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A slow-release organophosphate-filled trilayer polyolefin film

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A dissertation submitted in partial fulfilment of the requirements for the
Degree of
Master of Engineering (Chemical Engineering)
In the
Faculty of Engineering, Built Environment and Information Technology
University of Pretoria, Pretoria

2017

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Degree: MEng (Chemical Engineering)

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Synopsis

The development of pyrethroid resistance in mosquitoes threatens the goal of malaria elimination in Africa. Alternative insecticides, e.g. organophosphates, can be considered to control pyrethroid resistant mosquitoes. The problem associated with the deployment of organophosphate-based insecticides is their high volatility. Conventional application forms have a fairly short residual efficacy. This study aimed at extending the residual efficacy of an organophosphate insecticide by using a polymer matrix as a slow release device. A multilayer film blower was used to produce a trilayer film. The middle layer comprised poly(ethylene-co-vinyl acetate), i.e. EVA polymer, impregnated with malathion. This was sandwiched by two low density polyethylene (LDPE) outer layers. These acted as semi-permeable membrane-like barriers that slowed down the release of the contact insecticide to the surfaces of the film. In theory, such a film could be deployed as a long-lasting insecticide-treated wall lining in pyrethroid resistant settings.

Scanning electron microscopy (SEM) confirmed the trilayer film structure of the blown film. The malathion release from the film was tracked with Fourier transform infrared spectroscopy (FTIR). The malathion absorption band in the FTIR spectra disappeared gradually over time. Confocal Raman analysis showed a malathion concentration gradient across the thickness of the polyethylene layers. These results suggested diffusion-controlled transport through the LDPE membranes. Bioassays indicated that the residual efficacy of the malathion, against mosquitoes, was increased to about six months. This means that trilayer films, impregnated

with an organophosphate, may have potential as alternative mosquito control interventions in pyrethroid resistant settings.

Key words: malaria; mosquitoes; insecticide; trilayer film; controlled release

DECLARATION

I, Tatenda Panashe Madzorera, with student number 12222934, do hereby declare that this research is my original work and that to the best of my knowledge and belief, it has not been previously in its entirety or in part been submitted and is not currently being submitted either in whole or in part at any university for a degree or diploma, and that all references are acknowledged.

SIGNED on this 13th day of November 2017.

T.P. Madzorera

Acknowledgements

I would like to thank the Lord almighty for giving me the strength and the grace to complete this project. I also extend my appreciation to the following for their respective contributions in making this project a success:

Prof Walter W. Focke (Project supervisor)
Dr Mthokozisi M. Sibanda (Project co-supervisor)
Dr Homa Izadi (Thermal analysis)
Dr Jack Madito (Confocal Raman Imaging)
Dr Eudri Venter (Microscopy)
Mr Joseph Sebekedi (Film Blowing)
Mr Benjamim Mapossa (Microscopy)
Mr Alcides Sitoe (Microscopy)
Ms Isbe van der Westhuizen (Thermal analysis)
Mr Gerard Puts (Thermal Analysis)
Ms Suzette Seymore (Administration)
Ms Allison Gilbert (Bioassay testing)
UP ISMC (Funding)
NRF (Funding)

I would like to extend my sincere gratitude to my parents, Tsitsi and Abraham, for encouraging me and affording me this opportunity to further my studies. My girlfriend, Chenai Ngorima, thank you very much for the unwavering support.

"Veni, Vidi, Deus Vicit"

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Nomenclature

Symbol	Property	Units
A	Frequency factor	[days^{-1}]
a_o	Initial peak area	[cts·cm^{-1}]
a_t	Peak area at time *t*	[cts·cm^{-1}]
a_∞	Peak at an infinite time	[cts·cm^{-1}]
E_a	Activation energy	[J·mol^{-1}]
m_{EVA}	Mass of EVA	[g]
m_{mal}	Mass of malathion	[g]
m_{ML}	Mass of middle layer	[g]
m_o	Initial mass	[g]
m_t	Mass at time *t*	[g]
m_∞	Mass at an infinite time	[g]
P_{ow}	Octanol water partition coefficient	[-]
ρ_{EVA}	Density of EVA	[g·cm^{-3}]
ρ_{LDPE}	Density of LDPE	[g·cm^{-3}]
ρ_{mal}	Density of malathion	[g·cm^{-3}]
ρ_{ML}	Density of middle layer	[g·cm^{-3}]
R	Gas constant	[J·mol^{-1}·K^{-1}]
T	Temperature	[K]
τ	Time constant	[days]
t	Time elapsed	[days]
t_{ML}	Thickness of middle layer	[µm]
t_{OL}	Thickness of outer layers	[µm]
v_{EVA}	Volume of EVA	[cm^3]
v_{mal}	Volume of malathion	[cm^3]
v_{ML}	Volume of middle layer	[cm^3]

List of acronyms and abbreviations

ATSB	attractive toxic sugar baits
Bti	*Bacillus thuringiensis*
Bs	*Bacillus sphaericus*
CAS	chemical abstract service
DDT	dichlorodiphenyltrichloroethane
DEET	N,N-Diethyl-meta-toluamide
DSC	differential scanning calorimetry
EVA	poly(ethylene-co-vinyl acetate)
FTIR	Fourier-transform infrared spectroscopy
IRS	indoor residual spray
ITN	insecticide treated nets
ITWL	insecticide treated wall lining
IUPAC	International Union of Pure and Applied Chemistry
KD	knockdown
LD_{50}	lethal dose, 50%; the dose required to kill 50% of a test population after 14 days [$mg \cdot kg^{-1}$ body weight]
LDPE	low density polyethylene
LLIN	long lasting insecticide treated nets
LLRTN	long-lasting repellent-treated net
SEM	scanning electron microscope/microscopy
SIT	sterile insect technique
TGA	thermo gravimetric analysis
VA	vinyl acetate
WHO	World Health Organisation
wt.%	weight of component as a percentage of total weight

1. Introduction

1.1 Background

Malaria is a devastating disease in sub-Saharan Africa. In 2015, 207 million cases of malaria occurred globally resulting in 627 000 deaths (WHO, 2016). Almost 90 % of these deaths occurred in Africa with the majority being children under the age of five (Fang et al., 2011). Malaria largely affects under-privileged communities in poor African countries (Chima et al., 2003, Sachs and Malaney, 2002, Gallup and Sachs, 2001). Female *Anopheles* mosquitoes are the vectors responsible for malaria transmission. Therefore, decreasing their population should lead to a reduction of malaria morbidity and mortality, and eventually even the elimination of malaria (Pavela, 2015).

The most commonly used methods of mosquito control are indoor residual spray (IRS) and long lasting insecticide treated nets (LLINs) (Messenger et al., 2012b, Braack et al., 2015, Messenger et al., 2012a). IRS is deemed particularly effective. It is an annual activity that is widely applied in Southern Africa (WHO, 2007c). The World Health Organisation (WHO) has approved twelve insecticides for use in IRS. Six are pyrethroids (alphacypermethrin, betacyfluthrin, bifenthrin, deltamethrin, etofenprox, and lambdacyhalothrin), three are organophosphates (malathion, fenitrothion, pirimiphos-methyl), two are carbamates (propoxur, bendiocarb) and one is an organochlorine (DDT). Dichlorodiphenyltrichloroethane (DDT) is preferred for IRS because, depending on the application surface, it retains efficacy for twelve months. In contrast, the other insecticides only last for about six months. However DDT is a persistent organic pollutant which lasts for many years in the environment (Aneck-Hahn et al., 2007). It also has adverse effects on humans and animals (Coleman et al., 2008).

LLINs are cost effective and less technically demanding to implement. The main disadvantage of LLINs is that protection is only offered during sleeping time (Messenger et al., 2012a). It is possible to get infected at dusk when the mosquitoes start to be active and when occupants in the house are not subject to protection by LLINs (Bockarie et al., 1996, Meyers et al., 2016). Both LLIN and IRS vector control methods rely primarily on pyrethroid insecticides. They only provide some protection indoors by targeting mosquitoes resting after a blood meal (IRS) or preventing actual biting (LLINs). A limitation of these vector control methods is growing

insecticide resistance (Kikankie et al., 2010, Blanford et al., 2005), particularly to pyrethroids (Pates and Curtis, 2005, Moiroux et al., 2012).

Insecticide resistance is the reduction of insecticide activity in an insect population. It is indicated when an insecticide repeatedly fails to achieve the expected level of control when it is applied according to the specific recommendations for the insect species of interest. Permethrin resistance was found in mosquito populations of *An. arabiensis* in Gwave, a malaria endemic area in Zimbabwe (Munhenga et al., 2008). In Côte d'Ivoire, *An. gambiae* showed resistance towards permethrin, deltamethrin and λ-cyhalothrin (Ahoua Alou et al., 2012). In Sudan, WHO susceptibility tests on *An. arabiensis* showed resistance to DDT and pyrethroids. (Abdalla et al., 2014)

Resistance to insecticides develops when insects find ways to overcome the toxins. In biochemical resistance, enzyme detoxification deactivates the insecticide before it reaches the target site (Ranson et al., 2011). In physiological resistance, the toxin is not necessarily broken down but instead it is accommodated by altering one or more physiological functions, e.g. an increase in the rate of insect metabolism (Ranson et al., 2011). The growing trend of pyrethroid resistance constitutes a serious threat to malaria control programmes. If measures are not taken in time, the development of resistance may compromise future control efforts (Ahoua Alou et al., 2012). The use of alternative WHO-approved insecticides, e.g. organophosphates, can be used to overcome the problem of resistance to pyrethroids.

This study introduces the concept of trilayer polymer films impregnated with a suitable organophosphate insecticide. The idea is to trap a relatively large amount of liquid organophosphate in the middle layer. This layer is sandwiched by semipermeable sheath layers that act as diffusion barriers. This should slow down the rate at which the organophosphate is released thereby extending its residual effectiveness. Such a film may be deployed as wall or ceiling linings in areas where mosquitoes are resistant to pyrethroid insecticides. This concept could provide a suitable alternative intervention in pyrethroid resistant settings and contribute to the achievement of malaria elimination.

1.2 Aim

Produce a trilayer polymer film impregnated with a suitable organophosphate insecticide and extend the residual efficacy of the insecticide by using a polymer matrix as a slow release device.

1.3 Objectives

1. Assess the thermal stability of organophosphates.
2. Incorporate organophosphates into a suitable polymer matrix.
3. Blow trilayer films impregnated with organophosphate inescticides.
4. Characterise the trilayer films.
5. Perform WHO-recommended bioassay tests.

2. Literature review

2.1 The vector and disease

2.1.1 Mosquitoes

Mosquitoes are vectors of tropical diseases including malaria, Zika, dengue fever, chikungunya and filariases. There are about 3000 species of mosquitoes of which approximately one hundred of these are vectors (Rozendaal, 1997). The female *Anopheles* mosquitoes transmit malaria while the *Aedes* family transmit yellow fever, dengue fever and the Zika virus.

The life cycle of the mosquito consists of four stages namely the egg, larva, pupa and adult as illustrated in Figure 1. Under favourable conditions, the entire cycle, from egg to adult, takes about seven to thirteen days (Rozendaal, 1997). Female mosquitoes require a blood meal for them to be able to produce eggs throughout their lifetime. Male mosquitoes mainly feed on plant juices.

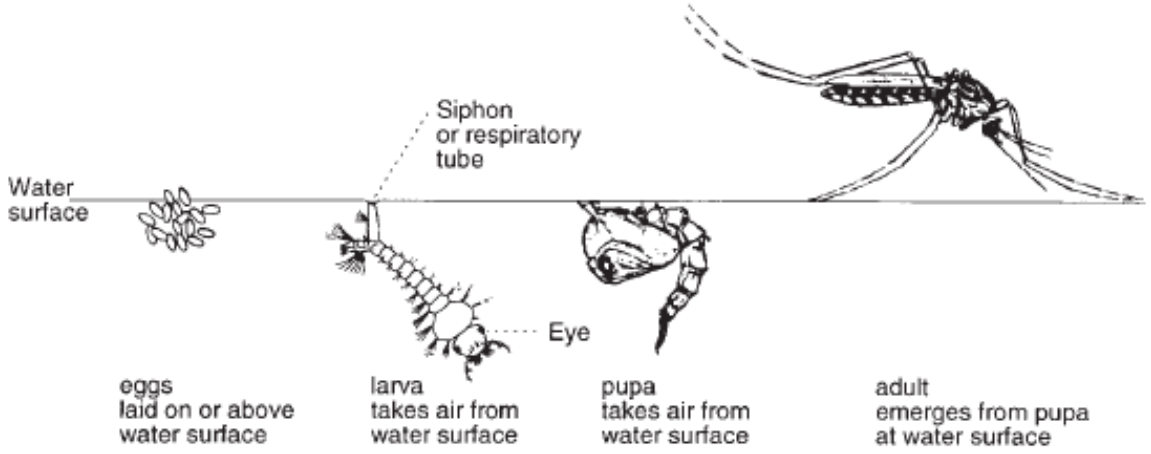

Figure 1: Life cycle of a mosquito (taken from (Rozendaal, 1997)).

One of the most prevalent tropical diseases transmitted by mosquitos is malaria. It is imperative to control mosquitoes because of the threat they pose. Several methods of vector control include:

- Preventing mosquitoes from biting
- Killing mosquitoes after they have bitten
- Environmental management to prevent mosquitoes from breeding
- House design

2.1.2 Malaria

Malaria is caused by single-celled protozoan parasites of the genus *Plasmodium*. Female *Anopheles* mosquitoes transmit the malaria parasite from one person to the next (Rozendaal, 1997). Figure 2 shows the lifecycle of the malaria parasite. When the female *Anopheles* mosquito bites an individual infected with malaria, it draws a small amount of blood which contains the malaria parasites. The parasites grow and mature in the gut of the mosquito and they then travel to the mosquito's salivary gland. The malaria parasites are transferred to the next victim when the infected mosquito bites the person. After an incubation period that ranges from eight days to several months, the victim starts showing malaria symptoms (Lund, 2005).

Malaria begins with an influenza-like illness with attacks of fever. The fever episodes coincide with the enlargement of the spleen and the liver. Death from malaria may occur when the parasitized red blood cells block the narrow blood vessels in the organs of the body. People who frequently get infected with malaria in endemic areas, especially in Africa, may end up developing immunity towards the disease (Rozendaal, 1997).

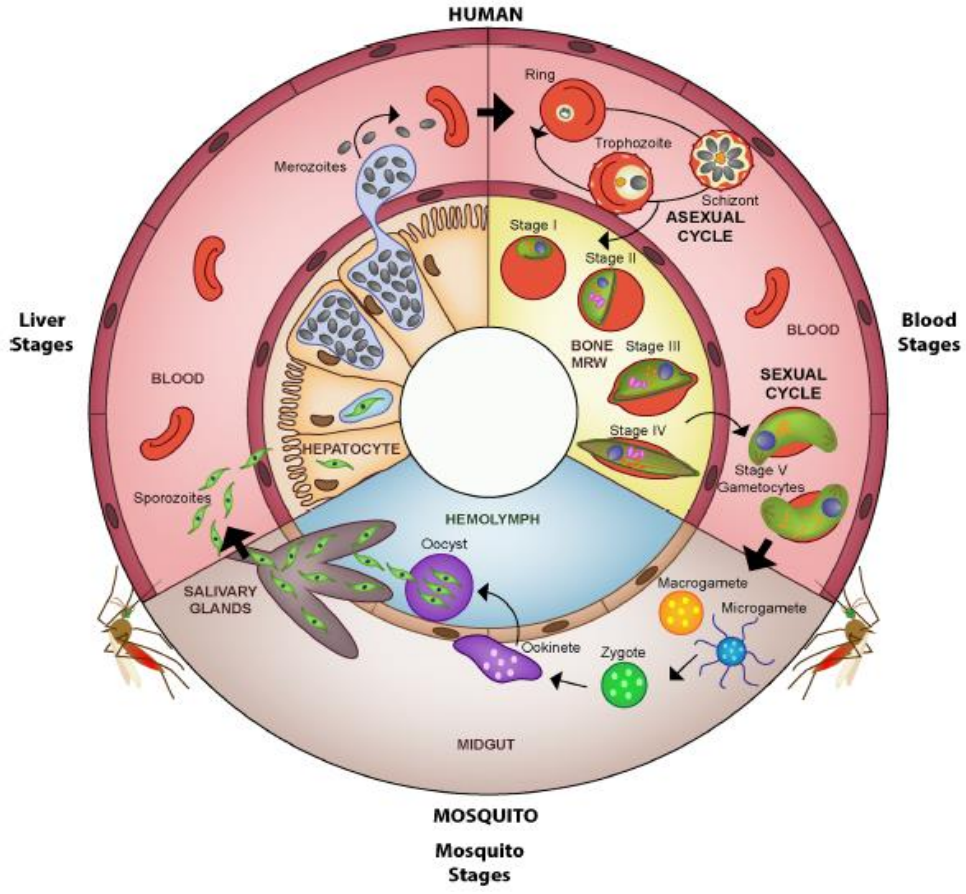

Figure 2: Lifecycle of the malaria parasite (taken from (Nilsson et al., 2015)).

2.1.3 Mosquito biting behaviour

The two main WHO recommended methods of preventing malaria transmission are IRS and LLINs. These methods help prevent indoor malaria transmission. However, increasingly there is a problem of outdoor mosquito bites from malaria vectors. A study conducted in rural Tanzania revealed that the fraction of malaria vector populations that engage in outdoor feeding is increasing. This change in behaviour is driven by the increased use of insecticide-treated nets in the area (Russell et al., 2011).

Braack *et al.* (2015) studied the biting behaviour of African malaria vectors to determine where they bite on the human body. The vectors used in the study were *An. arabiensis* from Malahlapanga in South Africa and *An. funestus* and *An. gambiae s.s.* from northern Uganda. The study was done outdoors with the human subjects either standing or seated. They had their ankles and feet exposed while the rest of their bodies were covered. There was a strong preference for feeding at the ankles and feet of the people. In fact, 98 % of bites from the vector *An. arabiensis* occurred below the mid-calf region. For *An. gambiae* and *An. funestus* species, the bites in the same region ranged from 81 % - 100 % and from 77 % - 100 % respectively (Braack et al., 2015). The reasons for this biting behaviour are manifold. The mosquito vectors are attracted to foot odour and also prefer to fly close to the ground. The portion of bites higher up the body did not increase when the study subjects covered their lower limbs. Instead, the mosquitoes flew away, presumably to find alternative hosts. Figure 3 shows the preferred bite sites for the main malaria vectors for standing and seated humans as well as when people are lying flat on the ground.

Some of the subjects in the study slept on mats on the floor. This was to determine the biting behaviour of the mosquitoes under such conditions. In this case, the vectors fed all over the body except for the area around the head. The implication is that people who sleep on the floor bear a disproportionate risk of being bitten by malaria vectors. This is especially true for children in Africa who usually do sleep on the floor.

This study showed that there is an opportunity to develop other vector control methods in order to prevent outdoor mosquito bites especially to cover the lower limbs. Examples of such products are inexpensive repellent impregnated anklets or sandals (Braack et al., 2015). These measures can complement IRS and LLINs, which help prevent indoor malaria transmission (Russell et al., 2011).

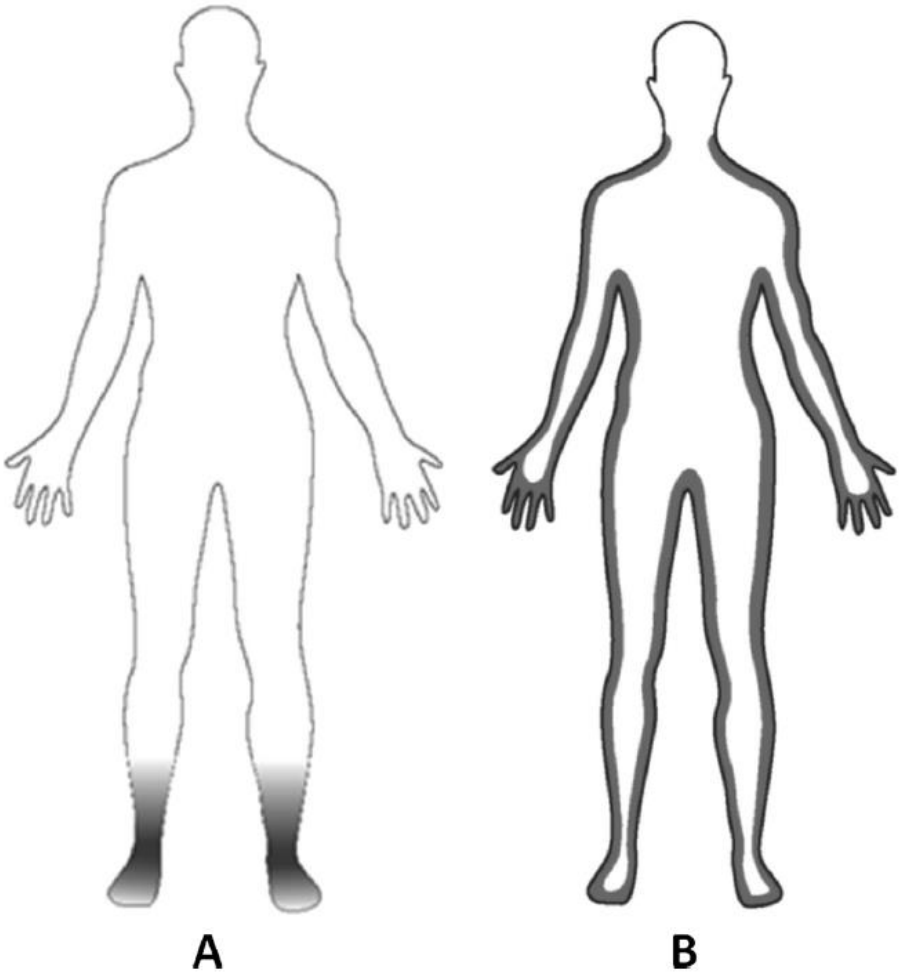

Figure 3: Preferred bite sites of *An. arabiensis*, *An. gambiae* and *An. funestus* on the human body. Darkened areas represent the preferred areas of all three species for biting on the human body, at (A) standing or seated humans and (B) at people lying flat on the ground (Braack et al., 2015).

2.2 Preventing mosquitoes from biting

2.2.1 Repellents

Repellents are the most commonly used method to prevent mosquito bites. They are applied to the skin or to clothing (Rozendaal, 1997). Repellents are available as aerosols, pump sprays, lotions, creams as well as powders (Katz et al., 2008). Natural and synthetic repellents are available.

2.2.1.1 Natural repellents

Citronella

Citronella oil is extracted from the leaves of a perennial grass that is native to tropical parts in Asia (Brown and Hebert, 1997). Before the 1940s citronella oil was the most widely used repellent (Katz et al., 2008). It is used at a concentration of 5-10 % because higher concentrations can result in skin sensitivity. A disadvantage of citronella based repellents is that they only give a residual efficacy of up to 2 hours (Maia and Moore, 2011).

Oil of Lemon Eucalyptus

Oil of lemon eucalyptus (*p*-menthane-3,8-diol) is extracted from the leaves of lemon eucalyptus (Diaz, 2016). It is available in pump sprays in concentrations that range from 10 % to 40 %. This natural repellent has been found to have the same efficacy as *N*,*N*-diethyl-m-toluamide (DEET) against mosquitoes when both are applied at low concentrations. It was found to last between four to seven hours (Katz et al., 2008).

2.2.1.2 Synthetic repellents

DEET

The synthetic insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET) is considered the most effective insect repellent. DEET provides long-lasting protection up to a period of 8 hours upon application on the skin (Logan et al., 2010). It is also available in different types of formulations such as lotions, liquids, aerosols as well as impregnated in materials e.g. in wrist-bands (Sudakin and Osimitz, 2010).

The mechanism of action of DEET is unknown but it is suspected that it works by forming a barrier of vapour which produces an odour and a taste that is offensive to insects (Sudakin and Osimitz, 2010).

There are concerns about the toxicity of DEET in humans. However, it has been discovered that DEET has a low order of acute toxicity via oral, dermal and inhalation routes of exposure. However, the United States Environmental Protection Agency (EPA) has concluded that insect repellent formulations containing DEET generally do not pose unreasonable risks to humans or the environment (Sudakin and Osimitz, 2010).

A major disadvantage of this repellent is that it is relatively expensive and hence the people in resource limited communities are not likely going to purchase this formulation. They will likely rely on cheaper alternatives which are not as effective as DEET. Table 1 shows the properties of DEET and Figure 4 shows its chemical structure.

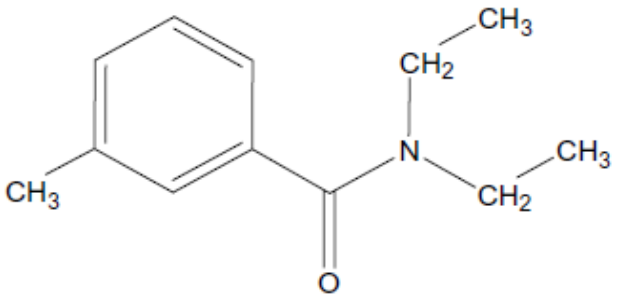

Figure 4: Chemical structure of DEET (Sudakin and Osimitz, 2010).

Table 1: Properties of DEET (Sudakin and Osimitz, 2010)

IUPAC name: *N*,*N*-diethyl-*m*-toluamide	
CAS Registry number	134-62-3
Molecular formula	$C_{12}H_{17}NO$
Molar Mass	191.26 $g \cdot mol^{-1}$
Density	0.998 ± 0.06 $g \cdot cm^{-3}$ at 20°C
Vapour Pressure	5.6×10^{-3} mmHg at 20 °C
Solubility in water	> 1.0 $g \cdot L^{-1}$
Octanol water partition coefficient	log P_{ow} = 2.02
Toxicity, LD_{50}	5000 $mg \cdot kg^{-1}$ body weight (male rat)

Ethyl butylacetyl aminopropionate (IR3535®)

Ethyl butylacetyl aminopropionate commonly known as insect repellent 3535 or IR3535® is a synthetic repellent which is used as an alternative to DEET. Like DEET it can be used in lotions and it can also be used to produce a novel long-lasting repellent-treated net (LLRTN) by coating the repellent onto the fibres of bed net fabric using a new polymer-coating technique (Faulde et al., 2010). There have not been any toxicity issues that have been reported due to the use of this repellent. It has been shown not to be harmful when inhaled, ingested or applied

onto the skin (Cilek et al., 2004). Figure 5 shows the chemical structure of IR3535® and Table 2 shows its properties.

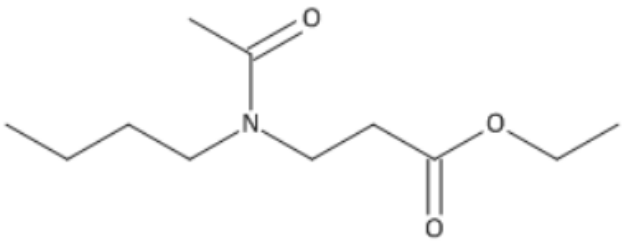

Figure 5: Chemical structure of IR3535® (WHO, 2006a) .

Table 2: Properties of IR3535® (WHO, 2006a)

IUPAC Name: 3-(*N*-acetyl-*N*-butyl)aminopropionic acid ethyl ester	
CAS Registry Number	52304-36-6
Molecular formula	$C_{11}H_{21}NO_3$
Molar Mass	215.3 $g \cdot mol^{-1}$
Vapour Pressure	0.15 ± 0.01 Pa at 20 °C
Solubility in water	70 $g \cdot L^{-1}$
Octanol water partition coefficient	log P_{ow} =1.7 at 23 °C
Thermal Properties	Decomposes at 141 °C
Toxicity, LD_{50}	> 5000 $mg \cdot kg^{-1}$ body weight (male rat)

Icaridin

Icaridin is an odourless liquid repellent is also known as Picaridin or Saltidin. It is used in topical applications and, unlike DEET, it is less likely to irritate the skin. The mechanism of action is unknown but it is suspected that it forms a vapour barrier which repels an insect because of the unpleasant smell (Katz et al., 2008). Icaridin has been found to have similar repellent efficacy to DEET (Antwi et al., 2008). The chemical structure of Icaridin is shown in Figure 6 and its properties are shown in Table 3.

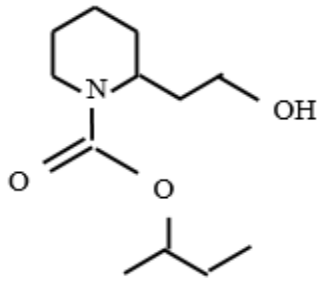

Figure 6: Chemical structure of Icaridin (WHO, 2004b).

Table 3: Properties of Icaridin (WHO, 2004b)

IUPAC Name: 1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropylester	
CAS Registry Number	119515-38-7
Molecular formula	$C_{12}H_{23}NO_3$
Molar mass	229.3 $g \cdot mol^{-1}$
Vapour Pressure	3.4 x 10^{-2} Pa at 20 °C at 97 % purity
Solubility in water	8.6 $g \cdot L^{-1}$ at 20 °C
Octanol water partition coefficient	log P_{ow} = 2.11 at 20 ˚C
Toxicity, LD_{50}	4743 $mg \cdot kg^{-1}$ body weight (male rat)

2.2.1.3 Other synthetic repellents

Indalone

Indalone (butyl-3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate) is a contact or a gustatory repellent. This is because it is slightly volatile and so the mosquito must be in contact with the treated surface before it is repelled (Brown and Hebert, 1997).

Dialkyl phthalates

Dialkyl phthalates were the earliest forms of synthetic repellents to be manufactured. Dimethyl phthalate was used as a solvent in which many solid repellents were tested. Initially dialkyl phthalates were thought to be non-toxic until 1988 when dimethyl and dibutyl phthalates were found to be toxic (Brown and Hebert, 1997).

2.2.2 Protective clothing

Normal clothing can offer protection against mosquito bites but this is dependent upon the thickness, texture and colour of the clothes. Fewer mosquitoes are attracted to brighter colours as compared to dark colours (Rozendaal, 1997). Boots can protect against bites around the ankle region.

According to Braack *et al.* (2015) malaria transmitting mosquitoes will bite around the ankle and foot region 93 % of the time. Insect repellent anklets and bands can also be made and these are effective because mosquitoes bite around the ankle and wrist area.

Clothing items can be treated with a repellent or an insecticide to improve the protection they offer. Applying the repellents on clothing is a preferable option because this reduces allergic reactions that might occur when the repellent is applied directly onto the skin (Rozendaal, 1997). Permethrin is an insecticide with repellent properties. It is the commonly used active ingredient to treat clothing items. This is because it is a solid and hence it can be easily applied as coating on fabrics.

A study was done in the Daab Refugee camp in Kenya to determine how effective permethrin treated clothing are as a protection against malaria infections (Kimani et al., 2006). The human subjects had their personal clothes and bedding treated with the insecticide. The clothing items were dipped in permethrin after every three weeks. This study showed that the use of insecticide treated clothing reduces malaria infection rates. The odds of contracting malaria by using insecticide treated clothing were reduced by up to 70 % (Kimani et al., 2006).

The use of repellent- or insecticide-treated clothing can be an effective method for malaria control. This applies in particular to situations where mosquito nets are inadequate, for example outdoors. However, they are not marketed as regularly as insecticide treated nets (Kimani et al., 2006). The current method of treating clothes with permethrin has its disadvantages. The protective efficacy is not long-lasting. The permethrin must be constantly reapplied every few weeks.

2.2.3 Insecticide vaporisers

2.2.3.1 Mosquito coils

Mosquito coils are one of the most commonly used insecticide vaporisers because they are inexpensive and easy to use. They are made from a paste of powdered insecticide plus a filler such as sawdust. This is pressed and punched into spiral shapes (Lawrance and Croft, 2004).

Once the mosquito coil is set alight, it releases smoke which acts as a carrier for the insecticide. Essentially mosquito coils function as fumigants and they achieve their effectiveness by killing resting mosquitoes and prevent them from biting. Mosquito coils will burn for a period of six to eight hours. A major advantage is that they are extremely easy to use and are inexpensive. There are concerns that the smoke from the coils may affect people with breathing problems and that the smoke may have particulate matter that acts as an air pollutant (Lawrance and Croft, 2004).

2.2.3.2 Vapour emitting mats

This method makes use of small electric heating plates that can be used to vaporise volatile insecticides. These insecticidal mats are porous paper pads impregnated with insecticides which are considered safe, e.g. the allethrin pyrethroids. A major disadvantage of these vaporising mats is that they require electricity which is often not available in poor regions that are affected by malaria (Rozendaal, 1997).

2.2.3.3 Electric liquid vaporiser

A liquid insecticide is placed in a reservoir bottle fitted with a porous wick. The insecticide is vaporised by an electric heater. The insecticide in the bottle lasts for up to 8 h (Rozendaal, 1997). Just like the vaporising mats the disadvantage of this method is that it requires the use of electricity which might not be available in resource limited communities.

2.2.4 Making houses and shelters insect proof

Mosquitoes can detect odours emitted by the hosts drifting out of the eaves and other openings found in houses. When *An. gambiae* mosquitoes reach a wall, they fly up and are then funnelled by the over-hanging eaves. They then enter into the house through gaps found at the top of the wall (Lindsay et al., 2002). Closing the eaves or installing ceilings should prevent mosquitoes from gaining access into the house and this leads to a reduction in malaria transmission. According to studies done by Lindsay *et al.* (2002), in 90 % of the cases, houses had closed eaves or a ceiling had fewer indoor mosquitoes than dwellings without these features.

Blocking the eaves may be unacceptable because this restricts the ventilation into the house. The installation of anti-mosquito screening is another option to consider. The netting used for the screening should have a size of 1.5 mm or less for it to be effective (Rozendaal, 1997). The screening can be used to cover openings of the house, for example doors and windows. Potential materials to make the anti-mosquito screening are cotton netting, metal screens and plastic screens. The screening can also be treated with insecticide in order to provide a toxic barrier for the mosquitoes (Rozendaal, 1997). The screens must be constantly checked for tears and holes. A disadvantage of the screenings is that they restrict the ventilation and mosquitoes are persistent with respect to finding openings.

Building houses on platforms or raised structures helps to prevent mosquitoes from gaining access into the house. This is because most mosquito species searching for a blood meal prefer to fly close to the ground (Rozendaal, 1997). These improvements might be too expensive to put in place, especially in the poorer parts of Africa.

2.2.5 Environmental Management

Malaria control measures built around environmental management are non-toxic, cost effective and sustainable. These methods revolve around installation, cleaning and maintenance of drains as well as the elimination of standing pools of water (Keiser et al., 2005). According to Keiser *et al.* (2005) these programmes have been highly effective in reducing the mortality rates due to malaria. Picking up, collecting and destroying litter such as cans, small containers, bottles and tyres, which can be used as mosquito breeding sites, is also an effective tool for environmental management to reduce the number of mosquitoes.

The filling of mosquito breeding sites with soil, rubble, stones and ash provides a permanent vector control solution. This helps reduce the breeding of mosquitoes in places such as standing water pools and abandoned ditches. This method is convenient because on a small scale it does not require much material. No expertise is required and the work can be done using shovels, wheelbarrows and carts (Rozendaal, 1997). On a large scale, tractors and motorised equipment are necessary.

Having proper drainage in areas where people live results in the reduction of mosquito breeding sites. This is especially true when the drainage system is properly designed and well maintained. The open earth drain is the best drainage system. This is because it is easy to construct. Such a drain prevents the accumulation of excess rain water and can prevent other accumulation of water on the surface (Rozendaal, 1997). Eucalyptus trees can improve the drainage in a certain area. This is because they dry the land by allowing water to evaporate through their leaves (Rozendaal, 1997).

Some mosquito species require shaded water and so clearing of vegetation may be effective in removing resting places for mosquitoes (Rozendaal, 1997). This can be achieved by removing undergrowth, forest and mangroves (Keiser et al., 2005). Clearing vegetation will result in faster evaporation of water pools and will also expose potential breeding sites for easy control. Removing water plants can also be effective. This is because this type of vegetation usually provides larvae with a safe hiding place from larvivorous fish (Rozendaal, 1997).

2.2.6 Biological control

2.2.6.1 Larviciding

Larval control can be an effective control tool and this is due to the low mobility of larval mosquitoes. Larvicides are used in breeding sites to kill mosquito larvae. They act as stomach poisons upon ingestion by the larvae or as contact poisons (Rozendaal, 1997). Larvicides are commonly used on mosquito breeding sites that cannot be easily drained or filled.

Petroleum oils as larvicides

Oil can be applied to water surfaces to kill larvae. This is considered an effective method. Diesel oils and kerosene are the commonly used oils for larviciding. The oil kills the larvae by two mechanisms which are:

- Suffocation when the larvae rise to the water surface to breathe
- Toxic vapours that emanate from the oil (Rozendaal, 1997)

The use of diesel oil and kerosene for mosquito control has decreased because of the high cost relative to other larvicidal approaches. The use of petroleum oils as larvicides is mostly recommended in cases where there is resistance to insecticides.

Plant Extracts

A berry extract known as *Solanum villosum* can be used as a larvicide. It has been shown to be effective against the *Stegomyia aegypti* mosquito which is a vector for dengue fever. The berry extract can be used in stagnant water bodies for the control of mosquitoes acting as vectors for many communicable diseases (Chowdhury et al., 2008).

Essential oils from plants can also be used as larvicides. Their mechanism of action on how they kill the larvae is not yet understood. Essential oils have the advantage that they are relatively safe substances. However, their use as larvicides is limited by the lack of availability of plant materials at affordable prices (Pavela, 2015).

2.2.6.2 Bacterial Agents

Bacterial agents can be used to control vector borne diseases. Examples of bacterial agents include *Bacillus thuringiensis (Bti)* and *Bacillus sphaericus (Bs)*. These are the most widely used bacterial larvicidal strains. Compared to insecticidal larvicides, *Bti* and *Bs* have been shown to have faster spreading abilities (Kamareddine, 2012). There are some concerns on whether these micro-organisms are highly effective, environmentally friendly and non-toxic.

2.2.6.3 Larvivorous fish

Fish that feed on mosquito larvae are introduced into mosquito breeding sites. Examples of larvivorous fish species are *Gambus affinis* and *Poecilia reticulata*. The fish should have the following properties:

- They should prefer mosquito larvae as food
- The fish should be small for them to have access to shallow water

- They should have a high reproduction rate
- They should have a high tolerance to water pollution and salinity (Rozendaal, 1997)

The method of using larvivorous fish is effective because of the low cost of introducing and maintaining the fish. However, the fish are ineffective during periods of heavy rainfall. This is because of the immediate hatching of the large numbers of mosquito eggs. There is also dilution of predatory fish densities due to the increase in water levels (Pates and Curtis, 2005).

2.2.6.4 Entomopathogenic Fungi

Fungal pathogens can be used as biopesticides against insects such as mosquitoes. This is because fungal pathogens commonly infect insects. An example is the entomopathogenic fungus *Beauveria bassiana* which has the potential for use as an alternative vector control tool (Kikankie et al., 2010). The fungus does not require ingestion by the host. Only the insect's cuticle must make contact with the fungi for an infection to occur (Kamareddine, 2012). Fungal spores can be applied on indoor house surfaces, bed nets and even curtains and they remain effective for months.

The major disadvantage of the use of the fungal pathogens is that the rate at which they penetrate and grow within an infected host is dependent on temperature. Fungal infections kill mosquitoes at slower rates as compared to insecticides. The fungi can be used alone or as a synergist with insecticides such as DDT. This is an advantage in areas where there is resistance towards insecticides (Kamareddine, 2012).

2.2.6.5 Attractive Toxic Sugar Bait

A different approach against malaria vectors is the use of attractive toxic sugar baits (ATSB). The method makes use of a fruit or a flower scent as an attractant, sugar solution as a feeding stimulant and an oral toxin to kill the mosquitoes (Muller et al., 2010). The ATSB solution is either sprayed on vegetation or suspended in simple baits. Mosquitoes that ingest then toxic solutions are then killed.

According to Muller *et al.* (2010) successful field trials on the effectiveness of the ATSB methods were carried out against *An. gambiae* mosquitoes in Mali. It was observed that a single

application of ATSB resulted in a substantial decrease in malaria vector populations. This method can be an effective tool considering that it is simple, inexpensive and environmentally safe.

2.2.6.6 Genetic control of mosquitoes

This refers to instances where a mechanism for vector control is introduced into a population through mating. Examples of genetic control include the Sterile Insect Technique (SIT) and the introduction of genetic factors into wild populations which result in the mosquitoes being harmless to humans (Pates and Curtis, 2005).

SIT is a technique by which male mosquitoes are sterilised by irradiation and then released to mate with the females. This then results in the females laying sterile eggs. The source of the radiation is gamma radiation from the isotopic sources ^{60}Co and ^{137}Cs. When biological material is irradiated, molecular bonds are broken which results in ions and free radicals being formed. The insect is then rendered reproductively sterile due to the damage of gonial cells specifically by the fragmentation of germ-cell chromosomes. This then leads to the production of imbalanced gametes which results in the death of fertilised eggs or embryos (Dyck et al., 2005). When a female mosquito mates with a sterile male, the female becomes infertile for the rest of her lifespan.

Mosquitoes should be irradiated at or near the completion of their developments into adults. SIT programmes have been successful in the elimination of the screw worm in the USA and the tsetse fly in Zanzibar. This technique can be a useful tool to reduce the population sizes of mosquitoes in selected areas (Dyck et al., 2005).

2.3. Killing mosquitoes after they have bitten

2.3.1 Indoor residual spray

This method of vector control is important because mosquito vectors typically rest on inner walls after feeding. This method acts by spraying residual insecticides on surfaces to kill insects that land or crawl over the treated surface. The duration of action is dependent upon the nature of the treated surface as well as the nature and the dosage of the insecticide used (Rozendaal, 1997).

The limitations of this method of insecticide residual spraying are as follows:

- The development of resistance to the insecticides by certain vector species
- Some vector species prefer to bite outdoors
- The surfaces in some houses are not adequate for spraying
- Some people prefer to sleep outdoors during hot seasons.

Insecticides used for IRS should be highly toxic to target insects, last long on the applied surface, be safe to humans and animals as well as being cost effective. The insecticides are rarely used in their pure forms but are supplied as formulations. The insecticides commonly used for IRS are as follows:

- Chlorinated hydrocarbons
- Organophosphates
- Carbamates
- Pyrethroids

2.3.1.1 Organochlorines

DDT is the only organochlorine used for indoor residual spraying. Previously used organochlorines were cyclodienes with examples being dieldrine and another insecticide known as β-hexachlorocyclohexane (HCH). Their use was discontinued because of their high toxicity to humans (Najera, 2001).

DDT has a low volatility and a very low solubility in water. In 1993, DDT was replaced by λ-cyhalothrin, a pyrethroid, but mosquitoes quickly developed resistance to this insecticide. The pyrethroid was phased out in the year 2000 (Coleman et al., 2008). The carbamate bendiocarb was then introduced as another alternative but resistance to the insecticide was then detected in both *An. funestus* and *An. arabiensis*.

Environmental issues arose due to the use of DDT. The insecticide is extremely stable in the environment and hence it is considered an organic pollutant. Carbamates and organophosphates are available for use in IRS but they have short half-lives which means that IRS must be repeated once or twice per year as compared to DDT which requires only one spray. This makes

the alternatives more expensive (Coleman et al., 2008). The molecular structure of DDT is shown in Figure 7 and its properties in Table 4.

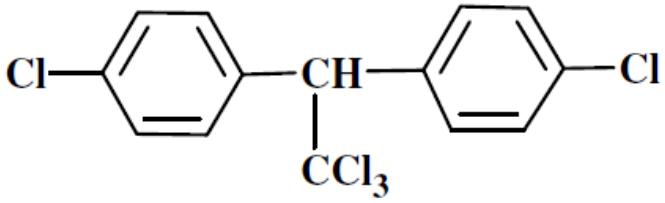

Figure 7: Molecular structure of DDT (WHO, 2009b).

Table 4: Properties of DDT (WHO, 2009b)

Mixture of *p,p'*-DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane and *o,p'*-DDT: 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane	
CAS Registry Number	50-29-3
Molecular formula	$C_{14}H_9Cl_5$
Molar Mass	354.5 $g \cdot mol^{-1}$
Melting point	109°C
Density	0.98 $g \cdot cm^{-3}$ at 20°C
Vapour Pressure	2.5×10^{-5} Pa at 25°C
Solubility in water	0.0055 $mg \cdot L^{-1}$ at 25°C
Octanol water partition coefficient	log P_{ow} = 6.15 at 25°C
Toxicity, LD_{50}	113 $mg \cdot kg^{-1}$ body weight (male rat)

2.3.1.2 Organophosphates

Organophosphates are insecticides that were developed after the organochlorines. When mosquitoes developed a resistance to DDT, organophosphates began to be used as an alternative. The most commonly used organophosphates are malathion, fenitrothion and pirimiphos-methyl. These insecticides are more expensive than DDT and have a shorter residual effectiveness (Rozendaal, 1997).

Malathion

Malathion is a liquid with a low vapour pressure. Malathion has been used for malaria control since the 1960s. It has low mammal toxicity. It is recommended that for indoor residual spray a dosage of 2 g m^{-2} be used and this gives a residual effect of two to three months. On some occasions people object to the spraying of Malathion because of its unpleasant odour (Najera, 2001). Figure 8 shows the molecular structure of Malathion while Table 5 has the physical and chemical properties of the insecticide.

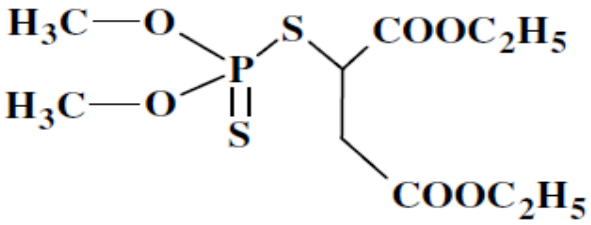

Figure 8: Molecular structure of Malathion (WHO, 2004c).

Table 5: Properties of Malathion (WHO, 2004c)

IUPAC Name: S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate	
CAS Registry Number	121-75-5
Molecular formula	$C_{10}H_{19}O_6PS_2$
Molar Mass	330.36 g·mol^{-1}
Thermal Properties	Decomposition > 174°C at 99.1 % purity
Density	1.272 ± 0.06 g·cm^{-3} at 20°C
Vapour Pressure	4.5×10^{-4} Pa at 25°C and 98.9 % purity
Solubility in water	148mg·L^{-1} at 25ºC and 98.4 % purity
Octanol water partition coefficient	log P_{ow} = 2.7 at 25ºC and 98 % purity
Toxicity, LD_{50}	1768mg·kg^{-1} body weight (male rat)

Pirimiphos-methyl

Pirimiphos-methyl is an orange, oily liquid which has a characteristic pungent smell. For indoor residual spray the insecticide should be sprayed at doses of between 1 and 2 g m^{-2}, giving a residual effect of two to three months (Najera, 2001). Figure 9 shows the molecular structure of pirimiphos-methyl while Table 6 has the physical and chemical properties of the insecticide.

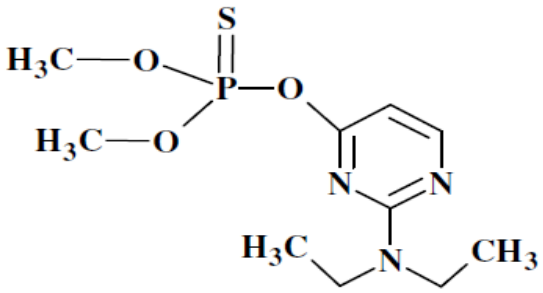

Figure 9: Molecular structure of pirimiphos-methyl (WHO, 2006b).

Table 6: Properties of Pirimiphos-methyl (WHO, 2006b)

IUPAC Name: O-2-diethylamino-6-methylpyrimidin-4-yl-o,o-dimethyl phosphorothioate	
CAS Registry Number	29232-93-7
Molecular formula	$C_{11}H_{20}N_3O_3PS_2$
Molar Mass	305.3 $g{\cdot}mol^{-1}$
Thermal Properties	Melting point is -20°C at 99.1%
Density	1.229 ± 0.06 $g{\cdot}cm^{-3}$ at 20°C
Vapour Pressure	2.0×10^{-6} at 20°C and 99% purity
Solubility in water	10$mg{\cdot}L^{-1}$ at pH 7, 20°C and 99% purity
Octanol water partition coefficient	log P_{ow} = 4.2 at pH 7, 25°C and 99% purity
Toxicity, LD_{50}	1414$mg{\cdot}kg^{-1}$ body weight (male rat)

Fenitrothion

Fenitrothion has been used for indoor residual spray since the 1970s. Fenitrothion is a reddish-brown, oily liquid with a characteristic pungent smell. This insecticide is toxic and protective clothing should be used when handling it. The recommended formulation for IRS is 2 g m^{-2} and it results in a residual efficacy of three to six months (Najera, 2001). Figure 10 shows the molecular structure of fenitrothion while Table 7 gives the physical and chemical properties of the insecticide.

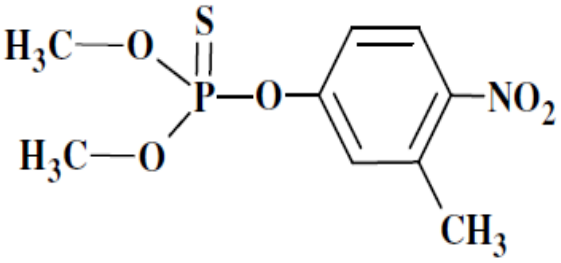

Figure 10: Molecular structure of Fenitrothion (FAO, 2007).

Table 7: Properties of Fenitrothion (FAO, 2007)

IUPAC Name: o,o-dimethyl o-4-nitro-m-tolyl phosphorothioate	
CAS Registry Number	112-14-5
Molecular formula	$C_9H_{12}NO_5PS$
Molar Mass	277.25 $g \cdot mol^{-1}$
Thermal Properties	Melting point is -1°C ± 1°C
Density	1.229 ± 0.06 $g.cm^{-3}$ at 20°C
Vapour Pressure	1.57×10^{-3} Pa at 25°C and 99.1% purity
Solubility in water	19.0 $mg \cdot L^{-1}$ at 20 ± 0.5°C and 99.1% purity
Octanol water partition coefficient	log P_{ow} is 3.319 ± 0.080 at 25°C and 99.3% purity
Toxicity, LD_{50}	1 700 $mg \cdot kg^{-1}$ body weight (male rat)

2.3.1.3 Carbamates

Carbamates are esters of carbamic acid. They are used as pesticides in agricultural applications. Carbamates are used more often than organophosphates because they are much safer to mammals. The commonly used carbamate insecticides are N-methyl carbamates (Gupta, 2006).

Propoxur

Propoxur is a very strong contact and stomach poison for insects. Propoxur is available as a wettable, white crystalline powder. It is neither sensitising nor an irritant to the skin and it is also not irritant to the eyes. Propoxur has an airborne effect inside and near houses for up to two months after spraying (Rozendaal, 1997). There is no evidence of propoxur being carcinogenic, however, it is highly toxic to birds and slightly toxic to fish (WHO, 2005). Figure

11 shows the molecular structure of propoxur while Table 8 gives the physical and chemical properties of the insecticide.

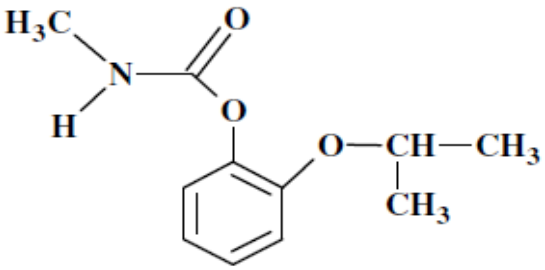

Figure 11: Molecular structure of propoxur (WHO, 2005).

Table 8: Properties of Propoxur (WHO, 2005)

IUPAC Name: 2-isopropoxyphenyl methylcarbamate	
CAS Registry Number	114-26-1
Molecular formula	$C_{11}H_{15}NO_3$
Molar Mass	209.25 $g \cdot mol^{-1}$
Melting point:	Crystal modification I: 87.5 °C at 99.9% purity Crystal modification II: 90.0 °C at 99.9% purity
Density	1.17 $g \cdot cm^{-3}$ at 20°C
Vapour Pressure	2.78×10^{-3} Pa at 20°C and 99.1% purity
Solubility in water	1.75 $g \cdot L^{-1}$ at 20°C and 99.8 % purity
Octanol water partition coefficient	log P_{ow} is 1.56 at 20°C and 99.8 % purity
Toxicity, LD_{50}	89.7 $mg \cdot kg^{-1}$ body weight (male rat)

Bendiocarb

Bendiocarb is a crystalline solid which has low solubility in water. Bendiocarb works by disrupting the normal functioning of an insect's nervous system. It acts as a contact or stomach poison (WHO, 2009b). The insecticide is classified as being moderately hazardous. The recommended dosage for IRS is 0.2 – 0.4 $g \cdot m^{-2}$ and the insecticide remains effective for two to three months after it has been sprayed on the walls (Rozendaal, 1997). Figure 12 shows the molecular structure of bendiocarb while Table 9 contains the physical and chemical properties of the insecticide.

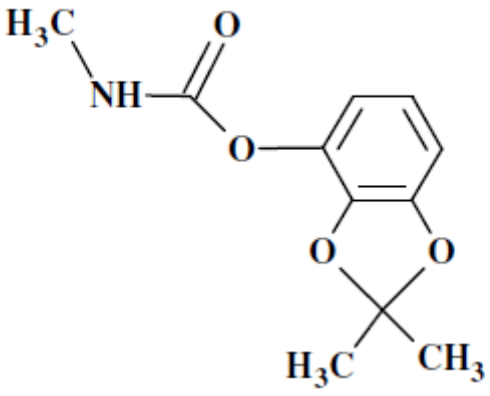

Figure 12: Molecular structure of Bendiocarb (WHO, 2009c).

Table 9: Properties of Bendiocarb (WHO, 2009b)

IUPAC Name: 2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate	
CAS Registry Number	22781-23-3
Molecular formula	$C_{11}H_{13}NO_4$
Molar Mass	223.4 $g \cdot mol^{-1}$
Melting point	129°C at 98.5% purity
Density	1.203 ± 0.06 $g \cdot cm^{-3}$ at 20°C
Vapour Pressure	4.6×10^{-3} Pa at 25°C and 99.8 % purity
Solubility in water	0.28 $g \cdot L^{-1}$ at pH 7 and 99.3% purity
Octanol water partition coefficient	log P_{ow} = 1.7 at 25°C and 99% purity
Toxicity, LD_{50}	45 – 48 $mg \cdot kg^{-1}$ body weight (male rat)

2.3.1.4 Pyrethroids

The term pyrethroid is a general name for pyrethrins which are insecticidal ingredients of pyrethrum and its synthetic analogs (Matsuo, 2012). Pyrethrum is obtained from the preparation of dried *Chrysanthemum cineum* flower heads that contain insecticidally active pyrethrins (Rozendaal, 1997). Synthetic pyrethroids have a similar structure to natural pyrethrins.

Pyrethroids exhibit quick action on insects even when applied at a low dosage. They show selective toxicity to insects over mammals and this makes them ideal for use as household

insecticides. They are environmentally friendly because they quickly breakdown in the soil. Pyrethroids are available as solutions called emulsifiable concentrates (Rozendaal, 1997).

Lambdacyhalothrin

Lambdacyhalothrin is a white wettable powder with a low vapour pressure, insoluble in water and has low volatility. The recommended dosage for IRS is 20 – 30 $mg \cdot m^{-2}$ with a residual efficacy of three to six months (Najera, 2001). Figure 13 shows the molecular structure of lambdacyhalothrin and Table 10 shows its properties.

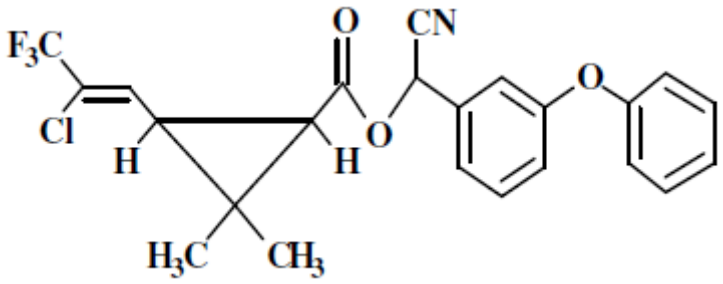

Figure 13: Molecular structure of lambdacyhalothrin (WHO, 2007d).

Table 10: Properties of Lambdacyhalothrin (WHO, 2007d)

CAS Registry Number	91465-08-6
Molecular formula	$C23H19ClF_3NO_5$
Molar Mass	449.9 $g \cdot mol^{-1}$
Thermal decomposition	228-230 °C at 87.63 % purity
Density	1.344 ± 0.06 $g \cdot cm^{-3}$ at 20°C
Vapour Pressure	2.8×10^{-7} Pa at 20 °C and 87.63 % purity
Solubility in water	0.001$mg \cdot L^{-1}$ at pH 7 and 87.63 % purity
Octanol water partition coefficient	log P_{ow} = 6.28 at 25°C and 87.63% purity
Toxicity, LD_{50}	91 $mg \cdot kg^{-1}$ body weight (male rat)

Etofenprox

Etofenprox has a high vapour pressure and very low solubility in water. It is the insecticide with the lowest acute toxicity to mammals of all the insecticides used for IRS. The recommended dosage for IRS is 100 – 300 $mg \cdot m^{-2}$ and this results in a residual efficacy of

three to six months (Najera, 2001). Figure 14 and Table 11 show the molecular structure and properties of etofenprox respectively.

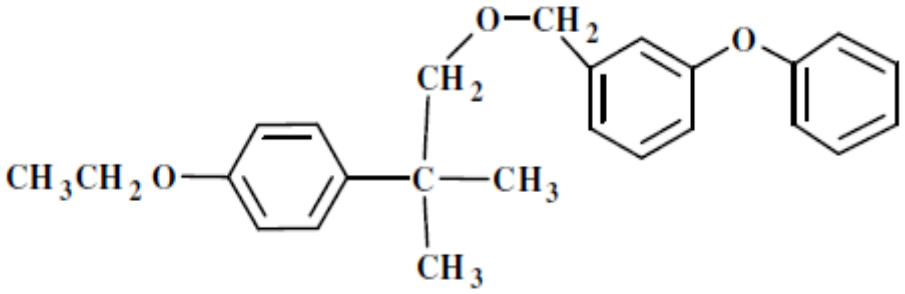

Figure 14: Molecular structure of etofenprox (WHO, 2007b).

Table 11: Properties of Etofenprox (WHO, 2007b)

IUPAC Name: 2-(4-ethoxyphenyl)-2-methylpropyl-3-phenoxybenzylether	
CAS Registry Number	80844-07-1
Molecular formula	$C_{25}H_{28}O_3$
Molar Mass	376.5 $g{\cdot}mol^{-1}$
Melting point	37.4 °C
Density	1.073 ± 0.06 $g{\cdot}cm^{-3}$ at 20°C
Vapour Pressure	8.13×10^{-7} Pa at 25°C and 99 % purity
Solubility in water	22.5×10^{-6} $g{\cdot}L^{-1}$ at pH 7 and 98 % purity
Octanol water partition coefficient	log P_{ow} = 6.9 at 25°C and 99 % purity
Toxicity, LD_{50}	> 2000 $mg{\cdot}kg^{-1}$ body weight (male rat)

Betacyfluthrin

Betacyfluthrin is a synthetic pyrethroid with a low vapour pressure. This insecticide has a high knockdown effect on insects and it has a low excito-repellency. The recommended dosage for IRS is 25 – 50 $mg{\cdot}m^{-2}$ and this gives a residual efficacy of three to six months (Najera, 2001). The insecticide has three chiral centres with eight enantiomer forms and this results in it having four pairs of diastereoisomers as illustrated in Figure 15 and Table 12.

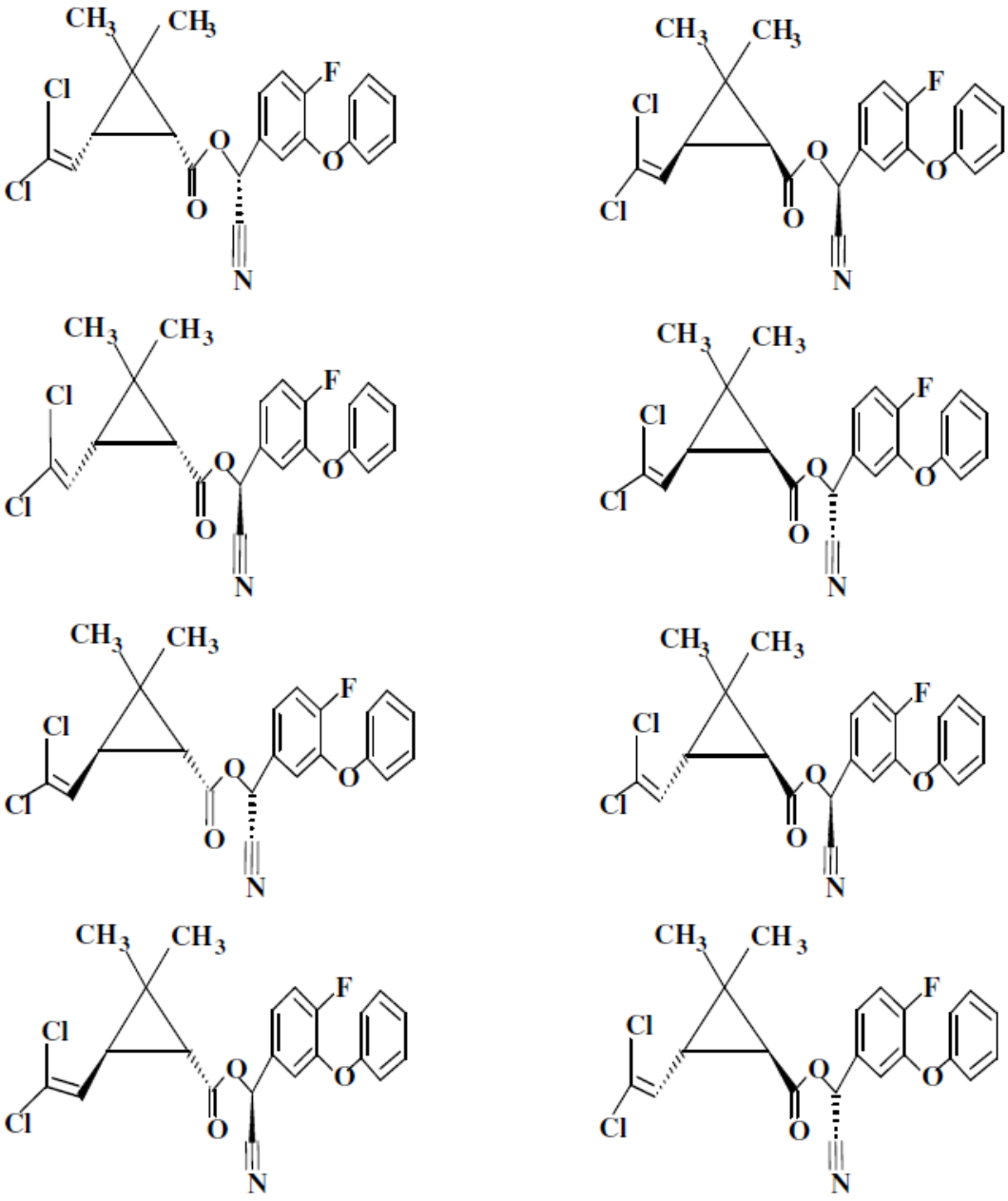

Figure 15: Molecular structure of betacyfluthrin (WHO, 2004a).

Table 12: Physical and chemical properties of Betacyfluthrin (WHO, 2004a)

IUPAC Name	(RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS, 3RS; 1RS,3SR)-3-(2, 2 dichlorovinyl)-2, 2-dimethylcyclopropanecarboxylate
CAS Registry Number	Diasteroisomer I: 86560-92-1 Diasteroisomer II: 86560-93-2 Diasteroisomer III: 86560-94-3 Diasteroisomer IV: 86560-93-5
Molecular formula	$C_{22}H_{18}Cl_2FNO_3$
Molar Mass	434.3 $g \cdot mol^{-1}$
Thermal Properties	Melting point is 77°C
Density ($g.cm^{-3}$ at 20 °C)	Diastereoisomer I: 1.46 Diastereoisomer II: 1.373, Diastereoisomer III: 1.316, Diastereoisomer IV: 1.356
Vapour Pressure at 25°C (at purity indicated)	Diasteroisomer I: 2.1×10^{-6} (98.8 %) Diasteroisomer II: 3.4×10^{-7} (97.4 %) Diasteroisomer III: 4.7×10^{-7} (97.8 %) Diasteroisomer IV: 2.0×10^{-7} (98.9 %)
Solubility in water in $g \cdot L^{-1}$ at pH 3 or pH 7	Diastereoisomer I: 2.5/2.2 Diastereoisomer II: 2.1/1.9 Diastereoisomer III: 3.2/2.2 Diastereoisomer IV: 4.3/2.9
Octanol water partition coefficient (Log P_{ow} at 20 °C):	Diastereoisomer I: 6.00, Diastereoisomer II: 5.94, Diastereoisomer III: 6.04 Diastereoisomer IV: 5.91
Toxicity, LD_{50}	20 $mg \cdot kg^{-1}$ body weight (male rat)

Alphacypermethrin

Alphacypermethrin is a synthetic pyrethroid and technical alphacypermethrin is a white wettable powder. This insecticide exhibits a high knockdown effect against mosquites and it also has a strong excito-repellent effect. When used for IRS the insecticide has a residual efficacy of four to six months (Najera, 2001). Alphacypermethrin occurs as a racemic mixture of (S)-α-cyano-3-phenoxybenzyl-(1R, 3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (R)-α-cyano-3-phenoxybenzyl-(1S,3S)-3(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate as shown in Figure 16. Table 13 contains the properties of the insecticide.

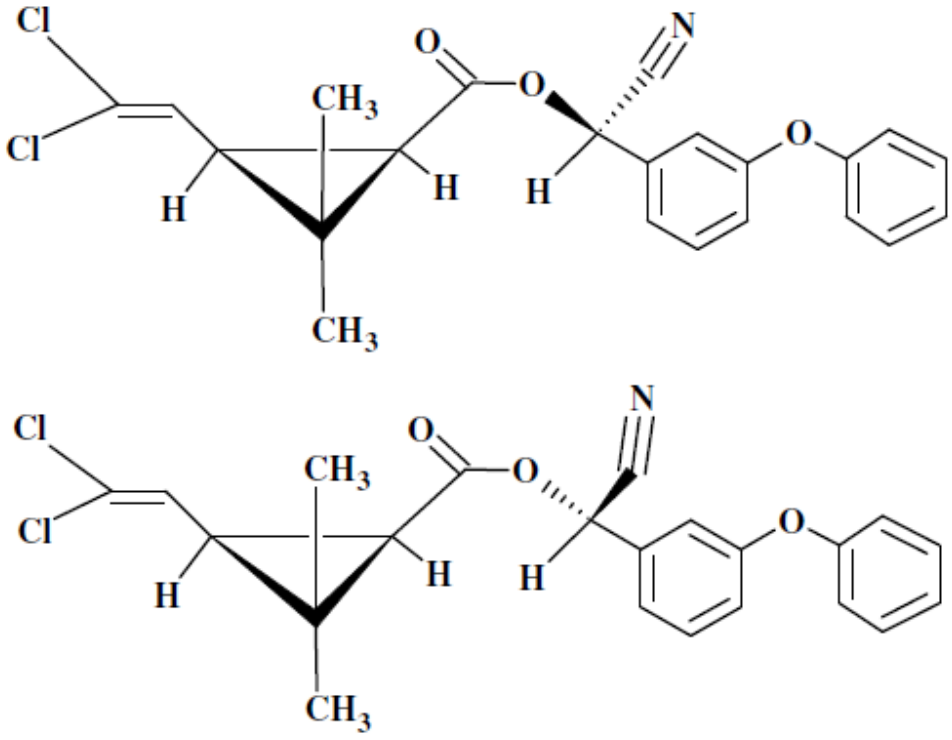

Figure 16: Molecular structure of alphacypermethrin (WHO, 2009a).

Table 13: Properties of Alphacypermethrin (WHO, 2009a)

CAS Registry Number	67375-30-8
Molecular formula	$C_{22}H_{19}Cl_2NO_3$
Molar Mass	416.3 $g \cdot mol^{-1}$
Melting point	81 – 83°C at 95 % purity

Density	1.329 ± 0.06 g·cm^{-3} at 20°C
Vapour Pressure	9 × 10^{-6} Pa at 25°C and 95 % purity
Solubility in water at pH 7 and 20°C	6 µg·L^{-1} at 97.8 % purity
Octanol water partition coefficient	log P_{ow} = 6.64 at 25°C and 95 % purity
Toxicity, LD_{50}	360 mg·kg^{-1} body weight (male rat)

Deltamethrin

Deltamethrin is a synthetic pyrethroid which has been used since the 1970s for malaria control. In addition to IRS it is also used to coat bed nets and curtains. The recommended dosage for IRS is 10 – 25 mg·m^{-2} and this results in a residual efficacy of three to six months. Deltamethrin is a toxic insecticide to mammals and it acts as a neuro-toxin which is primarily absorbed from the gastrointestinal tract (Najera, 2001). Figure 17 shows the molecular structure of deltamethrin and Table 14 shows the properties of the insecticide.

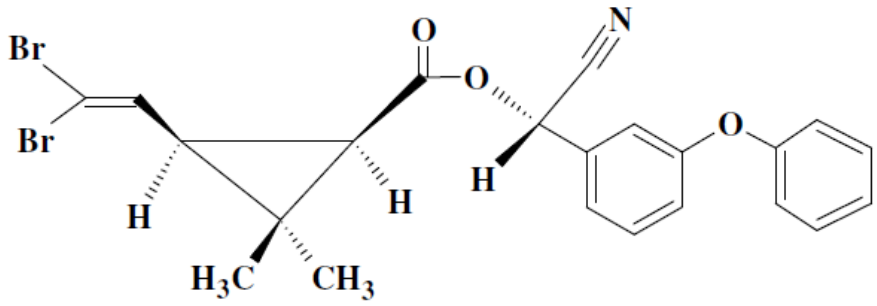

Figure 17: Molecular structure of deltamethrin (WHO, 2007a).

Table 14: Properties of Deltamethrin (WHO, 2007a)

IUPAC Name: (S)-α-cyano-3phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate	
CAS Registry Number	52918-63-5
Molecular formula	$C_{22}H_{19}Br_2NO_3$
Molar Mass	505.2 g·mol^{-1}
Thermal Properties	Melting point is 99 °C
Density	1.595 ± 0.06 g·cm^{-3} at 20°C
Vapour Pressure	1.24 × 10^{-7} Pa at 25°C and 99.1% purity

Solubility in water	19.0 $mg \cdot L^{-1}$ at 20 ± 0.5°C and 98% purity
Octanol water partition coefficient	log P_{ow} = 4.61 at 25°C and 98 % purity
Toxicity, LD_{50}	87.4 $mg \cdot kg^{-1}$ body weight (male rat)

2.3.2 Long Lasting Insecticide Treated Nets (LLINs)

The use of long lasting insecticide treated nets is one of the highly recommended interventions for vector control. LLINs typically have a mesh size of 1.2 – 1.5 mm and this is small enough to prevent mosquitoes from gaining access. However, they must be constantly checked for holes and tears. Nets have been used as a protection against malaria because of the late-night biting tendencies of mosquito species such as *An. funestus* and *An. gambiae* (Pates and Curtis, 2005). In a study done in South East China by Pates & Curtis (2005) it was found that, because of the overlap of mosquitoes biting rhythms and the sleep times of people (between 22h00 – 05h00), the use of mosquito nets reduces anopheline bites by about 75 %.

Mosquito nets are more effective when they are impregnated with insecticides. The insecticides commonly used to treat mosquito nets are pyrethroids. Pyrethroid-treated nets are much more effective in reducing the occurrence of malaria. This is because when mosquitoes make contact with the nets they are driven away before they have had the chance to penetrate through the nets since pyrethroids exhibit excito-repellency and have a rapid knockdown effect. Another reason is that mosquitoes that are attracted to the odour of sleeping humans may be killed in significant numbers after contacting the nets and this results in a reduction of the vector population and density (Pates and Curtis, 2005).

According to the WHO, insecticide treated nets should retain their biological activity for at least 20 washes or three years of use in field conditions (WHO, 2013). A limitation of nets is that resistance to pyrethroids has developed in certain mosquito strains. In a study in Tanzania showed that, when a person with an LLIN slept in the same room as a person without an LLIN, biting was diverted to the latter person (Pates and Curtis, 2005)

2.3.3 Insecticide treated wall linings

There are limitations associated with the recommended interventions for vector control. For example, some of the drawbacks associated with LLINs include personal confinement during

sleep as well as discomfort due to high indoor temperatures and humidity. A novel vector control intervention which has been developed is a durable wall lining impregnated with an insecticide. These wall linings are fixed to walls or ceilings.

The wall linings currently on the market go by the trade name ZeroVector® and it is manufactured by a company known as Vestergaard Frandsen in Switzerland. They are manufactured using high density polyethylene shade cloth with deltamethrin incorporated during production. The wall linings are designed to be effective for three to four years. In field studies conducted by Messenger *et al.* (2012) there was no decline in the effectiveness of these wall linings after 12 months of use.

In South Africa, there were field trials done to determine the effectiveness and user acceptability of a different type of insecticidal treated wall linings. The wall linings used were monofilament polyethylene linings produced by extruding and meshing the polyethylene in one step. These linings were produced at the Institute of Applied Materials (IAM) at the University of Pretoria, Pretoria, South Africa. The field trials were done in the Vhembe District of Limpopo, South Africa. In this study, there was very high perceived effectiveness of the linings amongst the participants with reports of observed mortality of mosquitoes (Kruger et al., 2015).

In field trials done in Angola and Nigeria on wall linings the participants reported a reduction in mosquito density of 93 % and reduction of biting by 82 %. The wall linings have the potential to become a viable alternative to indoor residual spraying and they may result in reduction of human exposure to insecticidal residue (Messenger et al., 2012b).

2.4 Limitations of current vector control methods

2.4.1 Insecticide resistance

One of the limitations of the current vector control methods is insecticide resistance. Insecticide resistance is the reduction of insecticide activity in an insect population. This resistance can be observed when an insecticide repeatedly fails to achieve the expected level of control when used according to the recommendations for the insect species. The growing development of insecticide resistance exhibited by various mosquito species poses a threat to malaria control programmes (Ahoua Alou et al., 2012).

Mosquitoes are developing resistance to groups of insecticides. In a study carried out on *An. arabiensis* from an area known as Gwave, a malaria endemic area in Zimbabwe, permethrin resistance in mosquito populations was discovered (Munhenga et al., 2008). In Côte d'Ivoire, resistance towards permethrin, deltamethrin and λ-cyhalothrin was observed to be largely present in *An. gambiae* (Ahoua Alou et al., 2012). In Sudan, WHO susceptibility tests with *An. arabiensis* showed resistance to DDT and pyrethroids (Abdalla et al., 2014).

Resistance to insecticides develops when insects find ways to overcome the toxins. In biochemical resistance, enzyme detoxification deactivates the insecticide before it reaches the target site (Ranson et al., 2011). In physiological resistance, the toxin is not necessarily broken down but instead it is accommodated by altering one or more physiological functions, e.g. an increase in the rate of insect metabolism. The growing trend of pyrethroid resistance constitutes a serious threat to malaria control programmes.

2.4.2 Outdoor biting behaviour of mosquitoes

IRS and ITNs are effective in reducing mosquito bites indoors hence reducing the rate of transfer of malaria. This method is limited because there is outdoor malaria transfer due to outdoor mosquito bites. As discussed in Section 2.2, in some areas in rural Tanzania there was a rise in outdoor malaria transmission (Russell et al., 2011). In hot seasons, some people in malaria endemic regions prefer to sleep outdoors rather than indoors. This aggravates the problem of outdoor malaria transmission. Insect repellents can be used to prevent outdoor mosquito bites but the problem with these repellents is that they are not effective for an extended period of time. Alternative vector control methods need to be developed to help prevent malaria transmission due to outdoor mosquito bites.

2.5 Concept of multi-layer polymer films and their applications

Multilayer polymer films can be used to enhance properties of materials. Examples of these properties are barrier improvement and chemical resistance. Co-extrusion techniques are used to produce the multi-layers. The main techniques are film blowing and the cast film process (Langhe and Ponting, 2016). Figure 18 is a schematic which shows the most commonly produced layered structures.

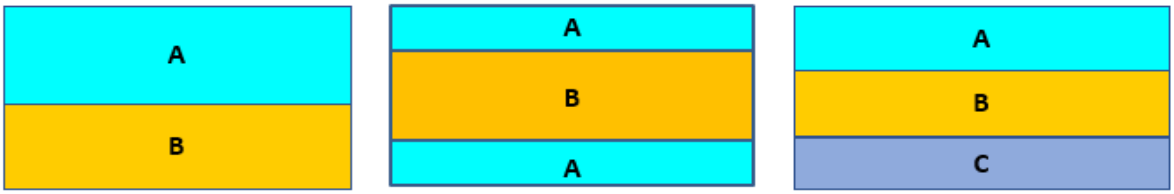

Figure 18: Different layered structures produced by co-extrusion techniques. A, B and C represent different types of polymers (Adapted from (Langhe and Ponting, 2016)).

Boumail *et al.* (2013) produced trilayer antimicrobial diffusion films. These films were developed to prevent food-borne microbial outbreaks. The films were to be used in vegetable packaging. The films consisted of two external layers made from polycaprolactone. The middle layer was made from a methylcellulose matrix. The matrix was reinforced with nanocrystalline cellulose. The antimicrobial mixtures were incorporated in the middle layer. Results from this study indicated that there was controlled release of the antimicrobials from the trilayer films. (Boumail et al., 2013)

In another study by Sonntag *et al*. (2004) a biocide liquid was incorporated into a protective film. The film was composed of a middle layer containing the liquid in droplet-like compartments. The middle layer was then sandwiched between two elastomeric boundary layers. The biocide would squirt out of the trilayer film when punctured. The application for this is in surgical gloves that squirt out a biocide when punctured during surgery (Sonntag et al., 2004). These studies suggest that it is possible to produce trilayer films that contain a liquid insecticide. This insecticide can be placed in the middle layer and sandwiched with outer layers that act as semi-permeable barriers. The insecticide is then slowly released to the surface of the trilayer film.

3. Experimental

3.1 Materials

Low density polyethylene (LDPE) (Sasol grade LT033, density 0.921 g·cm^{-3} and melt flow index (MFI) 0.33 g/10 min @ 190°C/2.16 kg) was used as the sheath polymer. It was chosen as it can be converted into a film at low extrusion temperatures and because of its medium crystallinity and low polarity. The latter properties should make it suitable as a semi-permeable membrane material for the polar organophosphate insecticides. Poly(ethylene-co-vinyl acetate) (EVA) was selected as the core polymer. Its high amorphous content and medium polarity should enable it to imbibe significant quantities of the polar insecticides. Three different grades of EVA were considered for use in carrying the organophosphate. They were Elvax 210W from Du Pont (vinyl acetate (VA) content of 28 %; MFI 400 g/10 min), Evatane 20-20 grade from Arkema (VA content ca. 20 %; MFI 20 g/10 min) and EV101 (VA content of 18 %; MFI 1.8 g/10 min) with all the MFI values obtained at 190°C/2.16 kg. Tris(2-chloroethyl) phosphate (97 %), sourced from Sigma-Aldrich, was used as a simulant for the insecticide. This compound is less toxic than the insecticide organophosphate and it is commonly used as a plasticizer and flame retardant in plastics. It was used in place of the insecticide to establish safe and stable operating conditions during initial film blowing trial runs. The organophosphate insecticides, malathion, fenitrothion and pirimiphos-methyl, were technical grade samples supplied by Avima.

3.2 Equipment and methods

3.2.1 Active absorption by EVA polymer matrix

The malathion was incorporated into the EVA by swelling on heating the pellets while submerged in the hot insecticide liquid. About 30 g of EVA pellets were placed in small glass bottles containing an excess amount of malathion. The glass bottles were placed in a convection oven and the temperatures were set to values just below the melting points of the various EVA grades. These were 58°C for EVA 28 % VA and 81°C for the other two EVA grades. The absorption tests were terminated after 1 h, 2 h, 12 h, 24 h, 48 h and 72 h. These experiments were done twice in order to get the mean values of the malathion absorbed by the pellets. Excess malathion was decanted from the bottles and ethanol was used to quickly rinse off any remaining traces of the insecticide on the surfaces of the EVA pellets. The change in mass of

the pellets in the glass bottles was determined to establish the amount malathion absorbed by the EVA.

3.2.2 Extrusion and film blowing

Initial film blowing trials were conducted with the less-toxic tris (2-chloroethyl) phosphate simulant to establish the safe processing window. Trilayer films were blown on a Labtech Engineering multilayer film blower fed by two extruders. The EVA, containing the simulant or insecticide, was extruded as the inner layer. EVA with a VA content of 28 % was chosen for the film blowing. The two outer layers consisted of the neat LDPE. Table 15 lists the temperature profiles for the two polymer streams. Zone 1 is the feed section.

Table 15: Film blower LDPE and EVA extruder temperature profiles expressed in °C

	Zone 1	**Zone 2**	**Zone 3**	**Zone 4**	**Screen**	**Ring Die**
LDPE extruder	160	170	175	180	180	180
EVA extruder	130	140	150	150	150	

3.2.3 Film thickness

The film thickness of the neat film and malathion-containing film sections were measured with a Mitutoyo Digimatic Indicator IDF-150 model. The probe from the instrument was placed on five various positions on the films and an average thickness is reported. It proved difficult to control the bubble pressure during the short film blowing trial run used to blow the insecticidal film samples. The consequence was that the film thickness varied along the length of the extruded tube. This extended to the relative thicknesses of the LDPE and EVA layers too as continuous adjustments were necessary. Figure 19 shows the micrometer used to measure film thickness.

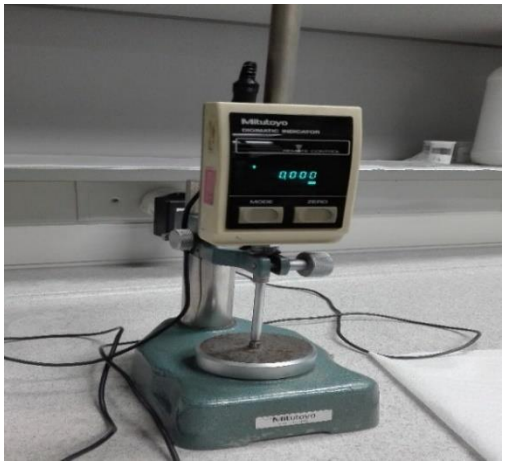

Figure 19: Mitutoyo micrometer to measure film thickness.

3.2.4 Microscopy

Scanning electron microscopy (SEM) images were recorded using a Zeiss Ultra Plus field emission microscope. The accelerating voltage used was 1 kV. A film sample was immersed in liquid nitrogen for a period of 5 min before fracturing with a set of pliers in the submerged state. The fractured samples were coated with carbon before being viewed in the SEM. Figure 20 shows the instrument used for SEM analysis. Figure 21 shows the film sample preparation for SEM.

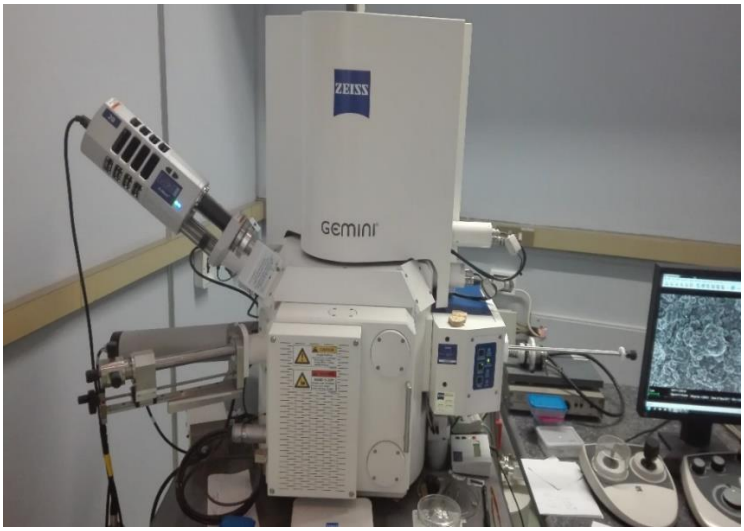

Figure 20: Zeiss Ultra Plus field SEM.

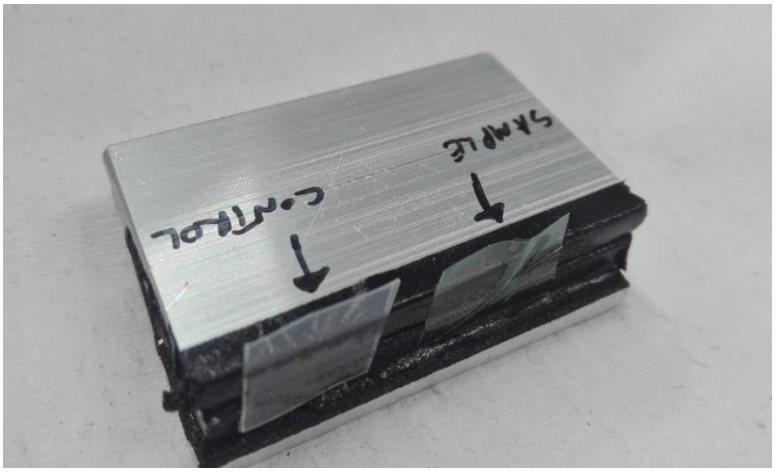

Figure 21: Film samples mounted on slabs in preparation for SEM.

3.2.5 Spectroscopy

FTIR

The organophosphate content of films was tracked as a function of time by FTIR. The spectra were recorded on a Perkin Elmer Spectrum 100 instrument at a resolution of 2 cm^{-1}. Reported spectra over the wavenumber range 4000 cm^{-1} to 550 cm^{-1} represent averages over 16 scans. Figure 22 shows the FTIR spectrometer.

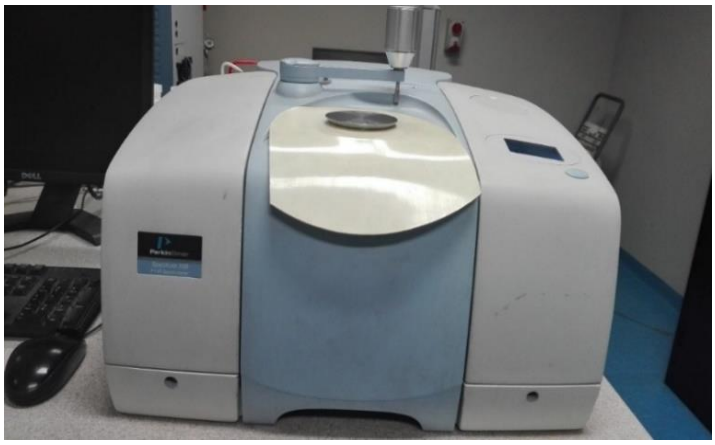

Figure 22: FTIR spectrometer.

Confocal Raman Imaging

Confocal Raman imaging was used to study the distribution of the malathion across the film thickness. The spectra were recorded with a WITec alpha300R confocal Raman microscope fitted with ×100 Zeiss objective. The excitation wavelength was 532 nm, the laser power was

30 mW and the integration time was 10 s. The maximum scan depth was 40 µm and the scan width and length was 40 µm. Figure 23 shows the Confocal Raman imaging microscope.

Figure 23: WiTec Confocal Raman imaging microscope.

3.2.6 Thermal analysis

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed on a Perkin Elmer TGA 4000 instrument. Temperature was scanned from ambient to 700°C at a scan rate of 10°C·min^{-1} with nitrogen flowing at a rate of 50 mL·min^{-1}. The thermal stability of the organophosphate liquids was investigated with thermogravimetric analysis. The total organophosphate content of the trilayer film samples was determined by oven ageing until mass loss ceased. Figure 24 shows the TGA instrument used for the analysis.

Figure 24: Perkin Elmer TGA 4000 instrument.

Differential scanning calorimetry

The melting and crystallisation characteristics of the trilayer films was studied by using differential scanning calorimetry (DSC) performed on a Perkin Elmer DSC 4000. The temperature was repeatedly scanned from 30°C to 160°C and back at a scan rate of 10°C·min^{-1} in a nitrogen gas atmosphere with the gas having a flowrate of 50 mL·min^{-1}. Figure 25 shows the instrument used for DSC.

Figure 25: Perkin Elmer differential scanning calorimeter.

Film oven aging tests

Oven ageing tests were done to track the mass loss from the trilayer films over time. The films had a length of 60 mm and a width of 40 mm. The films were suspended vertically in separate convection ovens set at three different temperatures: 30°C, 40°C and 50°C. There were three replicates for each temperature. The mass of the films was recorded daily. The setup for this experiment is seen in Figure 26.

Figure 26: Setup for film oven aging tests.

3.2.7 Bioassays

The insecticidal efficacy of the films was checked with cone bioassays. These tests were conducted at the Vector Control Unit of the National Institute for Communicable Diseases (NICD). The WHO bioassay protocol for cone tests was followed (WHO, 2013). Films measuring 12 cm × 12 cm were cut and stuck onto flat ceramic tiles. This was done to mimic the walls of dwellings that are treated by IRS. Adult female *An. arabiensis* mosquitoes were exposed for 30 minutes to the treated films as well as to an untreated film that was used as control. Each cone had 10 mosquitoes. At this point the mosquitoes were provided access to a sugar solution. Knockdown was recorded after 1 h and mortality 24 h after exposure commenced. In-between bioassays the tiles covered with the films were aged in a laboratory fume hood at ambient temperature (ca. 22°C). Bioassays were conducted monthly for seven months to track the efficacy of the insecticidal trilayer films. Figure 27 shows the samples used for the bioassay tests. Figure 28 shows the setup for the cone bioassays.

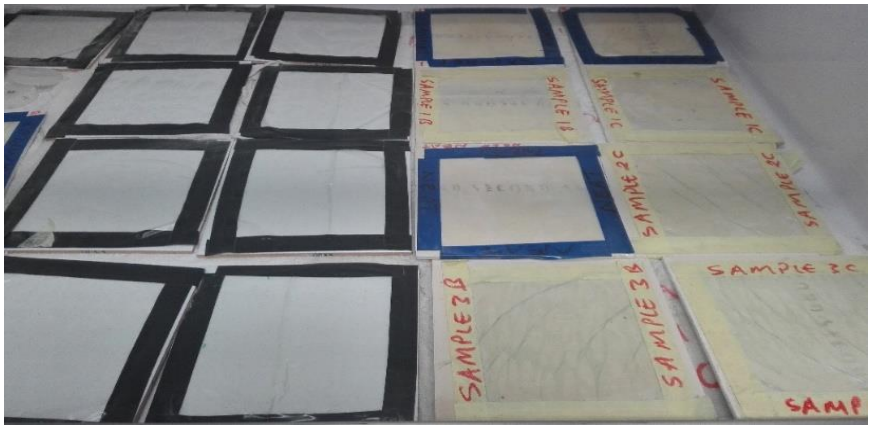

Figure 27: Malathion film samples glued to flat tiles and stored in fume hood.

Figure 28: Setup for film cone bioassay tests.

4. Results

4.1 Thermal analysis

4.1.1 TGA studies of pure organophosphates

Figure 29 shows TGA scans obtained for neat fenitrothion, pirimiphos-methyl and malathion. The temperatures at which the three insecticides reached 5 wt.% mass loss were 154, 175 and 176°C for pirimiphos-methyl, malathion and fenitrothion respectively. Considering these initial mass loss values, the relative temperature volatility of the three insecticides increases in the order fenitrothion ≈ malathion < pirimiphos-methyl. Polyethylene film blowing is usually conducted at elevated temperatures, i.e. above 180°C. It is therefore necessary need to select an organophosphate that is stable to exposure to higher temperatures. Malathion was selected because of its lower toxicity compared to the other two organophosphates. The LD_{50} for malathion is 1768 $mg \cdot kg^{-1}$ body weight (male rat) while the corresponding values for fenitrothion and pirimiphos-methyl are 1700 and 1414 $mg \cdot kg^{-1}$ body weight (male rat) respectively (WHO, 2004c, WHO, 2006b, Najera, 2001) .

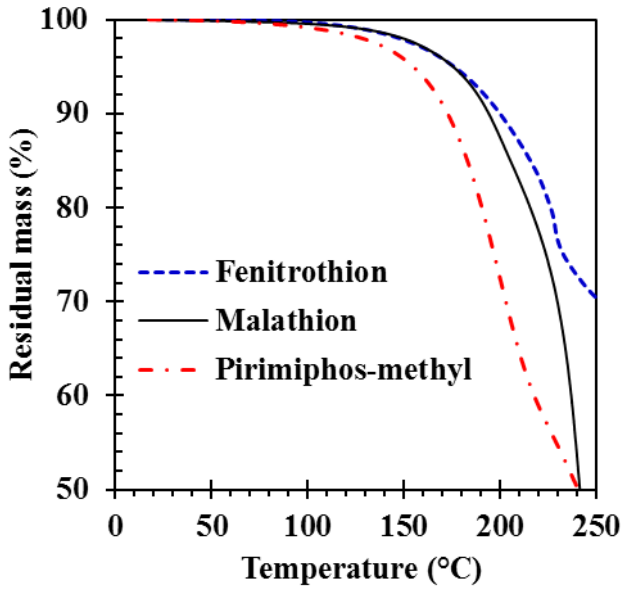

Figure 29: TGA mass loss curves for technical grade fenitrothion, pirimiphos-methyl and malathion samples. The temperature was scanned at $10°C \cdot min^{-1}$ and nitrogen flowed at 50 $mL \cdot min^{-1}$.

4.2 Active absorption studies

Figure 30 shows the progress of malathion absorption expressed as wt.% of the total mass. The equilibrium malathion absorption increases with the VA content of the poly(ethylene-co-vinyl acetate). The 20 % VA content EVA grade showed a marginally higher malathion absorption compared to the 18% VA grade. Higher VA substitution increases the fraction of the amorphous phase in the semi-crystalline polymer and tends to decrease the melt temperature. The malathion only dissolves in the amorphous regions, causing swelling of the polymer.

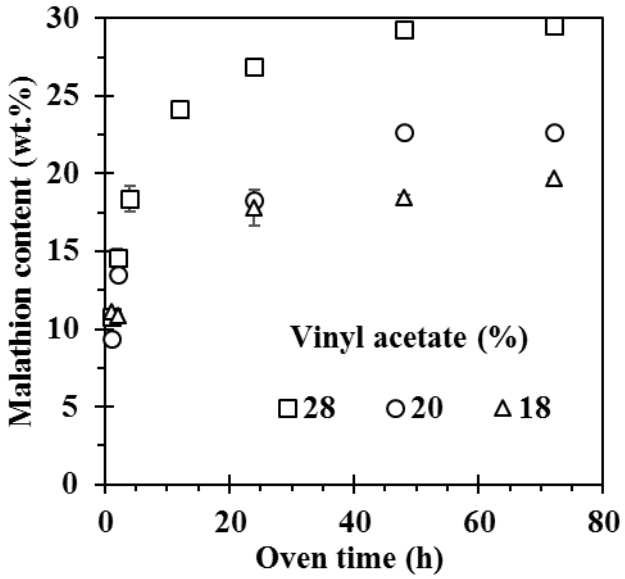

Figure 30: The effect of vinyl acetate content on the progress of the swelling of poly(ethylene-co-vinyl acetate) by malathion 58°C for EVA 28 % VA and 81 °C for the other two EVA grades.

4.3 TGA of trilayer films

The neat trilayer film had a thickness of 283 ± 2.1 μm whilst the malathion film had a thickness of 70 ± 2 μm. Figure 31 shows TGA plots for the trilayer films produced using neat EVA pellets as well as EVA pellets containing ca. 29 wt.% malathion. The Figure also shows the TGA trace for malathion liquid. The malathion-containing film commenced mass loss at a lower temperature than the neat film. This is attributed to vaporisation loss of the malathion. Oven ageing results for the insecticidal trilayer film indicated that the film contained ca. 5.8 ±

0.8 wt.% malathion. However, degradation reactions at the elevated temperatures in the TGA scan cannot be excluded because the mass loss at about 300°C exceeded this amount.

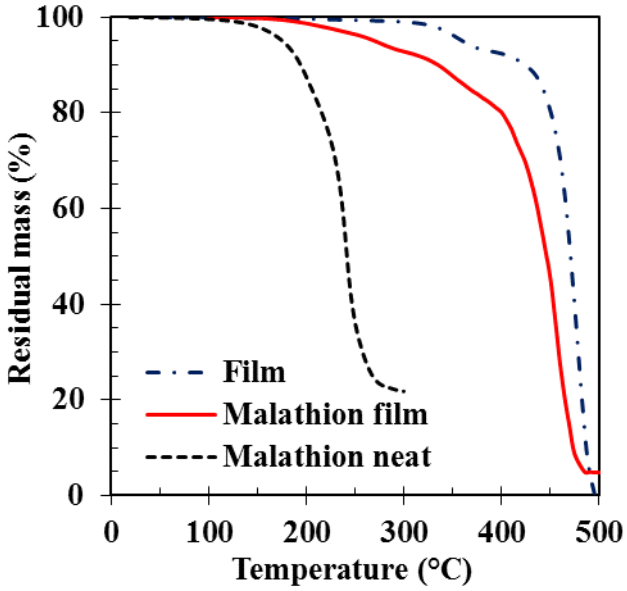

Figure 31: TGA traces of the neat films and insecticide trilayer films.

4.4 Differential scanning calorimetry

Figure 32 shows the DSC heating traces of neat EVA (28 % VA content) and EVA pellets containing malathion ca. 29 %. In both samples, the onset of melting endotherms is ca. 53°C. However, there is a decrease in the area under the melting peak of the EVA-containing malathion probably because less polymer was present. Figure 33 shows the DSC cooling curves of neat EVA (VA content 28 %) and swollen EVA pellets containing malathion ca. 29 %. In the neat EVA, the onset of crystallisation is ca. 48 °C. The onset of crystallisation for the EVA pellets with malathion is 50 °C. This means that the malathion nucleates the crystallisation of the EVA. As expected, there is also a decrease in the crystallisation peak area of the EVA containing malathion as compared to the neat EVA.

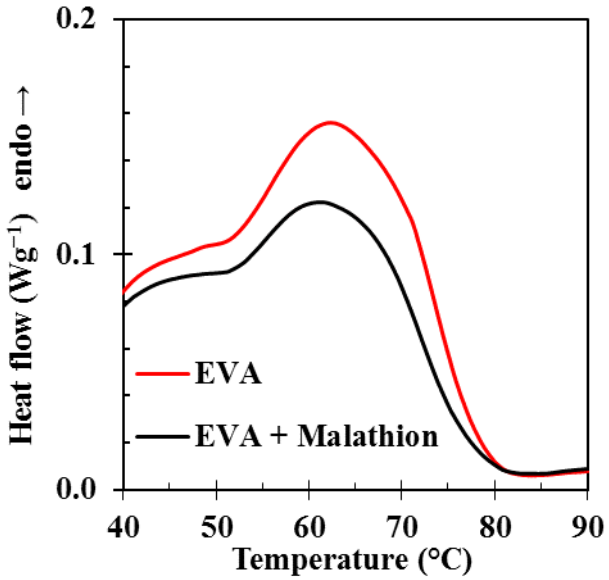

Figure 32: DSC heating curves of neat EVA (VA content 28 %) and swollen EVA pellets containing malathion ca. 29 %.

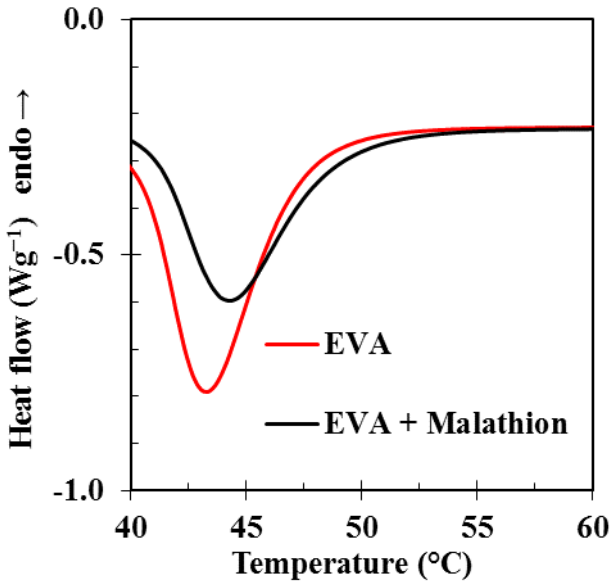

Figure 33: DSC cooling curves of neat EVA (VA content 28 %) and swollen EVA pellets containing malathion ca. 29 %.

Figure 34 and Figure 35 show the heating and cooling DSC traces for both the malathion trilayer film and the neat trilayer film respectively. In Figure 34, the onset of melting endotherms for both samples is ca. 100°C. In Figure 35, the onset of crystallisation for both samples is ca. 102°C. This means that the malathion did not alter the melting and crystallisation temperatures for the trilayer films. However, there is a decrease in the peak areas for both the

melting endotherms and crystallisation exotherm for the malathion trilayer film. This is in comparison to the neat trilayer film. The reason is that the malathion decreases the total amount of polymer present in the trilayer film and this resulted in a lower peak area.

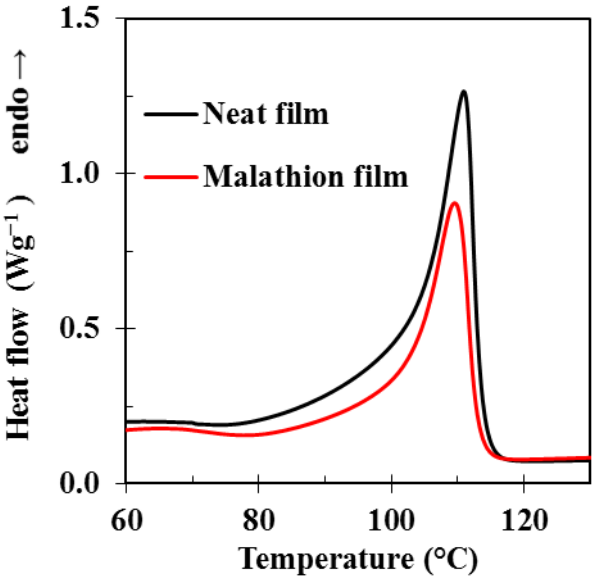

Figure 34: DSC heating curves of a neat trilayer film and a malathion-filled trilayer film.

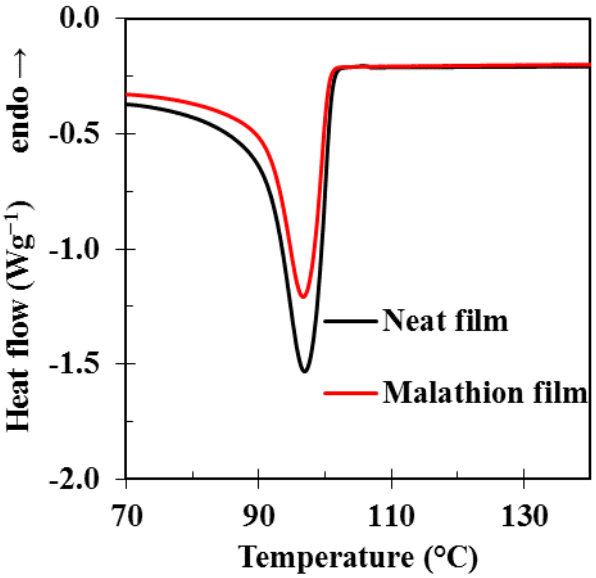

Figure 35: DSC cooling curves of neat trilayer film and a malathion-filled trilayer film.

4.5 Microscopy

Figure 36 shows the SEM micrographs of the trilayer films. The outer layers are LDPE while the middle layer is made up of neat EVA in (A) and EVA-containing insecticide in (B). The rough outer edges in both (A) and (B) are artefacts of the freeze fracturing done at liquid nitrogen temperatures. The impregnated film is thinner than the neat film because it was blown at a reduced throughput rate for safety reasons. The scale from the SEM data was used to evaluate the thicknesses of the layers in the film. In this sample, the two LDPE outer layers in the impregnated film have a thickness of approximately 57 µm. The middle layer has a thickness of approximately 29 µm. This means that the middle layer makes up 20 % of the overall film thickness. The middle layer contains approximately 29 wt.% malathion. This was determined from the absorption studies of malathion into EVA with 28 wt.% VA content. Malathion has a density of 1.272 $g \cdot cm^{-3}$ and the EVA has a density of 0.951 $g \cdot cm^{-3}$. This puts the density of the middle layer at 1.018 $g \cdot cm^{-3}$. The LDPE has a density of 0.921 $g \cdot cm^{-3}$ and this layer takes up 80 % of the overall film thickness. From these values, the middle layer makes up approximately 21.8 % of the total mass. The middle layer has 29 wt.% malathion. This means the impregnated film has a malathion content of *ca*. 6.3 %. This is slightly higher than the value obtained in the oven ageing tests. The difference is due to the partial loss of malathion during the film blowing process. A more detailed calculation is found in Appendix 2.

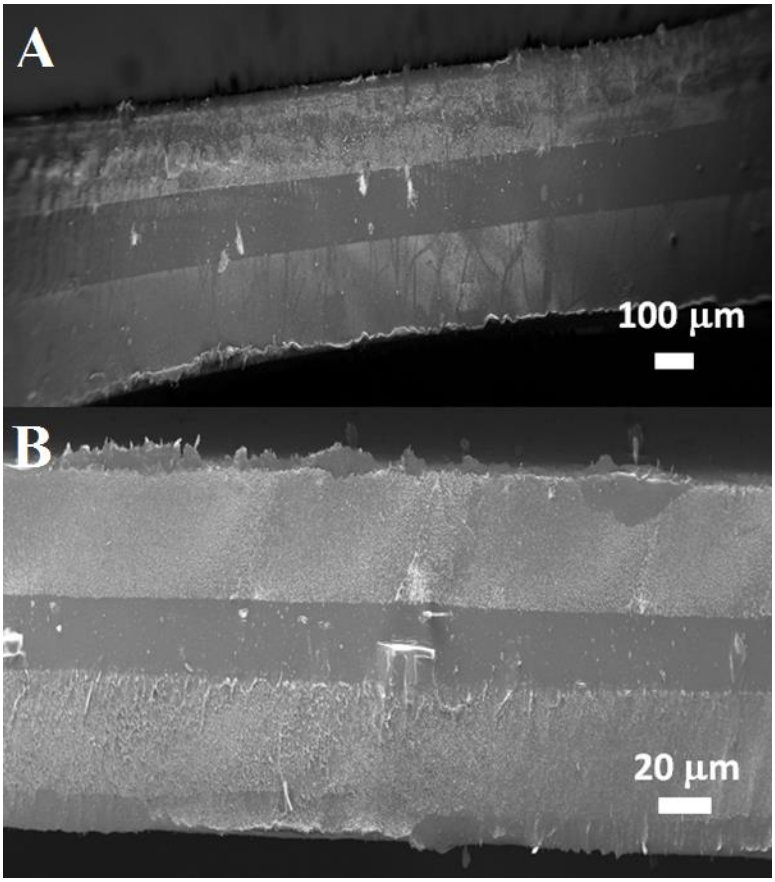

Figure 36: SEM micrographs of (A) neat trilayer film and (B) trilayer film impregnated with malathion.

4.6 Confocal Raman imaging

Figure 37 shows Raman spectra for neat malathion, the 28 % VA EVA and the neat LDPE. The intense absorption band located at *ca.* 660 cm^{-1} is a characteristic feature of the malathion spectrum corresponding to stretching vibrations of the P=S bond. It was used to quantify the distribution of the insecticide inside the trilayer film. To do this, the laser scanned an area measuring 40 × 40 μm and the average intensity of this Raman band is plotted as a function of depth and width in Figure 38. In Figure 38, the term CCD cts is a measure of the Raman intensity. The Raman depth scans shown in Figure 39 also show the variation of malathion concentration with distance from the top surface. The low concentration, with a well-defined concentration gradient, in the polyethylene layer confirms that it acted as a mass transfer barrier. The high concentration in the EVA layer confirms the swollen polymer acted as a reservoir for the malathion. The Raman scan also shows a very steep, almost stepwise, concentration drop across the interface of the two polymer layers (Figure 39b).

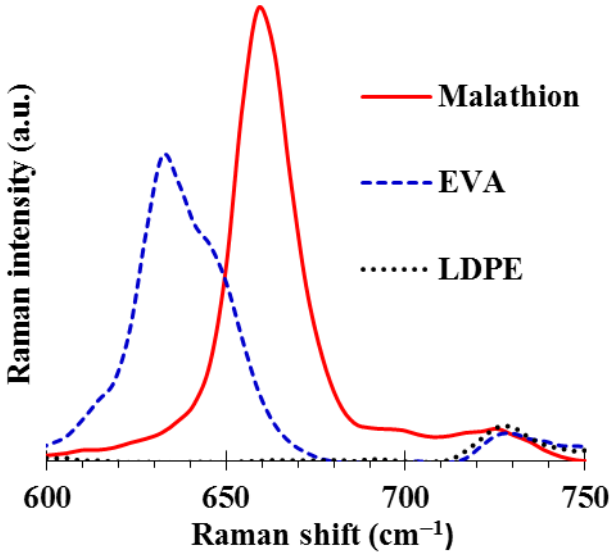

Figure 37: Raman spectra for malathion, EVA (28 % VA) and LDPE. The most intense absorption band at *ca.* 660 cm^{-1} is characteristic for the P=S bond in malathion.

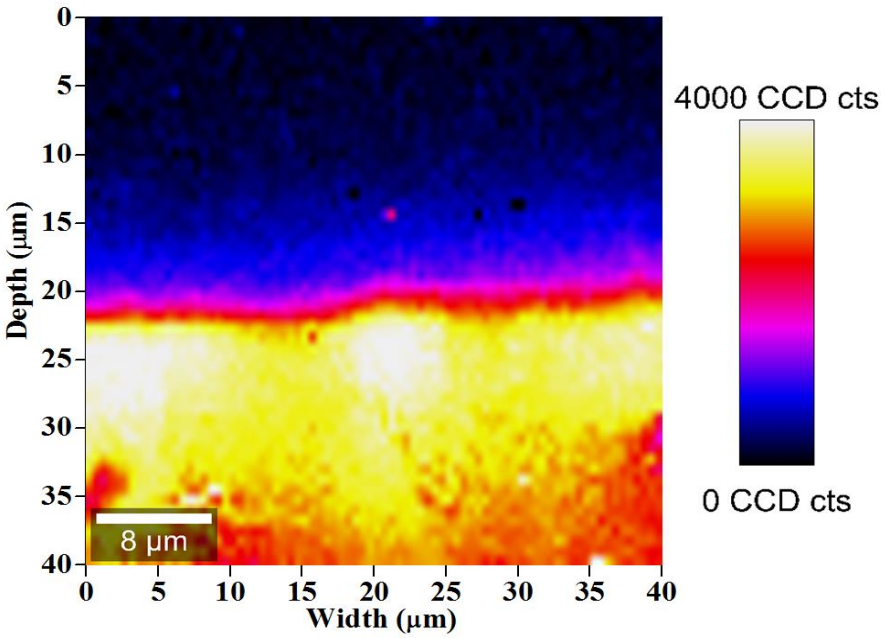

Figure 38: The distribution of malathion inside a trilayer polymer film estimated from the intensity of the 660 cm^{-1} band. The sample used had a thickness of 83 ± 3.7 µm. In this particular sample, the polyethylene layer was about 22 µm thick and the inner malathion-swollen EVA layer is 39 µm thick.

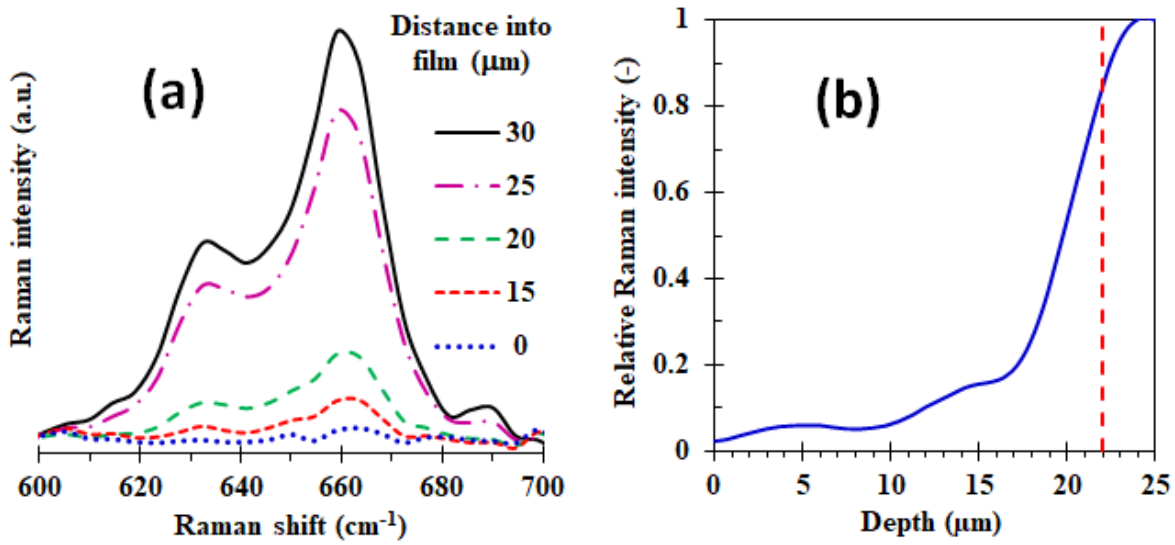

Figure 39: Variation of the Raman spectra with distance from the surface. (a) The Raman spectra recorded at different penetration depths. The bands at *ca.* 660 cm^{-1} and 632 cm^{-1} are attributed to the P=S in malathion and the carbonyl stretch deformation in EVA respectively. (b) The intensity of the 660 cm^{-1} Raman band as a proxy for the concentration of malathion inside the trilayer film. The red dotted line indicates the boundary between the LDPE and the EVA layers.

4.7 Film oven ageing tests

4.7.1 Mass loss

Figure 40 shows a plot of the residual mass of the insecticidal trilayer film at 30°C, 40°C and 50°C. The samples used had a thickness of 72.5 ± 7.42 µm. As expected, mass loss proceeds faster as the temperature is increased. This is expected because a higher temperature is supposed to result in an accelerated mass loss rate. The films aged in the oven lost mass much faster than the films that were glued onto the flat tiles. This happened because the temperatures were higher and because the mass loss occurred through both sides in the former case.

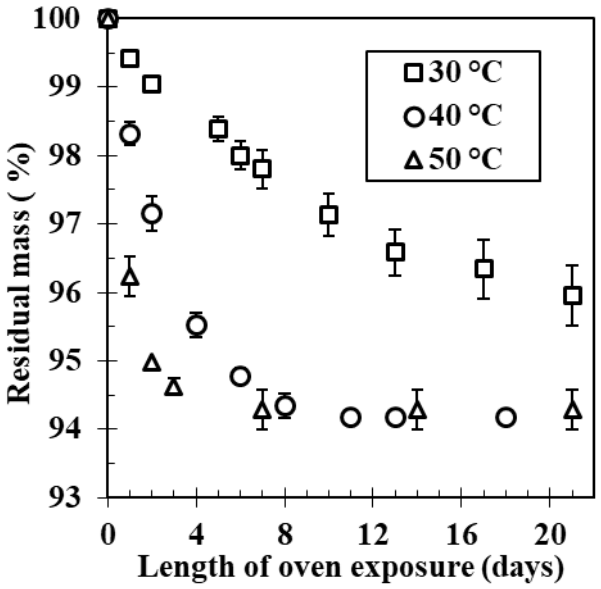

Figure 40: Plot of the residual mass of the trilayer films at different temperatures. The tests at 30°C had not reached equilibrium. These films were exposed to ambient air on both sides when they were in the oven. The films were hanging vertically in the oven.

The mass loss followed first order kinetics and hence the following model was used for fitting the data:

$$(m_t - m_\infty)/(m_o - m_\infty) = e^{-t/\tau} \qquad (1)$$

Where m_t is the mass at time t, m_o is the initial mass, m_∞ is the mass at an infinite time. The time constant, τ, is a measure of the rate at which the film losses mass. Figure 41 shows the normalised residual mass of the malathion films as a function of time and temperature. The time constant values were extracted using relative least squares regression. The τ values were found to be 9.17 days, 1.98 days and 1.01 days at 30°C, 40°C and 50°C respectively. As expected the τ value at 50°C was the smallest because the diffusion rate of the malathion trapped in the film is fastest at this temperature. The time constant at 30°C increases considerably.

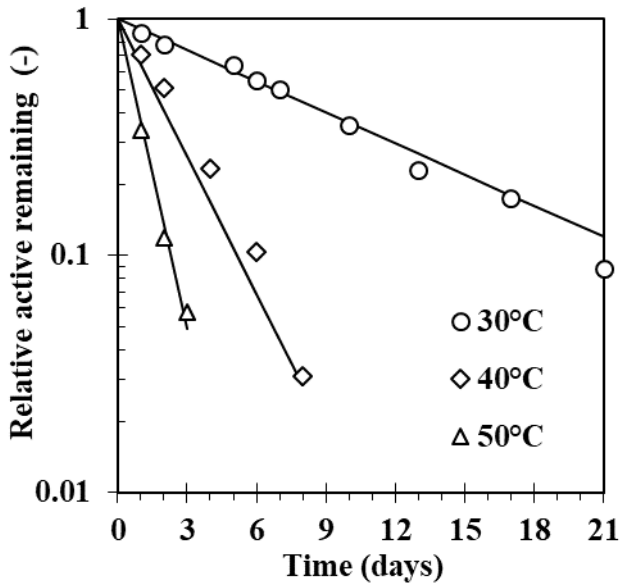

Figure 41: Effect of temperature on the normalised residual mass for the insecticidal films at different temperatures.

4.7.2 Infrared Spectroscopy

Figure 42(A) shows the FTIR spectra for a neat trilayer film, malathion insecticide and the insecticidal trilayer film. Two bands, uniquely associated with malathion, are located near 660 cm^{-1} and the two peaks near 830 cm^{-1} respectively. The band at 660 cm^{-1} is assigned to stretching vibration of the P=S bond. The doublet at 838 cm^{-1} and 822 cm^{-1} is associated with P−S bond stretching modes. The latter two bands together were broader and they were therefore selected for further analysis of the insecticidal films.

Figure 42(B) shows absorbance spectra recorded for the insecticidal films that was aged at 30°C in a convection oven. The malathion band diminishes in intensity over time as the insecticide is lost by evaporation from the film. Similar changes were observed for the films aged at the two higher ageing temperatures. The evaporation happened faster at the higher temperatures and therefore the malathion spectra recorded for the film aged at 50°C disappeared fastest. The areas under the peaks (780 – 860 cm^{-1}), obtained at 30°C, 40°C and 50°C, were assumed to be directly proportional to the amount of malathion present in the insecticidal film. This allowed estimation of the change in malathion content over time and the results are plotted in Figure 9.

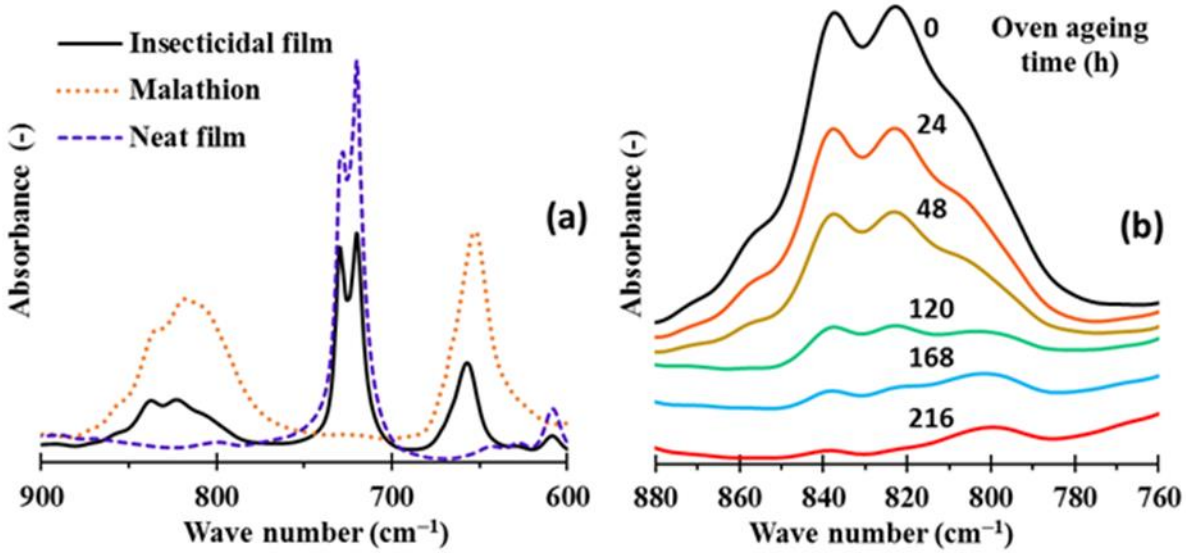

Figure 42: (a) FTIR absorbance spectra for malathion insecticide, a neat trilayer polymer film and an insecticidal film. **(b)** Time evolution of FTIR spectra for insecticidal film oven-aged at 30°C.

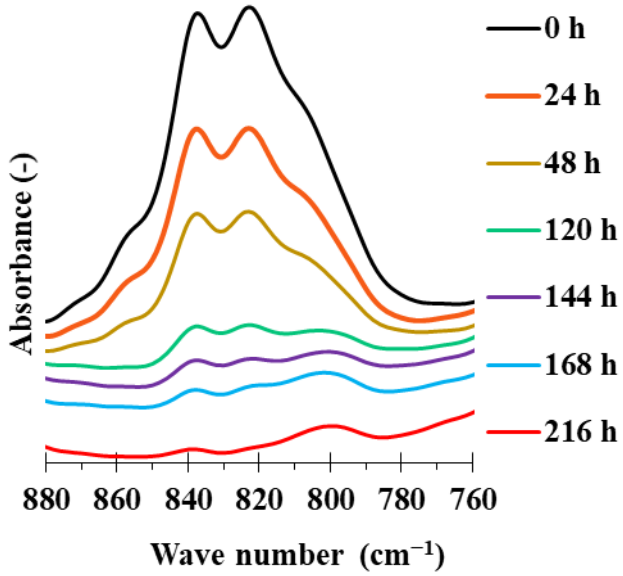

Figure 43: Time evolution of FTIR spectra for insecticidal film oven-aged at 40°C.

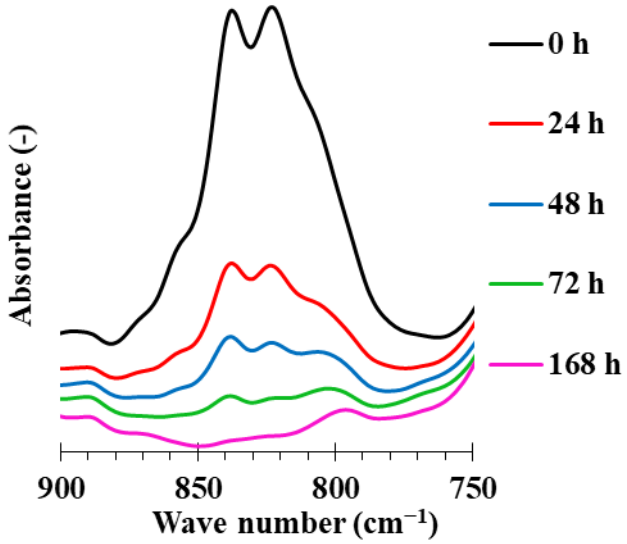

Figure 44: Time evolution of FTIR spectra for insecticidal film oven-aged at 50°C.

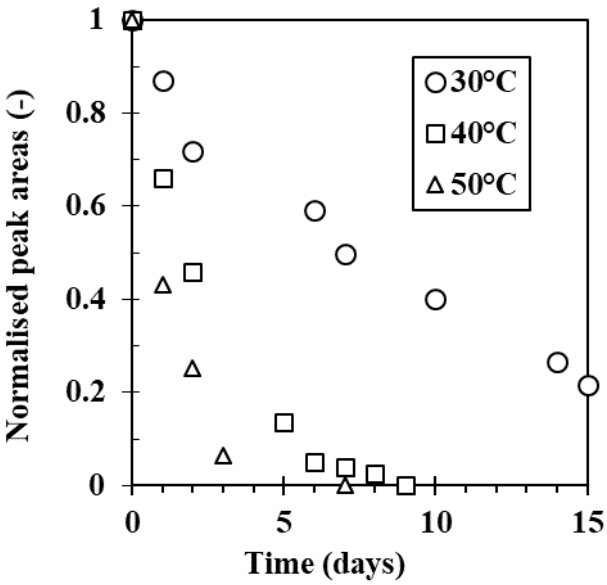

Figure 45: Normalised peak areas obtained from the FTIR absorbance spectra of the films.

The peak areas for the absorbance spectra at *ca.* 830 cm^{-1} for the spectra obtained at 30°C, 40°C and 50°C were evaluated using OriginPro® software. Figure 45 shows the plot of the normalised peak areas. The peak areas were then used to extract the kinetics of the diffusion of malathion from the trilayer film. The model described in Equation 1 was used but it was modified as a_t is the peak area at time t, a_0 is the intial peak area, a_∞ is the peak at an infinite time and τ is a time constant. The model is seen in Equation 2. Figure 46 shows the normalised residual active of the malathion films as a function of time and temperature. The data followed

first order kinetics. The τ values were 10 days, 2.2 days and 1.19 days at 30 °C, 40 °C and 50 °C.

$$(a_t - a_\infty)/(a_o - a_\infty) = e^{-t/\tau} \quad (2)$$

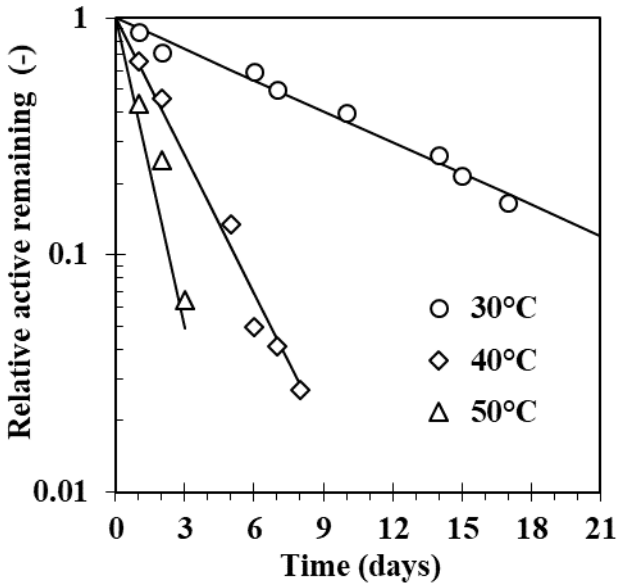

Figure 46: Release kinetics based on FTIR absorbance spectra for the insecticidal film at 30, 40 and 50°C.

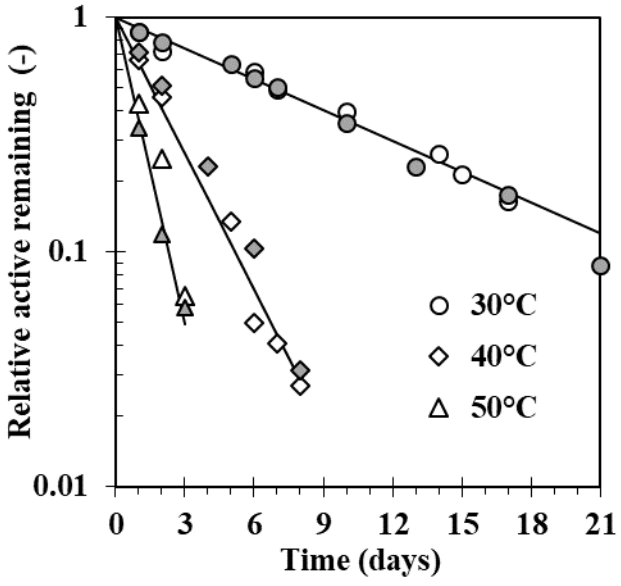

Figure 47: Plot of the residual malathion content of trilayer films oven-aged at different temperatures. The films were exposed to ambient air on both sides. The filled and open symbols correspond to results obtained from mass loss and FTIR data respectively.

The data from the kinetics obtained from the FTIR data and the oven aging mass loss results were combined as shown in Figure 47. The time constant values were estimated from relative least squares data reduction using Figure 47 and were found to be 9.93 days, 2.24 days and 1.00 days at 30°C, 40°C and 50°C respectively. If an Arrhenius-like temperature dependence is assumed for the time constant (τ), this corresponds to the Arrhenius plot shown in Figure 48. The slope of the graph it $\frac{E_a}{R}$, with R being the universal gas constant. This corresponds to an activation energy, E_a, of approximately 94 kJ.mol^{-1}. Appendix 3 shows the detailed calculations for the activation energy.

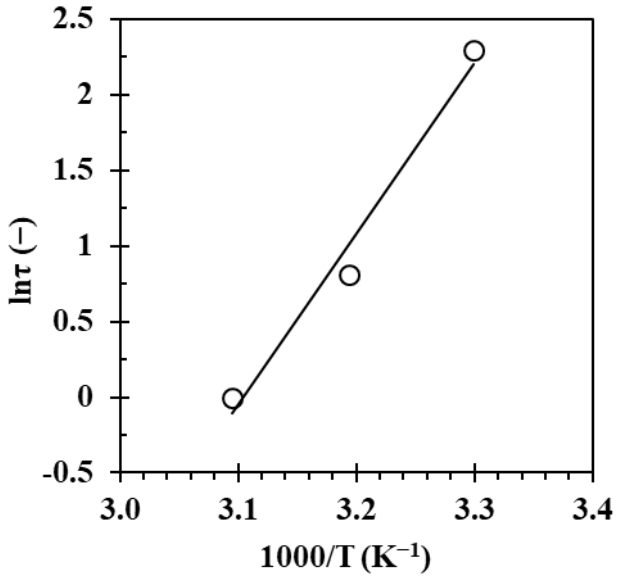

Figure 48: Arrhenius plot for the mass loss rate.

4.8 Bioassay results

Testing mosquito repellence activity of the film samples began a month after they were made. The films were aged in a fume hood for the duration of the tests. A neat trilayer film was used as the control. The insecticidal films had a thickness of 70 ± 2.58 µm. Figure 49 shows the results of the bioassays conducted over a period of seven months after preparation of the samples. The WHO criterion for insecticidal nets is mortality of 80 % after 24 h (WHO, 2013). The insecticidal films produced satisfactory efficacy results according to this criterion for the first 4 months of testing. Figure 48 shows that the malathion film failed the WHO mortality criterion after seven months.

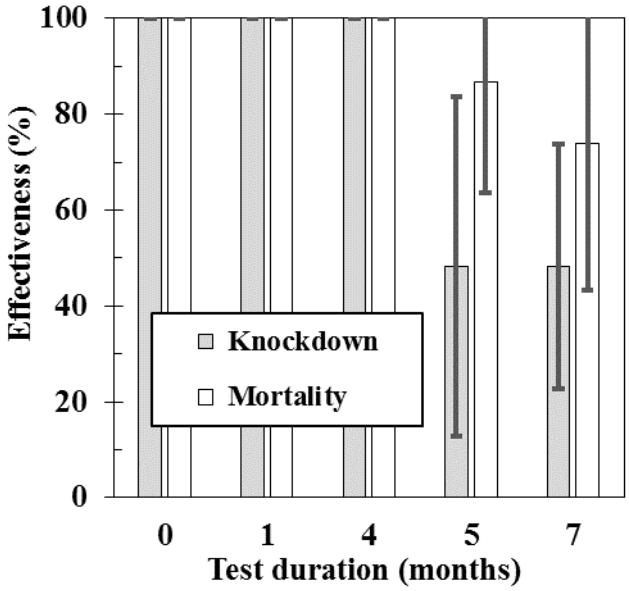

Figure 49: Efficacy results for the malathion film over time. The error bars show one standard error above and below the mean values.

5. Discussion

Malathion has a higher solubility in the hot molten polymer than in the film at ambient temperature. This is because solubility increases with an increase in temperature. When the film is cooled at the end of the film blowing process, the amorphous phase that is available to dissolve the malathion diminishes as crystallization of the polymer proceeds. If too high a malathion concentration was initially fed into the extruder, this would lead to a supersaturated state inside the EVA polymer on cooling back to ambient (Focke Walter and van Pareen, 2011). For the malathion to return to the equilibrium state, it would diffuse to the surface of the EVA film layer and this could lead to delamination of the trilayer structure. For this reason, the EVA pellets were fed saturated with the insecticide at more or less ambient conditions.

Malathion is a contact insecticide. This means that it must be available on the surface of the film in adequate amounts to kill the mosquitoes. It is also volatile and this means that the insecticide must be replenished constantly by the malathion diffusing to the surface of the polyethylene layers. The malathion trapped in the EVA core must diffuse from the core to the surface of the LDPE sheath. This means there must be a concentration gradient of malathion from the middle of the EVA core to the LDPE sheath. Raman depth profiling results showed the presence of the malathion concentration gradient across the film. This observation clearly shows a build-up of a concentration gradient. The FTIR results showed that the malathion absorption band disappeared over time. These results indicate that there is diffusion-controlled transport of the malathion to the LDPE membrane surfaces.

FTIR results confirmed that there was slow release of the malathion from the trilayer film. However, surprisingly the release rate followed first order kinetics rather than the zero-order kinetics that was desired. This suggests that the diffusion of the malathion out of the EVA layer was also diffusion controlled and actually rate limiting. This effectively negated the diffusion barrier posed by the polyethylene layers. The malathion shallow concentration gradient inside the EVA phase, and a sharper gradient in the LDPE layer, seen in Figure 39(B) provides at least some support for this hypothesis. Another possible complication is posed by the nature of the interfacial layer. Here the polyethylene and EVA are in an interpenetrated state and this could pose another non-linear diffusion barrier effect.

During the manufacturing of the trilayer films, some gassing was experienced. The gases were probably caused by either the presence of some moisture or the vaporisation of the malathion

at the high processing temperatures employed. Bubbles formed on one side of the film tube as seen in Figure 50. To curb this problem, the throughput of the layer containing malathion was reduced. This meant that the produced trilayer film contained less malathion than was originally envisaged. This stabilised the film blowing process and allowed insecticidal film samples to be collected. Further work can be done to determine better processing conditions that would allow for a stable process and for high quantities of malathion to be incorporated in the trilayer films. This should prolong the residual efficacy of the insecticidal film.

When the malathion is used in IRS, the insecticide is effective for two to three months (Najera, 2001). Trapping the malathion in the trilayer film increased the effectiveness of the insecticide to about six months. This result shows that the use of trilayer films is a promising way to increase the residual efficacy of organophosphate insecticides. More work should be done to prolong the duration and effectiveness of the malathion film against mosquitoes.

Figure 50: Trilayer film produced with bubbles due to the evaporation of malathion.

There are concerns about the toxicity of malathion to humans. However, the insecticide has a low toxicity and a very good safety record. In addition, malathion is approved for use in IRS and it is a requirement for the insecticide to pose a low risk for the spray workers and the inhabitants of the houses where the insecticide is applied (Najera, 2001). A disadvantage of malathion is that it has a strong and unpleasant odour and therefore people may object to the

insecticide being used. The production of these trilayer films is sustainable as the polymers used are relatively inexpensive and readily available.

6. Conclusions and recommendations

Malathion, a WHO-approved insecticide for IRS, was successfully incorporated into a polymer film matrix. Trilayer films containing malathion were produced by a conventional film blowing process. SEM results confirmed a trilayer film structure. Bioassay results showed that the residual effectiveness of the malathion was increased to six months from the usual two to three months stated in literature (Najera, 2001). Confocal Raman revealed a concentration gradient of the malathion across the polyethylene film layers. FTIR results confirmed diffusion-controlled slow-release of the malathion from the trilayer film. However, and rather surprisingly the release rate followed first order kinetics instead of the anticipated zero order kinetics that was desired. This means that the diffusion of the malathion out of the EVA layer was probably the diffusion limiting step which effectively negated the diffusion barrier posed by the polyethylene layers.

The production of the films is cost effective as this involves a simple trilayer film blowing process. These insecticidal films have the potential to be an alternative malaria vector control intervention in pyrethroid resistant settings. Further work needs to be done on prolonging the residual efficacy of the insecticidal films. Furthermore, there is a requirement for optimised processing conditions and superior ventilation considering the fumes emitted during the production of the trilayer films. This could allow for more insecticide to be incorporated in the trilayer film during the film blowing process. Further studies should also consider incorporation of fenitrothion and pirimiphos-methyl.

7. References

ABDALLA, H., WILDING, C. S., NARDINI, L., PIGNATELLI, P., KOEKEMOER, L. L., RANSON, H. & COETZEE, M. 2014. Insecticide resistance in Anopheles arabiensis in Sudan: Temporal trends and underlying mechanisms. *Parasites and Vectors,* 7.

AHOUA ALOU, L. P., KOFFI, A. A., ADJA, M. A., ASSI, S. B., KOUASSI, P. K. & N'GUESSAN, R. 2012. Status of pyrethroid resistance in Anopheles gambiae s. s. M form prior to the scaling up of Long Lasting Insecticidal Nets (LLINs) in Adzopé, Eastern Côte d'Ivoire. *Parasites and Vectors,* 5.

ANECK-HAHN, N. H., SCHULENBURG, G. W., BORNMAN, M. S., FARIAS, P. & DE JAGER, C. 2007. Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. *J Androl,* 28**,** 423-34.

ANTWI, F. B., SHAMA, L. M. & PETERSON, R. K. D. 2008. Risk assessments for the insect repellents DEET and picaridin. *Regulatory Toxicology and Pharmacology,* 51**,** 31-36.

BLANFORD, S., CHAN, B. H. K., JENKINS, N., SIM, D., TURNER, R. J., READ, A. F. & THOMAS, M. B. 2005. Fungal pathogen reduces potential for malaria transmission. *Science,* 308**,** 1638-1641.

BOCKARIE, M. J., ALEXANDER, N., BOCKARIE, F., IBAM, E., BARNISH, G. & ALPERS, M. 1996. The late biting habit of parous Anopheles mosquitoes and pre-bedtime exposure of humans to infective female mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 90**,** 23-25.

BOUMAIL, A., SALMIERI, S., KLIMAS, E., TAWEMA, P. O., BOUCHARD, J. & LACROIX, M. 2013. Characterization of Trilayer Antimicrobial Diffusion Films (ADFs) Based on Methylcellulose–Polycaprolactone Composites. *Journal of Agricultural and Food Chemistry,* 61**,** 811-821.

BRAACK, L., HUNT, R., KOEKEMOER, L. L., GERICKE, A., MUNHENGA, G., HADDOW, A. D., BECKER, P., OKIA, M., KIMERA, I. & COETZEE, M. 2015. Biting behaviour of African malaria vectors:1. Where do the main vector species bite on the human body? *Parasites and Vectors,* 8.

BROWN, M. & HEBERT, A. A. 1997. Insect repellents: An overview. *Journal of the American Academy of Dermatology,* 36**,** 243-249.

CHIMA, R. I., GOODMAN, C. A. & MILLS, A. 2003. The economic impact of malaria in Africa: A critical review of the evidence. *Health Policy,* 63**,** 17-36.

CHOWDHURY, N., GHOSH, A. & CHANDRA, G. 2008. Mosquito larvicidal activities of Solanum villosum berry extract against the dengue vector Stegomyia aegypti. *BMC Complementary and Alternative Medicine,* 8.

CILEK, J. E., PETERSEN, J. L. & HALLMON, C. E. 2004. Comparative efficacy of IR3535 and deet as repellents against adult Aedes aegypti and Culex quinquefasciatus. *J Am Mosq Control Assoc,* 20**,** 299-304.

COLEMAN, M., CASIMIRO, S., HEMINGWAY, J. & SHARP, B. 2008. Operational impact of DDT reintroduction for malaria control on Anopheles arabiensis in Mozambique. *Journal of Medical Entomology,* 45**,** 885-890.

DIAZ, J. H. 2016. Chemical and plant-based insect repellents: Efficacy, safety, and toxicity. *Wilderness and Environmental Medicine,* 27**,** 153-163.

DYCK, V. A., HENDRICHS, J. & ROBINSON, A. S. 2005. *Sterile insect technique: Principles and practice in area-wide integrated pest management*.

FANG, W., VEGA-RODRÍGUEZ, J., GHOSH, A. K., JACOBS-LORENA, M., KANG, A. & ST. LEGER, R. J. 2011. Development of transgenic fungi that kill human malaria parasites in mosquitoes. *Science,* 331**,** 1074-1077.

FAO 2007. FENITROTHION O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate. *FAO Specifications and Evaluations for Agricultural Pesticides.* Rome.

FAULDE, M. K., ALBIEZ, G. & NEHRING, O. 2010. Insecticidal, acaricidal and repellent effects of DEET-and IR3535-impregnated bed nets using a novel long-lasting polymer-coating technique. *Parasitology Research,* 106**,** 957-965.

FOCKE WALTER, W. & VAN PAREEN, W. 2011. Polypropylene-based long-life insecticide-treated mosquito netting. *Journal of Polymer Engineering.*

GALLUP, J. L. & SACHS, J. D. 2001. The economic burden of malaria. *American Journal of Tropical Medicine and Hygiene,* 64**,** 85-96.

GUPTA, R. C. 2006. CHAPTER 2 - Classification and Uses of Organophosphates and Carbamates. *Toxicology of Organophosphate & Carbamate Compounds.* Burlington: Academic Press.

KAMAREDDINE, L. 2012. The Biological Control of the Malaria Vector. *Toxins,* 4**,** 748-767.

KATZ, T. M., MILLER, J. H. & HEBERT, A. A. 2008. Insect repellents: Historical perspectives and new developments. *Journal of the American Academy of Dermatology,* 58**,** 865-871.

KEISER, J., SINGER, B. H. & UTZINGER, J. 2005. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: A systematic review. *Lancet Infectious Diseases,* 5**,** 695-708.

KIKANKIE, C. K., BROOKE, B. D., KNOLS, B. G., KOEKEMOER, L. L., FARENHORST, M., HUNT, R. H., THOMAS, M. B. & COETZEE, M. 2010. The infectivity of the entomopathogenic fungus Beauveria bassiana to insecticide-resistant and susceptible Anopheles arabiensis mosquitoes at two different temperatures. *Malaria Journal,* 9.

KIMANI, E. W., VULULE, J. M., KURIA, I. W. & MUGISHA, F. 2006. Use of insecticide-treated clothes for personal protection against malaria: A community trial. *Malaria Journal,* 5.

KRUGER, T., SIBANDA, M. M., FOCKE, W. W., BORNMAN, M. S. & DE JAGER, C. 2015. Acceptability and effectiveness of a monofilament, polyethylene insecticide-treated wall lining for malaria control after six months in dwellings in Vhembe District, Limpopo Province, South Africa. *Malaria Journal,* 14**,** 485.

LANGHE, D. & PONTING, M. 2016. 1 - Introduction to Multilayered Films. *Manufacturing and Novel Applications of Multilayer Polymer Films.* Boston: William Andrew Publishing.

LAWRANCE, C. E. & CROFT, A. M. 2004. Do mosquito coils prevent malaria? A systematic review of trials. *J Travel Med,* 11**,** 92-6.

LINDSAY, S. W., EMERSON, P. M. & CHARLWOOD, J. D. 2002. Reducing malaria by mosquito-proofing houses. *Trends in Parasitology,* 18**,** 510-514.

LOGAN, J. G., STANCZYK, N. M., HASSANALI, A., KEMEI, J., SANTANA, A. E. G., RIBEIRO, K. A. L., PICKETT, J. A. & MORDUE, A. J. 2010. Arm-in-cage testing of natural human-derived mosquito repellents. *Malaria Journal,* 9.

LUND, O., NIELSEN, M, LUNDEGAARD, C, KE¸SMIR, C AND BRUNAK, S 2005. *Immunological Bioinformatics,* Cambridge, Massachusetts, The MIT Press.

MAIA, M. F. & MOORE, S. J. 2011. Plant-based insect repellents: A review of their efficacy, development and testing. *Malaria Journal,* 10.

MATSUO, N. A. M., T 2012. *Pyrethroids: From Chrysanthemum to Modern Industrial Insecticide,* Berlin Heidelberg, Springer

MESSENGER, L. A., MATIAS, A., MANANA, A. N., STILES-OCRAN, J. B., KNOWLES, S., BOAKYE, D. A., COULIBALY, M. B., LARSEN, M. L., TRAORÉ, A. S., DIALLO, B., KONATÉ, M., GUINDO, A., TRAORÉ, S. F., MULDER, C. E. G., LE, H., KLEINSCHMIDT, I. & ROWLAND, M. 2012a. Multicentre studies of insecticide-

treated durable wall lining in Africa and South-East Asia: Entomological efficacy and household acceptability during one year of field use. *Malaria Journal,* 11.

MESSENGER, L. A., MILLER, N. P., ADEOGUN, A. O., AWOLOLA, T. S. & ROWLAND, M. 2012b. The development of insecticide-treated durable wall lining for malaria control: Insights from rural and urban populations in Angola and Nigeria. *Malaria Journal,* 11.

MEYERS, J. I., PATHIKONDA, S., POPKIN-HALL, Z. R., MEDEIROS, M. C., FUSEINI, G., MATIAS, A., GARCIA, G., OVERGAARD, H. J., KULKARNI, V., REDDY, V. P., SCHWABE, C., LINES, J., KLEINSCHMIDT, I. & SLOTMAN, M. A. 2016. Increasing outdoor host-seeking in Anopheles gambiae over 6 years of vector control on Bioko Island. *Malar J,* 15**,** 239.

MOIROUX, N., GOMEZ, M. B., PENNETIER, C., ELANGA, E., DJÈNONTIN, A., CHANDRE, F., DJÈGBÉ, I., GUIS, H. & CORBEL, V. 2012. Changes in anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in benin. *Journal of Infectious Diseases,* 206**,** 1622-1629.

MULLER, G. C., BEIER, J. C., TRAORE, S. F., TOURE, M. B., TRAORE, M. M., BAH, S., DOUMBIA, S. & SCHLEIN, Y. 2010. Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria vectors in the Anopheles gambiae complex in Mali, West Africa. *Malar J,* 9**,** 210.

MUNHENGA, G., MASENDU, H. T., BROOKE, B. D., HUNT, R. H. & KOEKEMOER, L. K. 2008. Pyrethroid resistance in the major malaria vector Anopheles arabiensis from Gwave, a malaria-endemic area in Zimbabwe. *Malaria Journal,* 7.

NAJERA, J. A. Z., M 2001. *Malaria vector control : insecticides for indoor residual spraying* Geneva : World Health Organization.

NILSSON, S. K., CHILDS, L. M., BUCKEE, C. & MARTI, M. 2015. Targeting Human Transmission Biology for Malaria Elimination. *PLoS Pathogens,* 11.

PATES, H. & CURTIS, C. 2005. Mosquito behavior and vector control. *Annual Review of Entomology.*

PAVELA, R. 2015. Essential oils for the development of eco-friendly mosquito larvicides: A review. *Industrial Crops and Products,* 76**,** 174-187.

RANSON, H., N'GUESSAN, R., LINES, J., MOIROUX, N., NKUNI, Z. & CORBEL, V. 2011. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in Parasitology,* 27**,** 91-98.

ROZENDAAL, J. A. 1997. *Vector control: Methods for use by individuals and communities,* Geneva, World Health Organisation.

RUSSELL, T. L., GOVELLA, N. J., AZIZI, S., DRAKELEY, C. J., KACHUR, S. P. & KILLEEN, G. F. 2011. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malaria Journal,* 10.

SACHS, J. & MALANEY, P. 2002. The economic and social burden of malaria. *Nature,* 415**,** 680-685.

SONNTAG, P., HOERNER, P., CHEYMOL, A., ARGY, G., RIESS, G. & REITER, G. 2004. *Biocide squirting from an elastomeric tri-layer film.*

SUDAKIN, D. L. & OSIMITZ, T. 2010. Chapter 98 - DEET A2 - Krieger, Robert. *Hayes' Handbook of Pesticide Toxicology (Third Edition).* New York: Academic Press.

WHO 2004a. CYFLUTHRIN (RS)-α-cyano-4-fluoro-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2004b. ICARIDIN 1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-

methylpropylester *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2004c. MALATHION S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl

phosphorodithioate. *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2005. PROPOXUR 2-isopropoxyphenyl methylcarbamate *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2006a. IR3535® 3-(N-acetyl-N-butyl)aminopropionic acid ethyl ester *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2006b. PIRIMIPHOS-METHYL O-2-diethylamino-6-methylpyrimidin-4-yl-O,Odimethyl

phosphorothioate. *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2007a. DELTAMETHRIN (S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylate *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2007b. ETOFENPROX 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether. *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2007c. Implementation of Indoor Residual Spraying of Insecticides for Malaria Control in the WHO African Region

WHO 2007d. LAMBDA-CYHALOTHRIN A reaction product comprising equal quantities of (S)-α-cyano-3-phenoxybenzyl (Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2- dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2009a. ALPHA-CYPERMETHRIN A racemic mixture of: (S)-α-cyano-3-phenoxybenzyl-(1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (R)-α-cyano-3-phenoxybenzyl-(1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2009b. DDT 1,1,1-trichloro-2,2-bis(chlorophenyl)ethane. *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2009c. Bendiocarb 2,2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate *WHO Specifications and Evaluations for Public Health Pesticides.* Geneva.

WHO 2013. Guidelines for laboratory and field-testing of long-lasting insecticidal nets. Geneva.

WHO 2016. World Malaria Report 2016. Geneva.

8. Appendices

8.1 Appendix 1: Raw bioassay results

Table A-1: Raw bioassay results

	30 Minutes kd	**60 Minutes kd**	**24 hr alive**	**24 hr dead**	**Number of mosquitoes**	**% knockdown**	**% mortality**
Initial Tests	19	20	0	20	20	100	100
	20	20	0	20	20	100	100
	10	10	0	10	10	100	100
After 1 month	19	20	0	20	20	100	100
	20	20	0	20	20	100	100
	10	10	0	10	10	100	100
After 4 months	--	10	0	10	10	100	100
	--	10	0	10	10	100	100
	--	9	0	9	9	100	100
After 5 months	--	6	11	0	11	54.5	100
	--	1	6	4	10	10	60
	--	8	10	0	10	80	100
After 7 months	0	7	0	10	10	70	100
	0	2	6	4	10	20	40
	0	6	2	9	11	55	81.8

Table A-2: Average knockdown and mortality for malathion film

months	Average kd (%)	Standard deviation	Average mortality (%)	Standard deviation
0	100	0	100	0
1	100	0	100	0
4	100	0	100	0
5	48	35	87	23
7	48	26	74	31

8.2 Appendix 2: Malathion trilayer film density calculations

Density of LDPE, ρ_{LDPE} , is 0.921 g·cm^{-3}, Density of EVA, ρ_{EVA} , is 0.951 g·cm^{-3} and Density of malathion, ρ_{mal} , is 1.272 g·cm^{-3}.

Density of middle layer: v_{ML} is the volume of the middle layer, v_{EVA} is the volume of the EVA and v_{mal} is the volume of the malathion.

$$\rho_{ML} v_{ML} = \rho_{EVA} v_{EVA} + \rho_{mal} v_{mal} \qquad (3)$$

Using a mass basis of 1g for the middle layer: m_{ML} is the mass of the middle layer and it is 1 g, Mass of EVA, m_{EVA} , is 0.71 g and mass of malathion, m_{mal} , is 0.29 g.

$$v_{ML} = \frac{m_{EVA}}{\rho_{EVA}} + \frac{m_{mal}}{\rho_{mal}} = 0.982 \text{ cm}^3 \qquad (4)$$

$$\rho_{ML} = \frac{m_{ML}}{v_{ML}} = 1.018 \text{ g·cm}^{-3} \qquad (5)$$

Layer thickness: Middle layer thickness, t_{ML} , is 28.57 µm and Outer layer thickness, t_{OL}, is 57 µm.

$$\frac{t_{ML}}{t_{ML} + 2 \times t_{OL}} \times 100\ \% = 20\ \% \qquad (6)$$

On a basis of total film volume, v_{FILM} , of 1 cm^3 this means that v_{ML} is 0.2 cm^3 and outer layer volume, v_{OL} , is 0.8 cm^3.

Mass calculations: Mass of the insecticidal film, m_{FILM} , is calculated as seen below.

$$m_{FILM} = \rho_{ML} v_{ML} + \rho_{LDPE} v_{LDPE} = 0.9426 \qquad (7)$$

The mass percentage of the middle layer is calculated as follows:

$$\frac{\rho_{ML} v_{ML}}{m_{FILM}} \times 100\ \% = 21.83\ \% \qquad (8)$$

From the absorption experiments, malathion makes up 29 % of the middle layer and therefore the total percentage of the malathion in the film is calculated as seen below:

$$0.29 \times 21.83\ \% = 6.33\ \% \qquad (9)$$

8.3 Appendix 3: Mass loss kinetics calculations

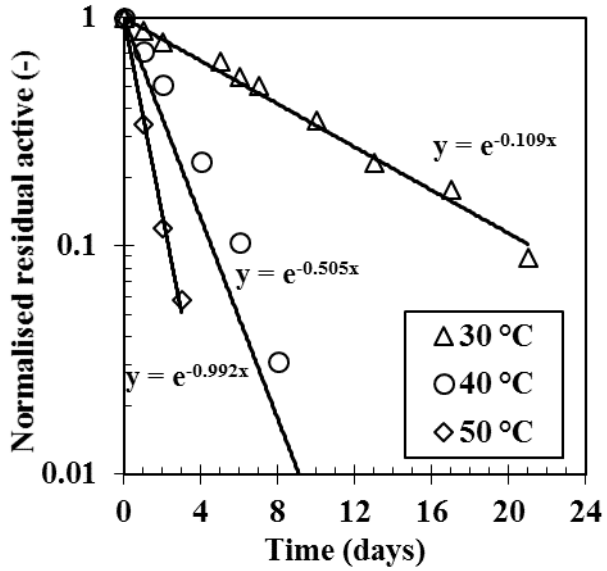

Figure A-1: Oven ageing mass loss kinetics for the films. The graph shows the equations for corresponding to the mass loss at 30°C, 40 °C and 50°C.

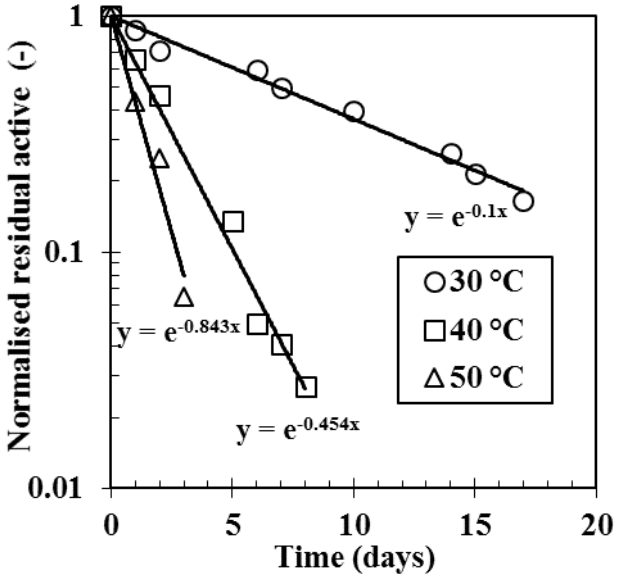

Figure A-2: FTIR kinetics for the films. The graph shows the equations for corresponding to the mass loss at 30°C, 40 °C and 50°C.

From Figures A-1 and A-2 the time constants can be calculated using Equation 10 and using the measurement at 30°C from Figure A-1 as an example.

$$\frac{1}{\tau} = 0.109 \qquad (10)$$

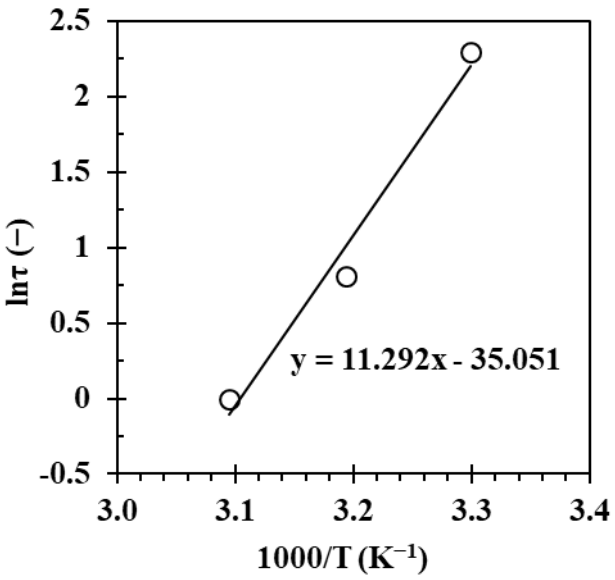

Figure A-3: Arrhenius plot for the mass loss kinetics with the equation

Figure A-3 shows the Arrhenius plot for the mass loss kinetics. The gradient of the plot is 11.292.

$$\frac{E_a}{R} = \text{slope} = 11.292 \quad (11)$$

$$R = 8.314\ \text{J.mol}^{-1}\text{K}^{-1} \quad (12)$$

$$E_a = 8.314 \times 11.292 = 94\ \text{kJ.mol}^{-1} \quad (13)$$

8.4 Appendix 4: Specification sheets of polymers considered in the study

Properties of LT033

Product data sheet

LT033

Low Density Polyethylene for film extrusion

sasol
reaching new frontiers

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

Sales office:
Sasol Polymers
PO Box 2525
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South Africa

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

Sasol Polymers
Polyolefins Business

Date of Issue: January 2012

www.sasol.com/polymers

Sasol Polymers LDPE: LT033 | Density: 0.921 g/cm³ | MFI: 0.33 g/10min

Features

- Tubular resin
- Good mechanical properties
- High impact strength
- High tear strength
- Wide processing range

Applications

- Heavy duty shrink film (>100µm)
- Heavy duty sacks
- Agricultural film
- Thick film

Additives

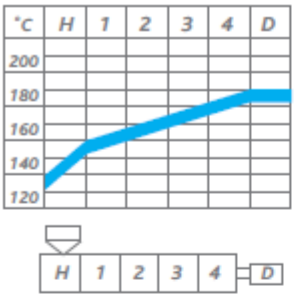
- Antioxidant

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

		Value	Unit	Test method	Based on
MFI (190°C/2.16kg)		0.33	g/10 min	PTM 058	ASTM D1238
Nominal density		0.921	g/cm³	PTM 002	ASTM D1505
Tensile strength at yield	MD	11	MPa	PTM 006	ASTM D882
	TD	10	MPa	PTM 006	ASTM D882
Tensile strength at break	MD	23	MPa	PTM 006	ASTM D882
	TD	20	MPa	PTM 006	ASTM D882
Elongation	MD	510	%	PTM 006	ASTM D882
	TD	610	%	PTM 006	ASTM D882
Elmendorf tear	MD	3	g/µm	PTM 009	ASTM D1922
	TD	5	g/µm	PTM 009	ASTM D1922
Impact strength		300	F_{50}g	PTM 066	ASTM D1709

The above values were measured on 100µm film produced on a 65mm Macchi extruder with a Macchi LDPE screw and a 250mm die, using 218°C melt temperature, 625mm FLH, 2.5:1 blow ratio and a die gap of 0.8mm.

Film Extrusion



Optimum melt temperature: 200°C - 220°C. Should be processed on a conventional LDPE extruder, but can be processed on a LLDPE extruder (wide die gap) with drawdown limitations, inferior mechanical and film shrinkage properties. The optimum blow ratio is 2:1. However excellent properties are obtained at a blow ratio of 1.4:1 (for > 100µm thick film). Recommended screen pack: 60/100/60 BS mesh.

Packaging

Sasol Polymers 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 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.

Properties of Elvax® 210W

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 industial applications. http://www2.dupont.com/Elvax/en_US/tech_info/index.html

Typical Properties

Physical	Nominal Values	Test Method(s)	
* Density ()	0.951 g/cm³	ASTM D792	ISO 1183
* Melt Flow Rate (190°C/2.16kg)	400 g/10 min	ASTM D1238	IS0 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

Properties of Evatane® 20-20

EVATANE® 20-20

Ethylene – Vinyl Acetate (VA) copolymer with high VA content

DESCRIPTION

EVATANE® 20-20 is a random copolymer of Ethylene and Vinyl Acetate made by high-pressure radicalar polymerization process.

TYPICAL PROPERTIES

Characteristics	Value	Unit	Test Method
Vinyl Acetate content	19-21	% Wt	FTIR (Internal Method)
Melt Index (190°C / 2.16 kg)	17-23	g/10min	ISO 1133 / ASTM D1238
Density (23°C)	0.95	g/cm³	ISO 1183
Melting point	83	°C	ISO 11357-3
Vicat softening point (10N)	46	°C	ISO 306 / ASTM D1525
Ring & Ball temperature	127	°C	ASTM E28 / NF EN 1238
Elongation at break	400-600	%	ISO 527 / ASTM D638
Tensile strength at break	17	MPa	ISO 527 / ASTM D638
Hardness Shore A	89	-	ISO 868 / ASTM D2240

APPLICATIONS

The high Vinyl Acetate content of EVATANE® 20-20 brings softness, flexibility and polarity. EVATANE® 20-20 is compatible with most tackifying resins and waxes. Combined with a high fluidity, it is an efficient and easy handling product for hot melt adhesives formulations. EVATANE® 20-20 can also be used as an additive for crude oil (pour point depressant) and for bitumen modification.

For more detailed information and recommendations regarding your specific application, please contact your local ARKEMA technical representative.

PROCESSING

EVATANE® 20-20 can be processed on most conventional equipments used for thermoplastics. It is recommended to avoid melt temperatures above 230°C and to purge the equipment after a run is completed.

EVATANE® 20-20

STORAGE, HANDLING AND SAFETY

EVATANE® 20-20 should be stored in standard conditions and protected from UV-light. Improper storage conditions may cause degradation and could have consequences on physical properties of the product.

Safety data sheet as well as information on handling and storage of the **EVATANE® 20-20** is available upon request to your ARKEMA representative or on the web site evatane.com.

SHELF LIFE

Two years from the date of delivery, in unopened packaging. For any use above this limit, please refer to our technical services.

Properties of EV101

Polymer-E EV101

Ethylene Vinyl Acetate Copolymer

Asia Polymer Corporation (APC)

PROSPECTOR®
www.ulprospector.com

Technical Data

Product Description

Polymer-E EV101 is an Ethylene Vinyl Acetate Copolymer (EVA) product. It can be processed by extrusion or foam processing and is available in Asia Pacific. Applications of Polymer-E EV101 include consumer goods, foam and sporting goods. Primary characteristic: good processability.

General

Material Status	• Commercial: Active		
Literature [1]	• Technical Datasheet (English)		
Availability	• Asia Pacific		
Features	• Good Processability		
Uses	• Foam	• Footwear	• Sporting Goods
Processing Method	• Extrusion	• Foam Processing	

Physical	Nominal Value	Unit	Test Method
Density	0.941	g/cm³	ASTM D1505
Melt Mass-Flow Rate (MFR) (190°C/2.16 kg)	1.8	g/10 min	ASTM D1238
Vinyl Acetate Content	18.0	wt%	Internal Method
Mechanical	**Nominal Value**	**Unit**	**Test Method**
Tensile Strength (Break)	20.6	MPa	ASTM D638
Tensile Elongation (Break)	730	%	ASTM D638
Hardness	**Nominal Value**	**Unit**	**Test Method**
Durometer Hardness (Shore D)	35		ASTM D2240
Thermal	**Nominal Value**	**Unit**	**Test Method**
Deflection Temperature Under Load			ASTM D648
0.45 MPa, Unannealed	40.0	°C	
Brittleness Temperature	< -70.0	°C	ASTM D746
Vicat Softening Temperature	65.0	°C	ASTM D1525
Melting Temperature	82.0	°C	Internal Method

Notes

[1] These links provide you with access to supplier literature. We work hard to keep them up to date; however you may find the most current literature from the supplier.

[2] Typical properties: these are not to be construed as specifications.