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## Malathion-filled trilayer polyolefin film for malaria vector control

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# **Highlights**

- Trilayer film sample containing an insecticide prepared by a film blowing process.
- Core polymer, EVA, acts as reservoir for insecticide.
- The LDPE semi-permeable barrier controls the rate of release of the insecticide thereby reducing the rate of evaporation.
- Bioassay tests showed excellent residual efficacy of the films against mosquitoes for several months.

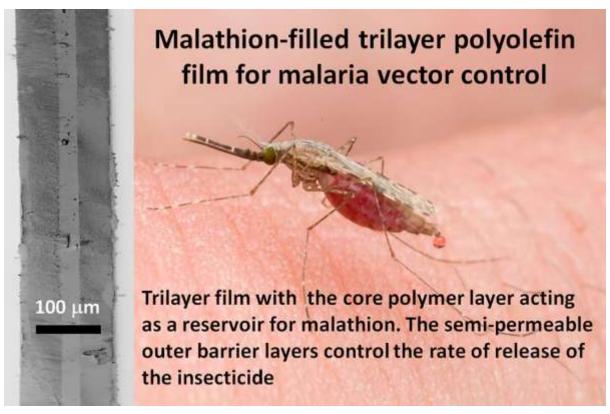
#### **Abstract**

The emergence of pyrethroid resistance in mosquitoes is complicating malaria elimination efforts in Africa and alternative insecticides have to be considered for indoor residual spray. Unfortunately, the high volatility of WHO-approved organophosphate alternatives, e.g. malathion, translates into an early loss of residual efficacy. This laboratory study explored the concept of trilayer films as potential wall or ceiling linings. In the proposed design, the fugitive liquid insecticide is trapped in an inner core layer. The two sheath layers act as low-permeability membranes controlling the release of the insecticide. The concept was explored using poly(ethylene-co-vinyl acetate) (EVA) and low density polyethylene (LDPE) as core and sheath polymers respectively. The polarity of the EVA polymer matrix allowed incorporation of substantial quantities (up to 30 wt.%) of malathion. The low polarity of the LDPE provided the necessary barrier properties and, in addition, allowed film blowing to be conducted at relatively low processing temperatures.

Trilayer films containing about 6 wt.% malathion were prepared on a film blowing line. Scanning electron microscopy confirmed the trilayer film structure. Confocal Raman microscopy studies revealed a malathion concentration gradient across the thickness of the polyethylene layers. Mass loss measurements and FTIR spectroscopy studies showed that the malathion release followed first-order kinetics. Bioassays, on samples aged at 22°C, indicated that the residual efficacy against mosquitoes can be maintained for up to about six months.

This suggests that trilayer films impregnated with organophosphates, may have potential as alternative mosquito control interventions in pyrethroid resistant settings.

# **Graphical abstract**



**Keywords**: malaria; mosquitoes; insecticide; trilayer film; controlled release

#### 1. Introduction

Malaria is a devastating infectious disease in sub-Saharan Africa. In 2015, 207 million cases of malaria occurred globally resulting in 429 000 deaths [1]. Almost 90 % of these deaths occurred in Africa with the majority being children under the age of five [2]. Malaria largely affects under-privileged communities in poor African countries [3-5]. Female *anopheles* mosquitoes are the vectors responsible for malaria transmission. Therefore, decreasing their population should lead to a reduction of morbidity and mortality, and eventually even the elimination of malaria [6].

The most commonly used mosquito control methods are indoor residual spray (IRS) and long lasting insecticide treated nets (LLINs) [7, 8]. IRS is deemed particularly effective. It is an annual activity that is widely applied in Southern Africa [9]. The World Health Organization (WHO) has approved thirteen insecticides for use in IRS. They include six different pyrethroids

(alphacypermethrin, betacyfluthrin, bifenthrin, deltamethrin, etofenprox, and lambdacyhalothrin), three organophosphates (malathion, fenitrothion, and pirimiphos-methyl), two carbamates (propoxur and bendiocarb), an organochlorine (DDT) and one neonicotinoid (clothianidin) [10]. DDT is often preferred for IRS because, depending on the application surface, it retains efficacy for twelve months or more. In contrast, the other insecticides only last for about six months at best. However, DDT is a persistent organic pollutant which lasts for many years in the environment [11]. It also has adverse health effects on humans and animals [12].

LLINs are cost effective and less technically demanding to implement. The main disadvantage of LLINs is that protection is only offered during sleeping time [13]. It is possible to be infected at dusk when the mosquitoes start to be active and when occupants in the house are not yet protected by LLINs [14, 15]. Both the LLINs and IRS vector control methods rely primarily on pyrethroid insecticides. They only provide some protection indoors by targeting mosquitoes either resting after a blood meal (IRS) or preventing actual biting (LLINs). A limitation of these vector control methods is growing insecticide resistance [16, 17], particularly to pyrethroids [18, 19].

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 *Anopheles arabiensis* in Gwave, a malaria endemic area in Zimbabwe [20]. In Côte d'Ivoire, *Anopheles gambiae* showed resistance towards permethrin, deltamethrin and lamdacyhalothrin [21]. In Sudan, WHO susceptibility tests on *Anopheles arabiensis* showed resistance to DDT and pyrethroids [22]. A study in western Kenya found high resistance to pyrethroids, mild resistance to carbamates but absence of resistance to organophosphates in *An. gambiae* and *An. Arabiensis* [23]. Generally organophosphates exhibit the least reported mosquito resistance compared the other WHO-approved insecticides [24]. The reason may be that their usage rates are lower due to having the shorter length of time of residual effectiveness compared to alternatives.

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 [25]. 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 development of pyrethroid resistance constitutes a serious threat to malaria control programs. If measures are not taken in time, the development of resistance may compromise future control efforts [21]. 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. Film blowing and the cast film process are the main co-extrusion techniques used to make such a multi-layer film [26]. Monolayer insecticidal films were previously prepared by incorporating a biopesticide in polyethylene (LDPE) films using supercritical CO<sub>2</sub>—assisted impregnation [27]. Trilayer antimicrobial films, designed to prevent food-borne microbial outbreaks, are known [28]. The idea, considered in this study, is to trap a large amount of liquid organophosphate in the middle layer of a trilayer film. 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 extend 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.

### 2. Experimental

#### 2.1. Materials

Low density polyethylene (LDPE) (Sasol grade LT033, density 0.921 g cm<sup>-3</sup> 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 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 phase content and medium polarity should enable it to imbibe significant quantities of the polar insecticides. Three different grades were considered for carrying the organophosphate [29]. They were Elvax 210W from Du Pont (vinyl acetate (VA) content 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 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.

### 2.2. Equipment and methods

## 2.2.1. Active absorption by EVA pellets

The malathion was incorporated into the EVA by submerging the polymer pellets in the hot, liquid insecticide. About 30 g of EVA pellets were placed in small glass bottles containing an excess quantity 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. Excess malathion was decanted from the bottles and ethanol 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 of malathion absorbed by the EVA.

### 2.2.2. Extrusion and film blowing

Initial film blowing trials were conducted with the less-toxic tris(2-chloroethyl) phosphate simulant in order 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 the 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 neat LDPE. Tables 1 lists the temperature profiles for the two polymer streams.

Table 1: 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	

#### 2.2.3. Film thickness

The film thicknesses of the neat film and malathion-containing film sections were measured with a Mitutoyo Digimatic Indicator. The probe from the instrument was placed at several different positions on the films and the average thickness is reported. The thickness of the neat trilayer film was  $283 \pm 2.1~\mu m$ . 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. However, unless otherwise stated, the results presented refer to malathion-containing film sections with a thickness of  $70 \pm 2~\mu m$ .

## 2.2.4. Scanning electron microscopy

Scanning electron microscopy images were recorded with 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 viewing in the SEM.

## 2.2.5. Fourier transform infrared spectroscopy (FTIR)

The organophosphate content of films was tracked as a function of time by FTIR. The FTIR spectra were recorded on a Perkin Elmer Spectrum 100 instrument at a resolution of 2 cm<sup>-1</sup>. Reported spectra recorded over the wavenumber range 4000 cm<sup>-1</sup> to 550 cm<sup>-1</sup> represent averages of 16 scans.

#### 2.2.6. High-resolution confocal Raman imaging

High-resolution 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  $\times 100$  Zeiss objective which gives a diffraction limited lateral and depth resolution of about 360 and 530 nm respectively. 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  $\mu$ m and the scan width and length was also 40  $\mu$ m with 100 points per line and 100 lines per image.

### 2.2.7. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was performed on a Perkin Elmer TGA 4000 instrument. The temperature was scanned from ambient to 700°C at a scan rate of 10°C min<sup>-1</sup> with nitrogen flowing at a rate of 50 mL min<sup>-1</sup>. 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.

#### 2.2.8. Film oven aging tests

Oven ageing tests were done to track the mass loss from the trilayer films over time. The films were suspended vertically in separate convection ovens set at three different temperatures: 30°C, 40°C and 50°C. The mass of the films was recorded daily.

### 2.2.9. Bioassays

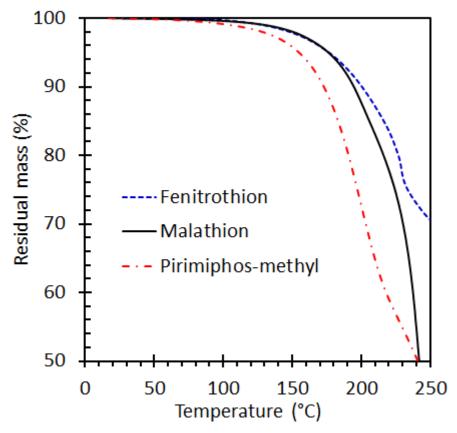
The insecticidal efficacy of the films was checked with cone bioassays. These tests were conducted at the Vector Control Unit of the National Institute for Communicable Diseases (NICD). The WHO bioassay protocol for cone tests was followed. Three films measuring 12 cm × 12 cm were cut and stuck onto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

### 3. Results

### 3.1. TGA studies of pure organophosphates

Figure 1 shows TGA scans obtained for the 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 stability/volatility of the three insecticides increased in the order fenitrothion < malathion ≈ pirimiphos-methyl. Polyethylene film blowing is usually conducted at elevated temperatures, i.e. above 180°C. It

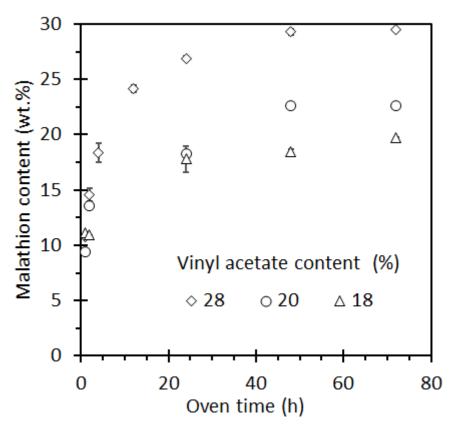
is therefore necessary to select an organophosphate that is stable to exposure to higher temperatures. Malathion was selected because of its lower toxicity compared to the other two organophosphates. The LD<sub>50</sub> for malathion is 1768 mg kg<sup>-1</sup> body weight (male rat) while the corresponding values for fenitrothion and pirimiphos-methyl are 1700 and 1414 mg kg<sup>-1</sup> body weight (male rat) respectively.



**Figure 1.** Thermogravimetric (TGA) mass loss curves for technical grade fenitrothion, pirimiphos-methyl and malathion samples. The temperature was scanned at 10 °C min<sup>-1</sup> and nitrogen flowed at 50 mL min<sup>-1</sup>.

### 3.2. Active absorption studies

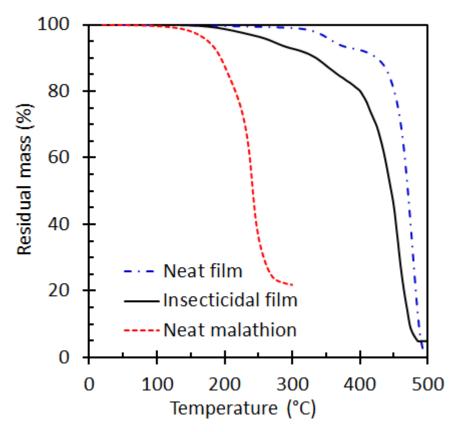
Figure 2 shows the progress of malathion absorption expressed as wt.% of the total mass. The equilibrium malathion absorption increased with the VA content of the poly(ethylene-co-vinyl acetate). It reached ca. 29 wt.% for the 28 %VA EVA grade. The 20% VA EVA grade showed a marginally higher malathion absorption compared to the 18% VA grade. Higher VA substitution increases the fraction of the amorphous phase in the semi-crystalline polymer and also tends to decrease the melt temperature [29]. The malathion only dissolves in the amorphous regions, causing swelling of the polymer.



**Figure 2.** The effect of vinyl acetate content on the progress of the swelling of poly(ethylene-co-vinyl acetate) by malathion at 58°C for the 28% VA EVA and at 81°C for the other two EVA grades.

## 3.3. TGA of trilayer films

Figure 3 shows TGA plots for the trilayer films prepared using either neat EVA pellets or EVA pellets containing ca. 29 wt.% malathion. Figure 3 also shows a TGA trace obtained for the neat malathion liquid. Mass loss, from the malathion-containing film, commenced at a lower temperature than for the neat film. This is attributed primarily to vaporization loss of the trapped malathion. Oven ageing results for the insecticidal trilayer film indicated that the film contained ca.  $5.8 \pm 0.8$  wt.% malathion. However, degradation reactions at the elevated temperatures in the TGA scan cannot be excluded because the mass loss at about  $300^{\circ}$ C exceeded this amount.



**Figure 3.** TGA traces of the neat films and insecticide trilayer films recorded at a scan rate of 10°C min<sup>-1</sup> and in nitrogen flowing at 50 mL min<sup>-1</sup>.

### 3.4. Scanning electron microscopy [30]

Figure 4 shows the SEM micrographs of trilayer film samples. The two outer LDPE layers sandwich a middle layer made up of either neat EVA or EVA containing insecticide. The rough outer edges in both micrographs are artefacts of the freeze fracturing process conducted at liquid nitrogen temperatures. The insecticide impregnated film is thinner than the neat film because it was blown at a reduced throughput rate for safety reasons. In this sample, the two LDPE outer layers of the impregnated film had thicknesses of approximately 57  $\mu$ m each and the middle layer was approximately 29  $\mu$ m thick. This means that the middle layer made up 20 % of the overall film thickness. The insecticide-swollen EVA pellets, used as feedstock for the film blowing trial, contained approximately 29 wt.% malathion. The density of the middle layer is estimated at 1.018 g cm<sup>-3</sup> considering the densities of malathion (1.272 g cm<sup>-3</sup>) and the EVA (0.951 g cm<sup>-3</sup>). The LDPE had a density of 0.921 g cm<sup>-3</sup> and this layer took up 80 % of the overall film thickness. This means that the middle layer made up approximately 22 % of the total mass and that the malathion content of the trilayer film should be ca. 6.3 %. This estimate is in fair agreement with the value of  $5.8 \pm 0.8$  wt.% measured in an oven-ageing test.

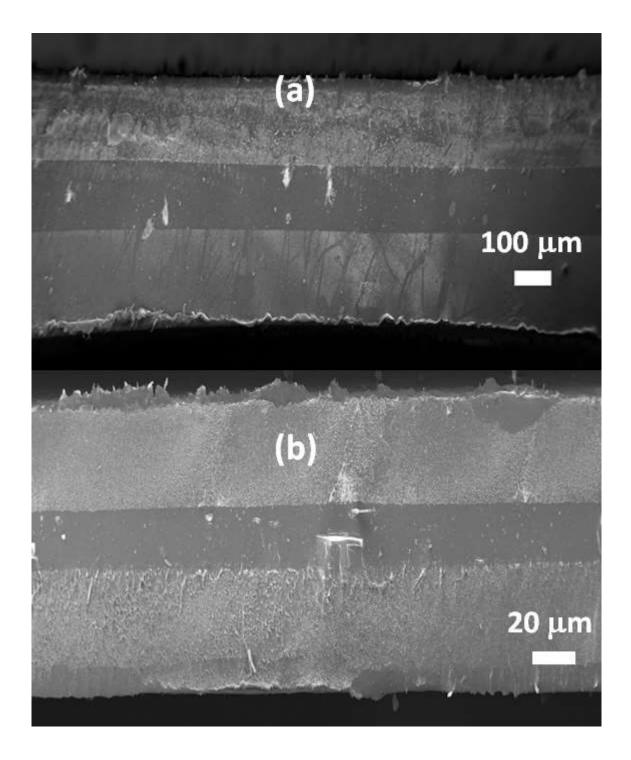
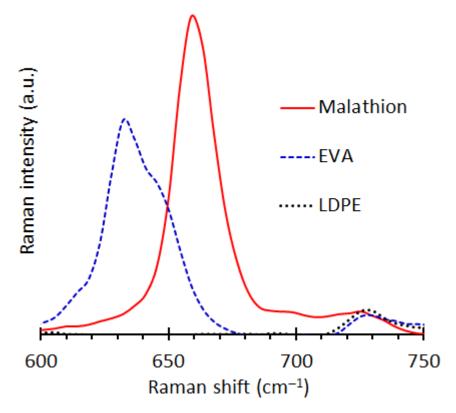


Figure 4. SEM micrographs of (a) a neat trilayer film, and (b) a trilayer film impregnated with malathion.

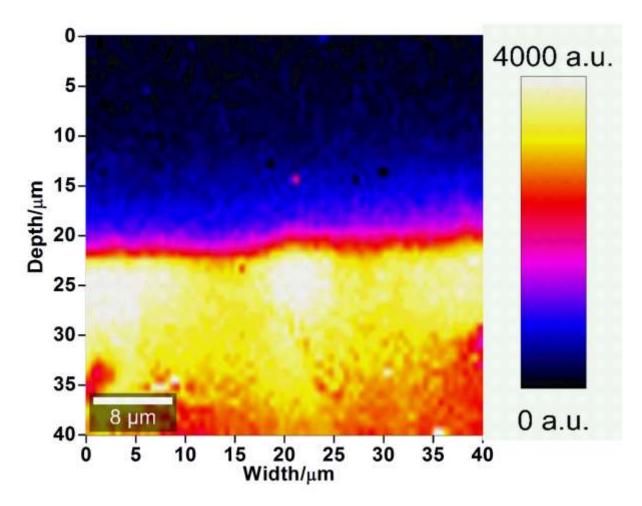
## 3.5. Confocal Raman imaging

Figure 5 shows Raman spectra for neat malathion, the 28 % VA EVA and the neat LDPE. The intense, carbonyl stretching, absorption band located at *ca.* 660 cm<sup>-1</sup> is a characteristic feature of the malathion spectrum corresponding to stretching vibrations of the P=S bond. It was used to quantifying the distribution of the insecticide inside the trilayer film. To do this, the laser

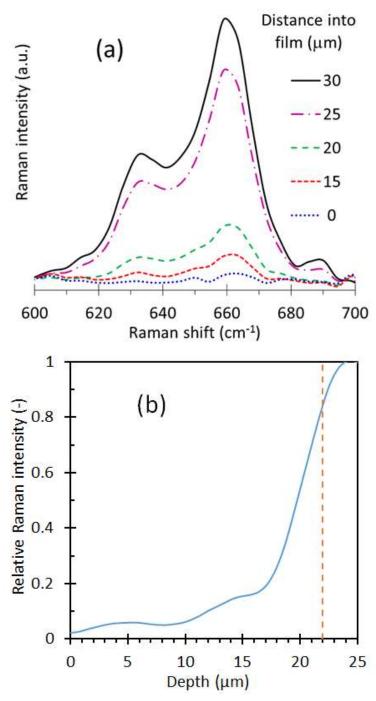
scanned an area measuring  $40 \, \mu m \times 40 \, \mu m$ . The average intensity of this Raman band was used to generate the depth profile image. It is plotted as a function of depth and width in Figure 6 that shows an increase in intensity with depth signifying that a malathion concentration increases with the film depth from the top surface. The Raman depth scans shown in Figure 7 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 between the two polymer layers (Figure 7B).



**Figure 5.** Raman spectra for malathion, EVA (28 % VA) and LDPE. The most intense absorption band at ca. 660 cm<sup>-1</sup> is characteristic for the P=S bond in malathion.



**Figure 6.** The distribution of malathion inside a trilayer polymer film estimated from the intensity of the  $660~\text{cm}^{-1}$  band. This sample had a thickness of  $83 \pm 4~\mu\text{m}$ , with the polyethylene layer and the inner malathionswollen EVA layer about  $22~\mu\text{m}$  and  $39~\mu\text{m}$  thick respectively.

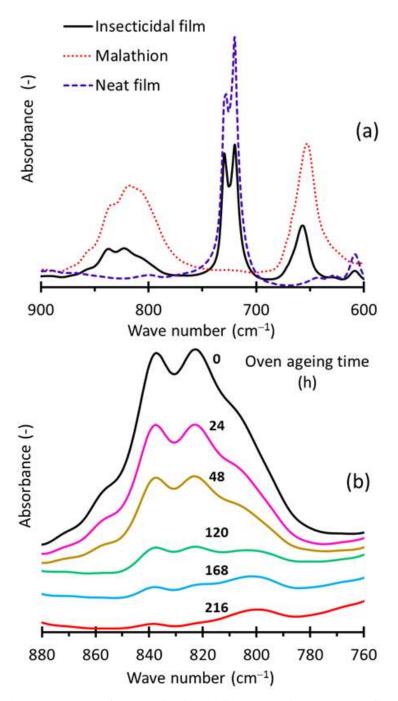


**Figure 7.** Variation of the Raman spectra with distance from the surface. (a) The Raman spectra recorded at different penetration depths. The bands at *ca.* 660 cm<sup>-1</sup> and 632 cm<sup>-1</sup> are attributed to the P=S in malathion and the carbonyl stretch deformation in EVA respectively. (b) The intensity of the 660 cm<sup>-1</sup> Raman band as a proxy for the concentration of malathion inside the trilayer film. The dotted line indicates the boundary between the LDPE and the EVA layers.

## 3.6. Infrared Spectroscopy

Figure 8(a) shows the FTIR spectra for a neat trilayer film, malathion insecticide and the insecticidal trilayer film. A band located near 660 cm<sup>-1</sup>, and the two peaks near 830 cm<sup>-1</sup>, are

uniquely associated with malathion. The band at 660 cm<sup>-1</sup> is assigned to stretching vibration of the P=S bond. The doublet at 838 cm<sup>-1</sup> and 822 cm<sup>-1</sup> 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 8.** (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.

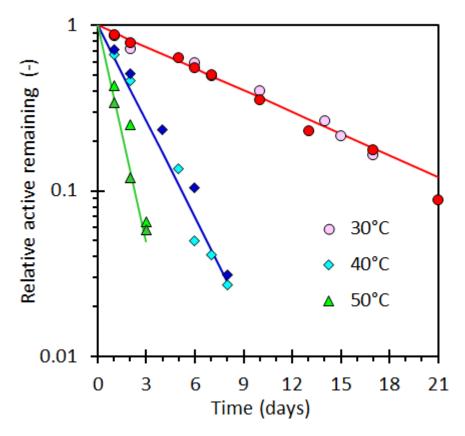
Figure 8(b) shows absorbance spectra recorded for the insecticidal films that were aged at 30°C in a convection oven. The malathion band diminishes in intensity over time as it is lost by evaporation from the film. Similar changes were observed for the films aged at the two higher ageing temperatures. The evaporation happened faster at the higher temperatures and therefore the malathion spectra recorded for the film aged at 50°C disappeared fastest. The area under the peaks (780 – 860 cm<sup>-1</sup>), obtained at 30°C, 40°C and 50°C, was 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.

## 3.7. Film Oven Aging Tests

Figure 9 shows a plot of the residual mass of the insecticidal trilayer films aged at  $30^{\circ}$ C,  $40^{\circ}$ C and  $50^{\circ}$ C. The samples used had thickness of  $73 \pm 7 \,\mu$ m. The mass loss data followed first-order kinetics. This means that the insecticide release showed an exponential decay over time. The mass loss, estimated from both mass measurements and FTIR results, was therefore modelled as follows:

$$(m(t) - m_{\infty})/(m_0 - m_{\infty}) = e^{-t/\tau}$$
 (1)

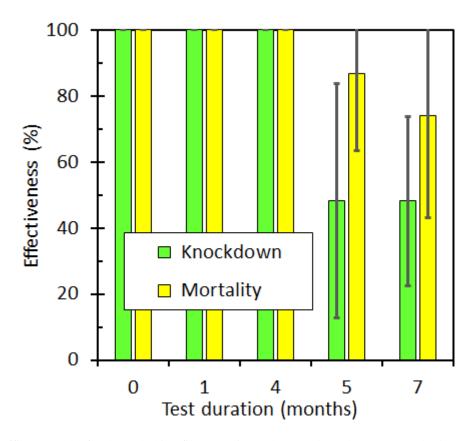
where m(t) is the mass at time t,  $m_0$  is the initial mass,  $m_\infty$  is the mass at an infinite time and  $\tau$  is a characteristic time constant. The time constant,  $\tau$ , provides a measure of the first order rate at which the film loses mass. The time constant values were estimated from least squares data reduction 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 ( $\tau$ ), this corresponds to an activation energy of approximately 94 J mol<sup>-1</sup> K<sup>-1</sup>.



**Figure 9.** 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 open and filled symbols correspond to results obtained from mass loss and FTIR data respectively.

## 3.8. Bioassays

Testing mosquito repellence activity of the film samples began soon after they were made. The films were aged in a fume hood for the whole duration of the tests. A neat trilayer film was used as the control. The insecticidal films had a thickness of  $70 \pm 3 \,\mu m$ . Figure 10 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. The insecticidal films produced satisfactory efficacy results according to this criterion for the first 4 months of testing. Figure 10 shows that the malathion film failed the WHO mortality criterion after seven months.



**Figure 10.** Efficacy results for the malathion film over time. The error bars show one standard error above and below the mean values.

#### 4. Discussion

Malathion only dissolves in the amorphous regions of the polymer matrices and the solubility is higher at elevated temperatures. The highest solubility occurs when the polymer is in the molten state. On cooling, following the film blowing process, the polymer partially crystallizes and the fraction amorphous phase available to dissolve the malathion diminishes. This means that the degree of saturation is higher at ambient conditions. At very low temperature the malathion liquid becomes supersaturated inside the polymer [31]. In the latter case, the malathion must diffuse to the surface of the film in order to return to an equilibrium state. Malathion is a contact insecticide and this means that it must be available on the outer surface of the trilayer film in adequate quantities to kill the mosquitoes. This means the liquid malathion that diffuses to the surface must replenish the insecticide constantly. 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 indicated that this is indeed the case. Ideally, if the diffusion through the LDPE layer is in fact the rate-controlling step, the release of

malathion should follow zero order kinetics, i.e. the release rate would remain constant over time until the insecticide was fully depleted.

However, FTIR results and mass loss data showed that the malathion release follows first-order kinetics despite the LDPE films acting as diffusion barriers. This dichotomy is not currently understood and further work is required to resolve this apparent contradiction. It is speculated that rate limiting diffusion out of the EVA matrix is the culprit. 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 7(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.

When the malathion is used in IRS, the insecticide remains effective for two to three months only [32]. This laboratory study showed that the residual effectiveness could be extended towards six months if the malathion is delivered via a trilayer film concept. It is likely that it will be possible to prolong the duration and effectiveness against mosquitoes of similar malathion films.

Based on the cost of the insecticide applied, the proposed intervention is more expensive. The recommended application dosage of malathion, in IRS format, is 2 g m<sup>-2</sup> and this provides a maximum efficacy period of three months. The concentration of the malathion in the present film is approximately 5.8 g m<sup>-2</sup>, i.e. almost three times more, yet it provided twice the protection period only. Clearly, there is need for further improvement in the length of residual efficacy of the proposed intervention as the typical malaria transmission season lasts about nine months. The major expense in conventional IRS is the implementation cost which typically accounts for 70 % of the total [33]. The aim should be to reduce the three spray cycles, required for the IRS of malathion, to a single annual intervention based on a trilayer film with a comparable residual effectiveness. In this study, we have simply provided laboratory-scale evidence that supports the concept of extending residual efficacy of malathion incorporated in a trilayer polymer film. Further development is necessary before actual field trials are considered to gauge user acceptability and feasible installation modes.

#### 5. Conclusions

Trilayer films containing ca. 6 wt.% malathion, a WHO insecticide approved for IRS, were prepared by film blowing. Laboratory bioassays indicate that, with this approach, the residual effectiveness of the malathion was increased to 6 months from the usual two to three months. Further work needs to be done to optimize the trilayer film delivery form. Nevertheless, this laboratory study has demonstrated that the idea has potential as an intervention to reduce the incidence of malaria in IRS-like applications.

### Acknowledgements

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