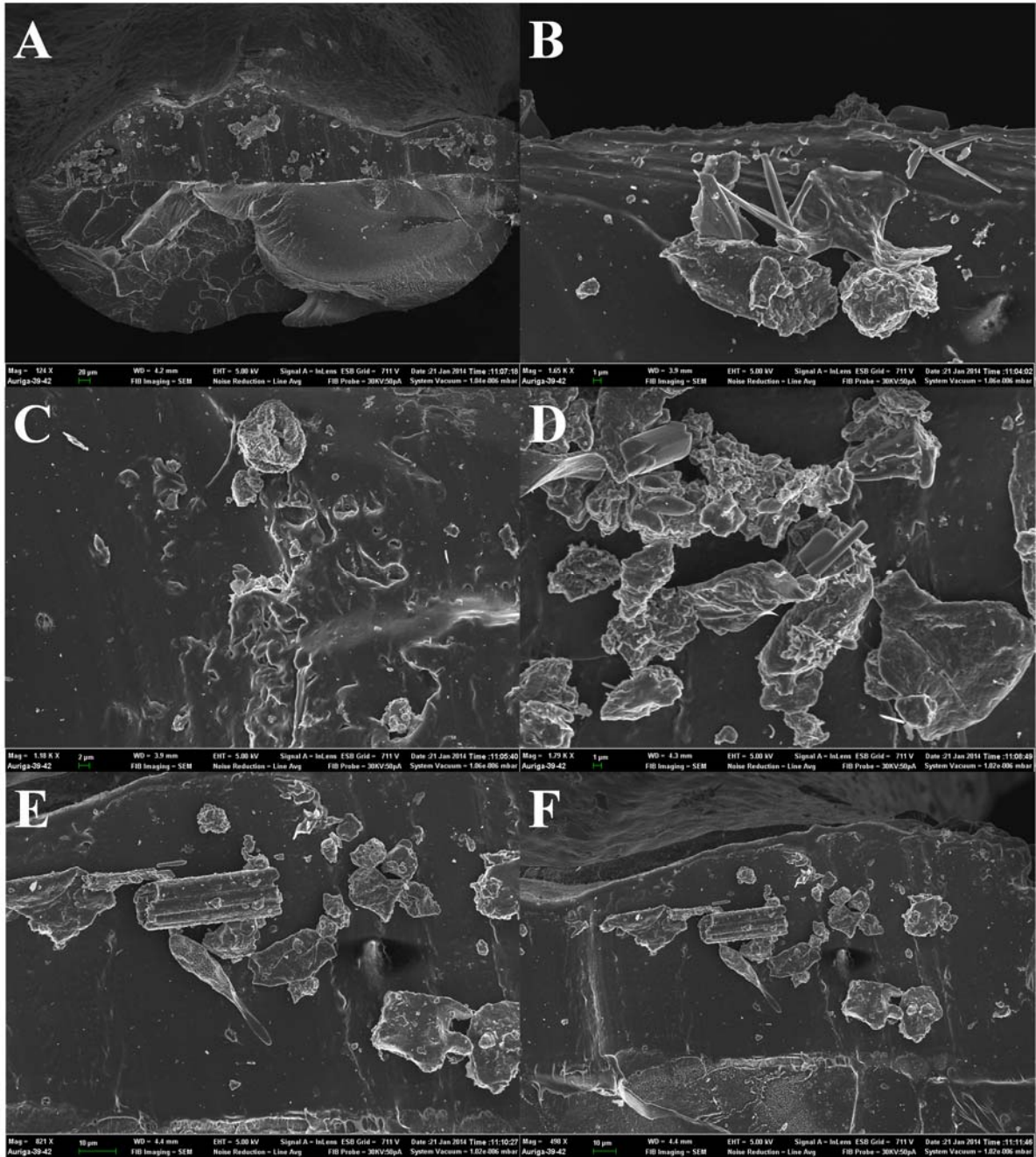
#### TEM micrographs of Netlon® loaded with 0.47wt.% alphacypermethrin



### TEM micrographs of Netlon® loaded with 0.52wt.% deltamethrin



TEM micrographs of Netlon® loaded with 0.85wt.% deltamethrin



## 10.4 Appendix 4: Field trial installation of Netlon®

### Netlon® installed in mud huts

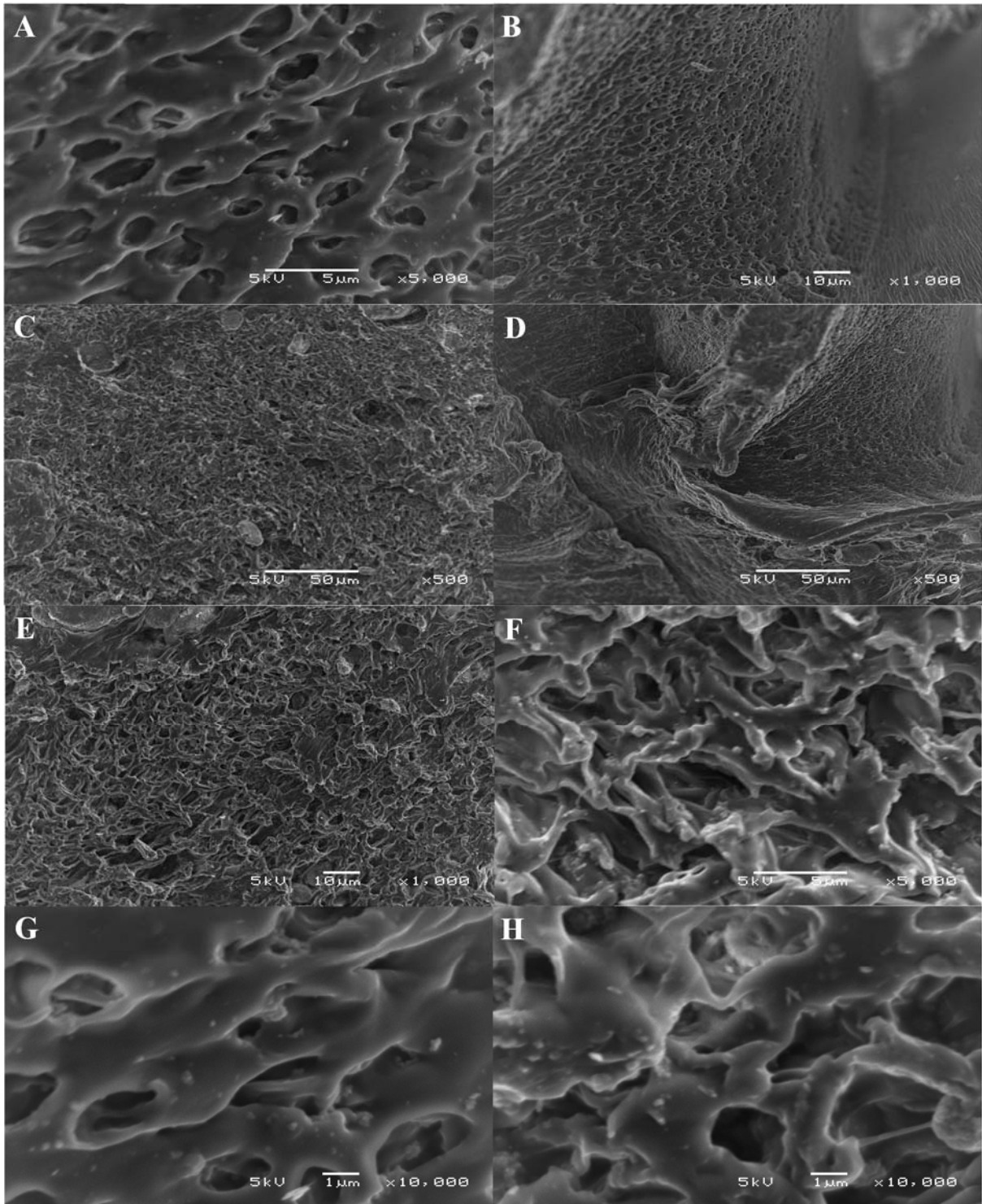


Netlon® installed in western style houses

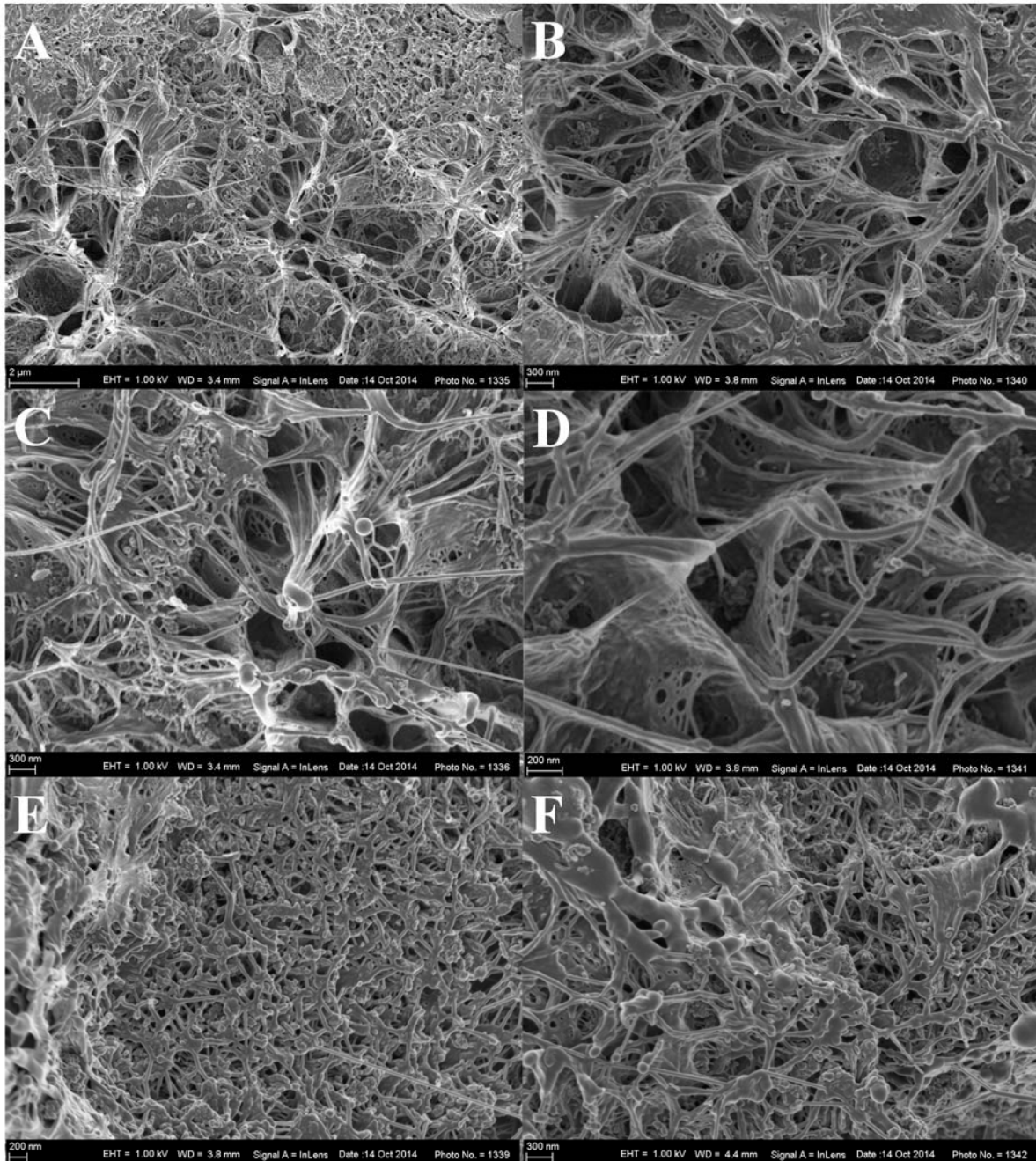




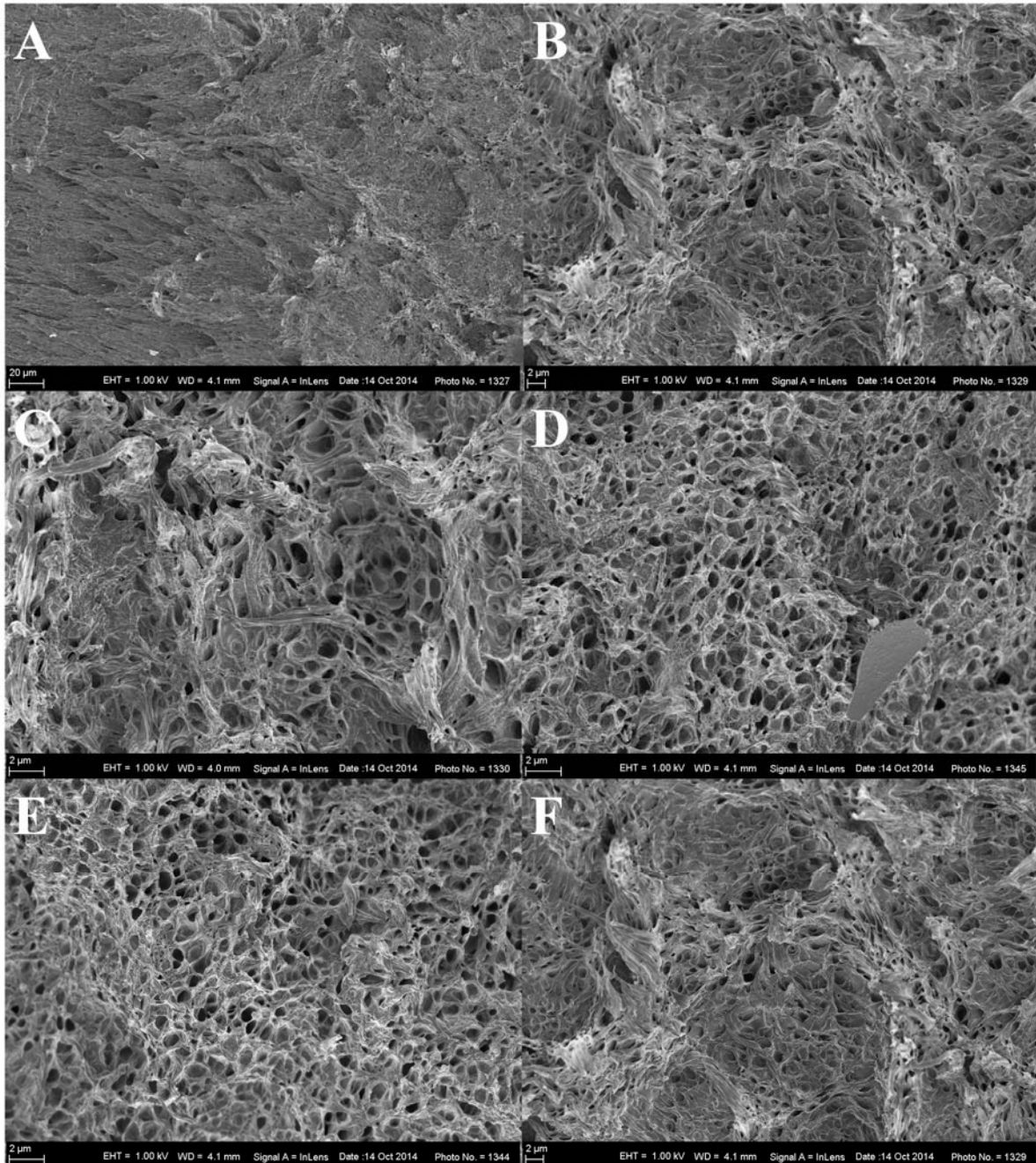
### 10.5 Appendix 5: SEM micrographs of micro-porous polymer EVA (18%) micro-porous surface morphology



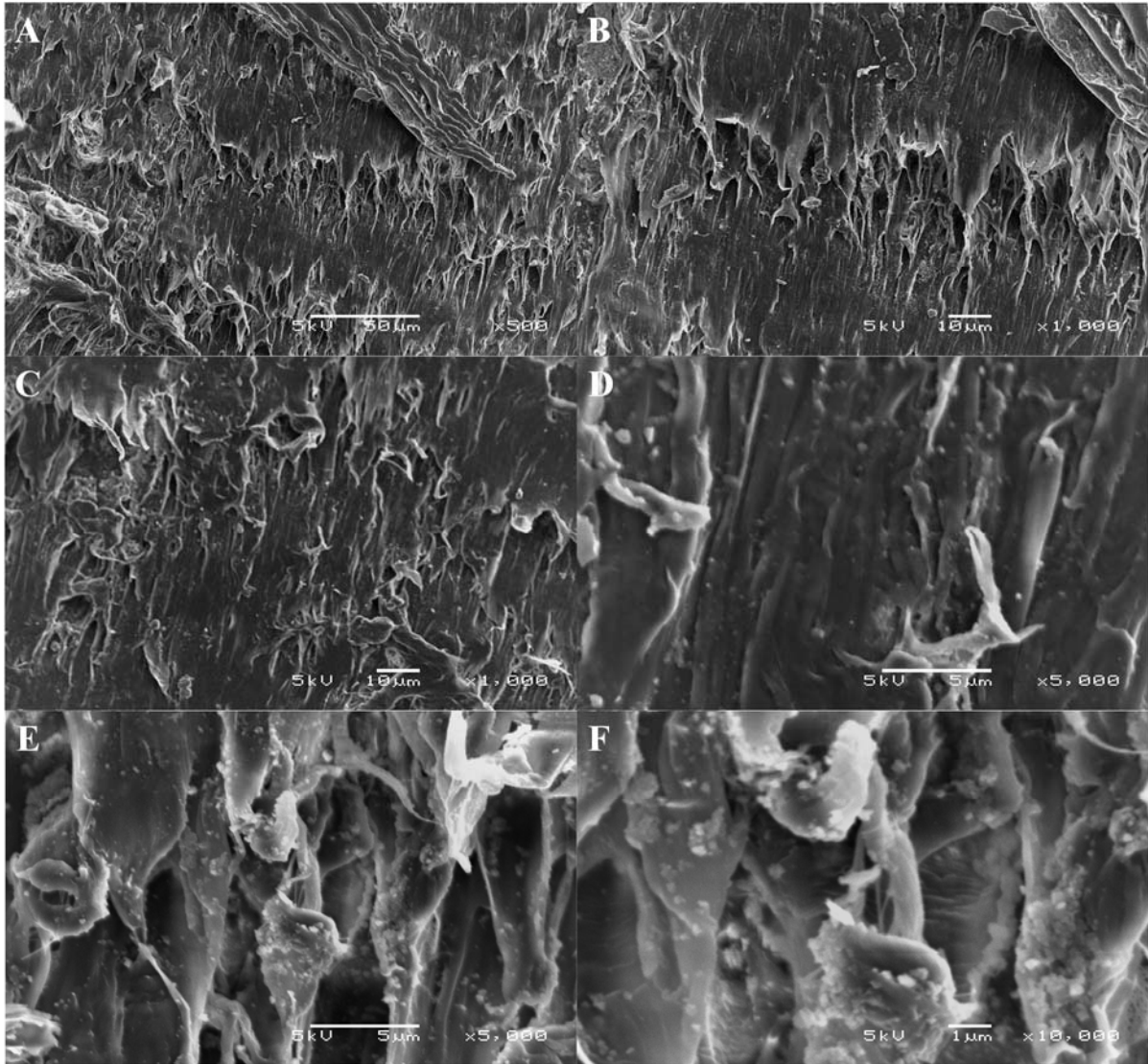
**LLDPE micro-porous polymer structure obtained with citronellal.** This structure was obtained using a micro-compounder which could recirculate the melt in the compounding barrel. This allowed for easy control of residence time which plays an important role in the production of micro-porous polymers from melt.



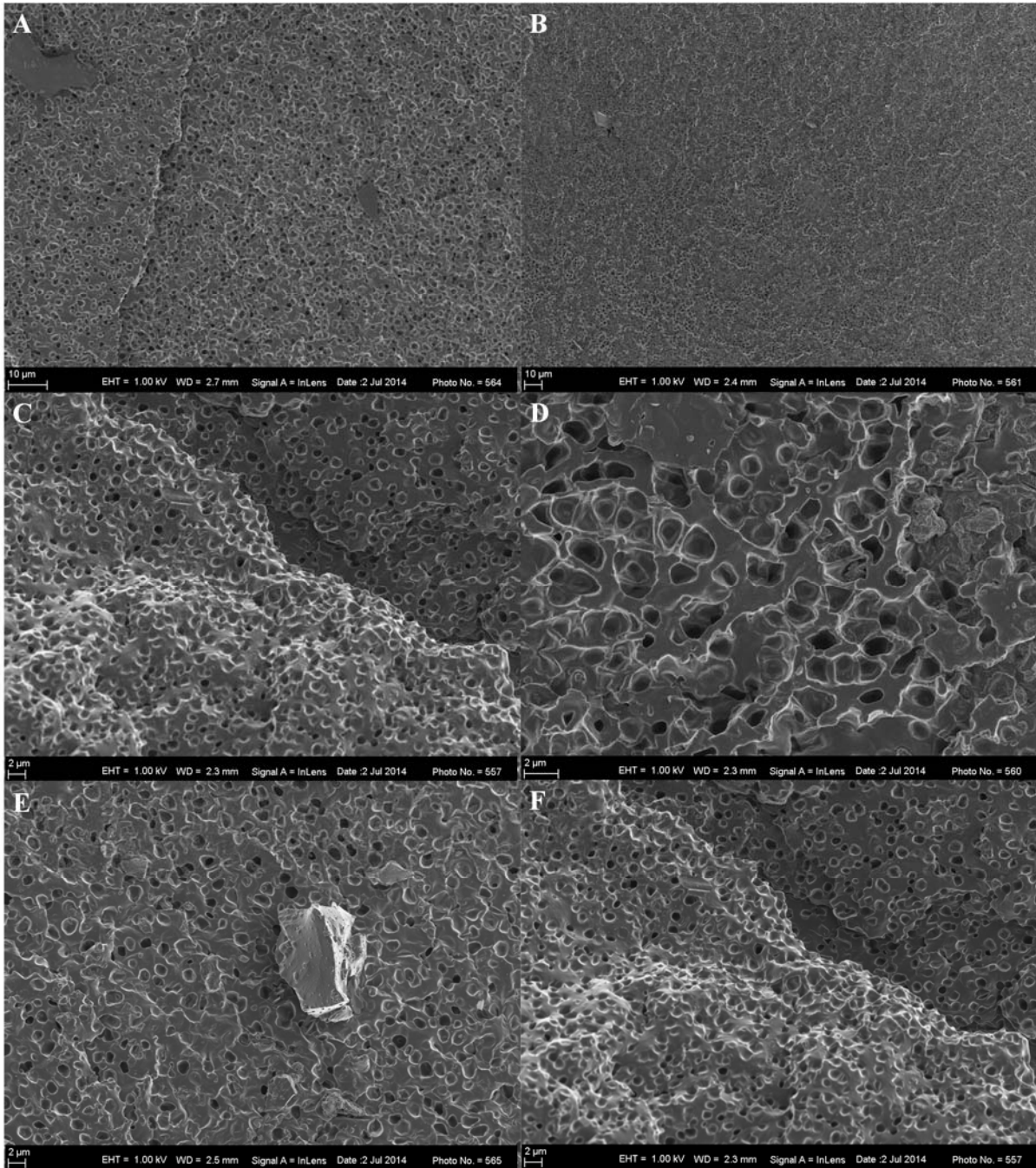
**LLDPE micro-porous polymer structure obtained with DOP.** This structure was obtained using a micro-compounder which could recirculate the melt in the compounding barrel. This allowed for easy control of residence time which plays an important role in the production of micro-porous polymers from melt.



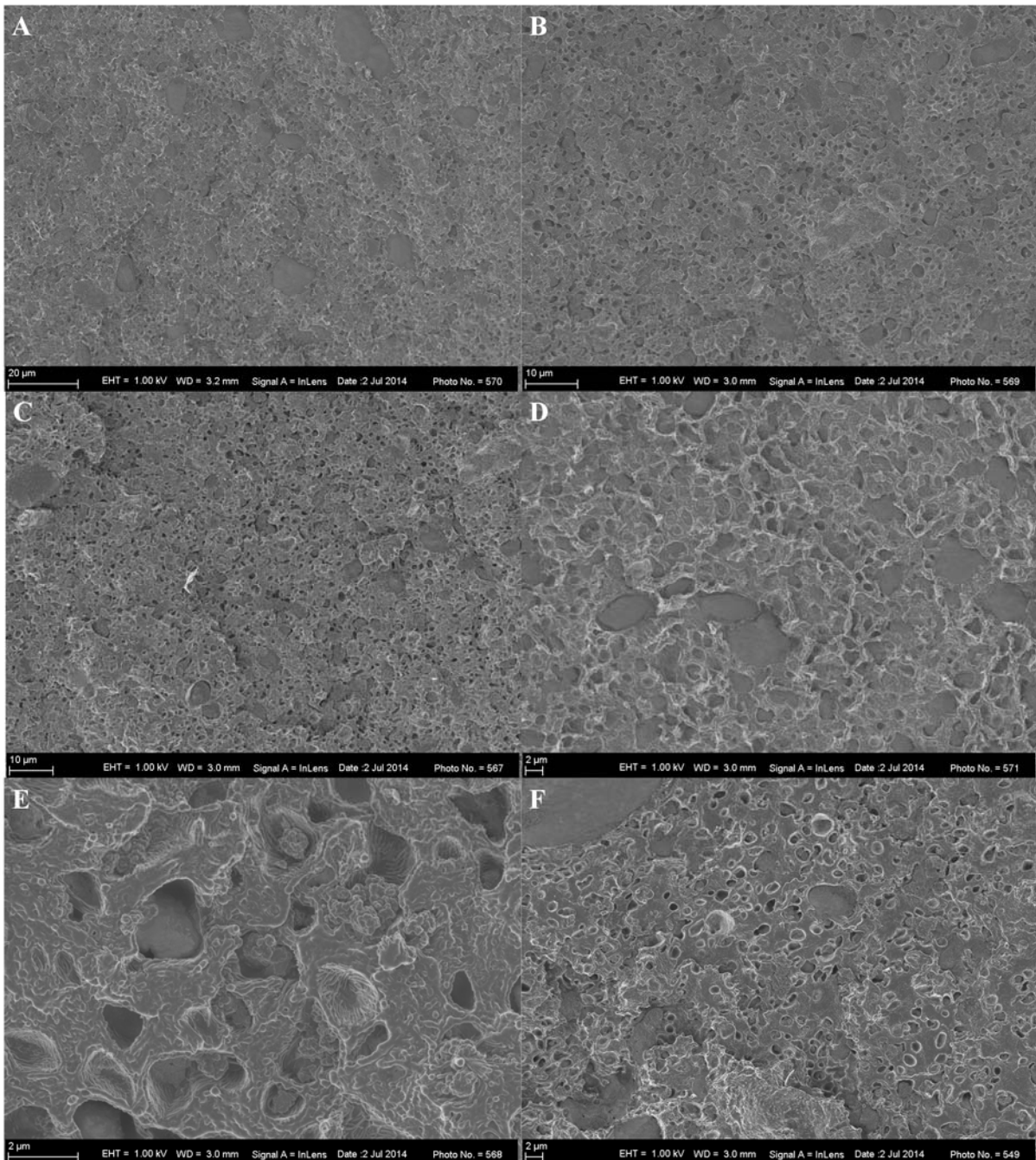
**Surface morphology of LLDPE compounded with ca. 60wt.% DEET.** The desired micro-porous polymer structure was not obtained. It was later established that LLDPE and DEET form a single phase at 320°C whilst the processing temperature was 180°C. It was concluded that failure to obtain a micro-porous polymer was due poor mixing of LLDPE and DEET.



**Surface morphology of EVA compounded with ca. 46.7wt.% DEET.** During this compounding trial a micro-porous polymer structure was not obtained. This is attributed to poor mixing during compounding due inadequate residence time in the screw compounder.




**Surface morphology of LLDPE compounded with ca. 60wt.% DOP.** The objective of this trial was to replace the DOP with DEET once the micro-porous polymer LLDPE was obtained using DOP. During this compounding trial a micro-porous polymer structure was not obtained. This is attributed to poor mixing during compounding due inadequate residence time in the compounder.





## 10.6 Appendix 6: Specification sheets of polymers considered in this study Specifications of EVA (18%)

13 Feb 06 10:22      Affirm Marketing Services 27 11 310 7705      p.3



**AFFIRM MARKETING SERVICES**  
***YOUR PARTNER IN ENGINEERING PLASTICS***

**AMS 3042 NATURAL**

**ETHYLENE VINYL ACETATE**


Applications:    **EXTRUTION**

PHYSICAL PROPERTIES	UNIT	TEST METHOD	VALUE
MFI (190°C / 2,16Kg)	g/10min	ASTM D1238	1.7
DENSITY	g/cm <sup>3</sup>	ASTM D1505	0.939
VA CONTENT	wt. %	HPC	18
HARDNESS (SHORE D)	Shore D	ASTM D2240	38
TENSILE STRENGTH AT BREAK	Kg/cm <sup>2</sup>	ASTM D638	140
VICAT SOFTENING TEMP.	°C	ASTM D1525	60
BRITTLINESS TEMP.	°C	ASTM 746	<-70
ELONGATION AT BREAK	%	ASTM 638	140

The properties shown are typical values

*This information not to be taken as a warranty or representation for which we assume legal responsibility nor as permission or recommendation to practice any*

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FAX: +2711310-7705  
E-MAIL: [sales@affirmmarketing.com](mailto:sales@affirmmarketing.com)



**ARMSA MEMBER**



## Specifications of EVA (28%) (Elvax 210)

DuPont Packaging & Industrial Polymers



The miracles of science™

DuPont™ Elvax® 210W

### Elvax® resins Product Data Sheet

#### Description

**Product Description** DuPont™ Elvax® 210W is an ethylene-vinyl acetate copolymer resin for use in industrial applications. This resin is supplied in pellet form and contains a "W" amide additive to improve pellet handling.

#### Restrictions

**Material Status** • Commercial: Active  
**Availability** • Globally

#### Typical Characteristics

**Composition** 28 % By Weight Vinyl Acetate comonomer content  
Contains a "W" amide additive to improve pellet handling.  
Thermal Stabilizer: BHT antioxidant

**Applications** Elvax® resins can be used in a variety of applications involving molding, compounding, extrusion, adhesives, sealants, and wax blends. For additional information and properties associated with specific applications, please refer to the Grade Selector Guides found on the Elvax® website for industrial applications. [http://www2.dupont.com/Elvax/en\\_US/tech\\_info/index.html](http://www2.dupont.com/Elvax/en_US/tech_info/index.html)

#### Typical Properties

Physical	Nominal Values	Test Method(s)	
• Density (g)	0.951 g/cm <sup>3</sup>	ASTM D792	ISO 1183
• Melt Flow Rate (190°C/2.16kg)	400 g/10 min	ASTM D1238	ISO 1133
Thermal	Nominal Values	Test Method(s)	
• Melting Point (DSC)	60°C (140°F)	ASTM D3418	ISO 3146
Freezing Point (DSC)	39°C (102°F)	ASTM D3418	ISO 3146

#### Processing Information

**General**

• Maximum Processing Temperature 230°C (446°F)

**General Processing Information** Elvax® resins can be processed by conventional thermoplastic processing techniques, including injection molding, structural foam molding, sheet and shape extrusion, blow molding and wire coating. They can also be processed using conventional rubber processing techniques such as Banbury, two-roll milling and compression molding.

Elvax can be used in conventional extrusion equipment designed to process polyethylene resins. However, corrosion-protected barrels, screws, adapters, and





dies are recommended, since, at sustained melt temperatures above 446°F (230°C), ethylene vinyl acetate (EVA) resins may thermally degrade and release corrosive by-products.

#### FDA Status Information

Elvax® 210W EVA Resin complies with Food and Drug Administration Regulation 21 CFR 177.1350(a)(1) -- Ethylene-vinyl acetate copolymers, subject to the limitations and requirements therein. This Regulation describes polymers that may be used in contact with food, subject to the finished food-contact article meeting the extractive limitations under the intended conditions of use, as shown in paragraph (b)(1) of the Regulation.

The information and certifications provided herein are based on data we believe to be reliable, to the best of our knowledge. The information and certifications apply only to the specific material designated herein as sold by DuPont and do not apply to use in any process or in combination with any other material. They are provided at the request of and without charge to our customers. Accordingly, DuPont cannot guarantee or warrant such certifications or information and assumes no liability for their use.

#### Safety & Handling

For information on appropriate Handling & Storage of this polymeric resin, please refer to the Material Safety Data Sheet.

A Product Safety Bulletin, Material Safety Data Sheet, and/or more detailed information on extrusion processing and/or compounding of this polymeric resin for specific applications are available from your DuPont Packaging and Industrial Polymers representative.

### Read and Understand the Material Safety Data Sheet (MSDS) before using this product

#### Regional Centres

DuPont operates in more than 70 countries. For help finding a local representative, please contact one of the following regional customer contact centers:

##### Americas

DuPont Company  
Chestnut Run Plaza – Bldg. 730  
974 Centre Road  
Wilmington, Delaware  
19805 U.S.A.  
Toll-Free (USA): 1-800-628-6208  
Telephone: 1-302-774-1000  
Fax: 1-302-355-4013

##### Asia Pacific

DuPont China Holding Co., Ltd.  
Shanghai Branch  
399 Keyuan Road, Bldg. 11  
Zhangjiang Hi-Tech Park  
Pudong New District, Shanghai  
P.R. China (Postcode: 201203)  
Telephone +86 21 3862 2888  
Fax +86-21-3862-2889

##### Europe / Middle East / Africa

DuPont de Nemours Int'l. S.A.  
2,Chemin du Pavillon Box 50  
CH-1218 Le Grand Saconnex  
Geneva, Switzerland  
Telephone +41 22 717 51 11  
Fax +41 22 717 55 00

DuPont do Brasil, S.A.  
Alameda Itapecuru, 506  
06454-080 Barueri, SP Brasil  
Telephone: +55 11 4166 8000  
Fax: +55 11 4166 8736

<http://elvax.dupont.com>

*The data listed here fall within the normal range of properties, but they should not be used to establish specification limits nor used alone as the basis of design. The DuPont Company assumes no obligations or liability for any advice furnished or for any results obtained with respect to this information. All such advice is given*

**CAUTION:** Do not use DuPont materials in medical applications involving implantations in the human body or contact with internal body fluids or tissues unless the material has been provided from DuPont under a written contract that is consistent with DuPont policy regarding medical applications and expressly acknowledges the contemplated



## Specifications of EVA (28%) (Repsol PA440)



### ALCUDIA® EVA PA-440

#### REPSOL YPF - Ethylene Vinyl Acetate Copolymer

Monday, February 02, 2015

#### General Information

##### Product Description

EVA resin ALCUDIA® PA-440 is recommended for hot melt adhesives applications, injection moulding and extrusion of profiles. It contains antioxidant and free flowing agent.

##### TYPICAL APPLICATIONS

- Injection moulding. Release agents containing silicone must be avoided.
- Hot Melt Adhesives
- Packaging
- Edge veneering
- Shoe industry
- Manufacture of masterbatches
- Microcellular foams
- Manufacture of sonic damping sheets

Recommended melt temperature below 200°C to avoid the decomposition of the polymer. Processing conditions should be optimised for each production line.

##### General

Material Status	• Commercial: Active		
Availability	• Europe	• North America	
Additive	• Antioxidant	• Free Flowing Agent	
Features	• Antioxidant	• Food Contact Acceptable	
Uses	• Acoustic Barrier	• Footwear	• Profiles
	• Adhesives	• Masterbatch	
	• Foam	• Packaging	
Agency Ratings	• EU Food Contact, Unspecified Rating		
Processing Method	• Extrusion	• Injection Molding	• Profile Extrusion

#### ASTM & ISO Properties <sup>1</sup>

Physical	Nominal Value	Unit	Test Method
Density (73°F)	0.960	g/cm <sup>3</sup>	ISO 1183
Melt Mass-Flow Rate (MFR) (190°C/2.16 kg)	7.0	g/10 min	ISO 1133
Vinyl Acetate Content	28.0	wt%	
Mechanical	Nominal Value	Unit	Test Method
Tensile Stress (Break)	3190	psi	ISO 527-2
Tensile Strain (Break)	760	%	ISO 527-2
Hardness	Nominal Value	Unit	Test Method
Shore Hardness			ISO 858
Shore A	80		
Shore D	28		
Thermal	Nominal Value	Unit	Test Method
Melting Temperature	167	°F	
Ring and Ball Softening Point	288	°F	ASTM E28
Fill Analysis	Nominal Value	Unit	Test Method
Brookfield Viscosity <sup>2</sup> (392°F)	1770000	mPa-s	Internal Method



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The information presented on this datasheet was acquired by UL Prospector from the producer of the material. UL Prospector makes substantial efforts to assure the accuracy of this data. However, UL Prospector assumes no responsibility for the data values and strongly encourages that upon final material selection, data points are validated with the material supplier.

Page: 1 of 2



**ALCUDIA® EVA PA-440**  
**REPSOL YPF - Ethylene Vinyl Acetate Copolymer**

Processing Information		
Injection	Nominal Value	Unit
Processing (Melt) Temp	392	°F
Extrusion	Nominal Value	Unit
Melt Temperature	392	°F

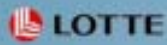
**Notes**

<sup>1</sup> Typical properties: these are not to be construed as specifications.

<sup>2</sup> Spindle SC4-27



## Specifications of EVA (19%) (SEETEC VS430)



Lotte International Co. Ltd.  
Kookmin 1 Bldg. 1009-5,  
Daechi-Dong, Kangnam-Gu,  
Seoul, 135-280 KOREA

### EVA – VS430

### Polyethylene

#### Description:

SEETEC VS430 resin is produced by ExxonMobil Chemical's autoclave process technology.  
SEETEC VS430 resin, with 19% VA content, is an excellent raw material in the application of foam.

#### Additives:

- Antioxidant

#### Applications:

- Shoesoles, Crosslinked foam-buggy wheels, Injection moulding, Hot melt adhesives, Flexible hoses

## PROPERTIES DATA SHEET

### Physical Properties

Properties	Test Method	Nominal Value	Unit
Melt index	ASTM D1238	2.5	g/10min
Vinyl acetate	HPC	19	wt. %
Density	ASTM D1505	0.939	g/cm <sup>3</sup>
Melting point	HPC	84	°C
Tensile strength at break	ASTM D638	140	kg/cm <sup>2</sup>
Elongation at break	ASTM D638	670	%
2% Secant modulus	ASTM D638	300	kg/cm <sup>2</sup>
Hardness	ASTM D2240	38	D scale
Vicat softening point	ASTM D1525	60	°C

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## Specifications of LDPE (LT1050/LT033)



Sasol Polymers Product Table

LDPE film				
Grade Name	MFI (g/10min)	Density (g/cm <sup>3</sup> )	Additives	Applications
LT033	0.33	0.921	Antioxidant	Heavy duty shrink film (>100µm) Heavy duty sacks Agricultural film Thick Film
LT159	0.75	0.922	Antioxidant	Non-slip shrink film Lamination film Blending resin to modify CoF
LT079	0.75	0.922	Antioxidant Medium Antiblock	Light duty shrink film (50µm to 80µm) Lamination film
LT750	0.75	0.922	Antioxidant Medium Antiblock Medium Slip	General packaging film (35µm to 80µm) Form fill and seal film Boutique bags
LT388	2	0.922	Antioxidant	Non-slip shrink film Lamination film Blending resin to modify CoF
LT660	2	0.922	Antioxidant Medium antiblock Medium slip	General packaging film (20µm to 50µm) Clarity film Thin film



## Specifications of HDPE (F7650)



**Safrene®** (High Density PolyEthylene)

**Technical Data Sheet**

### F 7650

High Density Polyethylene for Blow Moulding Applications

Safrene® F 7650 High Density Polyethylene Resin is a bimodal medium molecular mass grade with good processing properties. It exhibits good impact strength and excellent environmental stress-crack resistance.

Safrene® F 7650 High Density Polyethylene Resin is recommended for all general purpose applications up to 20 litre in volume.

#### Typical Applications

- Toiletry containers
- General purpose containers
- Household and industrial chemical containers

PROPERTIES <sup>(1)</sup>	VALUE	UNIT	TEST METHOD
<b>Physical</b>			
Melt Flow Rate, 190 °C/ 5 kg	1.7	g/10min	ISO 1133
Melt Flow Rate, 190 °C/ 2.16 kg	0.33	g/10min	ISO 1133
Density <sup>(2)</sup>	0.950	g/cm <sup>3</sup>	ISO 1183
<b>Mechanical <sup>(3)</sup></b>			
Hardness Shore D	62	Units	ISO 868
Tensile Yield <sup>(4)</sup>	23	MPa	ISO 527
Ultimate Tensile <sup>(4)</sup>	36	MPa	ISO 527
Ultimate Elongation <sup>(4)</sup>	>600	%	ISO 527
Flexural Modulus	1200	MPa	ISO 178
Environmental Stress-Crack Resistance <sup>(5)</sup>	>1000	hours	ASTM D1693
<b>Impact <sup>(3)</sup></b>			
Charpy Notched Impact Strength (23 °C)	11	kJ/m <sup>2</sup>	ISO 179
Charpy Notched Impact Strength (-30 °C)	8	kJ/m <sup>2</sup>	ISO 179
<b>Thermal</b>			
Vicat Softening Point (10N)	126	°C	ISO 306/A50
Vicat Softening Point (50N)	70	°C	ISO 306/B50
Crystalline Melting Range	130-133	°C	ISO 3146

<sup>(1)</sup> Typical values; not to be construed as specification limits.

<sup>(2)</sup> Unannealed

<sup>(3)</sup> Compression moulded samples

<sup>(4)</sup> Test speed 50 mm/min

<sup>(5)</sup> Ball test condition B, 50°C, 10% typical, F50

Typical processing conditions	Blow Moulding
Feed Zone (°C)	160 - 180
Zone1 (°C)	170 - 190
Zone 2 (°C)	180 - 200
Zone 3 (°C)	180 - 190
Die (°C)	180 - 190
Melt Temp (°C)	180 - 190



## Specifications of HDPE (F7650) continued



**Safrene®** (High Density PolyEthylene)

**Technical Data Sheet**

**F 7650**

High Density Polyethylene for Blow Moulding Applications

<b>Product Stewardship</b>	<p>At Safripol, protecting people and the environment will be a part of everything we do and every decision we make. Each employee has a primary responsibility in ensuring that our products and operations meet applicable government standards.</p> <p>Our goal is to eliminate all injuries, prevent adverse environment and health impacts, reduce wastes and emissions and promote resource conservation at every stage of the life cycle of our products. The success of this rests with each and every individual involved with Safripol products throughout the life cycle.</p>
<b>Food Contact Compliance</b>	<p>Safrene® F 7650 High Density Polyethylene Resin should comply with Commission Regulation (EU) No 1831/11 and with U.S. FDA 21 CFR 177.1520(c)(3), for food contact applications when used unmodified and processed according to good manufacturing practices for food contact applications. The purchaser remains responsible for determining whether the use complies with all relevant regulations.</p>
<b>Customer Notice</b>	<p>Safripol strongly encourages its customers to review both their manufacturing processes and their applications of Safripol products from the standpoint of human health and environmental quality to ensure that Safripol products are not used in ways for which they are not intended or tested. Safripol personnel are available to answer your questions and to provide reasonable technical support. Safripol product literature, including safety data sheets, should be consulted prior to use of Safripol products. Current safety data sheets are available from Safripol.</p>
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<b>Additional Information</b>	
<p><b>Our contact details</b></p> <p>Safripol (Pty) Ltd Private Bag X 52 Bryanston 2021 South Africa</p> <p>Website: <a href="http://www.safripol.com">www.safripol.com</a></p> <p>Published:</p>	<p><b>Gauteng:</b> Tel: +27 (0) 11 575 4549 Fax: +27 (0) 11 576 4549</p> <p><b>Kwa-Zulu Natal:</b> Tel: +27 (0) 31 469-0696 Fax: +27 (0) 31 469-0694</p> <p>Email: <a href="mailto:info@safripol.com">info@safripol.com</a></p> <p>June 2013</p>



## Specifications of LLDPE (HR486)

### Product data sheet

## HR486

Linear Low Density Polyethylene for rotational moulding



**Sasol Polymers**  
Polyolefins Business

#### Technical Support:

Polymer Technology Services Centre  
PO Box 72  
Modderfontein 1645  
South Africa

Tel: +27 (0)11 458 0700  
Fax: +27 (0)11 458 0734

Date of Issue: January 2012

#### Sales office:

Sasol Polymers  
PO Box 2525  
Randburg 2125  
South Africa

Tel: +27 (0)11 790 1413  
Fax: +27 (0)11 344 0287

[www.sasol.com/polymers](http://www.sasol.com/polymers)

**Sasol Polymers LLDPE: HR486 Density: 0.939 g/cm<sup>3</sup> Melt index: 3.5g/10min**

#### Features

- High rigidity
- Excellent impact strength
- Excellent chemical resistance
- Good ESCR
- Heat and UV resistant
- Tough and abrasion resistant
- Colourable
- Hexene copolymer

#### Applications

- Large agricultural tanks
- Large industrial tanks
- Solar panels
- Outdoor use

#### Additives

- Antioxidant
- UV stabiliser
- Internal mould release

#### Material properties (typical values not to be construed as specifications)

	Value	Unit	Test method	Based on
MFI (190°C/2.16kg)	3.5	g/10min	PTM058	ASTM D1238
Nominal density	0.939	g/cm <sup>3</sup>	PTM002	ASTM D1505
Tensile strength at yield	19	MPa	PTM006	ASTM D638 <sup>1)</sup>
Tensile strength at break	24	MPa	PTM006	ASTM D638 <sup>1)</sup>
Elongation at break	830	%	PTM006	ASTM D638 <sup>1)</sup>
Flexural modulus	837	MPa	PTM008	ASTM D790
ESCR F <sub>50</sub>	>500	hr	PTM001	ASTM D1693 <sup>2)</sup>
Impact energy at -40°C	35	J/mm	PTM044	ASTM D3029 <sup>3)</sup>
Shore D hardness	61	Shore D	PTM087	ASTM D2240
Vicat softening temperature	120	°C	PTM086	ASTM D1525

1) Crosshead speed 50mm/min

2) 100% Igepal CO630

3) Tested on 5mm rotomoulded product



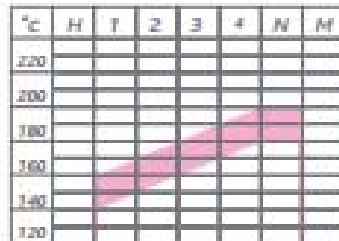
## Specifications of LLDPE continued

### Product data sheet - HR486

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#### Typical processing conditions



#### Processing

An air temperature of 270°C to 300°C is recommended for rotational moulding of HR 486. Temperatures above 300°C should be avoided as this would narrow the processing window considerably and could result in poor physical properties. Due to the material's excellent heat resistance it has very good colour stability even in adverse conditions.

#### Pigmentation for rotational moulded parts

For colouring purposes inorganic pigments should be added at the lowest possible concentration and mixed in using a high speed mixer or a tumble blender, prior to moulding. Pigment preparations should contain only minimal amounts of dispersants.

#### Packaging

Sasol Polymer polyolefin resins are supplied in pellet form packed in 25kg bags. Alternative packaging modes for polypropylene resins are available for selected grades.

#### Handling

Workers should be protected from the possibility of skin or eye contact with molten polymer. Safety glasses and heat resistant gloves are suggested as a minimal precaution to prevent possible mechanical or thermal injuries to the eyes and skin. Fabrication areas should be ventilated to carry away fumes or vapours.

Conveying equipment should be designed to prevent accumulation of fines or dust particles that are contained in all polyolefin resins. These fines and dust particles can, under certain conditions, pose an explosion hazard. Sasol Polymers recommend the conveying system used:

- be equipped with adequate filters
- is operated and maintained in such a manner to ensure no leaks develop
- that adequate grounding exists at all times

Sasol Polymers further recommend that good housekeeping be practised throughout the manufacturing facility. Polymer pellets may pose a slippage hazard if spilled.

#### Storage

As ultraviolet light may cause a change in the material properties, all polyolefin resins should be protected from direct sunlight during a storage. Under cool, dry, dark conditions Sasol Polymers polyolefin resins are expected to maintain their original material and processing properties for at least 18 months.

This information is based on Sasol Polymers' current knowledge and experience and is provided in good faith. In view of many factors that may affect processing and application, this data does not relieve processors from the responsibility of carrying out their own tests and experiments; neither does it imply any legally binding assurance of certain properties for a specific purpose. It is the responsibility of those to whom Sasol Polymers has supplied their products to ensure that any proprietary rights and existing laws and legislation are observed.



## Specifications of polycaprolactone (CAPA 6500)

Product data sheet

# Capa™ 6500



### Description

- ⇒ Capa™ 6500 is a high molecular weight thermoplastic linear polyester derived from caprolactone monomer.
- ⇒ It is supplied in granular form, approx. 3 mm pellets.

### Applications

- ⇒ Capa™ 6500 is used in a variety of adhesive applications.
- ⇒ Compatible with a wide range of common thermoplastics and soluble in several common solvents.

### Delivery forms

- ⇒ Bulk bag in a box
- ⇒ Bulk in 1000 kg bags
- ⇒ Bulk in 500 kg bags
- ⇒ 20 kg net paper sacks on 1000 kg pallets
- ⇒ 20 kg net paper sacks

### Storage

- ⇒ Undisturbed packaging as delivered until shortly before use. Storage area should be covered and protected from higher than ambient temperatures at all time

### Sales Specification

Melt flow index, g/10 min <sup>1</sup>	7.90-5.90*
Water content, % <sup>2</sup>	Max. 0.35
Colour, Hazen <sup>3</sup>	Max. 75

\* Melt flow index is tested with 2.16 kg, 1" PVC die at 160°C

### Typical properties

Mean molecular weight	50 000
Melting point, °C	58-60
Elongation at break, %	800

### Solubility parameters

The solubility parameter ( $\delta$ ) is 9.34 – 9.43 (cal/cm<sup>3</sup>)<sup>1/2</sup>.

#### Analytical method

<sup>1</sup> WC600/5V, <sup>2</sup> WC600/W/M40, <sup>3</sup> WC600C

Analytical methods available on request

CAS no.: 24980-41-4

HS No.: 3907 99

Valid from: August 1, 2013



## Specifications of polycaprolactone (CAPA 6800)

Product data sheet

# Capa™ 6800



### Description

- ⇒ Capa™ 6800 is a high molecular weight linear polyester derived from caprolactone monomer.
- ⇒ Supplied in granular form, approx. 3 mm pellets.

### Applications

- ⇒ Capa™ 6800 is used in a variety of adhesive applications.
- ⇒ Compatible with a wide range of common thermoplastics and soluble in several common solvents.

### Delivery forms

- ⇒ Bulk bag in a box
- ⇒ Bulk in 1000 kg bags
- ⇒ Bulk in 500 kg bags
- ⇒ 20 kg net paper sacks on 1000 kg pallets
- ⇒ 20 kg net paper sacks

### Storage

- ⇒ Undisturbed packaging as delivered until shortly before use. Storage area should be covered and protected from higher than ambient temperatures at all times.

### Sales Specification

Melt flow index, g/10 min <sup>1</sup>	4.03-2.01*
Water content, % <sup>2</sup>	Max. 0.35
Colour, Hazen <sup>3</sup>	Max. 75

\* Melt flow index is tested with 5 kg, 1" PVC die at 190°C

### Typical properties

Mean molecular weight	80 000
Melting point, °C	58-60
Elongation at break, %	800

### Solubility parameters

The solubility parameter (δ) is 9.34 – 9.43 (cal/cm<sup>3</sup>)<sup>1/2</sup>.

### Analytical method

<sup>1</sup> WC680/5V, <sup>2</sup> WC680/W/M40, <sup>3</sup> WC680C

Analytical methods available on request

CAS no.: 24980-43-4

HS No.: 2907 99

Valid from: August 1, 2013



## Specifications of Polyamide (Euremelt 2130)

Advanced Materials

**HUNTSMAN**

Surface Technologies

HOT MELT ADHESIVES

DATA SHEET

<b>Euremelt® 2130</b>																												
<b>Copolyamide</b>																												
<b>Applications</b>	Thermoplastic hot melt adhesive for bonding different substrates like plastics, metals, wood, packaging materials, leather																											
<b>Properties</b>	Good flexibility, widespread usefulness																											
<b>Key data</b>	<p><b>Specified key data</b></p> <table border="1"> <tr> <td>Colour (Gardner) (ISO 4630)</td> <td>≤ 10</td> <td></td> </tr> <tr> <td>Amine value (ISO 9702)</td> <td>≤ 7</td> <td>[mg KOH/g]</td> </tr> <tr> <td>Acid value (ISO 2114)</td> <td>≤ 7</td> <td>[mg KOH/g]</td> </tr> <tr> <td>Softening point R+B (ISO 4625)</td> <td>125 - 135</td> <td>[°C]</td> </tr> <tr> <td>Melt viscosity at 200 °C (ISO 2884)</td> <td>3.0 - 4.6</td> <td>[Pa s]</td> </tr> </table> <p>Specified key data are individually checked throughout and guaranteed.</p> <p><b>Typical key data</b></p> <table border="1"> <tr> <td>Open time – on silicon paper at 23 °C (application temperature: 200°C)</td> <td>26 - 34</td> <td>[sec.]</td> </tr> <tr> <td>Open time – canal method at 23 °C (application temperature: 200°C)</td> <td>58 - 70</td> <td>[sec.]</td> </tr> <tr> <td>Setting time – on Nora-Testrubber at 23 °C (application temperature: 200°C)</td> <td>2 - 4</td> <td>[sec.]</td> </tr> <tr> <td>Application temperature</td> <td>170 - 210</td> <td>[°C]</td> </tr> </table> <p>As-supplied form granulate in 25 kg paper bags</p> <p>Shelf life (dry environment, storage temperature between +2 and +40 °C) the quality of our hot melt adhesives remains stable for at least 2 years</p> <p>Hazardous decomposition products (when disposed of in fire) burning produces harmful and poisonous gases and vapours carbon oxides and nitrogen oxides</p> <p>Disposal simple disposal approved by local authorities</p> <p>Typical key data are spot checked; the values are typical for the product and are indicated for information only. The values are not guaranteed.</p>	Colour (Gardner) (ISO 4630)	≤ 10		Amine value (ISO 9702)	≤ 7	[mg KOH/g]	Acid value (ISO 2114)	≤ 7	[mg KOH/g]	Softening point R+B (ISO 4625)	125 - 135	[°C]	Melt viscosity at 200 °C (ISO 2884)	3.0 - 4.6	[Pa s]	Open time – on silicon paper at 23 °C (application temperature: 200°C)	26 - 34	[sec.]	Open time – canal method at 23 °C (application temperature: 200°C)	58 - 70	[sec.]	Setting time – on Nora-Testrubber at 23 °C (application temperature: 200°C)	2 - 4	[sec.]	Application temperature	170 - 210	[°C]
Colour (Gardner) (ISO 4630)	≤ 10																											
Amine value (ISO 9702)	≤ 7	[mg KOH/g]																										
Acid value (ISO 2114)	≤ 7	[mg KOH/g]																										
Softening point R+B (ISO 4625)	125 - 135	[°C]																										
Melt viscosity at 200 °C (ISO 2884)	3.0 - 4.6	[Pa s]																										
Open time – on silicon paper at 23 °C (application temperature: 200°C)	26 - 34	[sec.]																										
Open time – canal method at 23 °C (application temperature: 200°C)	58 - 70	[sec.]																										
Setting time – on Nora-Testrubber at 23 °C (application temperature: 200°C)	2 - 4	[sec.]																										
Application temperature	170 - 210	[°C]																										
<b>Storage</b>	Euremelt 2130 should be stored in a dry place, preferably in the sealed original paper bag, at temperatures between 2 and 40 °C.																											
<b>Handling precautions</b>	Mandatory and recommended industrial hygiene procedures should be followed whenever our products are being handled and processed. For additional information please consult the corresponding product safety data sheets.																											
<b>Note</b>	Euremelt® is a registered trademark of Huntsman LLC or an affiliate thereof.																											

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Registered trademark	

June 2004



## Specifications of Polyamide (Euremelt 2140)

Advanced Materials

**HUNTSMAN**

Surface Technologies

HOT MELT ADHESIVES

DATA SHEET

### Euremelt<sup>®</sup> 2140

Copolyamide

<b>Applications</b>	Thermoplastic hot melt adhesive for bonding of plastics or porous substrates		
<b>Properties</b>	Very good adhesion to many different substrates like plastics, metals, wood and leather; high flexibility		
<b>Key data</b>	Specified key data		
	Colour (Gardner) (ISO 4630)	≤ 6	
	Amine value (ISO 9702)	≤ 9	[mg KOH/g]
	Acid value (ISO 2114)	≤ 2	[mg KOH/g]
	Softening point R+B (ISO 4625)	135 - 145	[°C]
	Melt viscosity at 225 °C (ISO 2884)	7.6 - 10.0	[Pa s]
	Specified key data are individually checked throughout and guaranteed.		
	Typical key data		
	Open time – on silicon paper at 23 °C (application temperature: 200°C)	55 - 65	[sec.]
	Open time – canal method at 23 °C (application temperature: 200°C)	29 - 35	[sec.]
	Setting time – on Nora-Testrubber at 23 °C (application temperature: 200°C)	1 - 3	[sec.]
	Application temperature	160 - 230	[°C]
	As-supplied form	granulate in 25 kg paper bags	
	Shelf life (dry environment, storage temperature between +2 and +40 °C)	the quality of our hot melt adhesives remains stable for at least 2 years	
	Hazardous decomposition products (when disposed of in fire)	burning produces harmful and poisonous gases and vapours carbon oxides and nitrogen oxides	
	Disposal	simple disposal approved by local authorities	
	Typical key data are spot checked; the values are typical for the product and are indicated for information only. The values are not guaranteed.		
<b>Storage</b>	Euremelt 2140 should be stored in a dry place, preferably in the sealed original paper bag, at temperatures between 2 and 40 °C.		
<b>Handling precautions</b>	Mandatory and recommended industrial hygiene procedures should be followed whenever our products are being handled and processed. For additional information please consult the corresponding product safety data sheets.		
<b>Note</b>	Euremelt <sup>®</sup> is a registered trademark of Huntsman LLC or an affiliate thereof.		

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June 2004