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Abstract

In order to assess the possibility of adding an antimicrobial agent to insecticide treated nets already used for malaria prevention to expand their purpose and reduce in-home transmission of *Mycobacterium tuberculosis*, an extensive literature review was conducted. The review focused on the methodology of assessment of filtration efficacy of masks, antimicrobial effects of compounds that are commonly used in insecticide treated nets, generic biocidal compounds, and the use of electrostatic effects to attract *Mycobacterium tuberculosis*. Our hypothesis is that if a significant amount of the aerosolized bacteria can be arrested by the net, while still allowing for comfortable sleeping conditions and protection against malaria transmission, then tuberculosis incidence rates in malaria-endemic areas could be decreased. The findings from 16 relevant studies suggest that there is no universal standard method for testing filters and masks using bioaerosol challenge techniques. Permethrin, the most widely used insecticide for insecticide treated nets, has no known antimicrobial effects. The compounds with the highest potential for biocidal effects are the AM 500 surface agent and cupron-based compounds. Given that *Mycobacterium tuberculosis* has an overall negative charge, positively charged compounds, such as quaternary amines, have a potential to arrest the bacteria.

The systematic review of the literature will be used as a framework to move into the laboratory testing stage in order to determine the specific advantages and disadvantages of different approaches to develop the experimental net.

We plan to partner with North Carolina State University, the Centers for Disease Control and Prevention, and Badir Dar University in Ethiopia in order to

complete the final project. Our research is one of three separate efforts to create sustainable innovations in global health in order to advance the field and increase public health education capacity at Badir Dar University.

Acknowledgements

I would like to thank Dr. Annelies Van Rie and Dr. L.M. Ball for their continuous help, support, and encouragement throughout the research and the writing process. Without them, this project would not have been possible. I would also like to give a special thanks to Dr. Van Rie for her patience and desire to help me understand the complexities of academic research.

Introduction

Currently, one-third of the approximately 7 billion people on earth are infected with *Mycobacterium tuberculosis*.[13] Even though tuberculosis cases occur all around the world, a significant fraction of the disease burden falls in malaria-endemic areas. The maps on the next page indicate the overlap of these two diseases.

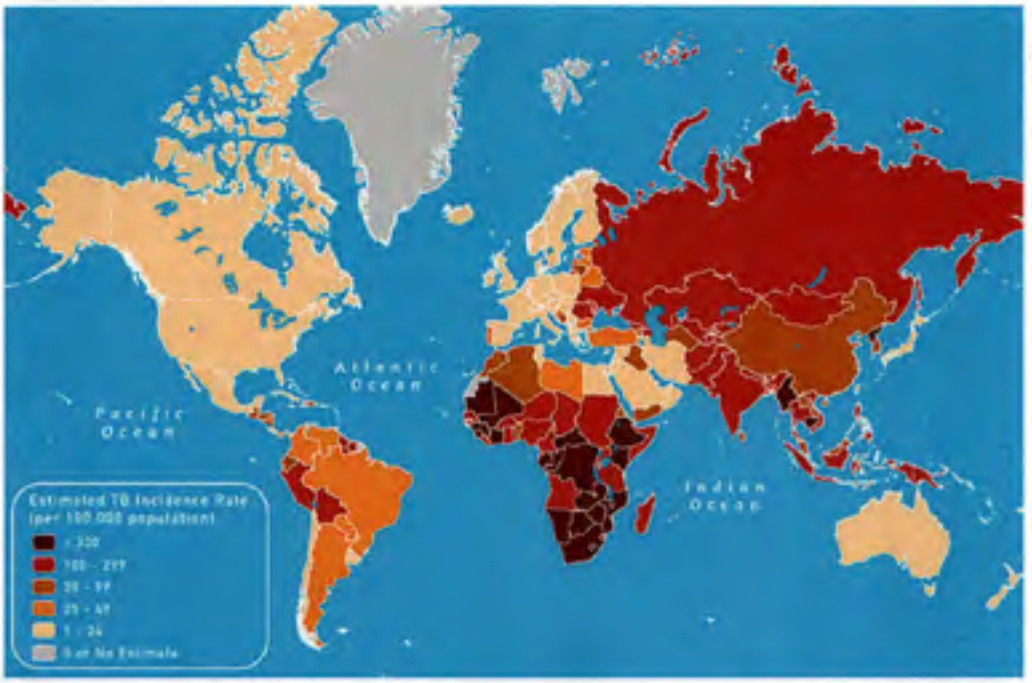


Figure 1: Map of tuberculosis incidence rate by country.[14]

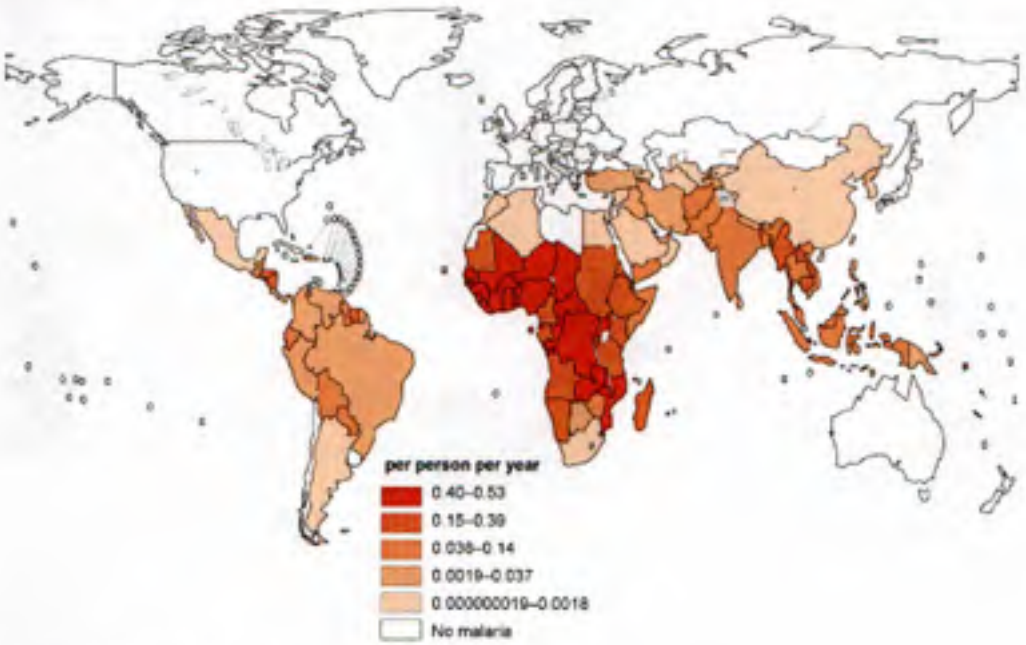


Figure 2: Map of estimated malaria incidence.[15]

Africa and South-East Asia are areas of particular interest given the overlap of the two diseases in these regions. The World Health Organization reports that in 2010 approximately 8.8 million people became ill and 1.4 million people died from tuberculosis. For malaria, the WHO estimates that there were about 216 million cases and 655,000 deaths in 2010.[13, 18]

Since tuberculosis is spread from person-to-person via aerosolized droplets, close contact is needed in order for an infectious person to spread the bacteria. Our research posits that a significant amount of transmission occurs indoors in areas with poor air circulation, such as in the home. Since the majority of time spent within the home occurs while inhabitants are asleep, we hypothesize that a substantial proportion of person-to-person transmission within the home occurs while individuals are sleeping. Through redesigning insecticide treated nets to expand their impact, our hope is to reduce the incidence of tuberculosis in malaria-endemic areas.

Tuberculosis is especially dangerous because of its often delayed diagnosis. Transmission to additional individuals normally occurs before diagnosis of the disease, and thus preventive measures must be put in place to reduce transmission and lower the disease burden. The focus on nets to decrease transmission will hopefully contain the disease more effectively through the reduction of in-home spreading even before an infected individual is diagnosed.

This literature review focused on four main aspects related to the use of an insecticide treated net in order to reduce in-home transmission of tuberculosis. The first goal was to determine the optimal methodology for the assessment of filtration efficacy of masks, filters, and netting materials. The second was to record commonly

used compounds in insecticide treated nets and to review any known antimicrobial activity associated with those compounds. Then a search for generic biocidal compounds was conducted with the goal of finding potential compounds to add to the insecticide treated nets. Finally, the electrostatic properties of *Mycobacterium tuberculosis* were considered in relation to potential materials that could be added to the nets. The overall goal of the literature review was to provide the evidence required for the design of the experiment to develop the experimental nets and evaluate their efficacy.

Materials and Methods

The literature review was conducted using online databases available through the University of North Carolina University Libraries. The primary databases used to collect relevant studies were *PubMed, Web of Science,* and *PubChem*. Key terms used in database searches included tuberculosis, biocidal, filtration efficacy, electrostatic properties, insecticide treated nets, and WHO pesticides.

An example of *PubMed* search parameters for this review is shown below.

("drugs, generic"[MeSH Terms] OR ("drugs"[All Fields] AND "generic"[All Fields]) OR "generic drugs"[All Fields] OR "generic"[All Fields]) AND biocidal[All Fields] AND compounds[All Fields]

Figure 3: Sample *PubMed* search criteria.

Articles considered to be a priority were published within the last ten years and related directly to the assessment of filtration efficacy in masks or filters,

insecticides that are commonly used in insecticide treated nets, generic biocidal compounds, or the electrostatic properties and susceptibility of *Mycobacterium tuberculosis*. The parameters of the search were expanded to include a wider range of mask and filter materials and testing methodology so that a standard could be assessed.

Level of importance and degree of relevance to the research goals were determined through review of study titles and abstracts. Studies deemed relevant were read carefully and the salient methods and findings were recorded. Editorials, overviews, and documents published by the Centers for Disease Control and Prevention and the World Health Organization were also considered. Studies published in other languages were not included in the literature review.

Relevant data was also gathered through consultation of experts in the field. Dr. David Weber, Department of Epidemiology in the Gillings School of Global Public Health at UNC, suggested considering AM 500 as a potential generic biocidal surface compound for use with the nets. Dr. Marian McCord, College of Textiles at North Carolina State University, was the driving force behind considering NEEM oil and provided feedback on the limitations of developing the physical net. Information on the feasibility of use for these compounds was primarily gathered from the product websites, as well as from any available literature data. Dr. Steven Meshnick, Department of Epidemiology in the Gillings School of Global Public Health at UNC, was consulted in regard to the potential biocidal capacity of insecticides already in use in insecticide treated nets. Dr. Paul Jensen and Garry Blackwelder, Centers for

Disease Control and Prevention, helped guide the study parameters and provided feedback on the achievability of our goals.

Results

Mask testing methodology

In the Mardimae et al. study, a plastic manifold with a one-way aspirating valve with an oxygen inlet was used to study the fractional inspired concentrations of oxygen allowed by the N95 mask at multiple flow rates and the capacity of the mask to filter and hold microparticles from the air.[1] These methods are helpful on the scale of a mask, but are slightly difficult to relate for the larger scale needed to test a mosquito net.

Another study of N95 respirators focused primarily on their filtration efficiency over a range aerosolized particle sizes. Using a Collison nebulizer, Qian et al. challenged the masks with three types of solid test particles. The group used a laser aerosol size spectrometer and an aerodynamic size spectrometer to measure the particle size with the most penetration. Their findings showed that filtration efficiency does, as suspected, increase with particle size. It is approximately 99.5% or higher at 0.75 micrometers. This data is a decent representation of *Mycobacterium tuberculosis*, which is around 2-4 micrometers in length and 0.2-0.5 micrometers in width.[2]

McCullough et al. studied the effect of varying flow rate and relative humidity on the penetrating ability of *Mycobacterium abscessus*, *Staphylococcus epidermis*, and *Bacillus subtilis* on multiple respirators and masks. The two flow rates generated

using the Collison nebulizer were 45 and 85 L/min and the two humidity levels were 30% and 70%. Aerosol penetration was detected using a total-particle, direct-readying spectrophotometer and a viable particle cascade impactor both upstream and downstream of the filter. The set-up used a 6.5-inch diameter vertical steel tube for the aerosol particles to challenge the filter. An Andersen six stage viable particle sampler was used to collect aerosols and sample and lower flow rates. It was found that increased flow rate yielded more penetration from each bacterium, but that relative humidity had a variable impact on the three different microbes.[3] The flow rate and humidity findings are helpful in our analysis of the effect of varying environmental conditions on bacterial penetration.

The Borkow et al. study considered the use of copper oxide as a disinfectant in respiratory protection devices. The masks passed bacterial filtration efficacy, differential pressure, latex particle challenge, and resistance to penetration by synthetic blood tests. These results indicate the masks adherence to NIOSH standards, while also providing a potent anti-influenza barrier thanks to the copper oxide. It was found that no infectious influenza A virus could be recovered from the masks after 30 minutes.[11]

Dharmadhikari et al. studied the effectiveness of surgical facemasks worn by multidrug-resistant tuberculosis patients in a hospital ward. The ward air was pulled into two identical chambers with 90 healthy guinea pigs, and the rate of infection of an intervention and a control group was determined. The investigators found a 56% decrease in guinea pig infection rate when the patients wore masks compared to when

they did not.[21] This study provides helpful methodology for field-testing our experimental nets.

Mesh testing methodology

The most relevant data from the search on bioaerosol challenge methodology came from the Davidson et al. study on pore size and bacterial arrestance. This investigation modified techniques for assessing the retention efficacy of respiratory protection equipment, such as masks, to assess the level of arrestance provided by a mesh net when challenged with bioaerosols. Using a Collison nebulizer, the prophylactic mesh barriers were challenged in a bioaerosol chamber and samples were taken with sixteen All-Glass Impinger samplers at four precise distances downwind from the nebulizer. Each of the two nets was tested in a bioaerosol chamber with airflow set at 9.9 L/sec resulting in air flowing through the chamber at 0.016 m/s. Four AGI-30 samplers, sixteen total, were placed in the bioaerosol chamber at distances of 0.25, 0.8, 1.2, and 1.75 meters downwind from the bioaerosol source. The mesh barrier was placed 1.0 meter from the source. The figure on the following page shows the experimental set-up.

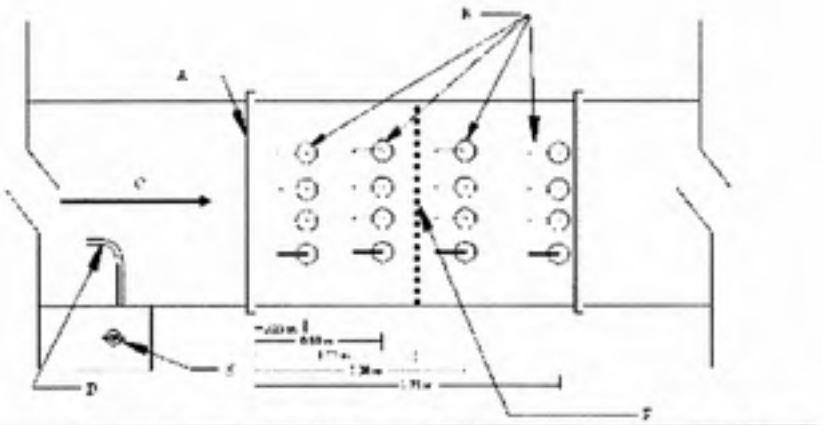


Fig 1. Experimental setup in bioaerosol chamber. (A) Baffle. (B) AGI-30 samplers, (C) airflow (velocity 0.016 m/s). (D) aerosol inlet. (E) nebulizer encased in polycarbonate housing. (F) prophylactic barrier.

Figure 4: Experimental setup from Davidson et al. study

They found that a pore size of 0.8 mm provided insignificant arrestance of aerosols, but that a 0.25 mm pore size gave an 8% arrestance for vegetative cells and a 13% for endospores. No arrestance was seen for particles less than 0.6 micrometers in size.[12] These findings are not very encouraging for *Mycobacterium tuberculosis* because it is 0.2-0.5 micrometers in width. However, Dr. McCord believes that smaller pore sizes could be created and would still provide adequate airflow. This testing methodology provides very valuable insight for how we plan to test our net in the laboratory.

Commonly used insecticidal compounds and nets

From the focus on typical net and filter designs, as well insecticides commonly used to treat them, it was found many different net models were effective, but that regional variability played an important role in vector resistance to insecticides. Refer to Appendix A for the WHO recommended insecticide products

treatment of mosquito nets document and to Appendix B for the long lasting insecticidal mosquito nets document.

The Kawanda et al. study took place in a highly malaria endemic area of western Kenya where pyrethroid resistance is one of the primary issues in adequate vector control. They examined pyrethroid susceptibility and the mechanism by which species developed resistance. The investigators found that Anopheles arabiensis and Anopheles funestus were not resistant to a combination of permethrin and DDT, while Anopheles gambiae was often resistant to both. They concluded that the mechanism for this resistance was most likely a high frequency kdr mutation that could be selected for due to frequent insecticide exposure.[4] This study elucidates the serious issue of pyrethroid resistance and directs us to consider the use of insecticides other than permethrin alone for our experimental net.

Consideration during the literature review was also given to novel designs of insecticide treated nets. The Djènontin et al. study focused on the use of combination carbamate-treated materials and pyrethroid-treated bed nets to combat pyrethroid resistance in the Anopheles gambiae vector. While the study considered four total carbamate-treated alternatives, the polypropylene mesh, in combination with the pyrethroid-treated bed net, provided superior protection and resistance to wear during washing.[7] These findings reinforce the necessity of moving beyond pyrethroid-treated bed nets alone in malaria prevention. Furthermore, the addition of a variety of carbamate-treated wall plastic sheeting may provide an opportunity for the additional use of a surface protectant to minimize tuberculosis transmission. A biocidal chemical

could potentially be applied to this plastic sheeting in order to block transmission and inactivate the bacteria, thus avoiding the issue of pore size with the mesh.

Another net comparison study considered the differences between PermaNet® 3.0 and PermaNet® 2.0 in their ability to prevent transmission of malaria. PermaNet® 3.0 differs from PermaNet® 2.0 in that the 3.0 version has stronger side panels and deltamethrin and PBO incorporated into the polyethylene roof. The study was conducted in multiple sites across Western and Central Africa and focused on pyrethroid resistance in the Anopheles gambiae vector. It was found that PermaNet® 3.0 consistently performed as well as or better than PermaNet® 2.0. This difference was attributed to the significantly higher deltamethrin content in the newer net. After twenty washes, only PermaNet® 3.0 fulfilled the WHO standards for long lasting insecticide treated nets, but did show decreased efficacy.[5] This study informs our consideration of newer technology in the laboratory testing process.

Biocidal agents

Much of the available literature related to surface protecting chemicals, such as Hulkower et al., has focused on protecting hard surfaces or has targeted short-term disinfectants, but there were some promising findings that could be relevant to our aim of finding a durable fabric surface protectant.[19] Baxa et al. assessed the capacity of Goldshield 5 to decrease the bacterial level on contaminated surfaces. The study considered both hard surface and fabrics, but found that the viability of the compound was only adequate for 14 days.[9] If the longevity of the substance can be improved, then Goldshield 5 could have potential to add bactericidal effects to insecticidal nets.

Incorporating copper oxide or a similar metal into a mosquito net could provide significant protection against the transmission of aerosolized pathogens. Borkow et al. considered the antiviral action of copper oxide in respiratory masks in order to reduce the spread of the influenza A virus. Given that no infectious influenza A could be detected in the mask after 30 minutes, this option is very promising for our purposes with mosquito nets as well. If a cupron-like material can be impregnated into the net, then it could provide significant biocidal properties as this study demonstrated.

At present, AM 500 biostatic surface protection appears to be the most promising option. Although there seems to be no literature available on its effectiveness, the list of the susceptible microorganisms is extensive and includes *Mycobacterium tuberculosis* along with many more aerosolized organisms. Through the use of positively charged quaternary amines, this compound attracts negatively charged microbes to its surface and binds them. The compounds are then inactivated through the breakage of their cell wall by the positively charged nitrogen atom. The use of organo-silane technology will hopefully make this compound more durable and effective in the field. See Appendix C for the full list of susceptible microbes and Appendix D for the mechanism of action for AM 500.

Biocidal effects of current insecticides

Pyrethroids, the category of insecticides from which permethrin is the most used, only work against arthropods. Their success against such insects is due to their

ability to target a specific sodium channel found in the arthropod nervous system. Single-cell organisms, such as the tuberculosis bacterium, do not have nervous systems, and thus are not affected by the insecticide.[20]

Electrostatic effects and net design

Since *Mycobacterium tuberculosis* has been indentified as having an overall negative charge, the negatively charged bacteria could be attracted to the positively charged surface compound.[17] This opposite charge attraction ought to allow for slightly larger pore sizes because of the interaction. If there were no charge interaction, then pores would need to be smaller than the bacterium in order to effectively capture it. If there is a significant charge interaction, slightly larger pores could still capture bacteria smaller than the pore size. This factor will be an advantage because of the importance of maximizing pore size to provide adequate airflow and increase net usage.

Discussion

Previous studies of prophylactic barriers to aerosolized microorganisms have focused on either larger pore size mesh (greater than 0.25 mm) or very fine masks and respirators. Neither one of these categories are very easily correlated to the study that we wish to perform. Since nets typically have larger pore size, they tend to allow particles the size of *Mycobacterium tuberculosis* through, while respirators have a pore size that is too small to allow adequate airflow for comfortable sleeping. We plan to tests pore sizes smaller than 0.25 mm in order to decrease the amount of

bacteria that are able to move through the net, but keep the net breathable enough to allow comfortable sleeping conditions. We plan to test multiple net designs with bioaerosol challenge techniques and to determine the best performing net for our intervention.

Multiple biocidal compounds will be tested on the nets for their ability to attract and inactivate *Mycobacterium tuberculosis*. Ideally, these products will be effective against other airborne microbes as well. Other commercially available biocidal chemicals and natural oils will be considered during the testing process as well. We will also consider any significant interaction between applied biocidal compounds and insecticides. If a compound inhibits an insecticide in any manner, then it will not be useful. Given the information about insecticide resistance, it is very likely that laboratory testing will include consideration of new chemicals, as well as combination approaches.

The potential global health impact of this project is very large. If such a net can be successfully developed, then the disease burden of tuberculosis in malaria-endemic areas could be significantly reduced. The WHO estimates that there are over 350 cases per 100,000 people in sub-Saharan Africa, and most of this area is malaria-endemic as well.[13] Of course, there are ever-present barriers to the effectiveness of mosquito nets, but research continues to be done as to how to improve cooperation with the suggestions of local and international health officials.

Through the partnership with Badir Dar University in Ethiopia, we hope to improve public health infrastructure in the region and to build the university's capacity to begin innovative projects in the future to serve the surrounding areas and

population. The project will move into the laboratory testing stages as soon as possible. Following laboratory testing, field trials will begin at multiple sites yet to be determined. Possible study areas include northern Ethiopia and South Africa.

Appendix

Appendix A

Updated December 2007

WHO recommended insecticide products treatment of mosquito nets for malaria vector control

1. **Conventional treatment:**

INSECTICIDE	*FORMULATION*[1]	*DOSAGE*[2]
Alpha-cypermethrin	SC 10%	20-40
Cyfluthrin	EW 5%	50
Deltamethrin	SC 1%; WT 25%; and WT 25% + binder[3]	15-25
Etofenprox	EW 10%	200
Lambda-cyhalothrin	CS 2.5%	10-15
Permethrin	EC 10%	200-500

2. **Long-lasting treatment:**

Product name	*Product type*	*Status of WHO recommendation*
ICON® MAXX	Lambda-cyhalothrin 10% CS + binder Target dose of 50 mg/m[2]	Interim

[1] EC = emulsifiable concentrate; EW = emulsion, oil in water; CS = capsule suspension; SC= suspension concentrate; WT = water dispersible tablet.
[2] Milligrams of active ingredient per square metre of netting.
[3] K-O TAB 1-2-3®

Note: WHO recommendations on the use of pesticides in public health are valid **ONLY** if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/en/.

http://www.who.int/whopes/Insecticides_ITN_Malaria_ok3.pdf

Appendix B

Updated July 2011

WHO recommended long-lasting insecticidal mosquito nets

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

Notes:

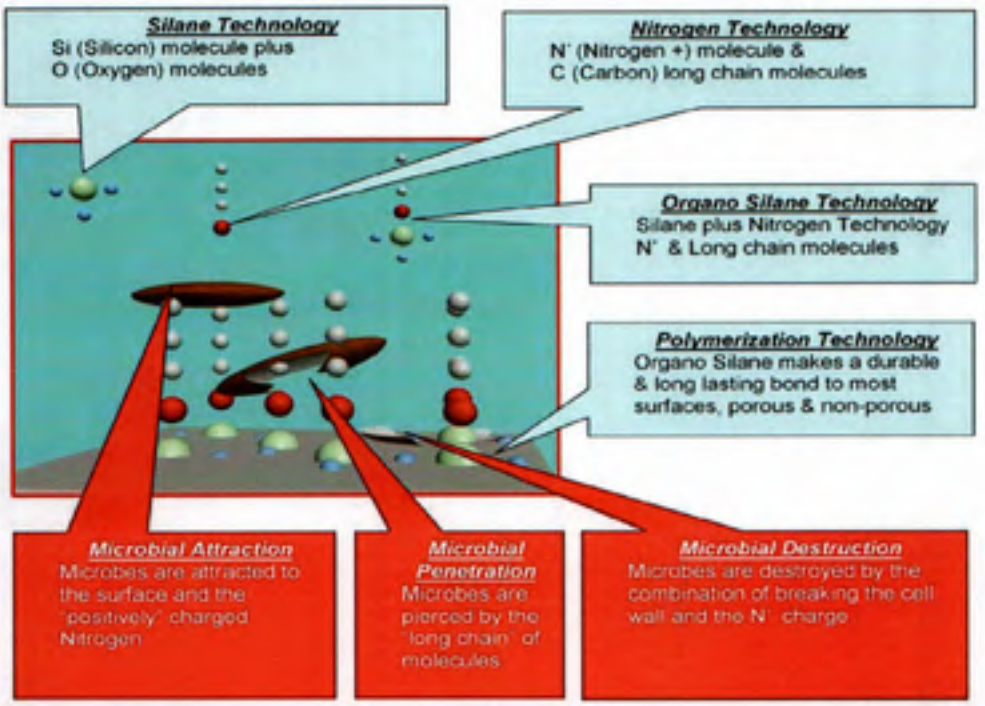
1. Reports of the WHOPES Working Group Meetings should be consulted for detailed guidance on use and recommendations. These reports are available on the WHO homepage on the Internet at http://www.who.int/whopes/recommendations/wgm/en/); and
2. WHO recommendations on the use of pesticides in public health are valid **ONLY** if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available on the WHO homepage on the Internet at http://www.who.int/whopes/quality/newspecif.en/.

http://www.who.int/whopes/Long_lasting_insecticidal_nets_Jan_2011.pdf

Appendix C

Slide from Indusco Distribution of America, Inc. Powerpoint presentation. Received through Dr. David Weber.

Appendix D



Slide from Indusco Distribution of America, Inc. Powerpoint presentation. Received through Dr. David Weber.

Appendix E

Parameter	Technical Consideration	Importance (1-5)	Metric	Unit	Marginal Value	Ideal Value
Protection	User	5	Filtration Efficiency - Fabric structure - Mesh count - Hole diameter - Electrostatic effects (ability to repel or attract droplets)	% efficiency holes/in^2 mm ?????	50 156 2	>50 200 <2
	Community	3	Insecticidal activity	% mosquito's killed	95%	>95%
	Community	3	Bactericidal activity	% bacteria killed	??	??
Comfort	Air Circulation	4	Denier (linear density) Convection flow Gas diffusion Evaporation heat loss	grams/9000m ??? CO2 build-up ???	135	<135
	Moisture vapor permeability	3	???	????	??	??
	Moisture Absorbance	3	Moisture gain of fibers	%	7	<7
Durability	Withstand wear & tear	5	2 years of typical wear and tear due to normal use, laundry, sun rays, ...	Bursting strength in KPa	250	>250
	Dimensional Stability	2	Shrinkage/ stretch	%	5	<5
Small Storage Size	Collapsed Dimensions	2	Collapsed Volume	Length x width x height	18 in x 12 in x 3 in	The lower the better
Affordability	Price	4	Cost	USD	$5	<$5 – 10
Ease of Use	Overall Design	4	time to set up	minute	2	<2
Color	Dye ability Visibility	1 4	color See-through	color	dyed	non-white or patterns

Table describing possible net parameters created by Dr. Annelies Van Rie and Dr. Marian McCord.

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