Fight against malaria: investigating the impact of physical and insecticidal durability on epidemiological outcomes, as well as assessing community acceptability of, and preferences for dual-active ingredient long-lasting insecticidal nets in Tanzania

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Declaration of own work

I **ELIUD ANDREA LUKOLE**, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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The main funding for the randomized controlled trials (RCTs) was provided by the Medical Research Council, Wellcome Trust, and the UK Department for International Development through the joint Global Health Trials scheme, which supported the research activities. The Bill and Melinda Gates Foundation (Seattle, USA) funded my flights to the UK, a stipend in London, and four years of PhD fees. The projects were awarded to Prof. Mark Rowland (Muleba project) and Prof. Natacha Protopopoff (Misungwi project). I extend my gratitude to all for their dedicated efforts.

There are many to thank, but I will be brief. I owe a deep debt of gratitude to my supervisors: Dr. Jackie Cook, Prof. Natacha Protopopoff, and Prof. Immo Kleinschmidt. Their guidance and support have profoundly shaped my journey. A special thanks to Prof. Natacha Protopopoff, who served as my primary supervisor until January 2024, when she concluded her tenure at LSHTM. Her immense support and mentorship introduced me to the malaria field and guided me in project and people management, data handling, and the realities of fieldwork. My heartfelt thanks to Dr. Jackie Cook for her unwavering support, encouragement, and kindness, and to Prof. Immo Kleinschmidt for his critical insights into study analysis and interpretation, always ready to assist. I also appreciate the advice and encouragement from my advisory committee: Prof. Mark Rowland, Dr. Manisha Kulkarni, Dr. Jackline Mosha, and Prof. Jayne Webster. My thanks also go to my upgrading committee, Lucy Paintain, John Bradley, and Thomas Walker, for their valuable guidance.

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Abstract

Since 2017, there has been a considerable scale-up of dual-active ingredient Long-Lasting Insecticidal Nets (LLINs) [dual-AI ITNs], gradually replacing the standard of care (pyrethroid-only LLINs) for the prevention of malaria transmitted by pyrethroid-resistant malaria vectors in Africa. As with other LLINs, dual-AI ITNs are likely to face challenges with their physical and insecticidal durability, which in turn, impacts net coverage and usage over time, ultimately affecting malaria control efforts. This thesis investigates the impact of physical and insecticidal durability on epidemiological outcomes, as well as assessing community acceptability of, and preferences for the dual AI LLINs namely: Royal Guard [containing pyriproxyfen and alpha(α)-cypermethrin], Interceptor G2 [chlorfenapyr and α-cypermethrin], and Olyset Plus [piperonyl-butoxide (PBO) and permethrin]. Data from two cluster-randomised controlled trials (RCTs) conducted in the rural districts of Muleba and Misungwi, Tanzania were used to assess the physical durability of dual-AI ITNs, the impact of LLIN damage on malaria infection, and perceptions of dual-AI LLIN compared to standard LLINs (pyrethroid-only nets) over the recommended lifespan of the net. Eleven cross-sectional surveys (six in Muleba & five in Misungwi) of 26,816 children aged 0.5-14 years (N=4,337 in Muleba & N=22,479 in Misungwi) from 14,433 households (N=1,779 in Muleba & N=12,654 in Misungwi) took place between January 2015 and February 2022. A total of 36 focus group discussions (FGDs) (N=17 in Muleba & N=19 in Misungwi) and 44 In-depth Interviews (N=14 in Muleba & N=30 in Misungwi) were conducted as part of qualitative data collection.

The data showed that:

- There was an association between fabric condition and malaria case incidence- though this differed by net type. For instance, in Olyset Plus and Interceptor G2 in poor condition, malaria incidence was lower than for intact/good pyrethroid-only LLINs during the first year after their distribution. However, this association was not present in the second year after their distribution, suggesting that the effectiveness of the dual-AI ITNs decreased with waning insecticide concentrations on nets over time.

- The net attrition rate was very high in both trials with approximately 83% of the LLINs lost after 3 years, with a functional survival time far below the expected 3 years for all net types (1.6-1.9 years). Insecticidal content declined rapidly with only 3% of PBO and 45% of permethrin in Olyset Plus; 8% of chlorfenapyr and 28% of alpha-cypermethrin in Interceptor G2; and 27.8% of pyriproxyfen and 62% of alpha-cypermethrin remaining in the LLINs after 3 years.

- Community-wide protection (benefits to others in the community not using nets) was found when community net use was above 40% across dual-AI study arms. Lower malaria risk was also observed in non-users living in villages with low usage of Interceptor G2 compared to high coverage of pyrethroid-only nets.
- In both trials, LLIN effectiveness and perceived physical characteristics (fabric durability, fibers, texture) influenced opinions towards LLIN. The communities preferred polyester nets to polyethylene nets. Polyethylene LLINs were more prone to misuse than polyester nets, with higher misuse in Misungwi compared to Muleba.

It was found that users of LLINs are much better protected than non-users regardless of net age, physical condition, or type. However, for better protection and upon the availability of resources, dual-AI ITNs should be considered as they provide superior protection to standard LLINs, even at low usage levels (Interceptor G2) or when they are torn (Interceptor G2 and Olyset Plus). For the sustainability of malaria control reliant on dual-AI ITNs, should be made more durable than the current ones which do not last for 3 years. Blue, rectangular, and polyester LLINs are preferred by the communities. While pilot studies to assess preferences before mass distribution could optimize outcomes, practical limitations in procurement timelines and availability of net types may make such studies infeasible for each campaign. Instead, integrating periodic evaluations of preferences within broader malaria control efforts may provide an actionable alternative. Despite challenges, findings suggest that dual-AI ITNs provide superior protection and are generally well-accepted. Recommendations for improving durability and aligning community preferences with net design are critical for sustaining malaria control efforts.

Thesis structure

This thesis adheres to the research paper format, encompassing four results chapters. Chapter one provides background. Chapter two outlines the aim, objectives, and hypothesis. Chapter three presents material and methods that include the study design, development of data collection tools, data management, and analysis strategy, offering additional methodological details beyond those in the results papers. Chapter four evaluates data from the Muleba Randomised Controlled Trial (RCT), examining the median survival of Olyset Plus and Olyset nets, the impact of holed and aging LLINs on malaria prevalence, and the durability of LLINs. This chapter is based on a peer-reviewed article published in PLOS Global Public Health (October 22, 2022, https://doi.org/10.1371/journal.pgph.0000453). Chapter five investigates the relationship between the physical condition of dual-AI ITNs and malaria prevalence/incidence, published in Malaria Journal (June 28, 2024, https://doi.org/10.1186/s12936-024-05020-y). Chapter six assesses the community impact of mass distribution of dual-AI ITNs on malaria prevalence (submitted to BMC Public Health). Chapter seven uses data from Muleba and Misungwi RCTs to analyse users' acceptability of, and preferences for dual-AI ITNs (to be submitted to PLOS one). Chapter eight presents the general discussion that synthesizes the study findings within the context of existing research, underscoring their contribution to the field. Supplementary materials, including ethical approvals, data collection tools, and additional results, are provided in the Appendix.

The Candidate's role

This thesis is part of the Pan-African Malaria Vector Research Consortium (PAMVERC) Malaria Prevention Trials conducted in Muleba district, Kagera region, and Misungwi district, Mwanza region; in Tanzania, East Africa. For my PhD, I conducted epidemiological and malaria behaviour survey analyses across both trials. I spent two years in Muleba as a field supervisor and another two years as deputy trial manager under the supervision of Prof. Natacha Protopopoff, overseeing the planning and execution of the study. When I joined the project in August 2014, the study protocols and sub-protocols were already established, pilot work completed, and census mapping finished.

My initial task involved community mobilization and sensitization to inform village leaders about the project. I also contributed to amending sub-protocols where necessary. Under Prof. Protopopoff supervision, I led all five rounds of malaria prevalence cross-sectional surveys and all rounds of entomological surveillance, specifically overseeing morning resting, light trap, and tent trap mosquito collections, as well as on-site laboratory testing and a Phase III LLIN durability study. I managed the financial, logistical, and operational aspects of the field station.

Following training by Mr. Ramadhan Hashim, the senior data manager at Mwanza Intervention Trial Unit (MITU), and with Prof. Protopopoff support, I designed the project database using Microsoft Access and Open Data Kit. I piloted household questionnaires, data collection forms, and consent forms for epidemiological and entomological surveys and trained all fieldworkers on these protocols under Prof. Protopopoff guidance. Clinical staff were trained and supervised by Senior Laboratory Manager Ms. Alexandra Wright and Senior Epidemiologist Dr. Jackline Mosha. I coordinated the preparation and day-to-day activities of the surveys with support from Dr. Mosha, Ms. Wright, and Prof. Protopopoff.

From 2018 to 2022, I served as the project manager for the Misungwi Randomised Controlled Trial (RCT), overseeing all research activities from inception to completion. I led the mapping of critical areas, including schools, health facilities, and villages, and coordinated a mosquito surveillance pilot in April-May 2018, examining the distribution and abundance of Anopheles mosquitoes across all 116 villages in the district to inform the selection of the study villages.

My contributions to each results chapter are detailed at the beginning of those chapters. I supervised the data collection, analysed the data, wrote the initial drafts of all papers included in this thesis, and incorporated feedback from co-authors. Detailed comments and writing support were provided by Prof. Natacha Protopopoff, Dr. Jackie Cook, and Prof. Immo Kleinschmidt.

Acronyms and abbreviations

AI	Active Ingredient	NIMR	National Institute for Medical Research
ANC	Antenatal care	NMCP	National Malaria Control Program
CHW	Community health worker	ODK	Open Data Kit
cRCT	Cluster Randomised Controlled Trial	PA	Public advertisement
CRF	Case report form	PBO	Piperonyl Butoxide
DDT	Dichloro-diphenyl-trichloroethane	PCA	Principal component analysis
DRC	Democratic Republic of the Congo	PDA	Personal Data Assistant
EPI	Expanded program on immunization	pHI	proportionate hole index
FGD	Focus Group Discussion	PMI	President's Malaria Initiative
GEE	Generalized Estimation Equations	PPF	Pyriproxyfen
GF	Global Fund	PQ	Prequalification
GMEP	Global Malaria Eradication Programme	PQT/VCP	Vector Control Product Assessment Team in the Prequalification Unit
GPS	Global Positioning System	PY	Pyrethroid
HAS	Hole surface area	RBM	Roll Back Malaria
HPLC	High-Performance Liquid Chromatography	RCT	Randomised Controlled Trial
ID	Identification	REA	Rural Energy Agency
IDI	In-depth Interview	s.l	sensu lato
IEC	Education, and communication	s.s	sensu stricto
IRS	Indoor Residual Spraying	SBCC	Social and Behavioural communication change activities
ITN	Insecticidal Treated nets	SES	Socio-economic status
KAP	Knowledge, Attitude, and Practice	SNP	School Net Program
KCMUCO	Kilimanjaro Christian Medical University College	SSA	Sub-Saharan Africa
LLIN	Long-lasting insecticidal nets	TCDC	Tanzania Communication and Development Centre
LMIC	Low- and middle-income countries	UK	United Kingdom of Great Britain and Northern Ireland
LSHTM	London School of Hygiene and Tropical Medicine	USAID	United States Agency for International Development
MIS	Malaria Indicator Surveys	VCAG	Vector Control Advisory Group
VCP	Vector Control Product	WHO	World Health Organisation
MRDT	Malaria Rapid Diagnostic Test	WHOPES	WHO Pesticide Evaluation Scheme

1 Chapter 1: Background

Malaria, one of the most ancient diseases afflicting humankind, remains a significant global health threat, responsible for an estimated 608,000 deaths in 2023 [1], predominantly affecting children under five years old (Figure 1.1). The disease's burden is disproportionately concentrated in four African nations: Nigeria (31.1%), the Democratic Republic of the Congo (11.6%), Niger (5.6%), and Tanzania (4.4%) [1]. Although malaria is both preventable and treatable [2], the adaptability of the parasite and its anopheline vector necessitates continuous advancements in treatment and prevention strategies [3]. The primary prevention strategy focuses on preventing mosquito bites that transmit the parasite to humans. This can be achieved through physical barriers such as house screening [4-10] and bed nets [11], and by incorporating larvicidal [12, 13] or insecticidal agents to reduce mosquito populations or their longevity. Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have been core tools in malaria vector control for decades [1, 14]. Despite the development of various mosquito control tools, none offer complete protection, and vector adaptation has rendered some methods less effective over time. Therefore, vector control strategies must be multifaceted, employing multiple tools simultaneously or integrating novel approaches that combine different insecticides [3].

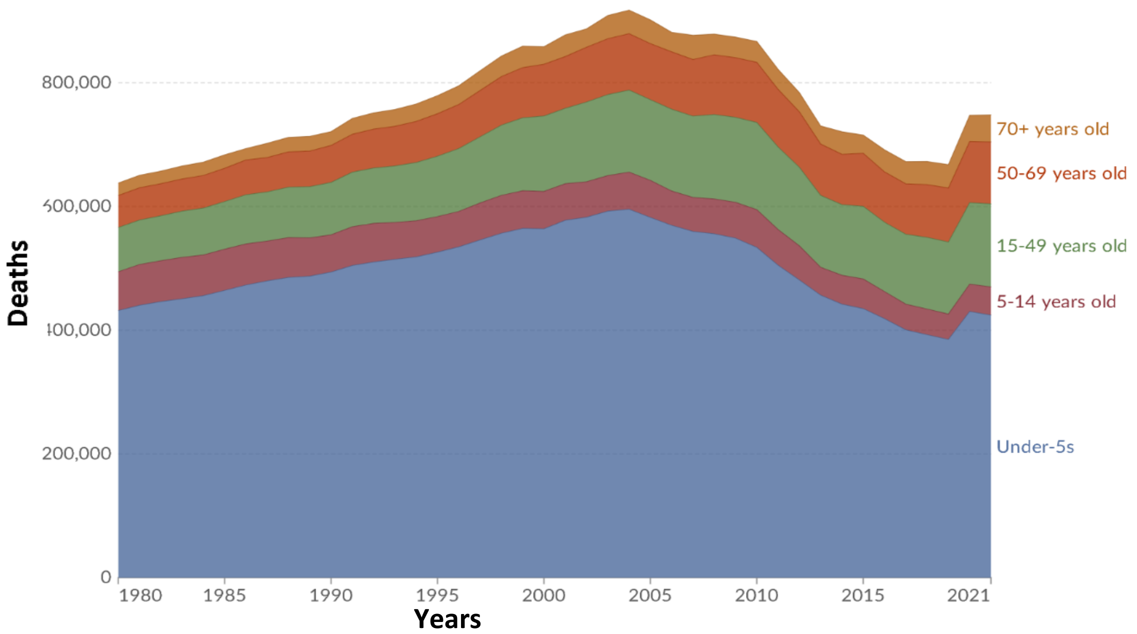

Figure 1.1: World Malaria deaths by age (estimated annual number of deaths from malaria) (Source: IHME, Global Burden of Disease, 2024)

1.1 The global burden of malaria cases and deaths

Between 2000-2015, there was a 29% and 18% decline in malaria deaths and cases globally [1]. However, since 2015 there has been a rebound in malaria cases and deaths with an increase of 8% in cases and 4% deaths globally by 2022 [1]. This is far from the aims of the Global Technical Strategy for Malaria 2016-2030, which had the ambitious goal of reducing the malaria mortality rate and case incidence by 40% in 2020 compared with 2015 [15, 16]. The rebound in malaria transmission is attributed to several factors, including pyrethroid-insecticide resistance, the COVID-19 pandemic, plateauing of funding, drug resistance, and climate change [1]. Among the strategies expected to mitigate the impact of pyrethroid resistance was the scaling-up of new-generation insecticide-treated nets, including dual-active ingredient long-lasting insecticidal nets (dual-AI ITNs) and nets combining a pyrethroid insecticide and a synergist [3].

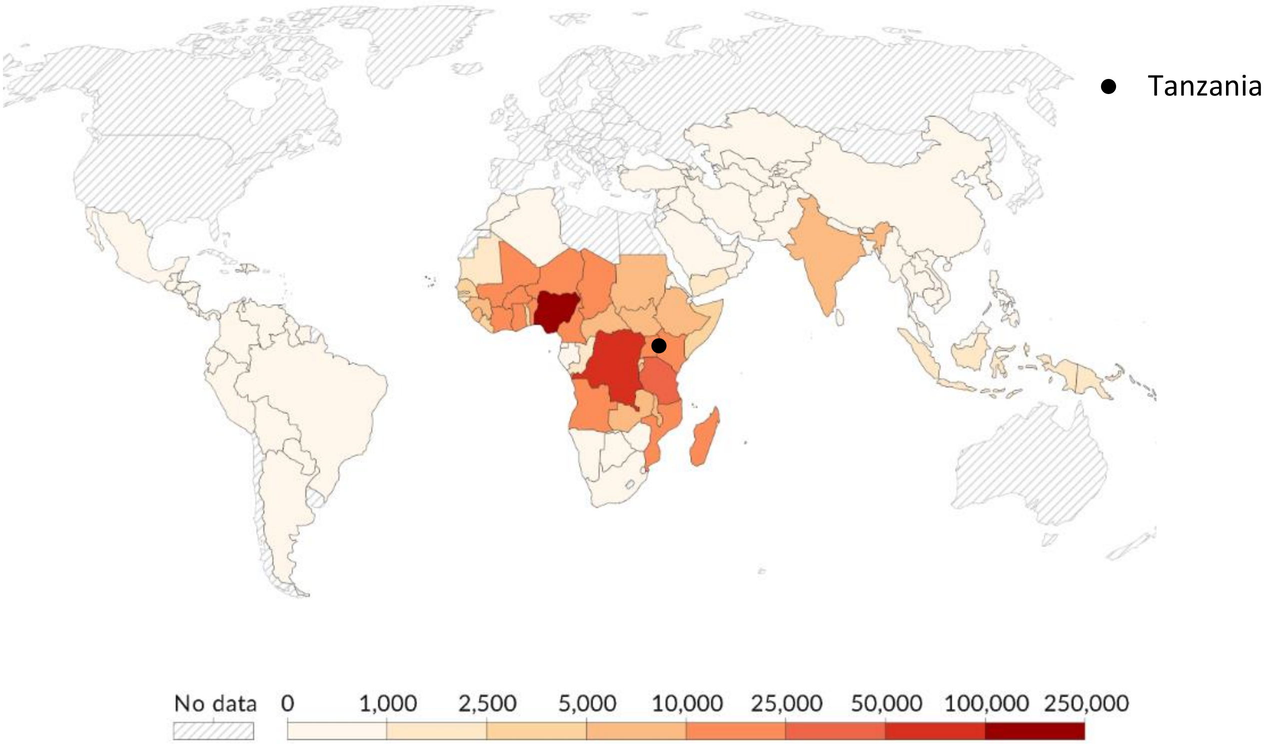

Figure 1.2: Estimated annual number of deaths from malaria. [Source: World Health Organization - Global Health Observatory, 2024]

1.2 Economic burden of malaria in low- and middle-income countries (LMIC)

Malaria is intrinsically linked to poverty, disproportionately affecting the most impoverished communities and significantly hindering development efforts [17-19]. In endemic African countries, malaria is estimated to impose an economic growth penalty of up to 1.3% [20]. For instance, Tanzania allocates approximately US$ 131.9 million annually to malaria control and treatment efforts [21]. Low- and middle-income countries encounter numerous challenges in malaria control, including insufficient human, financial, and material resources; inefficiencies within healthcare systems that compromise the quality of services and limit access to timely diagnosis and treatment; the absence of an effective disease surveillance system; and a deficient health education infrastructure [22].

There are both direct and indirect costs related to malaria. Directly, malaria can affect both personal and public expenditures. Public expenditures include spending by the government on maintaining health facilities and health care infrastructure, publicly managed vector control, education, and research which can cost up to $125.2 per person per episode [20]. Family/household expenditures ranging between $38.1 and $182 [20] include individual or family doctors' fees, antimalarial drugs, transport to health facilities, money to support the patient, and sometimes an accompanying family member during hospital stays [20]. Indirectly, it causes time loss in terms of family time spent caring for the sick [up to 9.2 days of caregiver absenteeism] [20], school absenteeism [23], loss of productive time [24], time spent by families and communities to grieve for the dead, and funeral costs. As the foremost cause of illness in many rural areas of sub-Saharan Africa, malaria undermines agricultural productivity and incomes, especially because the peak period of transmission often coincides with the peak period of agricultural activity and labour operations. The combined effects of malaria-caused mortality, morbidity, and debility on the household labour force and on community members as a whole manifest in reduced quantity and quality of labour inputs, reduced economic output, and resource under-utilization.

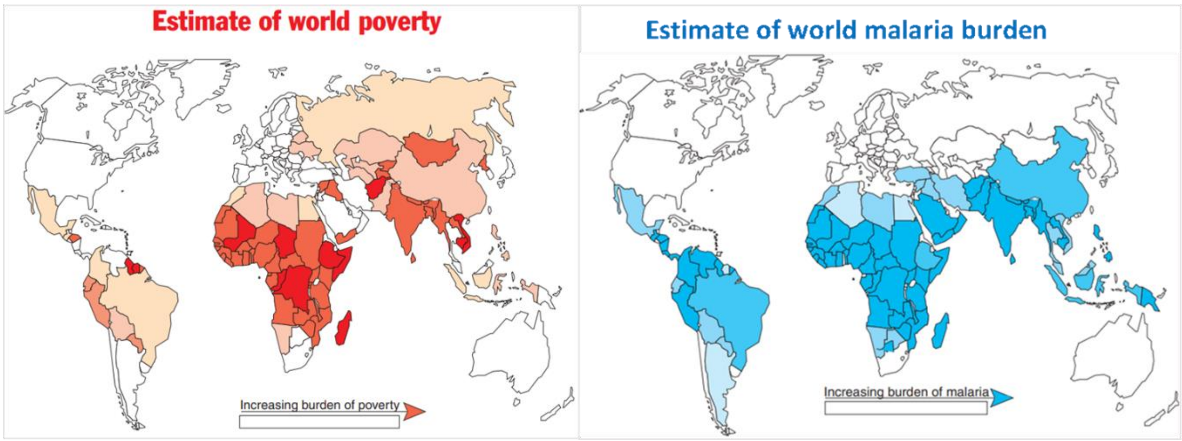

Figure 1.3: Correlation between malaria and poverty [Source: RBM data/J. Sachs 1999]

1.3 Malaria transmission

Malaria is caused by a protozoan parasite of the genus *Plasmodium,* which is transmitted to human hosts exclusively by an infectious bite of female *Anopheles* mosquitoes [25, 26]. *Plasmodium* species that can infect humans include *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. The most prevalent and dangerous malaria parasite in sub-Saharan Africa (SSA) is *P. falciparum* and it is responsible for 99.7% of estimated malaria cases in SSA[1]. The lifecycle of *P. falciparum* parasites occurs in distinct stages, two of which occur in the human host and one in the mosquito vector [25].

1.3.1 The parasite- lifecycle within vectors

The gametocytes, male (microgametocytes) and female (macrogametocytes) are ingested by an *Anopheles* mosquito during a blood meal from an infected human [26]. The parasites' multiplication in the mosquito is known as the sporogonic cycle [27]. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes. The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle. *Plasmodium* parasites require at least 8-12 days to develop from gametocytes into mature sporozoites within the vector mosquito's salivary glands (however this is highly dependent on temperature) [28]. This means that most malaria transmission is carried out by mosquitoes that are at least 10 days old and have taken several previous blood meals at intervals of 2 to 5 days [29].

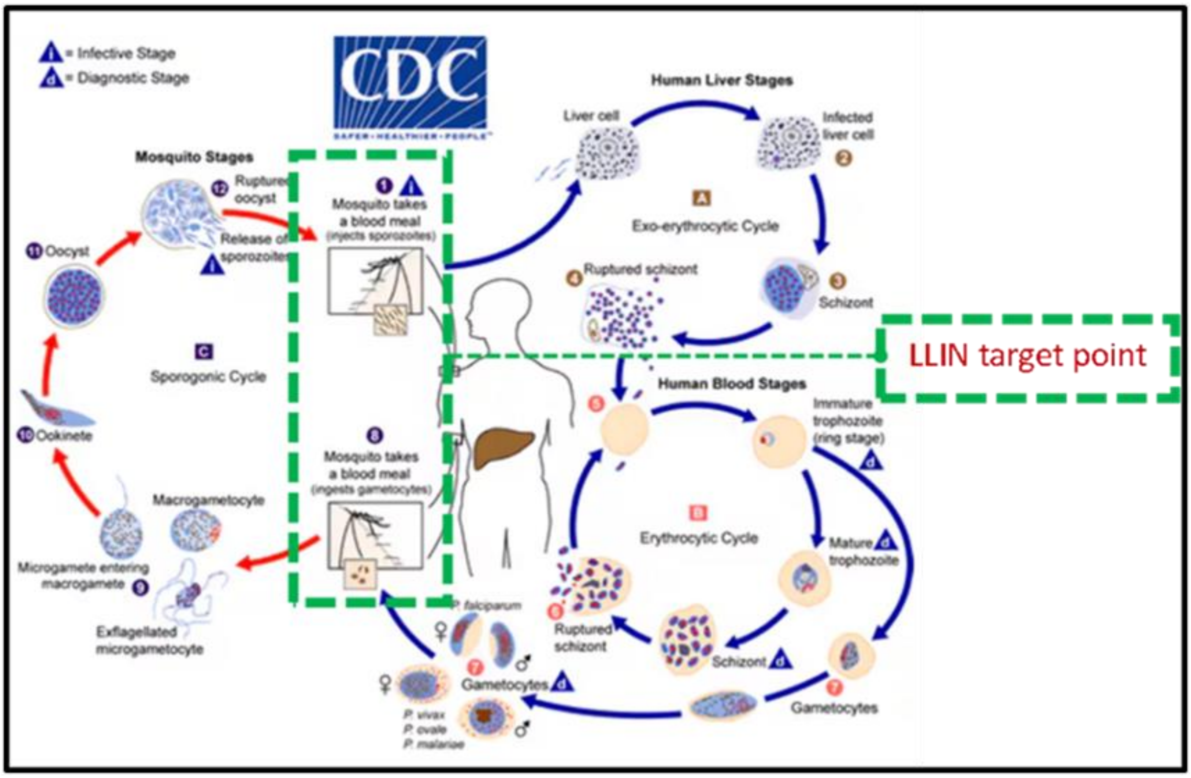

Figure 1.4: Malaria cycle [Source: Center for Disease Control and Prevention]

1.3.2 The parasite- lifecycle within humans

During a blood meal, a malaria-infected female *Anopheles* mosquito injects saliva containing sporozoites into the human host [30-32]. Sporozoites infect liver cells and mature into schizonts, which rupture and release merozoites [33]. After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony) and develop into merozoites, which go on to infect red blood cells[31]. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites. Some parasites differentiate into sexual erythrocytic stages (gametocytes). Blood-stage parasites are responsible for the clinical manifestations of the disease [34, 35].

There is overwhelming evidence of asymptomatic malaria transmission, where humans carry the malaria parasite without exhibiting clinical symptoms of the disease and hence do not seek treatment [36-39]. Asymptomatic individuals are critical in the spread and persistence of malaria. It complicates disease control efforts as they do not show clinical symptoms of malaria and cannot be detected through fever-based methods. Since they do not seek treatment, they serve as hidden reservoirs for malaria parasites, which can be picked up by mosquitoes and transmitted to other humans.

The study by Rodriguez-Barraquer et al. (2018) [40] highlight the pivotal role of asymptomatic parasitaemia in sustaining malaria transmission, particularly in high-transmission regions. Asymptomatic infections, facilitated by naturally acquired immunity, allow individuals especially older children to harbour parasites without clinical symptoms, serving as a significant hidden reservoir. The development of immunity to malaria, both anti-parasite immunity (ability to control parasite densities) and anti-disease immunity (ability to tolerate parasite presence without fever) is age-dependent and shaped by the intensity of malaria transmission. In high-transmission areas, symptomatic malaria incidence peaks in young children, who have not yet developed immunity. Older children, having acquired significant anti-disease immunity through repeated exposure, exhibit the highest prevalence of asymptomatic infections. Interestingly, children in low-transmission areas acquire immunity more efficiently than those in moderate-transmission settings, likely due to reduced interference from frequent high-density infections. These findings underscore the need for advanced diagnostics and community-based strategies to address asymptomatic carriers and disrupt transmission cycles effectively.

1.3.3 Transmission of malaria by *Anopheles* vectors

The intensity of transmission depends on factors related to the parasite, the vector, the human host, and the environment. Transmission is more intense in areas where the mosquitoes have a longer lifespan, to favour the completion of the gonotrophic cycle and the acquisition of a second blood meal from a different individual [41]. The most important *Anopheles* species responsible for transmitting malaria in SSA include *Anopheles gambiae* and *An. funestus* complexes. Transmission intensity is dependent on the distribution and survival of these *Anopheles* mosquitoes and temperatures need to be high enough for the sporogonic cycle to be completed within the mosquito, but not so high that the mosquito life-span is too short for sporogony.

1.4 Vector control: Bed nets

1.4.1 History of bed nets, Insecticidal Treated Nets (ITNs) and Long-Lasting Insecticidal Nets (LLINs)

Insecticidal Treated Nets (ITNs) and Long-Lasting Insecticidal Nets (LLIN) are the principal malaria vector control tools in Africa [42-44]. The practice of treating nets with insecticide goes back as far as World War II when the Soviet, German, and U.S. armies started impregnating bed net materials using insecticide (pyrethrum) to prevent vector-borne diseases [42]. Pyrethrum is derived from the dried and crushed flower heads of [45]. It is expensive as it is limited in natural supply [46]. Because of that, a synthetic insecticide from pyrethrum called pyrethroid was developed in 1949 [46]. Synthetic pyrethroids are more stable to light, more toxic, and last longer in the environment than natural pyrethrum [47]. Pyrethroid insecticides have high insecticidal activity and low mammalian toxicity [42, 47]. Bed nets were retreated every 6 to 12 months with a pyrethroid class of insecticides (permethrin, deltamethrin, lambda-cyhalothrin, or alpha-cypermethrin) to maintain an appropriate level of protection. However, re-treatment was difficult to implement and eventually resulted in low ITN ownership as many nets remained untreated [48]. Because of these logistical constraints, net distribution was limited to vulnerable populations, such as pregnant women and children <5 years [49]. At the beginning of the 2000s, LLINs were developed to counteract the challenges posed by the need to retreat bed nets [50, 51]. After the development of long-lasting nets which were treated directly by the manufacturers, net distributions were scaled to the wider population [52].

Box 1.1: Basic differences between a net, ITN, LLIN, and Dual-AI LLIN

Nets (Bed nets)	Insecticide-treated net (ITN)	Long Lasting Insecticidal Treated Net (LLIN)	Dual Active Ingredient Long Lasting Insecticidal Treated Net (Dual-AI LLIN)
Nets (bed nets) are made from open-meshed fabric intricately twisted, knotted, or woven at consistent intervals.	ITNs are bed nets that have been treated with a single insecticide (pyrethroid class), that can either	LLIN is an ITN that is specifically designed to maintain their insecticidal properties for an extended period	Dual-AI ITNs are LLINs that contain a combination of insecticides that work differently

They were first used as far back as the 5th century by Egyptian fishermen to protect themselves from gnats and nocturnal biting flies [53].	physically block mosquitoes and/or kill or repel them. When the term was first used, historically they referred to nets that required re-treatment every 6-12 months. However, the term is now sometimes used interchangeably with LLIN.	without the need for re-treatment as they were permanently treated by the manufacturer. They are supposed to retain their efficacy for about 3-5 years or withstand 20 washes.	from traditional pyrethroids. They are designed to address the challenges of insecticide resistance in mosquito populations. Some contain synergists, which enhance the potency of the insecticide.

In this thesis, I use 'LLINs' for pyrethroid-only nets, and 'ITNs' for all dual-AI nets, including pyrethroid-PBO, pyrethroid-pyriproxyfen, and pyrethroid-chlorfenapyr nets as per WHO guidelines for malaria [3].

1.4.2 How do LLINs work?

ITN, and now LLINs, treated with pyrethroids have been the core intervention for malaria control for over 30 years in malaria-endemic countries [54]. Mosquitoes are killed and/or have a reduced lifespan when they come into contact with insecticide on the netting. If the lifespan is reduced below the 10 days necessary for the sporogonic cycle to complete, this will likely reduce malaria transmission [11]. As nearly 90% of the exposure occurs indoors [55], ITNs/LLINs are designed to mainly target mosquitoes who feed and rest indoors at night when people are sleeping- they work by reducing human-vector contact. This can be done by blocking physical contact as mosquitoes cannot penetrate the mesh. Also, some pyrethroid insecticides in the ITNs/LLINs have excito-repellency properties that can reduce the frequency with which endophilic (mosquitoes that rest indoors, inside human dwellings) and anthropophilic (mosquitoes who prefer human hosts) mosquitoes successfully acquire human blood, by diverting them to feed on non-human hosts, resulting in a reduction in transmission [56].

1.4.3 Personal protection vs community-wide effects of LLINs

The proportion of individuals using LLINs within a community impacts whether these LLINs offer solely personal protection or extend to broader community-level effects. LLINs provide personal protection by reducing human-vector contact, as the nets inhibit mosquito-biting success. Mosquitoes require a blood meal to develop eggs; therefore, by preventing them from acquiring blood, LLINs effectively block egg production unless mosquitoes seek non-human hosts. Additionally, for the cycle to continue, mosquitoes need to feed on infected human blood to further transmit the malaria parasite to uninfected individuals; thus, by physically blocking, repelling, or killing mosquitoes during their blood meal attempts, LLINs lower the transmission rate[29]. When LLIN coverage is sufficiently high within a community, leading to a substantial proportion of the population sleeping under LLINs, a cumulative area-wide reduction in transmission is expected due to decreased mosquito density and feeding frequency[57]. This effect benefits even those who do not use nets [49, 57, 58].

This concept ties back to the foundational work of Ross and Macdonald, who developed a theory for the dynamics and control of mosquito-transmitted pathogens [59]. Their models elucidate how interrupting the mosquito-human transmission cycle can reduce the basic reproduction number (R0) of malaria, ultimately leading to the decline of the disease within a community. However, determining a specific threshold at which the community effect begins or ends is challenging due to the variability in contextual factors influencing the relationship between LLIN usage and malaria transmission impact [60]. Some findings argue that there is no minimum threshold for community effect [61], while others report its presence even at low usage levels, such as 15%, continuing up to at least 85% usage [60]. For indoor residual spraying (IRS), which does not provide personal protection, WHO recommends that at least 80% of target households must be sprayed to achieve community-wide protection[62, 63]. However, the threshold for LLINs remains contentious. A model by Killeen et al. suggests that a community usage rate between 35% and 65% may be sufficient to achieve a community effect [49]. Similarly, a large field trial in Kenya indicated that a minimum of over 50% population usage of LLINs is necessary to realize community protection [57]. The WHO and other programs emphasize the importance of high LLIN usage levels, but the critical question remains: what coverage level is sufficient?

1.4.4 LLIN efficacy

Since the introduction of pyrethroid insecticides for treating nets in the 1980s, several randomized controlled trials of ITNs and LLINs have been conducted to evaluate the superior efficacy of pyrethroid treatment on nets, compared to untreated nets. The first comprehensive review of these trials, published in 1998 [44] and another in 2004 [43], concluded that insecticide-treated nets could reduce child mortality by one-fifth and halve malaria episodes in sub-Saharan Africa compared to no net. In 2015, Bhatt and colleagues analysed malaria coverage trends from 2000 to 2015 using data from Malaria Indicator Surveys (MIS) [14]. They quantified the attributable effects of malaria control efforts, concluding that LLINs were twice as effective as untreated nets, offering greater than 70% protection to users compared to non-users [14]. Furthermore, their models predicted that between 2000 and 2015, 68% of malaria cases were averted through the use of LLINs [14]. However, the effectiveness of LLINs is compromised by the widespread emergence of pyrethroid resistance among mosquito populations [64].

1.5 Challenges with LLINs

1.5.1 LLIN coverage

In the early 2000s, ITN distributions were targeted to vulnerable groups, such as pregnant women and infants [65]. Distributions were subsequently expanded to include children less than 5 years old. As a result, the nets delivered in SSA increased from 5.6 million in 2004 to 229.5 million in 2010. And, people using ITNs in SSA rose steadily from 1.8% in 2000 to 18.5% in 2007 and reached 30% in 2010 [66]. Before 2010, the majority of nets distributed were conventional ITNs, which required regular re-treatment with insecticides to maintain their effectiveness. From 2010 onward, mass distribution campaigns began targeting the entire at-risk population, accelerating the replacement of traditional ITNs with more durable and maintenance-free LLINs [66]. This translated, for example, into an increase in the net coverage indicators. During the 2010-2015 period, the estimated proportion of the population at risk sleeping under LLINs increased from 30% in 2010 to 53% in 2015 and remained at this level until 2019 in SSA [67]. The net coverage indicators show very promising progress from 2010, but no remarkable changes since 2015, despite bulk purchases of LLIN in the years afterward.

For instance, the household ownership of at least one LLIN in SSA which stood at 5% in 2000 [67], increased to 72% in 2018 and remained unchanged afterwards (68% in 2019 [68] and 72% in 2023 [1]). The proportion of people with access to nets (assuming one LLIN protects two persons) increased significantly from 1% in 2000 to 57% in 2018, but then declined to 36% in 2019, with a slight recovery to 40% in 2023 [1]. Similarly, the percentage of people who slept under LLINs the previous night rose from 2% in 2000 to 50% in 2018, before dropping to 46% in 2019, and then inching up to 49% in 2023 [1, 67-69]. These figures suggest that regular usage of LLINs across populations remains below 50%. The decline in both access and usage of LLINs could be attributed to funding gaps, which may have contributed to the reduced coverage of these interventions [1].

However, gaps persist in translating ITN/LLINs access into consistent use. The ITN use:access ratio, which measures the proportion of individuals using ITNs among those with access within their household, highlights this challenge. For instance, recent reports show that across SSA, the ITN use:access ratio varies widely ranging from 0.79 in 2010 to 0.87 in 2022, with some countries achieving only 60% usage despite high access [70]. This indicates that even when nets are available, behavioural, cultural, and logistical barriers prevent optimal usage. Addressing these factors is critical, as disparities in ITN use exist based on geographic location, age, gender, urban/rural residence, and wealth quintiles. Enhanced behavioural interventions and targeted campaigns are essential to bridge this gap and maximize the protective effects of ITNs/LLINs.

1.5.2 Acceptability of, and preferences for, LLINs

The acceptability, and preference for different types of LLINs in SSA are shaped by a complex interplay of sociocultural, economic, environmental, and LLIN-related factors like material texture (polyethylene/polyester), mesh size, net size, shape, and colour [58]. The success of LLIN programs depends in part on the type of net that is being distributed. A program that identifies community preferences in advance is most likely to succeed [71].

Studies have shown that the type of net plays a crucial role in determining its acceptability. For example, Grietens et al. (2013) found that certain undesirable attributes of the Olyset Net, such as large mesh size, transparency, and perceived ineffectiveness in protecting against mosquitoes and other insects, significantly impaired net use [72]. In Benin, it was observed that users replaced study-issued polyethylene ITNs with non-study polyester LLINs, indicating a clear preference for specific textile types [73]. Despite these findings, a review by Koenker and Yukich (2017) concluded that net characteristics were generally not the primary reason for non-use. Instead, they identified factors such as mosquito biting nuisance and heat discomfort as the main deterrents [74]. Nonetheless, the perception of malaria risk, traditional beliefs, and community norms also play significant roles in influencing LLIN usage. Communities with a heightened perception of malaria risk tend to use LLINs consistently, regardless of their acceptability and preference [71]. Conversely, in some areas, traditional beliefs regarding the causes and seriousness of malaria can reduce the perceived need for LLINs, resulting in lower usage rates [75]. Moreover, socioeconomic status appears to shape preferences for LLINs/ITNs within communities, with wealthier households often preferring conical nets [74], and white nets for their aesthetic appeal, while less affluent individuals tend to favour dark colours [71].

1.5.3 LLIN physical state and durability

Polyethylene and polyester fibres are commonly used for making LLINs due to their strength and resistance to tears and wear [76, 77]. However, inconsistencies in manufacturing processes have led to variations in net quality, which affects their longevity and bio-efficacy [78]. LLIN functional survival, as termed by WHO, is characterised by the LLIN/ITN remaining in the household, in an acceptable physical condition, and retaining biological activity (giving protection against mosquitoes). The physical functional survival of LLINs (whether pyrethroid-only LLINs or dual-AI ITNs) is recommended to be at least 3 years under field conditions [79, 80]. While the pyrethroids on the LLINs may maintain biological activities for 3 years [50], there have been studies suggesting that not many nets stay in good physical condition for the same amount of time, meaning that the effectiveness of LLINs/ITNs is compromised. There is huge variability in the median survival (lifespan) of the LLIN/ITN between and within locations in Africa (Figure 1.5). The median survival of LLINs ranges from approximately 0.9 to 5.3 years, with notable variability within the same LLIN types. When comparing fabrics, polyester LLINs have slightly longer median survival (2.8 years) than polyethylene LLIN/ITNs (2.3 years), though there is significant overlap (Figure 1.5). The LLIN/ITN durability also varies significantly by country, with LLINs in Nigeria showing a median survival of around 4.0 years [81, 82], Mozambique [83] and Burkina Faso [84] (2.73 years), Senegal (2.71 years) [85], Tanzania (2.07 years) [86-89], DRC (1.9 years) [90], and the lowest in Ethiopia (1.68 years) [91, 92].

The durability of LLINs directly impacts their effectiveness in controlling malaria [93, 94]. When LLINs degrade prematurely, their protective efficacy diminishes, leading to increased exposure to mosquito bites and a higher risk of malaria transmission [93]. This not only undermines the health benefits of LLINs but also requires a significant amount of resources, as LLINs need to be replaced or replenished more frequently than planned [95, 96]. Moreover, the perception of LLIN durability among users can affect their acceptance and proper usage. If communities perceive that nets do not last as long as expected, they may be less likely to use them consistently [97], further weakening malaria control efforts. To enhance the durability of LLINs, it is reported that educating individuals on the proper use and maintenance of LLINs-such as correct methods of washing, drying, caring for, and repairing LLINs, can significantly extend their lifespan [82].

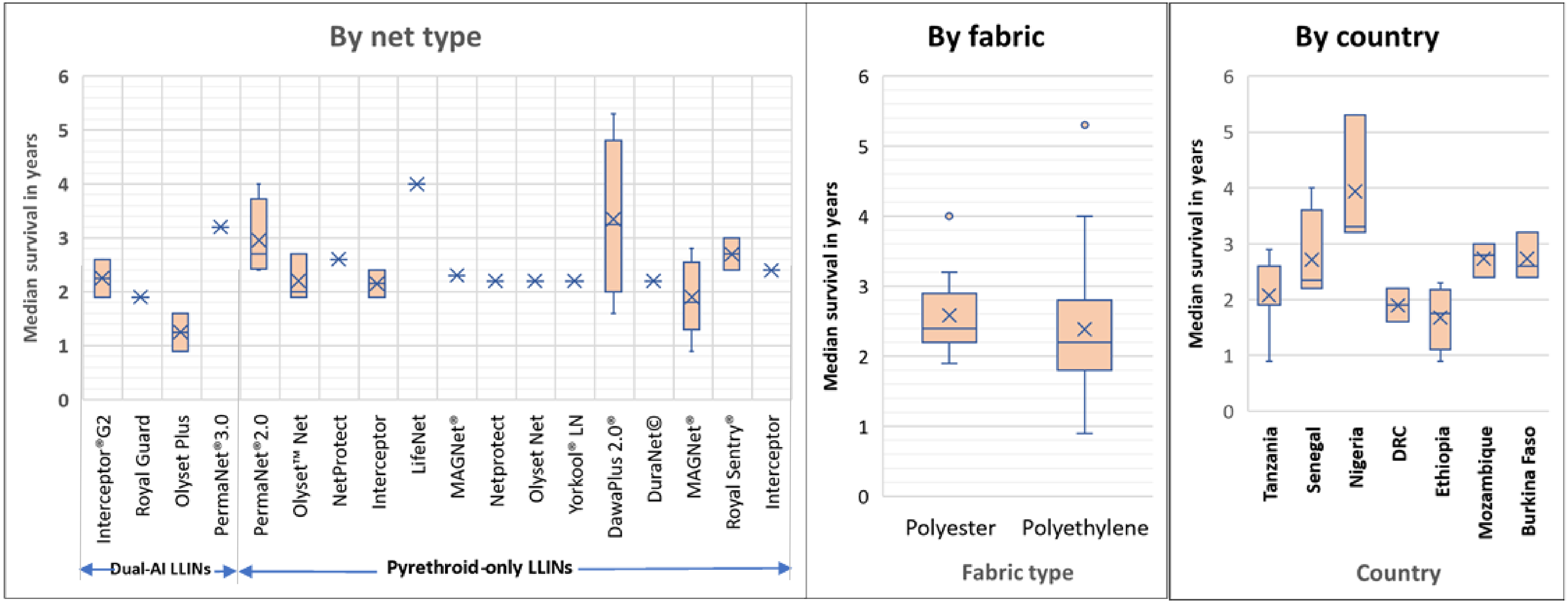

Figure 1.5: Box and Whiskers plots illustrating variations in median survival in year between LLIN products in Africa: by country, net type and fabric (created by Eliud Lukole using results from Tanzania [86-88, 97], Nigeria [81, 82, 97], DRC [90, 97], Mozambique[83, 97], Senegal [85], Benin [73], Burkina Faso [84], and Ethiopia [91, 92]

Legend of the figure

1. X =mean (average)
2. _= the median
3. Box=the interquartile range (IQR),
4. The dots = outliers (survival significantly higher than the rest of the net)

1.5.4 Pyrethroids and insecticide resistance: impact on LLINs

Pyrethroids were the only class of insecticide deemed safe and effective to use for treating bed nets for many years [47]. Reports of growing pyrethroid-resistant mosquito populations in recent years have raised concerns that the effectiveness of LLINs might be compromised [98]. The rapid development and spread of high-intensity pyrethroid resistance called for the deployment of new tools that combine more than one insecticide [99]. Pyrethroid resistance poses a significant problem to the long-term management and control of malaria [1, 99]. In 2024, more than 43 countries in SSA had reported resistance to pyrethroids and the number of *Anopheles* populations that are now susceptible to pyrethroids continues to decline [100, 101].

The impact of insecticide resistance on malaria transmission is challenging to quantify [102], and few studies have tried doing so. Kleinschmidt et al. [103] (2018) presented a multi-country study that examined the implications for malaria vector control with pyrethroid-only LLINs in the presence of insecticide resistance. They found no evidence that the level of insecticide resistance was associated with the prevalence or incidence of infection; instead, net users were more protected than non-users, irrespective of the intensity of resistance. In Malawi [104], an area of high insecticide resistance, there was reduced malaria incidence in children below five years who used pyrethroid-only LLINs. A meta-analysis by Strode et al., [105] showed that LLINs continued to reduce blood-feeding success compared with untreated nets in high pyrethroid resistance settings. Conversely, Churcher et al. (2016) highlighted that while LLINs remain a key tool in malaria control, their effectiveness is significantly reduced in areas with high levels of pyrethroid resistance, leading to less reduction in mosquito populations and, consequently, higher malaria transmission [106]. Their model showed that in settings with moderate to high resistance, the protective efficacy of pyrethroid-treated nets could be reduced by up to 66% [106]. This implies that the efficacy of pyrethroid-only LLINs has continued to decrease as one compares their impact on wild versus susceptible mosquitoes, a decline due to increased insecticide resistance [100, 107].

1.5.5 Co-deployment of IRS and LLINs for the control of malaria

LLINs are sometimes used in conjunction with other vector control methods. In particular, they have been combined with the IRS [108]. IRS involves applying insecticides with longer residual effects to the interior walls of houses, these can remain effective for six to twelve months depending on the insecticide and wall substrate [63]. Many malaria vectors preferentially feed indoors (endophagic) and rest indoors after feeding (endophilic) [109, 110]. The insecticide on the walls or ceiling surfaces once taken up by mosquitoes when resting reduces mosquito lifespan. IRS coverage needs to be high enough (>80%) as it does not provide personal protection but works by reducing vector populations and therefore transmission [94]. Research has shown varying outcomes when combining IRS with LLINs. For example, a study in Kenya demonstrated that co-deployment of IRS and LLINs significantly reduced malaria incidence compared to LLINs alone, but the impact was variable depending on local resistance patterns and coverage levels [111]. Another study in Tanzania (Muleba) found that while IRS combined with LLINs led to a substantial reduction in malaria transmission [108], the additional benefit over LLINs alone was modest and did not always justify the increased cost and complexity of co-interventions [112]. Consequently, WHO generally does not recommend routine co-deployment of IRS and LLINs solely for malaria prevention and control [3]. Instead, it is advised to optimize the coverage of either intervention based on effectiveness in the specific setting and for insecticide resistance management [3].

1.6 How are new LLIN classes evaluated?

The LLIN evaluation process is a rigorous, multi-phase procedure designed to ensure that LLINs meet the necessary standards of safety, efficacy, and public health impact before they can be recommended for widespread use. This process is initiated by manufacturers, who must first engage with the WHO Pre-Submission Coordination Committee to determine the class and pathway [113]. This committee plays a crucial role in determining the product's eligibility and establishing the appropriate evaluation pathway. There are two main evaluation pathways: the prequalification pathway and the new intervention pathway, each tailored to different categories of vector control products [113-115] (Figure 1.6).

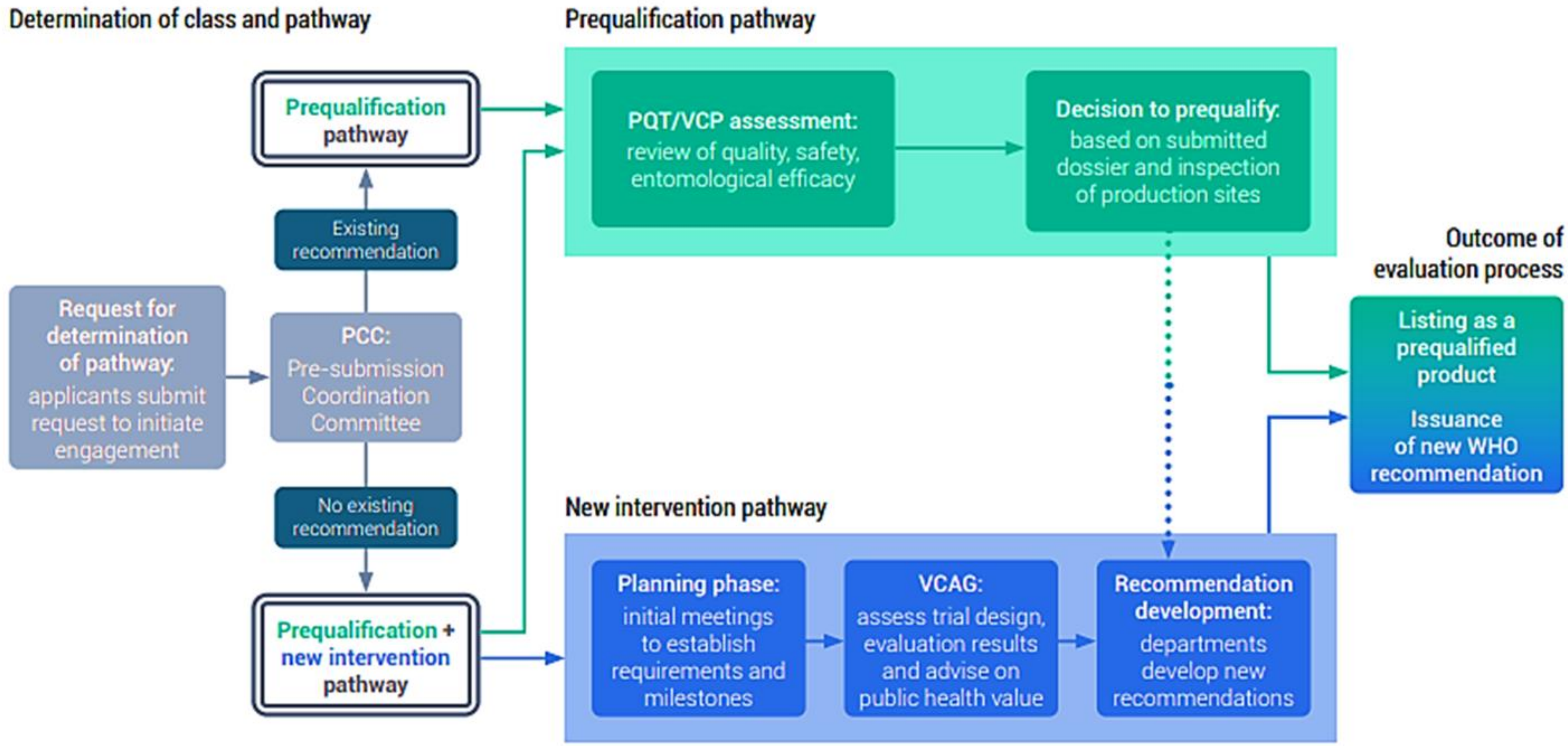

Figure 1.6: Pathway to prequalification[figure from WHO Prequalification of Vector Control Products [115]]

1.6.1 Prequalification Pathway

This pathway is for vector control tools, including LLINs, that already have a WHO policy recommendation [available research and evidence to help end-users make informed decisions on whether, when, and how to undertake specific decisions]. For example, pyrethroid-only LLINs fall under this category [47]. These products have already demonstrated a certain level of public health value and safety as assessed by WHO [50, 51, 116-119]. The prequalification pathway focuses on confirming that these products continue to meet established standards through rigorous testing (Phase I to Phase III).

1.6.1.1 Phase I: Laboratory testing

This initial phase focuses on assessing the bio-efficacy of the LLIN in a controlled laboratory environment. Key indicators evaluated include the LLIN's ability to kill or repel mosquitoes, its wash resistance (after 20 WHO standard washes), and the dynamics of the insecticide used in the net. This phase is crucial for understanding the basic performance characteristics of the LLIN [120].

1.6.1.2 Phase II: Semi-field trials (Experimental Huts)

In this phase, the LLINs are tested in experimental huts to simulate more realistic conditions. The nets are evaluated for their ability to inhibit mosquito blood-feeding, cause mortality, and any potential side effects. Both washed and unwashed nets are compared to assess the impact of washing on the effectiveness of the LLIN[120].

1.6.1.3 Phase III: Community follow-up (large-scale community trials)

This final phase involves a long-term follow-up of the LLIN bio-efficacy in a real-world community setting over a period of three years. This phase is critical for understanding how the LLIN performs under actual use conditions, including how it holds up over time and after repeated washing [120].

1.6.1.4 WHO prequalification recommendation

A product through the prequalification pathway that satisfactorily completes both Phase I and Phase II testing may receive WHO prequalification, indicating that it meets the necessary standards for bio-efficacy. However, the final results of the Phase III evaluation (at least entomological outcomes) are necessary to obtain full approval for product prequalification, which is crucial for the LLIN's widespread adoption and use [120].

1.6.2 New Intervention Pathway

This pathway is designed for innovative tools that have a novel mode of action and do not yet have a WHO policy recommendation [121]. Products in this category represent new classes of vector control interventions that have not been widely used or evaluated previously. These products must undergo more comprehensive assessments to demonstrate not only their safety and efficacy but also their public health impact [122]. This is particularly important as donors, who are the primary purchasers of LLINs, will only procure products that have received a WHO recommendation [123].

For LLINs or other insecticides that do not have a WHO policy recommendation, additional requirements are imposed [121]. In addition to undergoing the standard WHO prequalification process, these new product classes must demonstrate their efficacy through well-designed and well-conducted trials [randomised controlled trials (RCTs)] with epidemiological endpoints to demonstrate the public health value of the intervention [121, 124]. In addition, two 24-month RCTs are to be conducted in two geographically separate settings, enabling independent replication of study outcomes [121]. This additional level of scrutiny ensures that novel interventions are not only effective in theory but also make a meaningful contribution to malaria control in practice. This comprehensive evaluation process is essential for maintaining the integrity of malaria control programs and ensuring that only the most effective and safe LLINs are deployed in the fight against malaria [121].

As of August 2024, no other class of net is currently under assessment for public health value by the Vector Control Advisory Group (VCAG) [a key advisory body established by the World Health Organization (WHO)]. Over 15 LLIN products that combine either a PBO synergist or a second active ingredient have been developed with some already prequalified, while others have been recommended to be implemented based on the available local evidence [102] (*Table 1.1*). By March 2024, 78% (N=195,375,167) of the LLINs distributed in SSA were dual-AI ITNs (including PBO ITNs) [60].

Table 1.1: List of pre-qualified, evaluated and recommended dual-AI LLIN products and those still under assessment by July 2024

Status	Product name	Active Ingredient/Synergist	Manufacturer
Pre-qualified	OLYSET PLUS	Permethrin + Piperonyl Butoxide (PBO)	Sumitomo Chemical Co., Ltd
	Vector Guard	Alpha-cypermethrin + PBO	Disease Control Technology LLC
	VEERALIN	Alpha-cypermethrin + PBO	V.K.A. Polymers Pvt. Ltd
	DuraNet Plus	Alpha-cypermethrin + PBO	Shobikaa Impex Private Limited
	PermaNet 3.0	Deltamethrin + PBO	Vestergaard Sarl
	Tsara Boost	Deltamethrin + PBO	PPP Hollandi DMCC
	Tsara Plus	Deltamethrin + PBO	PPP Hollandi DMCC
	Yorkool G3 LN	Deltamethrin + PBO	Tianjin Yorkool International Trading Co., Ltd
	Royal Guard	Alpha-cypermethrin + Pyriproxyfen (PPF)	Disease Control Technology LLC
	Interceptor G2	Alpha-cypermethrin+ chlorfenapyr (CFP)	BASF AGRO B.V. Arnhem (NL) Freienbach Branch
	PermaNet Dual	Deltamethrin + CFP	Vestergaard Sarl
Under prequalification pipeline	YAHE 4.0	Alpha-cypermethrin + PBO	Fujian Yamei Industry & Trade Co. Ltd
	MiraNet Combi	Alpha-cypermethrin + PBO + PPF	A to Z Textile Mills Limited
	DuraActive 2.0	Alpha-cypermethrin + PPF	Shobikaa Impex Private Limited
	PRONet Duo	Bifenthrin + CFP	V.K.A. Polymers Pvt. Ltd

1.7 Evidence of bio-efficacy for dual AI LLINs against entomological outcomes in Phase I, II, and III studies

In response to the 2012 WHO [99] call for developing new LLIN products to counteract the resistance challenge, several manufacturers developed novel LLIN products, and several have completed full evaluations.

1.7.1 Pyrethroid + Piperonyl butoxide (Py-PBO) LLINs

Piperonyl butoxide (PBO) [125] is a chemical synergist commonly used to enhance pyrethroid insecticides, particularly in household aerosols. While PBO lacks insecticidal activity, it inhibits detoxifying enzymes in insects, increasing the potency of pyrethroids [126]. This combination is more effective against pyrethroid-resistant mosquitoes. Olyset® Plus (Sumitomo Chemicals) is an LLIN combining PBO (400mg/m²) and the repellent pyrethroid permethrin (800 mg/m²) incorporated into the polyethylene fibers [116]. In semi-field experimental hut trials in West Africa, Olyset Plus demonstrated a strong capacity to withstand repeated washing, with higher retention rates of permethrin (64%) and PBO (45%) after 25 washes [116, 127]. Permanet 3.0 is an LLIN consisting of a roof panel made of monofilament polyethylene containing deltamethrin at 4 g/kg and PBO at 25 g/kg, with side panels made of multifilament polyester treated with deltamethrin only at 2.8 g/kg and a strengthened border. Initial studies conducted in 2008 and 2009 reported to WHOPES Permanet 3.0 was not superior to Permanet 2.0 (standard LLIN) on resistant mosquitoes [118, 119]. In 2010, in Tanzania, Tungu and colleagues [128] evaluated Permanet 3.0 in experimental huts and found that there was no difference in *An. gambiae* mortality compared to unwashed Permanet 2.0. However, the blood-feeding rate was lower in Permanet 3.0 (3%) compared to Permanet 2.0 (10%). Subsequent studies demonstrated the improved efficacy of PermaNet® 3.0 against pyrethroid-resistant mosquito populations.

In laboratory studies, in Ethiopia in 2012 [129], Uganda in 2013 [130], Mozambique in 2015 [131], and Benin in 2017 [132] found that PermaNet 3.0 achieved over 80% mortality in resistant populations compared to lower rates with standard LLINs. In experimental hut trials, Corbel et al. (2010) conducted a multi-center study across Western and Central Africa, showing that PermaNet 3.0 achieved significantly higher mortality rates, ranging from 50% to

80%, compared to 20% to 40% for PermaNet 2.0 [133]. In Burkina Faso, a high mortality rate of 45% was recorded in highly resistant *An. gambiae* populations in PermaNet 3.0 compared to 20% for standard LLIN [134], consistent with Togo where PermaNet 3.0 reduced the blood-feeding rate to 5% compared to 25% for untreated nets [135].

1.7.2 Pyrethroid + chlorfenapyr LLINs

Chlorfenapyr, a pyrrole, was launched by the BASF Crop Protection division in 1995 [136]. It was mainly for Pest Control use (including use in kitchens and food storage) and later was repurposed for use in Public Health as a contact insecticide to control mosquitoes [136]. Unlike other adulticides (insecticides used to kill adult mosquitoes) in vector control, chlorfenapyr is not neurotoxic. Chlorfenapyr works by interfering with the insect's ability to produce energy. Specifically, it disrupts the electron transport chain in mitochondria, which is essential for adenosine triphosphate production. By blocking this process, chlorfenapyr inhibits the insect's cellular respiration and energy metabolism, leading to reduced energy levels, impaired physiological functions, and ultimately death [137]. Interceptor® G2 is an LLIN made of polyester, coated with a wash-resistant formulation of 200 mg/m² chlorfenapyr and 100 mg/m² alpha-cypermethrin. It was evaluated in Benin in a Phase II trial against wild-resistant *An. gambiae*, showing a significantly greater killing effect (71%) compared to the pyrethroid-only LLIN (20%) [122, 138]. Other Phase II trials in Burkina Faso (78% vs 17%) [139], and, Tanzania (70% vs 37%) [140] reported consistent results. Additionally, experimental hut trials conducted in north-eastern Tanzania demonstrated the efficacy of Interceptor G2 against *An. funestus*, with a mortality rate of 47.9% compared to 16.5% for the standard pyrethroid-only LLIN [141].

1.7.3 Pyrethroid + pyriproxyfen (PPF) LLINs

Pyriproxyfen (PPF) mimics natural hormones in insects and is used as a larvicide. In adults, it functions as an insect growth regulator (or insect sterilant) that disrupts female mosquito reproduction and fertility, preventing the production of viable eggs and stopping the next generation of mosquitoes [142]. Two types of LLIN have been developed using PPF; Olyset Duo combining permethrin and PPF; and Royal Guard, a mixture of alpha-cypermethrin and PPF. Royal Guard (Disease Control Technologies, LLC) is a mixture LLIN made of polyethylene incorporating 225 mg/m² pyriproxyfen and 261 mg/m² alpha-cypermethrin. Olyset Duo, another mixture of PPF with pyrethroid, showed higher mosquito-killing effects in phase II trials of up to 95% reduction in reproductive rate [reduction in fertility of mosquitoes] in Benin [143] and Côte d'Ivoire [144]. Royal Guard, induced an 83% reduction in oviposition and 95% reduction in offspring before washing, and 25% reduction in oviposition, and 50% reduction in offspring after 20 washes in an experimental hut trial against wild free-flying pyrethroid-resistant *An. gambiae s.l.* in Benin [145].

1.8 Epidemiological evidence for the effectiveness of dual-AI LLIN through RCTs

The WHO Vector Control Advisory Group (VCAG) extended the trial duration for new vector control products from 18 to 24 months to ensure comprehensive public health evaluation before endorsement [124]. At the start of the Misungwi trial, a similar 18-month study on pyriproxyfen LLINs in Burkina Faso had already concluded [146] but did not meet the new 24-month VCAG requirement.

1.8.1 Olyset Plus (Pyrethroid + Piperonyl butoxide (PBO))

A total of 3 RCTS have been conducted to assess the efficacy of PBO LLINs on epidemiological indicators, 2 in Tanzania evaluating Olyset plus [147, 148] and one in Uganda evaluating both Olyset plus and Permanet 3.0 [149]. In Tanzania, malaria infection prevalence after 9 months was lower in the groups that received PBO LLINs compared to the ones who received standard LLINs (Olyset net) with 63% lower odds of malaria infection after 9 months and 60% lower odds in children aged 0.5-14 years after 21 months [148]. A trial in Uganda by Staedke et al. (2020) followed at 6, 12, and 18 months, found that malaria prevalence in PBO arms vs non-PBO arms were 11% vs 15%, 11% vs 13%, and 12% vs 14%, respectively [149]. Olyset Plus has PBO and permethrin on all sides [116]. It has been suggested that the efficacy of Olyset Plus is better than Olyset Net because of an increased release rate of permethrin [116, 148] and surface concentration of permethrin compared with Olyset Net since the slow insecticide release rate is a known problem for Olyset Nets [78]. Olyset Plus showed better efficacy compared to Olyset Net and received a WHO recommendation for deployment in 2017 [150].

1.8.2 Interceptor G2 (Pyrethroid + chlorfenapyr LLIN)

Interceptor G2 was evaluated in two RCTs conducted in Tanzania and Benin. In Tanzania, Interceptor G2 provided significantly better protection in children aged 0.5 to 14 years over three years than pyrethroid-only LLINs with 55% lower odds of malaria prevalence and 44% lower rates of malaria incidence (in children aged 0.5-10 years) after two years; and 43% lower odds of malaria infection was observed after three years [147, 151]. Similarly, in Benin, a significant reduction in odds of malaria infection prevalence was detected in Interceptor G2 compared to pyrethroid-only LLINs by 53% at 6 months, 39% at 18 months; and 46% reduction of malaria incidence after 2 years [152]. However, this reduction in malaria incidence and prevalence was not sustained by 30 months in Benin [153].

1.8.3 Royal Guard (Pyrethroid + pyriproxyfen (PPF))

Royal Guard was evaluated in the cluster randomised controlled trial (RCT) conducted in Tanzania and Benin. In Tanzania, Royal Guard provided significantly better protection in children aged 0.5 to 14 years compared to pyrethroid-only LLINs with 41% lower odds of having malaria prevalence [147, 151], but there was no evidence of added protection after one year. In Benin, no significant reduction in the odds of malaria infection prevalence was detected in Royal Guard compared to pyrethroid-only nets over the study period [152]. In Burkina Faso, a cluster-randomised controlled trial found that PPF-treated LLINs provide greater protection against the incidence of clinical malaria: 2 episodes per child-year in the standard LLIN group versus 1.5 episodes per child-year in the PPF-treated LLIN group, and the entomological inoculation rate was 85 infective bites per transmission season in the standard LLIN group versus 42 infective bites per transmission season in the PPF-treated LLIN group [146].

1.9 Tanzania malaria epidemiology and vector control

Tanzania is the largest country of all eight East African countries with a population of over 61 million [154]. Malaria is mainly prevalent in mainland Tanzania, and 93% of the mainland population lives in areas where malaria is transmitted [155] (Figure 1.7). Malaria epidemics are largely concentrated around Lake Victoria and the south of Tanzania [156]. Malaria remains a major public health threat, and it is a leading cause of hospital admissions in Tanzania. In 2015, Tanzania recorded a 45% reduction in all-cause under-five mortality as compared to 1999 [156]. Scaling up malaria interventions in the country has likely contributed to this reduction. These efforts include vector control using LLINs, improved case management, and prevention and control of malaria in pregnancy. Tanzania is at a crossroads and is challenged with the need to deploy dual-AI ITNs in places where insecticide resistance is confirmed (Figure 1.8) and where malaria transmission is high (Figure 1.7).

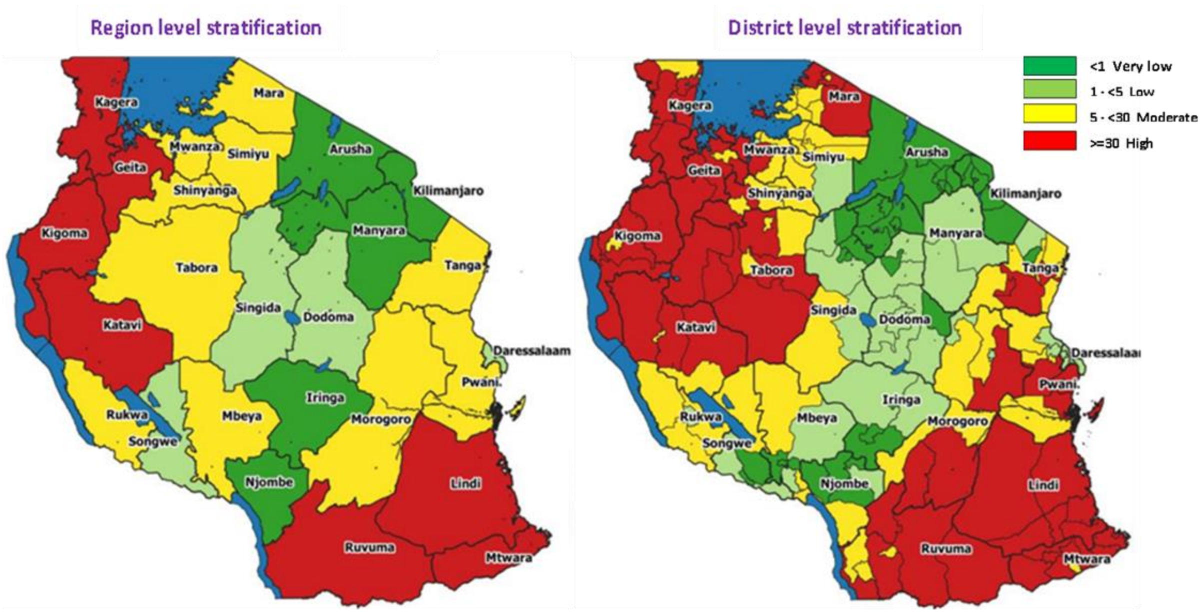

Figure 1.7: Malaria prevalence by regions and districts in Tanzania (Source: NMCP-strategic plan Tanzania 2021-2025)

P. falciparum is the most common species and accounts for 96% of malaria infections in Tanzania [157]. The principal malaria vector in the country is *An. gambiae* complex *(An. gambiae sensu stricto (s.s.), An. arabiensis,* and *An. funestus* [157, 158]. Recently, there has been a shift in vector composition with *An. arabiensis* becoming more prevalent than *An. gambiae* s.s. [157, 159] due to the scaling-up of indoor insecticidal interventions such as LLINs and IRS [160, 161]. *An. funestus* has also become the main vector in some places in the country [159]. In the western and north-western zones of Tanzania, including Lake Victoria, the population of *An. gambiae* s.s. has persisted despite the large-scale use of LLINs and IRS. This may be attributed to high levels of insecticide resistance in the population [148]. In most eastern and northern regions, the main vector species, *An. Arabiensis* presents a challenge to vector control as these vectors tend to feed and rest outdoors and can feed on non-human hosts [160].

In Tanzania, pyrethroid insecticide resistance in *An. gambiae* was first reported in 2006 and by 2017 was widespread [162] (Figure 1.8). The use of agricultural pesticides and the scaled-up of pyrethroids in LLINs and IRS for malaria vector control is likely responsible for the selection of resistance [163].

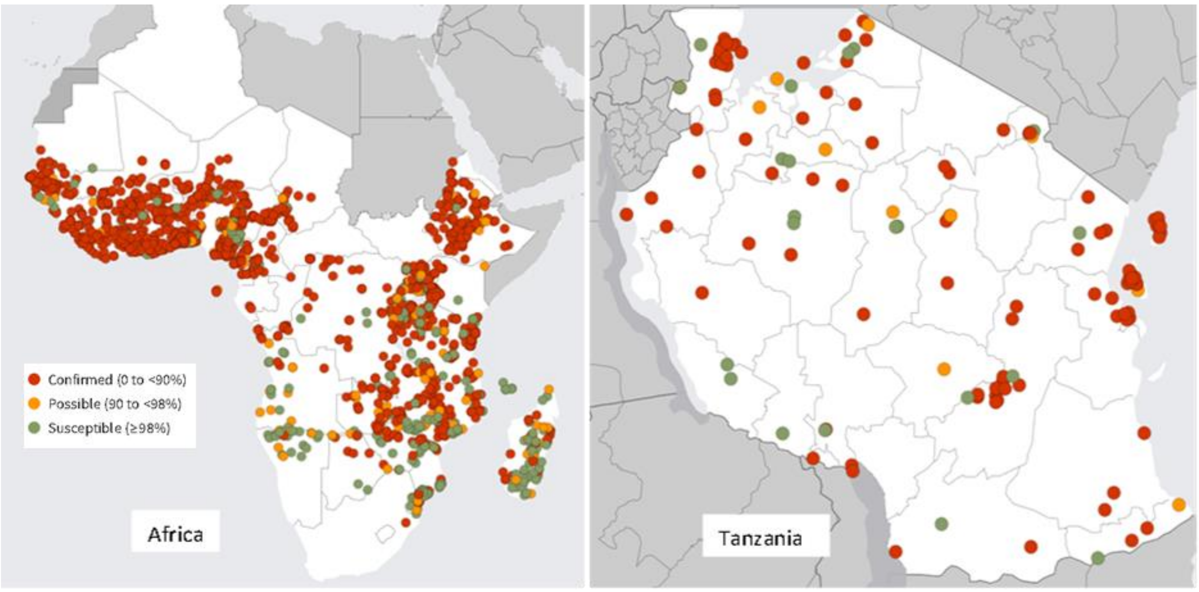

Figure 1.8: Widespread pyrethroid resistance in Africa and Tanzania from 2000-2024

In Tanzania, malaria control efforts began during the colonial era with the Germans in the late 1890s and continued under British rule from 1914 [164] (Figure 1.10). The colonial governments implemented meticulously planned malaria intervention programs, including environmental management, house screening, oil application to open water bodies, larviciding, and the use of antimalarial drugs like quinine and chloroquine. These interventions were integrated and conducted simultaneously [164]. Between 1955 and 1969, Tanzania participated in the Global Malaria Eradication Programme (GMEP), which focused on indoor residual spraying (IRS) with DDT (dichloro-diphenyl-trichloroethane) and mass drug administration [165]. Despite initial success, with a significant reduction in malaria cases in targeted areas, the campaign was eventually abandoned due to logistical challenges, emerging insecticide resistance, and financial constraints [164]. By 1972, Tanzania's health system deteriorated due to economic depression and decentralization policies [166], reversing much of the progress made against malaria [164]. Consequently, malaria interventions were reduced to chemotherapy treatment alone [167]. In recent decades, despite setbacks, Tanzania has made notable progress [168] (Figure 1.11). The distribution of nets increased significantly, from covering just 10% of households in 2004 to over 80% by 2012 [163]. This widespread coverage contributed to a 50% reduction in malaria prevalence among children under five between 2008 and 2015 [163]. However, IRS coverage has fluctuated. At its peak in 2010, IRS was implemented in 60 districts, protecting about 6 million people. But by 2024, due to funding cuts from the President's Malaria Initiative, IRS implementation had decreased from 26 districts in 2015 to just 2 districts (in the Kigoma region) [169, 170](Figure 1.9) in 2024, leaving millions unprotected by IRS. Despite these challenges, the combined use of LLINs and IRS during their peak coverage periods helped reduce malaria prevalence in Tanzania from 18% in 2007 to 9.5% in 2017 [168].

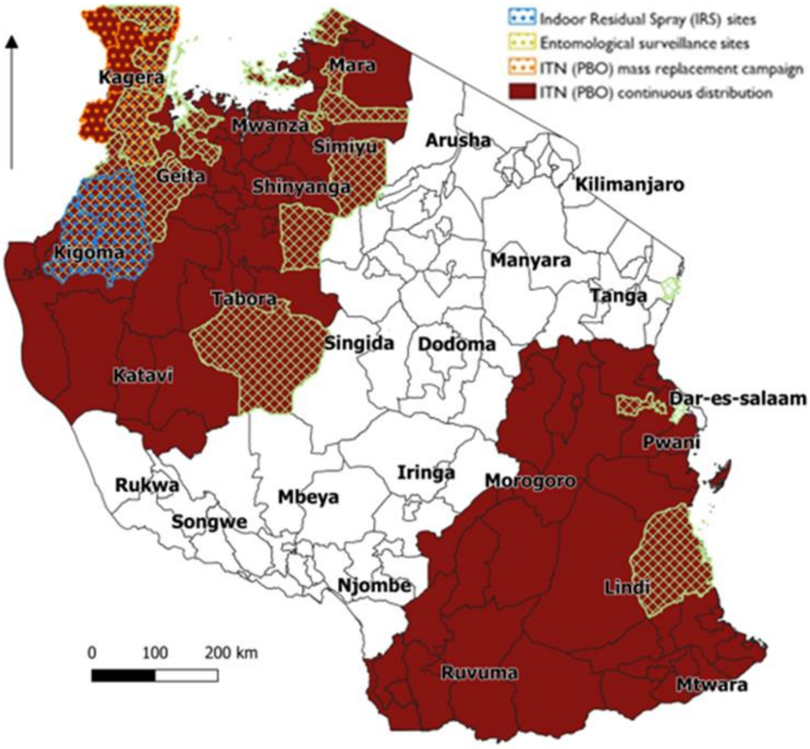

Figure 1.9: Map of Vector Control Activities in Tanzania in 2021 (Source: President's Malaria Initiative (PMI) FY 2023)[169, 170]

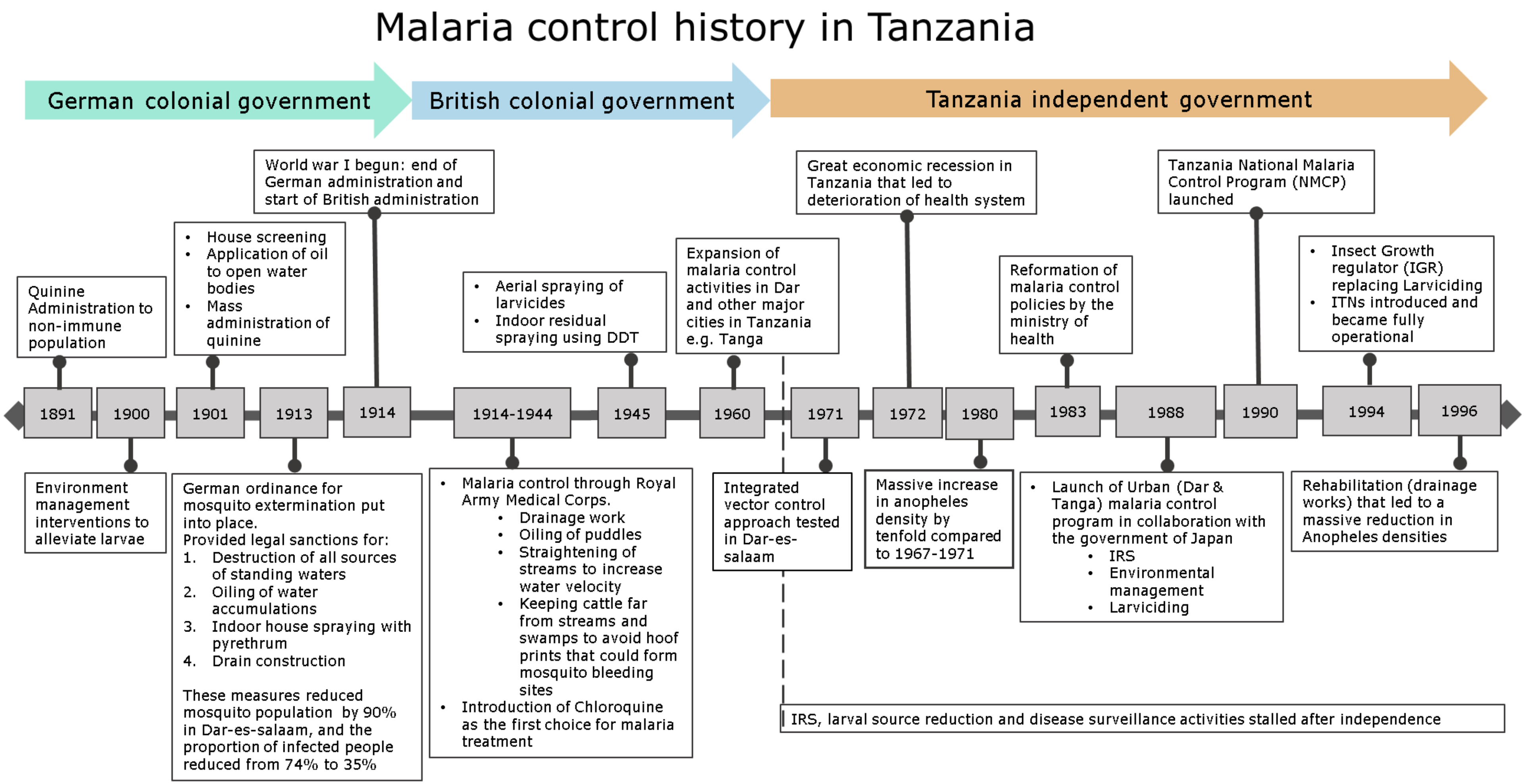

Figure 1.10: History of malaria control in Tanzania: before and after independence (Created by Eliud Lukole)

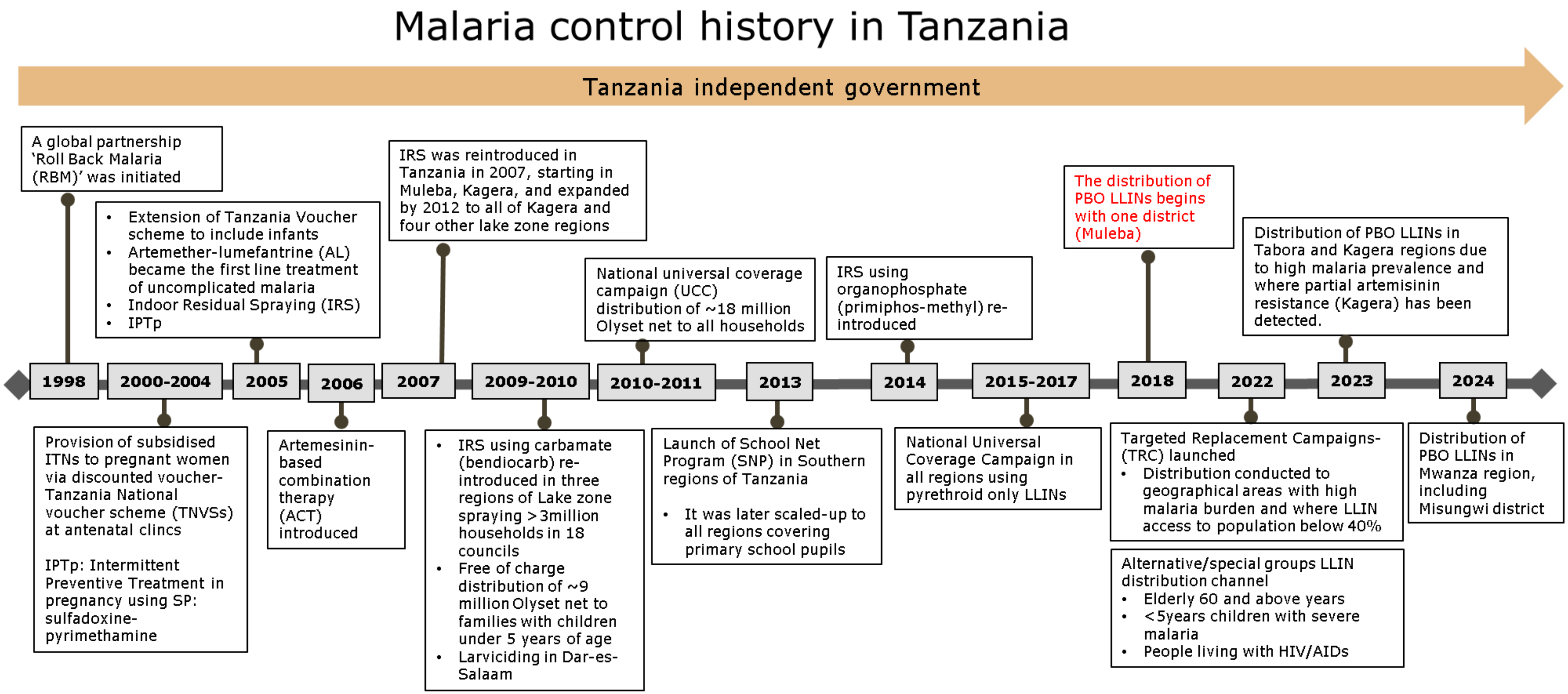

Figure 1.11: History of malaria control in Tanzania: 1998 onwards (Created by Eliud Lukole)

2 Chapter 2: Aim and Objectives

2.1 Overall aim:

To investigate the effectiveness, durability, acceptability, and user preferences for dual-AI ITNs [Royal Guard [containing pyriproxyfen and alpha(α)-cypermethrin], Interceptor G2 [chlorfenapyr and α-cypermethrin], and Olyset Plus [piperonyl-butoxide (PBO) and permethrin] in two different settings around Lake Victoria in North-Western Tanzania

2.2 Specific objectives

1. To evaluate the protective efficacy of physically damaged and aging Olyset Plus LLINs on malaria infection prevalence and determine the median functional lifespan of Olyset Plus in field conditions in Muleba.

2. To assess the impact of physical integrity deterioration and aging of dual-AI ITNs on malaria prevalence and incidence rates in Misungwi.

3. To quantify the personal and community-level protective effects of dual-AI ITNs over three years in Misungwi.

4. To investigate the acceptability and user preferences for dual-AI ITNs in comparison to standard LLINs in both Muleba and Misungwi districts.

2.3 Hypothesis

1. It is hypothesized that holed dual-AI ITNs in use will continue to offer protection to users, with children sleeping under dual-AI ITNs having lower odds of malaria infection compared to those using standard LLINs, even at the same level of physical deterioration.

2. All three dual-AI ITNs are expected to exhibit uniform physical durability and provide a similar protective effect as they develop holes.

3. The community effect, which benefits individuals not using LLINs, is anticipated to be greater in clusters with dual-AI ITNs, regardless of the usage levels within those clusters.

4. Both users and non-users of dual-AI ITNs will likely experience similar levels of protection regardless of net age

2.4 Study outcomes

The study employed a rigorous, multi-site approach with distinct objectives, focusing on the efficacy and durability of LLINs, as outlined in Table 2.1. In Muleba, the investigation measured the protective efficacy of aging and damaged Olyset Plus LLINs, with key outcomes including malaria prevalence and fabric integrity measured through the proportionate hole index (pHI). LLIN functional survival was selected as the primary outcome due to its utility as a proxy for estimating the minimum lifespan of a net in household settings [80]. This metric, frequently employed in WHOPES evaluations, is essential for assessing net longevity and differentiating physical strength [79, 120], facilitating cross-study comparisons and risk factor identification for LLIN integrity across various settings. While longitudinal surveys carry potential bias like the Hawthorne effect [171-173], the study mitigated this by conducting follow-ups at 12-month intervals, minimizing the chance of prolonged behaviour modification. Cross-sectional surveys further strengthened the study by reducing selection bias through random household and LLIN selection, ensuring that outcomes were both robust and reflective of typical conditions across diverse settings.

In Misungwi, the study examined the impact of LLIN deterioration on malaria prevalence and incidence, with surveys and cohort follow-ups scheduled from 12 to 36 months post-intervention. This methodical timing provided a robust dataset for evaluating both immediate and prolonged outcomes. Additionally, the study quantified the personal and community-level protective effects of dual-AI ITNs over three years, with regular malaria prevalence assessments. User preferences and acceptability of dual-AI versus standard LLINs were also explored in Muleba and Misungwi through Knowledge, Attitude, and Practice (KAP) surveys, complemented by qualitative methods such as FGDs and IDIs.

Table 2.1: Study objectives, outcomes, and data collection frequencies across sites

<table>
<tr><th>No.</th><th>PhD Objective</th><th>Survey</th><th>Study site</th><th>Main outcomes</th><th>Frequency of surveys</th></tr>
<tr><td rowspan="2">1</td><td rowspan="2">To evaluate the protective efficacy of physically damaged and aging Olyset Plus LLINs on malaria infection prevalence, and determine the median functional lifespan of Olyset Plus in field conditions</td><td>Prevalence cross-sectional surveys</td><td rowspan="2">Muleba</td><td>1. Malaria infection prevalence
2. Fabric integrity by proportionate hole index (pHI)</td><td>4, 9, 16, 21, 28, and 33 months post-intervention between 2015 and 2017</td></tr>
<tr><td>Cohort study</td><td>1. Median survival of LLINs in years
2. Survivorship/attrition of LLIN
3. LLIN fabric integrity</td><td>0, 12, 24, and 36 months post-intervention between 2015 and 2018</td></tr>
<tr><td rowspan="2">2</td><td rowspan="2">To assess the impact of physical integrity deterioration and aging of dual-AI ITNs on malaria prevalence and incidence rates</td><td>Prevalence cross-sectional surveys</td><td rowspan="2">Misungwi</td><td>1. Malaria infection prevalence
2. LLIN fabric integrity</td><td>12, 18, 24, 30, and 36 months post-intervention between 2019 and 2022</td></tr>
<tr><td>Cohort study</td><td>1. Malaria case incidence
2. LLIN fabric integrity</td><td>1. Every two weeks over two years between 2019 and 2021
2. At the end of each annual cohort in January 2020 and 2021</td></tr>
<tr><td>3</td><td>To quantify the personal and community-level protective effects of dual-AI ITNs over three years</td><td>Prevalence cross-sectional surveys</td><td>Misungwi</td><td>Malaria infection prevalence</td><td>12, 18, 24, 30, and 36 months post-intervention between 2019 and 2022</td></tr>
<tr><td rowspan="4">4</td><td rowspan="4">To investigate the acceptability and user preferences for dual-AI ITNs in comparison to standard LLINs</td><td>Knowledge, Attitude, and Practice (KAP)</td><td rowspan="2">Muleba</td><td rowspan="4">Users’ acceptability and preferences</td><td>Pre-intervention (November 2014) and 3 months post-intervention in April 2015</td></tr>
<tr><td>(Focus Group Discussions (FGDs) and In-depth Interviews (IDIs)</td><td>Pre-intervention survey between November and December 2014</td></tr>
<tr><td>KAP survey</td><td rowspan="2">Misungwi</td><td>3, 12, 18, 24, 30, and 36 months post-intervention between 2019 and 2022</td></tr>
<tr><td>FGDs and IDIs</td><td>34 months post-intervention</td></tr>
</table>

2.5 Conceptual framework: the interplay between PhD objectives

LLIN effectiveness is intrinsically linked to physical durability, community-level impact, and user acceptability. LLINs provide optimal malaria protection when they maintain physical integrity and insecticidal efficacy over time. The communal benefit, achieved through widespread use, significantly reduces mosquito populations and transmission rates. However, this effect hinges on community acceptance; if LLINs are perceived as uncomfortable or unsuitable, usage declines, compromising both individual and community protection. Thus, the sustained effectiveness and public health impact of LLINs depends critically on their durability and community acceptance (Figure 2.1).

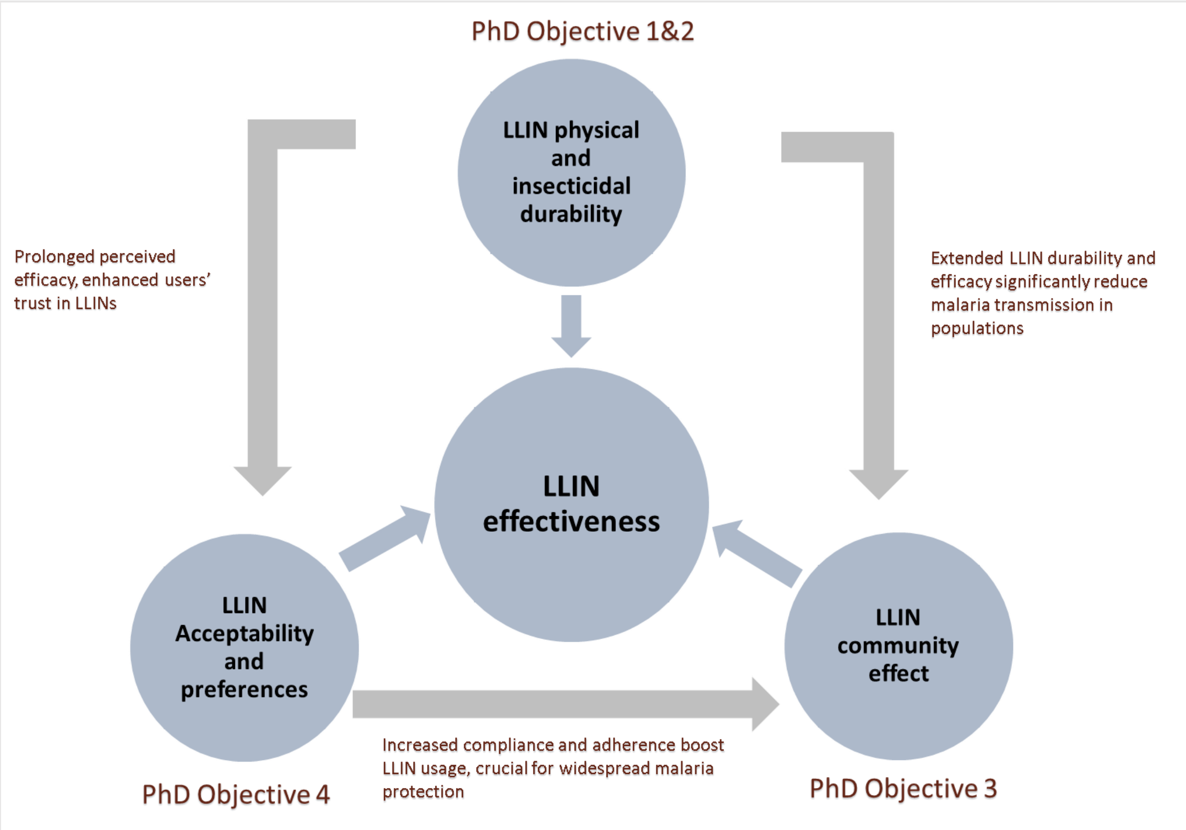

Figure 2.1: Conceptual framework: the interplay between PhD objectives

2.6 Funders, collaborators, and Principal investigator (PI)

2.6.1 Muleba RCT

The Muleba RCT was funded by: The Medical Research Council, Wellcome Trust, and the UK Department for International Development under the joint Global Health Trials scheme. The collaborators in the Muleba RCT included: the London School of Hygiene and Tropical Medicine (LSHTM)- London, UK; National Institute for Medical Research (NIMR), Mwanza, Tanzania; and Kilimanjaro Christian Medical University College (KCMUco), Moshi Tanzania. The principal investigator was Prof Mark Rowland from the London School of Hygiene and Tropical Medicine (LSHTM)- London, UK.

2.6.2 Misungwi RCT

The RCT was funded by the Department of Health and Social Care, the Department for International Development, the Medical Research Council, and the Wellcome Trust supported the research activities; the Bill and Melinda Gates Foundation, Seattle, USA supported flights to the UK, stipend in London, and 4 years Ph.D. fees. The collaborators in the Muleba RCT included: LSHTM-London, UK; University of Ottawa, Ottawa, Canada; NIMR-Mwanza, Tanzania; and KCMUco-Moshi Tanzania. The principal investigator of Muleba RCT was Prof Natacha Protopopoff from the London School of Hygiene and Tropical Medicine (LSHTM)-London, UK.

2.7 Ethical approval and RCT registration

Both RCTs were registered with ClinicalTrials.gov, number (NCT02288637) in Muleba and (NCT03554616) in Misungwi. Ethical approval for this was obtained from the institutional review boards of the Tanzanian National Institute for Medical Research (reference number: NIMR/HQ/R.8a/Vol.IX/2743), Kilimanjaro Christian Medical University College (2267), London School of Hygiene and Tropical Medicine (14952, 14952-1), and University of Ottawa (H-05-19-4411). The PhD project was approved by the ethics review committees of the London School of Hygiene & Tropical Medicine (21389), and the Tanzanian Medical Research Coordinating Committee. Informed consent/assent to participate in the study was obtained from a parent or guardian or any adult present in the house.

3 Chapter 3: Methods

Detailed methods are included in each results chapter (chapters 4 to 7- this section adds some additional detail and comparisons between the two settings.

3.1 Study sites and setting

The project was conducted in Muleba and Misungwi districts in Tanzania as part of two separate trials (Figure 3.1). The districts are approximately 351 km apart. Images of the study sites, villages, and housing are depicted in Figure 3.2. The geographical and demographic characteristics of the two districts are provided in **Table 3.1**. A detailed description of the study designs is provided in Table 3.2, and the data collection timeline is provided in Figure 3.3.

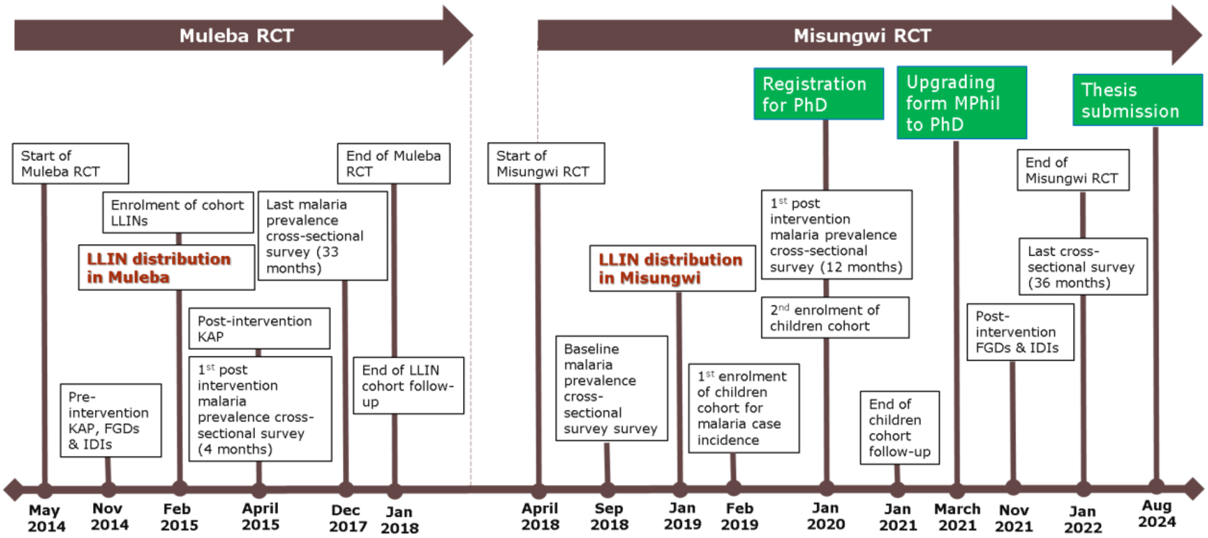

*Figure **3.3** Study timeline*

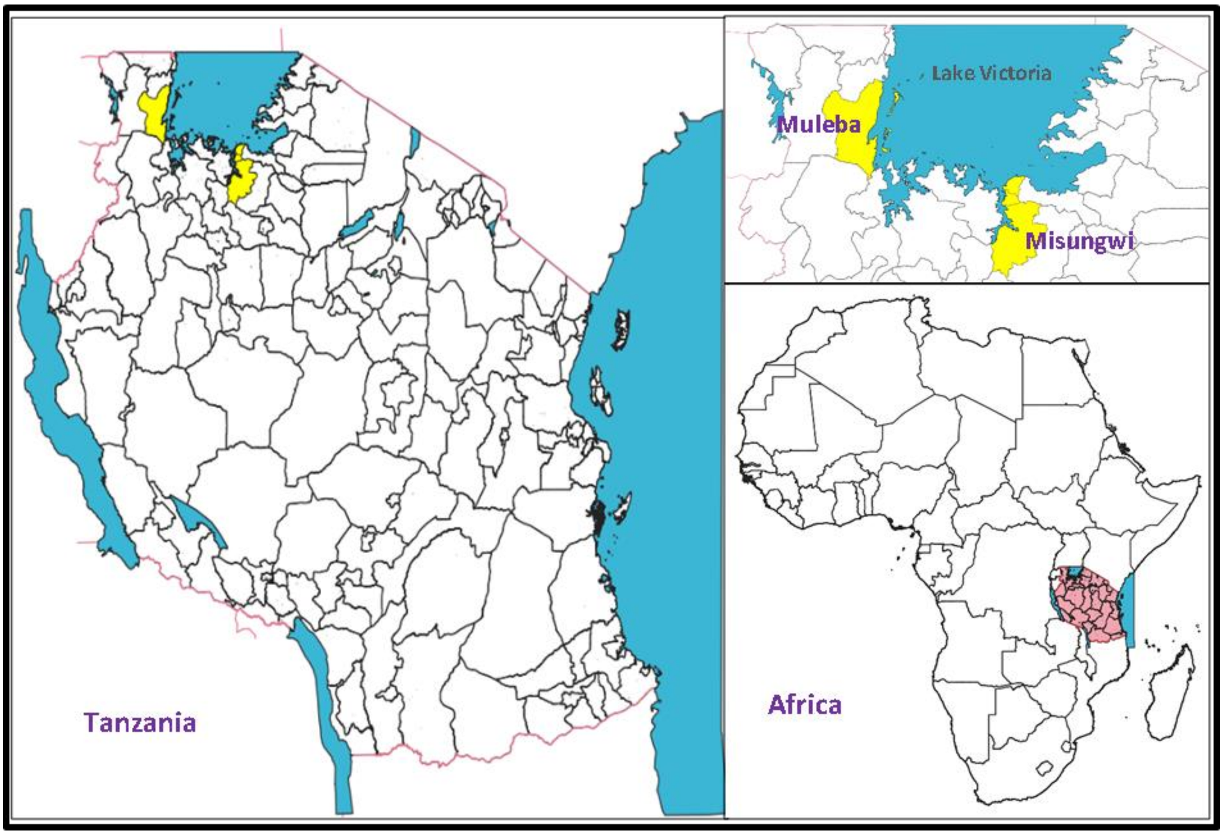

Figure 3.1: Study sites

	Muleba	Misungwi
Housing		
Landscape		
Farms		

Figure 3.2: Images of study areas in Muleba (Pictures by Eliud Lukole)

Table 3.1: Geographical and demographic differences between Muleba and Misungwi

Muleba	Misungwi
Muleba is located in the Kagera region along the northwestern shore of Lake Victoria, and it spans 10,739 km², with 3,502 km² of dry land and 7,237 km² of water.	Misungwi is situated in the Mwanza region along the southern shore of Lake Victoria and spans 2,122 km², with 1,947 km² of dry land and 175 km² of water.
The district has a population of 637,659 (2020 census), with 43% of its inhabitants under the age of 15 [154].	Misungwi has a smaller population of 467,867, with a higher percentage (48%) of its population under 15 years old [154].
The district is administratively divided into 43 wards, 166 villages, and 46 health facilities, including three hospitals: Rubya, Kagondo, and Ndolage	Administratively, the district is divided into 27 wards and 78 villages, with 38 dispensaries, 4 health centers, and 2 hospitals: Misungwi and Bukumbi
Annual rainfall averages between 850 mm and 1,500 mm, with two rainy seasons: a long season from March to May and a shorter one from September to December, interrupted by a dry season from June to July. A secondary dry season may occur unpredictably in January or February. Elevation ranges from 1,100 to 1,600 meters above sea level.	Annual rainfall averages between 700 mm and 1,000 mm, with two rainy seasons: a peak season from March to May and a shorter season from November to January. The district experiences a prolonged dry season from June to September/October. Elevation ranges from 1,000 to 1,250 meters above sea level.

3.2 Malaria transmission and vectors

Malaria transmission at each site occurs throughout the year, with peaks following the two rainy seasons: a high peak in June-July and a lower peak in January-February. The primary vectors are An. gambiae s.s., An. arabiensis, and An. funestus.

3.3 Malaria control in Muleba and Misungwi

Malaria control in Muleba has received focused attention due to its high prevalence and recurrent epidemics. Significant malaria outbreaks were reported in 1998, 2006, and 2010 [163, 174, 175]. In response to outbreaks in Muleba and Karagwe (both in the Kagera region), IRS was first introduced in Mainland Tanzania in 2007, initiated by the President's Malaria Initiative (PMI) at the request of the Tanzanian government to curb malaria epidemics [176]. Muleba, with its frequent outbreaks, was the first district in Tanzania to receive PBO LLINs (Olyset Plus), with over 225,000 LLINs distributed through the School Net Program (SNP) between 2018 and 2019 [177, 178]. It remains the only district where IRS and LLINs were regularly deployed until the IRS withdrawal in 2015 [177, 179]. In both Muleba and Misungwi, LLINs continue as the primary malaria intervention following the withdrawal of the IRS. Larviciding was partially implemented in 2017, following the establishment of a large bio-larvicide factory in Kibaha, Coastal region [180], and the government's ambition to finance larviciding in all the districts in Tanzania [181]. However, budget constraints halted the initiative after just one year, and the factory now exports its products due to a lack of local demand. Moreover, larviciding proved impractical in village settings due to the non-fixed and widespread nature of breeding sites.

3.4 Study design

The two trials were conducted in Muleba between March 2014 and December 2017, and in Misungwi between April 2018 and February 2022 (

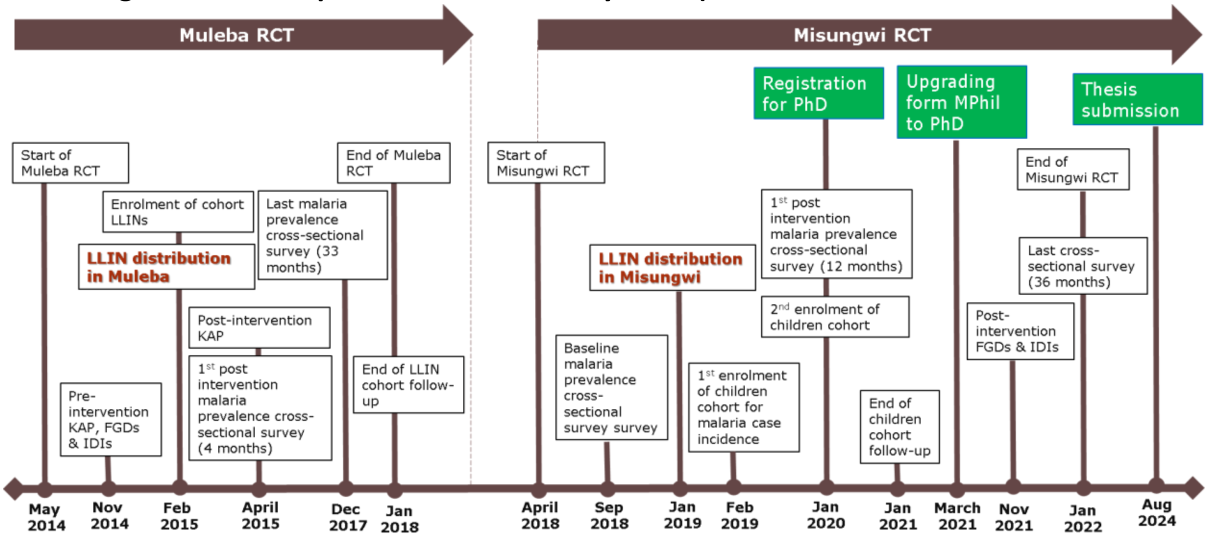

Figure 3.3). The interventions (LLINs) assessed were Olyset plus (both RCTs), Interceptor G2, and Royal Guard and compared with Olyset (Muleba) and Interceptor (Misungwi). The study design for each RCT is summarised in Table 3.2, with the details of the materials and methods for each study presented in the subsequent chapters (Chapters 4-7).

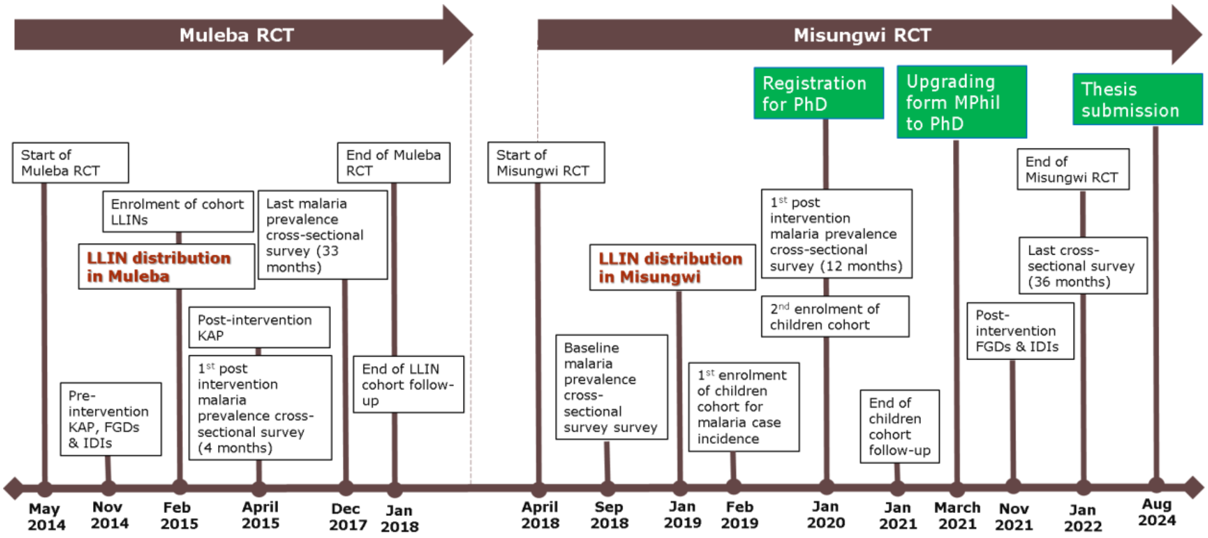

Figure 3.3: Data collection timeline in Muleba and Misungwi

Table 3.2: Description of study designs [cluster randomised controlled trial (RCT)] in each study site

Study areas	**Muleba (Kagera, Tanzania) March 2014-December 2017**	**Misungwi (Mwanza, Tanzania) April 2018-Feb 2022**
Study design	A four-arm, factorial design, RCT with 48 clusters and village/hamlet as the unit of randomization	A four-arm, superiority design, single-blinded, RCT with 84 clusters with village/hamlet as the unit of randomization
Study arms	Olyset Plus Olyset Plus + IRS Olyset net + IRS Control arm: Olyset net	Interceptor G2 Royal Guard Olyset Plus Control arm: Interceptor
Interventions implementation	A total of 90,000 LLINs (45,000 Olyset Plus and 45,000 Olyset Net) were distributed across all study clusters from February 6th to 8th, 2015, following a ratio of 1 net per 2 persons. IRS was done only once in year 1 in February 2015. In January 2016 (12 months post-intervention), a top-up of 10,000 LLINs (5,000 of each brand) was conducted to address a gap observed in ownership and access.	In January 2019 between the 26th to the 28th, a total of 147,230 LLINs from four different brands were distributed across all study villages/clusters based on 1 net per 2 persons, there was no IRS. In September/October 2021 (33 months post net distribution), the Tanzanian National Malaria Control Programme (NMCP) distributed 40,000 Olyset Plus in the study area via the school net program (SNP) in children in

		primary schools' grades 1, 3, 5, and 7.
Data collection methods	Malaria prevalence cross-sectional surveys were conducted at 4, 9, 16, 21, 28, and 33 months post-intervention. LLIN longitudinal survey at 0, 12, 24, and 36 months post-intervention. Knowledge, Attitude, and Practice (KAP) survey conducted pre- and 3 months post-intervention A pre-intervention qualitative survey involving Focus Group Discussions (FGDs) and In-depth Interviews (IDIs)	Malaria prevalence cross-sectional surveys were conducted at 12, 18, 24, 30, and 36 months post-intervention. A cohort study of children aged 0.5-10 years to assess malaria incidence over two years post-intervention. Knowledge, Attitude, and Practice (KAP) survey embedded within the malaria prevalence cross-sectional surveys The post-intervention qualitative study involving Focus Group Discussions (FGDs) and In-depth Interviews (IDIs)

The location of clusters where qualitative elements (FGDs and IDIs) and KAP surveys were conducted are presented in Figure 3.4.

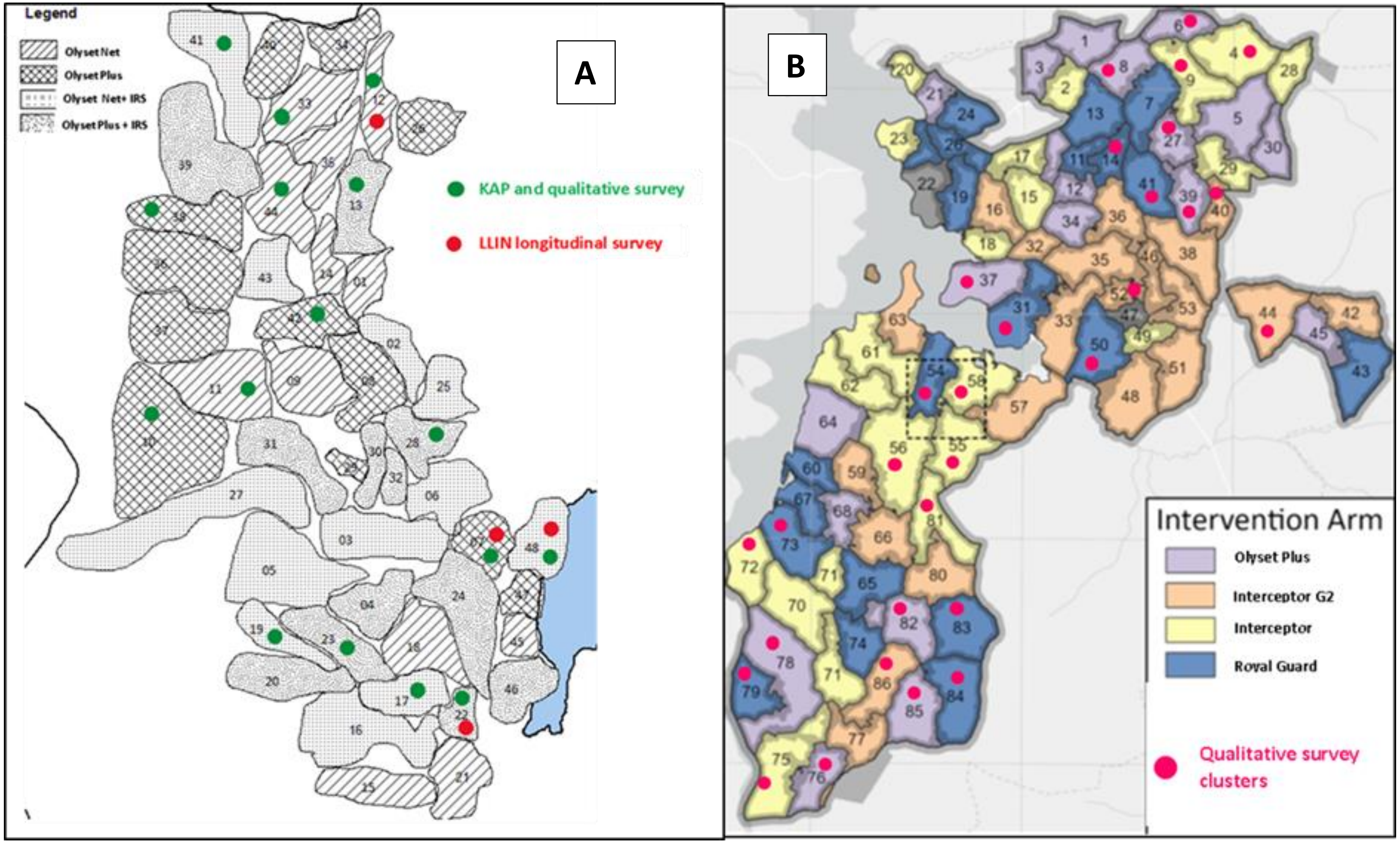

Figure 3.4: Cluster delineation and distribution of clusters where KAP, FGDs, and IDI were conducted per study arm in Muleba (A) and Misungwi (B)

3.5 LLIN characteristics

All LLINs used in the RCTs were blue, rectangular, and shared similar dimensions: 180 cm in length, 180 cm in height, and 160 cm in width. When new, they also had uniform dimensional stability, with shrinkage or expansion in any direction not exceeding 5% after washing. The differences between them are detailed in Table 3.3.

Table 3.3: Characteristics of LLINs distributed in Muleba and Misungwi

LLIN brand	AI and concentration (mg/m^2)	Physical properties		
		Fibre (Denier)	Bursting strength (kPa)	Netting mesh size (holes/cm^2)
Interceptor	Alpha-cypermethrin 200	Polyester (100)	≥405	24
Interceptor G2	Alpha-cypermethrin 100 + Chlorfenapyr 200	Polyester (100)	≥405	24
Royal Guard	Alpha-cypermethrin 220 + Pyriproxyfen 220	Polyethylene (120)	≥400	20
Olyset Plus	Permethrin 800 mg + PBO 400	Polyethylene (150)	≥250	6
Olyset Net	Permethrin 800	Polyethylene (150)	≥250	6

3.6 Sampling of households, children, and LLINs in Muleba and Misungwi

Before the surveys, the location of each household in the study areas was mapped, geolocated, and enumerated, and a census of the total number of people and their age groups was taken. Households were randomly sampled (55 or 45 households per cluster) and approximately 80 or 50 children were tested after allowing refusal, ineligible, and non-response at the household and individual levels in Muleba and Misungwi, respectively (Figure 3.5). In each site, only households with children aged 6 months to 14 years were eligible for inclusion in the study. Households that could not be included were not replaced (sampling without replacement). Within each household, a maximum of three children in Muleba and two in Misungwi aged 6 months to 14 years were randomly selected by the field workers for parasitological testing. Children who were severely ill were also excluded from sampling but given a special coupon (i.e. pink coupon) to go see the project nurses/clinicians for treatment, their results were not included in the study results. Within each household, in each study site, a maximum of three LLINs were selected for physical durability assessment [a priority given to the LLIN used by the selected children, and the rest of the LLINs were randomly selected with the aid of a random numbers table.

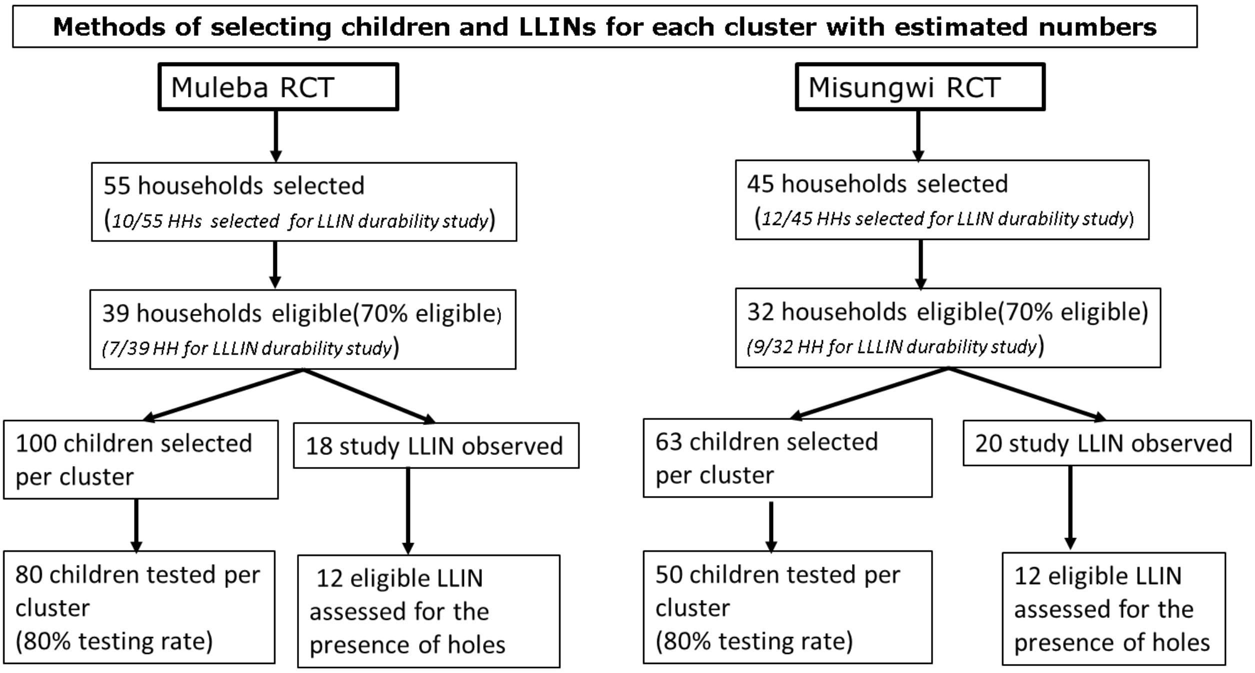

Figure 3.5: RCTs' profile plan

3.7 Organization of data collection activities

3.7.1 Malaria prevalence cross-sectional surveys organization

Each cross-sectional survey was composed of two teams of clinicians/nurses and field workers and lasted for five to six weeks in Muleba and Misungwi with two days spent in each cluster.

3.7.2 Day one-household component

Field workers in both sites visited the selected households with the aid of a handheld Global Positioning System (GPS), provided informed consent, completed the household questionnaire, selected ages 6 months to 14 years to be included in the study, and filled the case report form (CRF) for the selected children (Figure 3.6). In Muleba, 55 households per cluster were randomly selected, with the goal of testing at least 80 children per cluster (maximum of three children per household). Four teams (A-D) conducted the survey, with Team D allocated to fewer households for handling LLIN durability assessments. In Misungwi, 45 households per cluster were selected, aiming to test 50 children per cluster (maximum of two children per household). Six teams (A-F) conducted the survey, with Teams E-F covering fewer households due to additional LLIN assessment duties.

3.7.3 Day two-parasitological component

During the household survey, selected children were given a duplicate of the paper Case Report Form (CRF), which included their unique identifier, the head of the household's name, and the children's names, ages, and sexes. They were instructed to visit a central location the following day for malaria and anaemia testing. The clinical team retained the original CRF for each selected child, where they recorded clinical parameters such as fever, Rapid Diagnostic Test (RDT) results, hematocrit levels, and others.

The following parameters were assessed: presence of fever or history of fever in the past 48 hours, and body temperature using an ear thermometer. All selected children underwent testing for malaria parasites using malaria Rapid Diagnostic Tests (mRDTs). If an mRDT result was positive, the child was provided with free malaria treatment according to national guidelines. Children with severe malaria or other conditions beyond the team's capacity were referred to the nearest health facility. Anaemia was measured using a Haemocue device, though the results of anaemia testing are not included in this thesis. This procedure was uniformly applied across both sites.

3.7.4 Modification of testing and meeting procedures during the COVID-19 pandemic in Misungwi

During the high peak of COVID-19, significant changes were made to the testing and meeting procedures in Misungwi to adhere to safety protocols. In July/August 2020, (an 18-month survey) was conducted under strict gathering restrictions. To comply with safety protocols, the two-day survey components were condensed into one day. Clinical teams were doubled, and two meeting points were established in each cluster to minimize crowding. Both household visits and parasitological assessments were conducted on the same day. When a field worker visited a household and selected a child for testing, they were immediately directed to the nearest meeting point, a process facilitated by school closures that kept children at home. This approach helped prevent congestion at testing centres. To address low participation rates, especially in clusters where more than 50% of selected children were absent, village leaders were tasked with locating and escorting children to the testing site. This measure was necessary due to community suspicions linking the testing to COVID-19 testing and vaccination efforts. Additionally, to prevent contamination, body temperatures were measured using infrared thermometers.

3.7.5 Retrospective LLIN study

Comprehensive data on LLIN physical integrity were collected from a minimum of 10 households per cluster, resulting in at least 12 LLINs assessed per cluster. The teams responsible for LLIN durability assessments were assigned fewer, nearby, and easily accessible households, as they needed to carry an 8kg collapsible net frame for mounting LLINs during hole assessments (Appendix 3.5). A more detailed description of the study can be found in the results section, specifically in chapters 4 and 5.

3.7.6 Longitudinal LLIN study: survivorship, physical integrity, and chemical content

In Muleba, the LLIN longitudinal survey was conducted in four clusters (one cluster per study arm). A detailed description of the study has been presented in the Result paper (Chapter 4). Deliberate efforts were made until the cohort net was observed by doing the following: the houses that were closed or no eligible adult to present, it was visited a couple of times/days until we found them. For the houses that were demolished and rebuilt in a different position in the same hamlet/village, the field worker followed them and assessed the net if it was still in possession. In Misungwi, the LLIN longitudinal survey was conducted in 20 clusters (5 clusters per study arm), following similar follow-up procedures as in Muleba. The results were published in the Insects journal (Insects 2024, 15, 108. https://doi.org/10.3390/insects15020108) (Appendix 8) by my colleague. However, I led the data collection for three years and conducted the main outcome analysis (survival analysis).

3.7.7 Knowledge, Attitude, and Practices (KAP) and Qualitative surveys

KAP and Focus Group discussions (FGDs) and In-depth Interviews (IDIs), were conducted to assess users' acceptability of and preferences for dual-AI ITNs. Questions were asked to determine specifically what the villagers liked and disliked about the LLINs, whether they experienced any symptoms from the presence of insecticide, and whether they perceived any benefits from the different types of dual active ingredient LLINs. LLIN (pyrethroid-only) coverage was purportedly high in the districts at (>61% use) which appears to indicate a high level of adherence and user acceptance. Assessment of the social, cultural, and programmatic factors affecting the acceptability and uptake of the different LLINs was conducted using focus group discussions (FGD) and In-depth interviews (IDIs). A detailed description of the study has been presented in Chapter 6.

3.7.8 Information, education, and communication (IEC) and Social and Behavioural communication change activities (SBCC)

Information, Education, and Communication (IEC) and Social and Behaviour Change Communication (SBCC) efforts were supported by Johns Hopkins University (JHU) in Muleba and by PMI in Misungwi, with both initiatives implemented by the Tanzanian non-government organisation (NGO), Tanzania Communication and Development Centre (TCDC). In both districts, the IEC/SBCC strategies followed a similar approach, being implemented before, during, and immediately after net distribution to enhance awareness and uptake of the interventions. These initiatives mobilized leaders at all levels, from district to hamlet, and provided them with training on critical aspects of LLINs, including access, hanging, usage, care, and repair. The initial mobilization activities involved roadshows, cinema screenings, and public advertisements (PAs). In the final years of both trials, Muleba and Misungwi, a similar door-to-door mobilization campaign was launched in response to a significant decline in study net usage.

In Misungwi, despite extensive efforts to promote net usage following distribution, the desired level of net usage was not achieved. Recipients were instructed to replace their old nets with the newly provided LLINs, and this directive was initially followed. However, within a week, widespread complaints about side effects led many to remove the nets, with some households hesitating to install them at all due to fears of these side effects. To better understand the situation, a pilot study in several villages revealed that most distributed nets remained unused. To address this, a new approach was quickly implemented to educate the community on mitigating these adverse effects, by advising the community to air out the nets or wash them once and dry them in the shade before use. However, this practice may have inadvertently led to some LLINs being over-washed, particularly those still causing side effects after airing or washing, and others being over-exposed to sunlight due to inadequate shaded drying areas in many households. Interestingly, a three-month survey showed study LLIN usage increased to 72%.

3.8 Data management

3.8.1 Household and LLIN questionnaires

In Muleba, data were collected by trained fieldworkers using Pendragon Forms (Pendragon Corporation Software, Libertyville, USA) on Personal Data Assistant (PDAs) and transferred into a Microsoft Access database each evening by the thesis author. Pendragon Forms were programmed to prevent duplicate unique identification (ID) numbers on PDAs, and any duplicate IDs from different PDAs were checked and corrected before being added to the main database.

In Misungwi, data were captured using electronic questionnaires on Android tablets/smartphones designed in Open Data Kit (ODK). Fieldworkers uploaded the completed forms directly to the London School of Hygiene and Tropical Medicine (LSHTM) server while still in the field, as the initial servers at NIMR and KCMUco were unstable. After all data were uploaded, the team supervisor informed the data manager, who downloaded the data in comma-separated value (CSV) format and ran STATA codes to check for mismatches, inconsistencies, duplicate unique ID (ODK did not support the unique identification to be validated at the device level), and other errors. By the time the team returned in the evening, error reports were generated, and responsible persons were called to correct them on the same day.

3.8.2 Clinical data

Data were recorded on carbonated CRF forms (Figure 3.6) by the household team (day one), with the original returned to the office and a carbon copy left with the household. The clinical team verified the carbon copy against the original the next day (day two). Data were double-entered into a Microsoft Access database by two clerks, and discrepancies were corrected by comparing the datasets with the original CRF forms.

To be filled by field assistant

Cluster number: __ __

Location of testing: ____________________

Date of clinical survey: ____/_____/2015

Information on the Household

HH Number (6 digits): _ _ _ _ _ _

Coupon id: **9988**

Head of the HH name:

Information on children

HH team : __________

Interviewer initial : ______

Clinical team: __________

Resident No. (2	**Name Children**	**Sex** F/M	**Age** Year	Month
_ _				

To be filled by clinical team

Bar Code:

POST B SURVEY

Results test

Temp °C	**HB** g/L	**RDT Result** Neg	Falc	Oth	Both	**Malaria** Y/N	**ACT** Y/N	**hours** Y/N
_ _ . _	_ _ _	0	1	2	3	_	_	_

Prescriptions Given/Comments and Consent:

Finger print/signature

Figure 3.6: Example of the selected children's coupon

Additional data management features, including creating unique identifiers, merging datasets, and data quality assurance components, are detailed in Appendix 3.6 and 3.7.

3.9 Statistical analysis

This section outlines the basic statistical analysis performed. Detailed methods and analyses are covered in Chapters 4-7 under the relevant sections. All analyses were performed using STATA release 15 (StataCorp, College Station, TX, USA). Household socioeconomic wealth indices were constructed using Principal Component Analysis (PCA) and divided into tertiles, while a household design index was similarly categorized by quality. The svy command, accounting for the clustered design, adjusted for sampling units, and Chi-square ($\chi 2$) tests were used for group comparisons. Kaplan-Meier estimators provided median net lifetimes, and hazard ratios for functional survival differences were calculated using Cox regression, adjusting for the clustered design. Generalized Estimation Equations (GEE) with a logit model analysed factors associated with poor physical condition, accounting for repeated measures and cluster correlations. Multivariable logistic regression was applied to cross-sectional data to investigate factors influencing poor net condition. The association between net condition and malaria prevalence was assessed using mixed-effects logistic regression for cross-sectional data and mixed-effects Poisson regression for cohort studies. Models were adjusted for key variables such as age group, sex, housing quality, socio-economic status (SES), household design index, net age, survey timepoint, and baseline cluster-level variables (net age, malaria prevalence, and SES). Interaction tests were conducted to assess effect modification by survey timepoint.

4 Chapter 4: Protective efficacy of holed and aging Olyset Plus on malaria infection prevalence

This article covers PhD objective 1 and was published as:

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(https://journals.plos.org/globalpublichealth/article?id=10.1371/journal.pgph.0000453).

4.1 Abstract

Two billion pyrethroid long-lasting insecticidal nets (LLINs) have been distributed since 2004 for malaria prevention in Sub-Saharan Africa. Current malaria control strategies rely on an assumed effective 3-year lifespan for LLINs. PBO synergist LLINs are a newly recommended class of net but there is limited information on their life span and long-term protective efficacy in communities. To assess their operational survival, a cohort of 390 PBO LLINs (Olyset Plus) and 367 standard pyrethroid LLINs (Olyset net) from 396 households were followed for 36 months in Western Tanzania. To assess the association between the condition of the LLIN and malaria infection, nets from at least 480 randomly selected households were assessed during malaria prevalence cross-sectional surveys at 4, 9, 16, 21, 28, and 33 months post-distribution. Information on the presence and condition of nets, and demographic information from the household, were collected to evaluate factors influencing net durability. After 3 years less than 17% of nets distributed remained in the households. The fabric condition was not associated with malaria infection in either type of net. The difference between the net types was highest when nets were between 1-2 years old, when PBO nets appeared to be similarly protective as nets less than a year old, whereas standard nets were considerably less protective as they aged, regardless of fabric condition. There was no statistical difference in the estimated median functional survival time between net types with 1.6 years (95% CI 1.38-1.87) for PBO LLIN and 1.9 years (95% CI 1.67-2.06) for standard LLINs. After 3 years, there was a loss of 55% of permethrin (pyrethroid) content for both nets, and 97% of PBO content was lost in PBO LLIN. These results highlight that functional survival is less than the recommended 3 years for both net types. However, even as the nets age, the PBO nets remain more protective than standard nets, regardless of their condition.

4.2 Background

Long-lasting insecticidal nets (LLINs) remain a cornerstone approach for malaria prevention [68] and 2 billion have been distributed between 2004 and 2021 in Sub-Saharan Africa [182, 183]. The functional survival of LLINs is recommended to be at least 3 years under field conditions [79, 80]. Functional survival is characterised by the LLIN remaining in the household, in an acceptable physical condition, and retaining biological activity (giving protection against mosquitoes). Early accumulation of holes and loss of adequate insecticide are listed among the factors hindering LLINs' functional survival and protective effects [94, 184]. For decades, LLINs have been treated with pyrethroid insecticides, due to their high insecticidal activity and low mammalian toxicity[42]. However, recent evidence suggests that insecticide resistance in mosquito vectors may have reduced the level of protection provided by pyrethroid-only LLINs [107, 148].

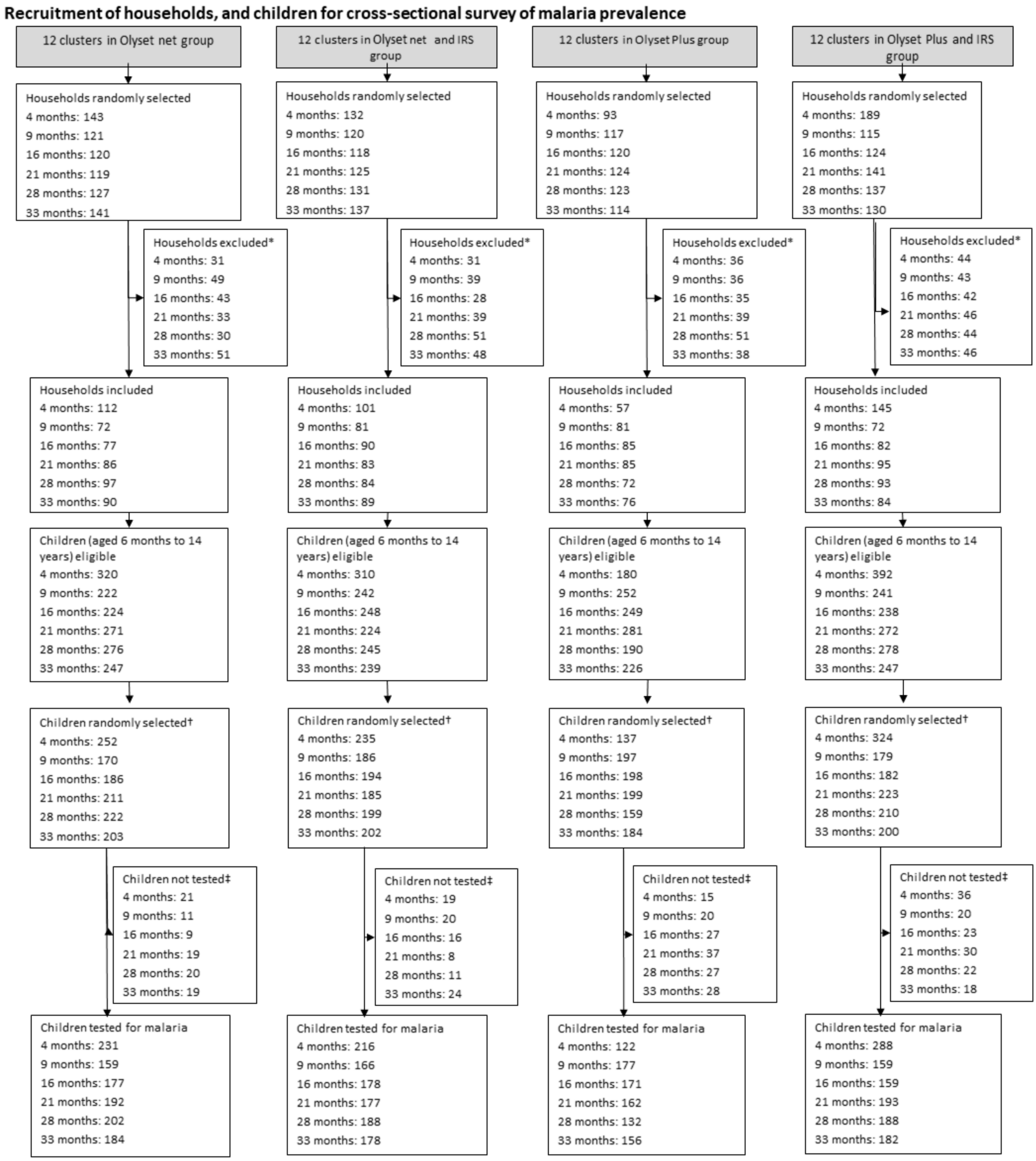
One alternative to pyrethroid-only LLINs is to treat nets with a combination of pyrethroid insecticides and a synergist, piperonyl butoxide (PBO) [150]. Piperonyl butoxide (PBO) is not designed to kill insects directly, but when mixed and applied with pyrethroids enhances their potency by inhibiting enzymes that normally act to detoxify insecticides in the insect [185], essentially rendering pyrethroids effective, even in pyrethroid-resistant insects[126]. PBO is designed to be effective against mosquitoes whose resistance is based on oxidative metabolism [148]. There are currently two brands of LLIN that incorporate PBO which has been assessed in epidemiological trials: Permanet 3.0 [149] and Olyset Plus [147, 148]. In these trials, the PBO nets showed better efficacy than standard pyrethroid nets for at least 12 months of monitoring [147-149]. However, there are mounting concerns that the efficacy of PBO LLINs may wane before 3 years because the PBO has been shown to degrade at a fast pace[186]. A recent study conducted in Uganda showed that in nets that had been in use in the community, mosquito mortality measured using a three-minute WHO cone bioassay decreased quickly however, remained higher for PBO LLIN compared to standard LLIN over two years [187].

Net durability is a key factor in the effectiveness of nets. As nets deteriorate there is likely to be more vector-human contact; and, in addition, owners are more likely to stop using, discard, or repurpose holed LLINs as they perceive them to be no longer useful [86, 188]. To investigate the durability of PBO LLINs, we performed a study to investigate the attrition (net loss), physical integrity (number of holes present), chemical content (amount of active ingredient remaining), and the association between holed and aged LLINs and malaria infection in children under 15 years, over three years of use in field conditions. The study took place within a 4-arm factorial cluster randomised control trial (RCT) to evaluate the efficacy of PBO LLIN compared to standard LLINs [148].

4.3 Methodology

4.3.1 Study area and study arms

The study was conducted in the Muleba district (1^0 45’ S 31^0 40’ E), in Kagera, in the northwest region of Tanzania on the Western shore of Lake Victoria. Muleba covers an area of approximately 3500 km^2 at an altitude ranging from 1,100m-1,600m above sea level. The district comprises 43 wards, 160 villages, and a population of 540,310 [189]. Rainfall occurs in two seasons: the short rains in October–December (average monthly rainfall 160 mm) and the long rains between March and May (average monthly rainfall 300 mm). Malaria transmission in the district occurs throughout the year with two distinct peaks, in June to July and November to January following the long and short rains, respectively. The study area is described in detail elsewhere [148]. Briefly, it comprised 48 clusters from 40 villages within 13 wards. A total of 30,000 households were included. The 48 clusters were randomly assigned to one of four arms: (1) conventional standard LLIN (pyrethroid only), (2) PBO LLIN (pyrethroid and PBO), (3) conventional standard LLIN + Indoor Residual Spraying (IRS, with pirimiphos-methyl CS at the dosage of 1–2 g AI/m^2 [117]), and (4) PBO LLIN + IRS (Figure 4.1). Indoor residual spraying (IRS) took place once in February/March 2015.



*Houses not consented due to refusal, not present or no eligible children. †From each household, up to three children were selected for rapid diagnostic testing. ‡ Children of eligible age who were selected for testing but did not show up

Figure 4.1: Durability Study Profile

4.3.2 Net treatment and distribution

As part of the trial, approximately 90,000 LLINs (45,000 PBO LLINs and 45,000 standard LLINs) were distributed to study clusters between the 6th and 8th of February 2015. The nets appeared similar and were blue, rectangular LLINs made from 150 denier polyethylene material. PBO LLIN (Sumitomo Chemicals, Japan) contains the pyrethroid permethrin (20 g/kg) as the active ingredient (AI) and the synergist Piperonyl Butoxide (PBO) (10 g/kg) [190]. The standard LLIN (Sumitomo Chemicals, Japan) contains permethrin (20 g/kg) incorporated in the yarn but no synergist [191].

4.3.3 Study Design

This is a secondary analysis of the clinical data from the trial registered with ClinicalTrials.gov (NCT02288637).

4.3.3.1 Longitudinal LLIN study

The durability of the nets was assessed first in a longitudinal survey, which followed a cohort of nets every year for 36 months. For this study, 100 households per cluster were randomly selected from two clusters receiving PBO LLIN (one with and one without IRS) and two receiving standard LLINs (one with and one without IRS).

At enrolment, all nets in selected households hung and in use received a unique ID number, to be easily identified at subsequent visits. At each 12, 24, and 36-month visit, the physical presence of the net was recorded (to assess survivorship) and nets were inspected for holes to estimate functional survival of the LLIN [80]. In addition, 10 PBO LLINs and 10 standard LLINs still in use were randomly sampled from the households for insecticide content analysis and if the selected nets were missing, alternative nets in the same household were sampled. All sampled nets were replaced with new nets of the same brand but were not subsequently followed up for fabric integrity or chemical analysis.

4.3.3.2 Cross-sectional LLIN study

Net physical integrity was also assessed in 480 households (10 households per cluster) during successive cross-sectional surveys done at 4, 9, 16, 22, 28, and 33 months post-distribution (*Table 4.1*).

At each timepoint, a random sample of 10 households per cluster was selected for LLIN physical integrity assessment. If the selected household had no study net, households were not replaced by another. In each household, field assistants randomly selected three study LLINs. In the same selected household, up to three children aged 6 months to 14 years were randomly selected to be tested for malaria infection using a rapid diagnostic test (CareStart Malaria HRP2/pLDH(pf/PAN) Combo, DiaSys, UK). Children diagnosed as malaria-positive by the rapid diagnostic test were treated with artemether-lumefantrine according to national guidelines. During the 21-month cross-sectional survey, 10 PBO LLINs and 10 standard LLINs were randomly selected to be used for insecticide content analysis.

4.3.3.3 LLIN fabric integrity assessment

To assess fabric integrity, nets were mounted onto a 170cm x 180cm x160cm collapsible frame to facilitate the visual assessment of the net [192]. The number of holes, location on the net, type of holes, and hole size were recorded. The size was classified into four categories as per WHO guidelines [79, 80]: size 1 =0.5–1.99 cm, size 2 =2–9.99 cm, size 3 =10–25 cm, and size 4 > 25 cm. The size of the holes was measured by superimposing transparent plastic with illustrations of hole sizes.

4.3.3.4 LLIN insecticidal content

For each net collected for chemical content, one 30 cm x 30 cm net piece was cut from each side of the net following standard WHO procedure [79]. These pieces were then uniquely labelled, packed in aluminium foil, stored at 4ºC; and sent to the Liverpool School of Tropical Medicine (United Kingdom) for chemical content analysis using High-Performance Liquid Chromatography (HPLC) [193].

Table 4.1. Measurement, frequency, and outcomes of durability components

Component	Definition	Data collection survey	Measurement	Outcome indicators	WHO criteria	Timing of measures	Clusters included
Attrition	Net no longer available in household due to discarding, alternative uses, given away, used elsewhere or stolen	Longitudinal survey: **Attrition rate-category1**	**Household follow-up survey:** all LLINs labelled and lost due to wear and tear	**Numerator**: Total number of each LLIN product no longer present in surveyed households due to wear and tear x 100		12, 24, 36 months	4
		Longitudinal survey: **Attrition rate-category2**	**Household follow-up survey:** all LLINs labelled and lost because they are given away, stolen, sold, used in another location, or withdrawn for chemical content analysis	**Numerator**: Total number of each LLIN product no longer present in surveyed households because they are given away, stolen, sold, used in another location, or withdrawn for chemical content analysis in surveyed households x 100			
		Longitudinal survey: **Attrition rate-category3**	**Household follow-up survey:** all LLINs labelled and lost because are being used for other purposes	**Numerator**: Total number of each LLIN product no longer present in surveyed households that are being used for other purposes in surveyed households x 100			
				Denominator: Total number of each LLIN product distributed to surveyed households			
Physical integrity	Physical state of the net	Longitudinal survey	Number, location, and size of hole(s) for all labelled nets in HH	Holed surface area (HS)	Good: HS < 80cm^2, Damaged: HS 80-789cm^2, extremely torn: HS ≥ 790cm^2	12, 24, 36 months	4
		Cross-sectional survey	Number, location, and size of hole(s) for 3 nets per HH			4, 9, 16, 21, 28, 33 months	48
Functional survival	Estimation of nets still in households and in good and damaged (serviceable) condition	Longitudinal survey	Median survival analysis	Total LLINs present and serviceable / (Total LLINs originally labelled at baseline - total net given away or not followed at each time point)	Median net survival in years = time point at which the estimate of functional survival crosses 50%	12, 24, 36 months	4
Holed nets protective effect	Ability of each torn LLIN product to provide protection against malaria infection	Cross-sectional survey	Children between 6 months and 14 years tested for malaria parasite by RDT	Odds ratio of malaria infection between users of holed standard LLIN and PBO LLIN in children between 6 month and 14 years, over three years of use in field conditions	Good: HS <80cm^2, Damaged: HS 80-789cm^2, extremely torn: HS ≥ 790cm^2	4, 9, 16, 21, 28, 33 months	48
Insecticide content	Amount of active ingredient (PBO and/or pyrethroid) per gram of the LLIN as determined by chemical assay	Cross-sectional survey	Permethrin and PBO content in g/kg in 10 pieces (30x30 cm pieces) per net	Concentration at baseline, and over study period as per WHO	Permethrin: 20 g/kg, PBO: 10g/kg	21 months	48
		Longitudinal survey				0, 12, 24, 36 months	4

4.3.4 Data analysis

Data collection was done on Personal Digital Assistants (PDAs) using Pendragon Software. The primary outcome was LLIN functional survival (LLIN present and in serviceable condition). Secondary outcomes included (1) malaria infection in children sleeping under LLINs of different physical conditions, and (2) chemical content. All statistical analyses were conducted using STATA release 15 (StataCorp, College Station, TX, USA). Household social and economic wealth indices were constructed and analysed by Principal component analysis (PCA), and households were subdivided into wealth tertiles. The *svy* command using cluster as a sampling unit was used to account for the clustered design of the study. Comparisons between the two types of nets were tested for significance using the Chi-square (χ^2) test allowing for the clustered design.

A sum of the holes identified in the nets was weighted [120] to calculate a hole surface area (HS) = (1.23 x no. of size1 holes) + (28.28 x no. of size2 holes) + (240.56 x no. of size3 holes) + (706.95 x no. size4 holes)[120]. Linear regression allowing for survey design was used to compare hole surface area between net products, using log-transformed data to normalise the distribution. Based on the hole surface area, LLINs were assigned to different categories (good < 80 cm^2; damaged=80-789 cm^2, and extremely torn ≥790 cm^2) to determine risk factors associated with the physical state of each LLIN product.

Survivorship, attrition, and functional survival were estimated using the longitudinal data and compared between net types and over the three visits using Chi-square (χ^2), accounting for the clustered design. The median lifetime of nets was estimated using Kaplan-Meier estimators and a hazard ratio for difference in functional survival was calculated using Cox regression, adjusting for the clustered nature of the survey design. To aid in functional survival calculations, a questionnaire was used to capture information on net condition and usage, as well as “how many months ago the net was lost/discarded” if the net was no longer in the household at each follow-up point. Then, the number of months was subtracted from the current date to determine the actual month the net was lost. For the longitudinal data, where nets were sampled at multiple timepoints, generalized estimation equations (GEE) using a logit model were used to determine factors associated with the poor physical condition of the 2 net brands accounting for the repeated measures on individual nets and correlations within clusters. In addition, we assessed the factors that were associated with bad net conditions using multivariable logistic regression with the cross-sectional data. Factors investigated included net type, net age, socio-economic status (SES) of household, hanging habits of nets, type of beds, type of mattress, net ever washed, indoor residual spraying (IRS), and use of open flames in the household. The association between net physical condition and malaria infection was assessed using multivariable logistic regression, controlling for SES, child age, indoor residual spraying (IRS), and the presence of eaves in the house.

4.3.5 Ethics

The trial was approved by the ethics review committees of the Kilimanjaro Christian Medical University College, the London School of Hygiene & Tropical Medicine, and the Tanzanian Medical Research Coordinating Committee (NIMR/HQ/R.8a/VolIX/1803). A trial steering committee reviewed progress. Written informed consent from parents or guardians was obtained for each survey.

4.4 Results

4.4.1 Nets included in the study

In total, 757 nets (397 PBO LLIN, 360 standard LLIN) from 396 households from four clusters were included in the longitudinal study and followed up for 3 years at 12-month intervals. The proportion of households that were lost to follow-up was 21% (3%-refused, 14%-dwelling vacant for survey duration, and 4% dwelling not found) over the 3 years of the trial. Cross-sectional surveys were conducted every year in June-July and November-December from 2015 to 2017. In total, 2104 LLINs (987 PBO LLINs, and 1117 standard LLINs) from 1,383 households were assessed during cross-sectional surveys. During cross-sectional surveys, the proportion of households with at least one study net for every two people decreased from 50% after 4 months to 7% after 33 months. The proportion of participants reporting using study nets the night before decreased from 71% at 4 months to 21% at 33 months post-intervention.

The demographics of households over surveys and study arms were relatively similar in terms of the average number of sleeping rooms, number of sleeping spaces, education status, and main housing materials (*Table 4.2*). There was an increase in the number of households with electricity over the study period, from 5% to 19% (*Table 4.2*). This was due to the government's mission to facilitate access to modern energy services in rural Mainland Tanzania through the Rural Energy Agency (REA).

Table 4.2. Household and socioeconomic characteristics of participating households in longitudinal and cross-sectional surveys

Covariates	**Longitudinal survey**	**Cross-sectional survey**					
Net Age		**4 months**	**9 months**	**16 months**	**21 months**	**28 months**	**33 months**
Mean number rooms (sd), N	4.7 (1.5), 350	5.1 (1.5), 405	5.0 (1.3), 298	5.2 (1.6), 326	5.4 (1.8), 340	5.5 (2.1), 339	5.0 (1.6), 331
Mean number sleeping spaces (sd), N	2.3 (1.0), 350	2.2 (0.8), 405	2.3 (0.8), 298	2.3 (0.9), 326	2.4 (0.9), 340	2.4 (0.9), 339	2.4 (0.8), 331
Mean number of people per household (sd), N	5.1 (2.5), 396	5.7 (1.9), 405	5.7 (1.8), 298	5.6 (2.3), 326	5.6 (1.9), 340	5.6 (1.9), 339	5.5 (1.8), 331
% Malaria infection (in children 0.5-14 years) (N), 95%CI		40 (948), 34-47	32 (732), 27-37	38 (760), 30-45	49 (818), 40-57	72 (790), 65-78	50 (789), 42-57
% literate heads of households (N), 95%CI	74 (351), 66-80	75 (405), 71-79	77 (298), 72-81	78 (326), 73-82	74 (340), 69-78	75 (339), 71-80	80 (331), 76-85
Proportion of households with at least one net for every two people (HH Access) % (N)							
Any LLIN		89 (460)	87 (349)	74 (371)	65 (447)	61 (441)	66 (407)
Study PBO LLIN (Olyset Plus)		51 (219)	46 (167)	26 (179)	18 (232)	10 (203)	8 (204)
Study standard LLIN (Olyset net)		49 (241)	40 (182)	22 (192)	13 (215)	12 (238)	6 (203)
Proportion of participants reporting using a net the night before % (N)							
Any LLIN		76 (2324)	76 (1749)	49 (1884)	53 (1968)	59 (1913)	48 (1879)
Study PBO LLIN (Olyset Plus)		72 (1134)	70 (894)	44 (926)	43 (1026)	38 (885)	23 (887)
Study standard LLIN (Olyset net)		69 (1190)	71 (855)	44 (958)	39 (942)	44 (1028)	20 (992)
Household possessions							
% electricity (N), 95%CI	8 (351), 3-18	5(405), 3-8	5 (298), 3-10	8 (326), 5-13	9 (340), 6-14	12 (339), 8-18	19 (331), 14-25
% mobile phones (N), 95%CI	70 (351), 58-81	69 (405), 64-74	69 (298), 61-75	71 (326), 65-76	73 (340), 67-78	69 (339), 62-76	79 (331), 75-84
% open eaves: (N), 95%CI	69 (351), 60-77	59 (405), 54-64	61 (298), 54-68	67 (326), 61-73	63 (340), 56-70	46 (339), 40-53	47 (331), 40-54
Main housing materials							
% floor: earth/sand (N), 95%CI	88 (349), 74-95	89 (405), 83-92	96 (298), 92-98	91 (326), 88-94	89 (340), 84-93	85 (339), 79-89	83 (331), 76-88
% roof: tin (N), 95%CI	86 (350), 77-91	82 (405), 76-87	83 (298), 78-87	91 (326), 86-94	92 (340), 87-95	93 (339), 90-95	92 (331), 87-95
% walls: mud (N), 95%CI	61 (350), 27-87	78 (405), 71-84	79 (298), 72-85	73 (326), 66-80	76 (340), 69-83	72 (339), 63-89	65 (331), 57-72

sd: standard deviation; CI: confidence intervals; N: total number of observations; LLIN: Long-lasting insecticidal net

4.4.2 LLIN survivorship and attrition

Three years after net distribution, survivorship (defined as nets present in surveyed households and available for sleeping under) was 28.8% (95% CI 11.8–54.9) for PBO LLIN and 25.6% (95% CI 22.2–29.3) for standard LLIN. A small proportion of nets were given away, stolen, sold, or used in other locations at each timepoint and these also did not differ significantly per arm (*Table 4.3*). Attrition due to wear and tear (poor net condition) increased from 3% (95% CI 0.3–23.6) at 12 months to 56% (95% CI 37.6–72.4) at 36 months in PBO LLIN and from 4% (95% CI 3.5–4.5) to 63% (95% CI 57.1–68.7) in standard LLINs (*Table 4.3*). There were no significant differences in attrition between PBO LLIN and standard LLINs over the study period ($p = 0.6816$). Comparing survivorship between arms that received IRS and those that did not, revealed a significant difference in survivorship at 24 months ($p = 0.044$) and 36 months ($p = 0.050$); where arms without IRS recorded higher survivorship of nets (Appendix 4.1). There was no significant difference in functional survival at 3 years (defined as the presence of serviceable LLIN) of the 2 net products; 15.4% (95% CI 4.8–39.7) for PBO LLIN and 17.9% (95% CI 14.7–21.5) for standard LLIN ($p = 0.6929$). Estimated median functional survival was (1.9 years (95% CI 1.67–2.06) for standard LLIN and 1.6 years (95% CI 1.38–1.87)) for the PBO LLIN (Appendix 4.2). There was a statistical difference in median lifetime between nets (regardless of net type) in IRS and non-IRS arms, with a lower median lifetime in IRS arms ($p = 0.0103$) (Appendix 4.3)

Table 4.3. Survivorship and attrition of cohort nets by net type and age (data from longitudinal survey)

LLIN type	**Standard LLIN (Olyset net) (N=360)**			**PBO LLIN (Olyset Plus) (N=397)**		
Net age	**12 months**	**24 months**	**36 months**	**12 months**	**24 months**	**36 months**
Nets labelled in visited households	N=329	N=308	N=309	N=326	N=313	N=309
Attrition rate-category1: % (95% CI)	4.0 (3.5-4.5)	34.4 (30.0-39.1)	63.1 (57.1-68.7)	3.1 (0.3-23.6)	31.9 (11.6-62.7)	55.7 (37.6-72.4)
Attrition rate-category2: % (95% CI)	6.1 (4.4-8.4)	10.1 (4.8-20.0)	11.3 (9.3-13.8)	6.7 (4.7-9.7)	14.4 (6.7-28.1)	15.5 (11.6-20.5)
Attrition rate-category3: % (95% CI)	0.3 (0.0-3.7)	1.0 (0.4-2.2)	0 (0-0)	0.3 (0.0-4.5)	0.6 (0.6-0.7)	0 (0-0)
Missing nets* (n)	31	52	51	71	84	88

*Attrition rate-category1: for nets that have been destroyed or disposed of due to wear and tear (poor condition) in surveyed households; Attrition rate-category2: for nets not available for sleeping under for reasons other than poor fabric integrity (given away, stolen, sold or used in another location, withdrawn by PAMVERC staff) in surveyed households; Attrition rate-category3: for nets used for other purposes in surveyed households; * Missing nets from households that were not interviewed due to either (dwelling vacant, a dwelling not found, and refused) and were not included in the denominator when calculating survivorship and attrition*

4.4.3 Net physical integrity

After 3 years of use, on average, 93% of standard LLINs and 94% of PBO LLINs had at least one hole of any size in the longitudinal survey; while on average, 63% of standard LLINs and 57% of PBO LLINs had at least one hole in the cross-sectional data (*Table 4.4*). The damage to PBO LLIN was more severe than to standard LLINs across the study period in the longitudinal survey but differences were only significant at 36 months. Based on WHO categories, 55% of PBO LLINs and 37% of standard LLINs were considered extremely torn after 3 years of field use in the longitudinal survey; whereas in the cross-sectional survey data, it was 24% of PBO LLINs and 33% of standard LLINs at 33 months post-distribution. Of the nets present in the households but no longer in use as they were perceived unprotective by users after 3 years, 32% were in good or damaged condition (7%-intact, 5%-good, and 20%-damaged). In both surveys, the lower half of the fabric of both net types was more vulnerable to tear than other parts of the net (Appendix 4.4).

Table 4.4. LLIN physical integrity expressed by hole surface area by survey (cross-sectional and longitudinal) and net age

Net type	**Standard LLIN (Olyset Net)**				**PBO LLIN (Olyset Plus)**				p-values for the comparison of mean hole surface area between PBO LLIN and Standard LLIN
	Total Net assessed	% net with at least 1 hole	% extremely torn LLIN (HS ≥790cm²)	Hole surface area (cm²): Median (IQR)	Total Net assessed	% net with at least 1 hole	% extremely torn LLIN (HS ≥790cm²)	Hole surface area (cm²): Median (IQR)	
Longitudinal survey									
12 months	296	91	19	89 (6-577)	294	87	29	234 (5-1116)	0.5763
24 months	171	93	41	368 (39-1840)	168	98	49	770 (92-2284)	0.1015
36 months	79	95	37	322 (31-1520)	89	97	55	925 (212-2464)	0.0462
Cross sectional survey									
4 months	289	39	4	31 (4-138)	273	24	2	30 (2-490)	0.9328
9 months	224	42	13	271 (58-994)	182	46	14	180 (28-1247)	0.3982
16 months	188	67	24	270 (60-1311)	162	65	26	402 (28-1421)	0.7701
21 months	150	70	26	375 (87-1326)	170	63	22	321 (25-1077)	0.1257
28 months	178	78	22	398 (111-911)	120	73	24	196 (33-1349)	0.0703

** net considered as extremely torn if hole surface area ≥790 cm^2; CI: confidence interval*

4.4.4 Factors associated with net physical integrity

Factors that could be associated with net fabric integrity were assessed. These included net type, net age, SES, type of bed, type of mattress, folding/unfolding the net in the morning, and washing of the net (*Table 4.5*). The influence of these factors on fabric integrity differed by survey (longitudinal and cross-sectional survey). In the cross-sectional data, factors that were significantly associated with increased net damage included net age, lowest SES, unfolding the net in the morning, sleeping under grass or reed mats, and washing the net. In the longitudinal data; net age, unfolding the net in the morning, and sleeping without a bed frame were significantly associated with increased net damage.

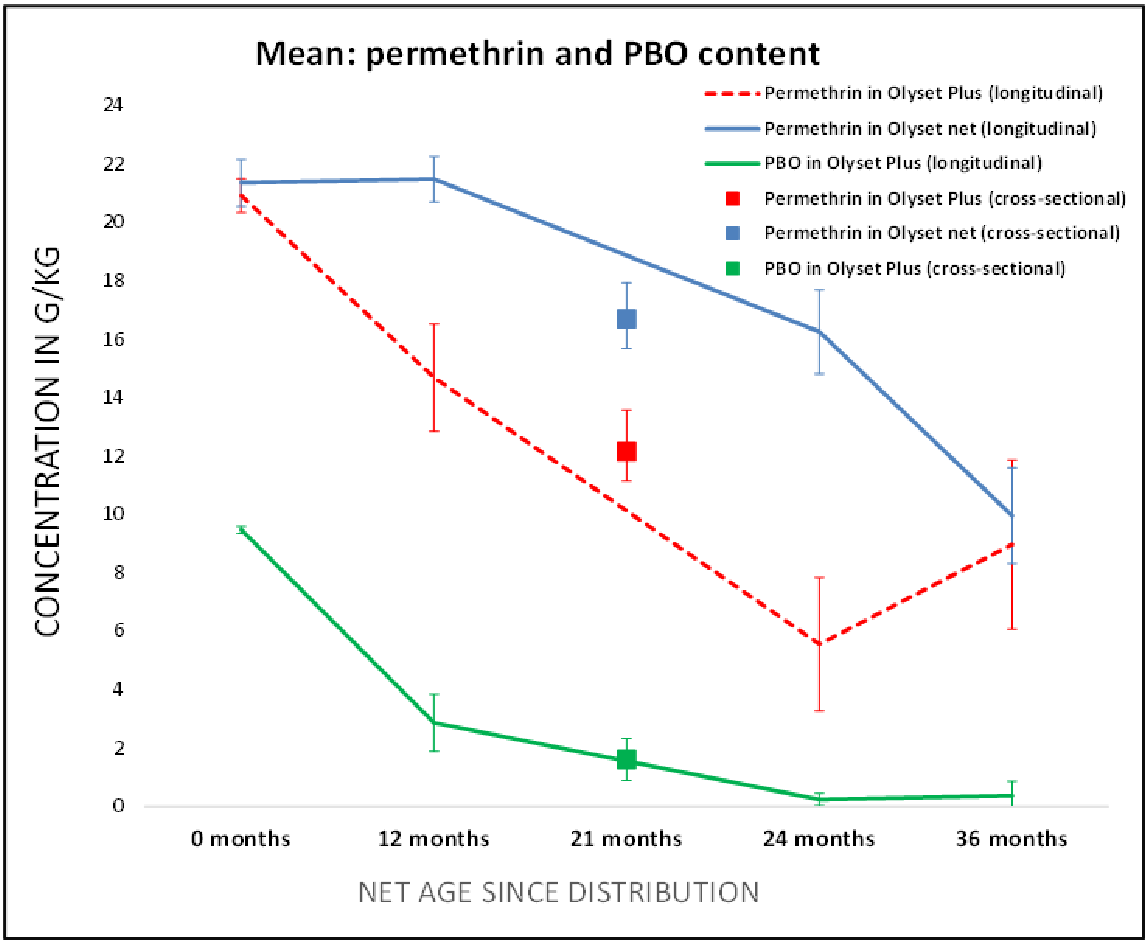
Table 4.5. Factors associated with LLIN being classed as unserviceable (HS: ≥790cm^2) (data from longitudinal and cross-sectional survey)

	Longitudinal survey[a]				Cross-sectional survey[b]			
Covariate	**%extremely torn (HS ≥ 790cm²) (N)**	**aOR**	**95% CI**	**p-value**	**%extremely torn (HS ≥ 790cm²) (N)**	**aOR**	**95% CI**	**p-value**
Net type								
Standard LLIN (Olyset net)	28.2 (546)	1 (Ref)			17.5 (1117)	1 (Ref)		
PBO LLIN (Olyset plus)	39.2 (551)	1.77	1.23-2.57	*<0.001*	16.1 (987)	0.81	0.60-1.09	0.160
Net age (years)								
Year 1	23.7 (590)	1 (Ref)			7.6 (968)	1 (Ref)		
Year 2	44.8 (339)	2.41	1.56-3.71	*<0.001*	24.5 (670)	3.60	2.60-4.97	*<0.001*
Year 3	46.4 (168)	1.68	0.95-2.97	0.080	24.9 (466)	3.62	2.50-5.24	*<0.001*
Socio economic status (SES)								
Lowest	38.4 (315)	1 (Ref)			20.5 (639)	1 (Ref)		
Medium	36.9 (393)	1.12	0.72-1.73	0.620	15.6 (716)	0.72	0.52-0.99	0.040
Highest	26.7 (389)	0.76	0.46-1.25	0.270	14.8 (749)	0.86	0.62-1.19	0.370
How was the net found								
Hanging loose over sleeping space	35.3 (309)	1 (Ref)			20.7 (781)	1 (Ref)		
Hanging folded	21.6 (393)	0.56	0.39-0.79	*<0.001*	14.3 (1216)	0.75	0.58-0.97	0.030
Type of bed								
No bed frame	35.5 (248)	1 (Ref)			18.8 (640)	1 (Ref)		
Stick	27.4 (237)	0.65	0.42-1.00	*0.050*	18.7 (646)	1.17	0.85-1.60	0.330
Wood/Iron	22 (287)	0.56	0.34-0.91	*0.020*	13.7 (736)	0.88	0.62-1.24	0.476
Type of mattress								
Grass/reed mat/clothes	33.5 (269)	1 (Ref)			22.9 (637)	1 (Ref)		
Foam/spring mattress	25.1 (502)	0.91	0.59-1.38	0.65	14.2 (1385)	0.69	0.51-0.92	0.010
Net ever washed								
No	32.6 (656)	1 (Ref)			12.9 (1441)	1 (Ref)		
Yes	37.8 (415)	0.89	0.60-1.34	0.59	25.1 (630)	1.40	1.06-1.85	0.020
Indoor residual spraying (IRS)								
No	30.7 (583)	1 (Ref)			18.5 (1026)	1 (Ref)		
Yes	37.2 (514)	1.44	1.00-2.07	0.05	15.2 (1078)	0.88	0.66-1.18	0.400

[a] Analysis was done using logistic generalized estimation equations (GEE); [b] analysis was done using multivariable logistic analysis; aOR: adjusted odds Ratios for all variables in the table; HS: Hole surface area

4.4.5 Chemical content

At baseline (0 months), all 10 PBO LLIN and 10 standard LLIN samples complied with their target doses of pyrethroid (20 ± 5 g AI/kg) and PBO concentration (10 ± 2.5 g PBO/kg) for PBO LLIN nets (***Figure 4.2***). At 12 months, 24 months, and 36 months mean permethrin content in PBO LLIN decreased to 15 g/kg, 6 g/kg, and 9 g/kg, corresponding to a loss of 29.8%, 73.4%, and 56.9% of the original dose, respectively. The mean permethrin content of standard LLINs was 21 g/kg at 12 months, 16 g/kg (23.8% loss of the original dose) at 24 months, and 10 g/kg (53.3% loss) at 36 months. Mean PBO content decreased to 3 g/kg (69.5%) at 12 months and less than 1g/kg (96% loss) after 36 months. Pyrethroid content of nets collected at 21 months during the cross-sectional survey was 12.2 g/kg for PBO LLIN and 16.7 g/kg for standard LLIN and PBO concentration in PBO LLIN was 1.4 g/kg.



Longitudinal nets to assess chemical content were collected at 0, 12, 24 and 36 months post net distribution. Cross-sectional nets were collected once at 21 months post net distribution

Figure 4.2: Insecticide content over 3 years of field use

4.4.6 Malaria infection and physical condition of LLIN

Malaria infection was measured in cross-sectional surveys only. Between years two and three, there was a significant increase in malaria infection in all arms. When controlling for other factors, the fabric condition was not associated with malaria infection in either type of net. However superior protection afforded by PBO nets depended on the age of nets: with the PBO LLIN providing particularly strong protection versus standard nets when both net types were aged between 1 and 2 years (*Table 4.6*) or nets that were still relatively new (<1 year), there was only weak evidence that PBO nets were better in protecting against infection; after 2 years there was also little evidence that PBO nets provided enhanced protection compared to standard nets.

Table 4.6. Multivariable adjusted odds ratios of malaria infection among 0.5–14-year olds using nets with different physical conditions

Covariates	Malaria Prevalence % (N)	Adjusted odd Ratios*	95% CI	p-value
Net types per condition of net				
Olyset plus vs Olyset net: Less than 1 year	32.8 (540) vs 39 (564)	0.80	0.46-1.38	0.4197
Olyset plus vs Olyset net: 1 year to < 2 years	32.7 (312) vs 50.3 (322)	0.44	0.26-0.75	*0.0028*
Olyset plus vs Olyset net: 2 years to < 3 years	57.1 (191) vs 64.6 (243)	0.88	0.49-1.60	0.6791
Net condition				
Good (HS: < 80 cm^2)	39.5 (1174)	1 (Ref)		
Damaged (HS: 80-789 cm^2)	45.9 (342)	0.99	0.71-1.36	0.9330
Extremely torn (HS: ≥ 790cm^2)	47.0 (387)	1.10	0.79-1.51	0.5790

**adjusting for survey rounds, socio-economic status (SES), children's age, indoor residual spraying (IRS), and presence of eaves in the house; There was evidence of interaction between net age and net type, p value= 0.0392; PBO LLIN (Olyset plus) vs Standard LLIN (Olyset net)*

4.5 Discussion

This study presents the durability of two types of nets with and without the synergist PBO over 3 years of field use and examines whether this was associated with malaria infection in an area of pyrethroid resistance. As has been seen with other studies [86, 194], the nets in this study did not remain in serviceable condition for three years which is required for 'long-lasting' nets, both in terms of fabric integrity and chemical content.

The majority of nets followed in the longitudinal study had at least one hole by 12 months with poor fabric integrity stated as the main reason for attrition. Nets collected via the cross-sectional surveys were generally less damaged, this is likely to be due to the fact that participants in the longitudinal study were asked to keep hold of their nets, whilst it appears that if this was not the case, users are more likely to get rid of the torn nets quickly. In addition, we do not have data on when the nets sampled cross-sectionally were first used, and some may have been stored for several months before being put into use, as has been seen elsewhere [195, 196]. This highlights the differential information the two methods (longitudinal and cross-sectional) obtain with regard to damage to fabric integrity over time, compared to the point when users realistically discard their nets. Indeed, a third of the nets that were considered unprotective by users that were examined by the field workers were still in serviceable condition per WHO guidelines. Community attitudes towards damaged nets may vary from place to place and may feed into differences in durability reported between countries [194].

Overall, PBO nets were more likely to be torn, although this was only evident in longitudinal survey nets and not in the cross-sectional nets. The higher survivorship (presence of nets in the household) despite fewer being classed as in serviceable condition per WHO guidelines of PBO nets by 12 months, suggests that users held onto them even if they were in bad condition. This could be due to the higher protective efficacy of the PBO nets, regardless of fabric condition [197].

Both net types lasted for less than 2 years, which is a shorter timespan than has been seen in other studies for similar nets [86, 186, 198]. Moreover, in this study, less than a third of the distributed nets remained in households after 3 years of community use, which is slightly lower than has been observed in other studies conducted in Tanzania (45%) [86], Mozambique (40%)[83], and Kenya (82%)[199]. Notably, a recent study in Kenya examining the durability of the same make of PBO LLIN showed extremely high survivorship of 91% after 3 years of use [186]. Differences in living conditions but also net care behavior could explain these variations [81]. Noticeably, net survivorship in the arms that did not receive indoor residual spraying (IRS) was significantly higher than in arms that received IRS, suggesting that people are more likely to care for their nets if there is no other preventive intervention in place. This is unlikely to be a chance finding since communities were randomly allocated to receiving IRS, and may therefore have implications for any future strategy that combines different interventions. A similar effect may have been seen in clusters where non-study nets were readily available to replace lightly damaged study nets- the durability of the nets therefore may be underestimated in settings where other interventions are abundant.

Several risk factors were identified for increased risk of damage to the net. Washing of the net and aging of the net increased the likelihood of net damage whereas folding the net every morning was observed to protect the nets against damage, as has been seen in other studies [83, 200, 201]. Other factors that could protect the net against damage included sleeping under bed frames and the use of foam mattresses. Targeted behavioural change communication (BCC) campaigns could increase awareness of the net care, repair, and net durability determinants thereby preventing LLINs from becoming extremely torn [200-203].

In this study, fabric condition was not associated with malaria infection in either type of net. However, older nets were associated with a higher risk of infection, with differential effects by net type depending on the age of the net. Notably, the difference between the net types was highest when nets were between 1-2 years old, when PBO nets appeared to be similarly protective as nets less than a year old, whereas standard nets were considerably less protective as they aged, regardless of fabric condition (Table 4.6). This suggests that a major benefit of PBO nets may be in mitigating the detrimental effect of aging of nets, but that this advantage relative to standard nets wanes after two years. Therefore, to protect the increased efficacy that PBO nets offer in combating malaria, programmes, and funding agencies should consider adapting net distribution strategies to the effective net lifespan or physical and chemical durability of the nets need to be improved to meet the three-year replacement cycle. Currently, it seems clear that swathes of the community are using considerably less effective nets for at least a year before the next net distribution cycle [97].

The results also suggest that much of the protection given by the nets is related to the insecticide (which declined in both nets over the three years), rather than the condition of the fabric [204]. Other studies have also found that malaria infection is not necessarily associated with the condition of the nets [94, 205], however, we would caution against interpreting this too strongly as study net usage considerably declined over the three years of this study and low community-level coverage of nets could be confounding the impact of fabric quality on malaria infection.

Both net types had chemical content within the manufacturer's specifications at the start of the study. However, PBO content was low at 12 months and almost gone by 24 months. The active ingredient (permethrin) was considerably reduced in both nets but reduced more quickly in PBO nets. Despite insecticide loss and aging of nets, PBO nets were more protective against malaria infection in the second year than standard LLINs, suggesting that people should continue using PBO LLINs even if they are aged and damaged[148]. This may also explain why there was a high proportion of extremely torn PBO LLINs compared to standard LLINs in use at the end of the study; as users may still find that they are effective, despite the physical condition and aging. These findings concur with the earlier findings on torn PBO LLIN, that they will continue protecting against blood-feeding *Anopheles* even if they are damaged or torn[197].

Access and usage of study nets decreased sharply over the study period likely due to the high attrition rate leading to too few study nets remaining in the households at the end of the study. However, the higher access and usage of non-study nets suggests that residents are keen to keep using nets if they are considered in good condition. In general, the nets that replaced study nets were obtained from periodic governmental net distributions in the study area. It is of key importance that manufacturers produce more durable nets, which could potentially be achieved with a small increase in unit price [86, 206] by reinforcing the seam and net lower mid-zone or bottom part which is vulnerable to abrasion.

The study had some limitations. The low study net usage by the third year may have confounded some of the results, though it also highlights the speed at which these nets are discarded in the community. In addition, the insecticide content was tested on a smaller number of nets than what is recommended by WHO, although, a study that assessed the insecticide content of PBO nets on a number of nets 4 times higher than this study, found a relatively similar reduction in chemical content[186]. Importantly, this study does not report on entomological bio-efficacy meaning that we do not know whether the reduced chemical content on the nets over time would have also resulted in reduced mosquito mortality. Finally, due to higher attrition than expected, textile integrity could only be assessed on a small sample of nets after 3 years. However, the high rate of attrition in this community is clear and highlights that the usable life of these two nets is well under the recommended lifespan of 3 years.

4.6 Conclusion

The findings of this study demonstrate that the nets' lifespan in this setting was below 2 years. Aged PBO LLINs still provided better protection against malaria infection than standard LLINs for up to 2 years. It is important that users are aware of the benefits of using nets, even if they become torn- and targeted behaviour change communication campaigns may help to reduce the attrition of torn nets. Moving forward, to ensure population coverage with effective nets, either manufacturers need to make nets that last for the recommended 3 years, in terms of fabric and chemical content, or distribution methods need to be increased in regularity to ensure that the population is able to sleep under effective bed nets.

5 Chapter 5: The effect of Dual-AI ITNs physical integrity on malaria prevalence and incidence

This article covers PhD objective 2 and has been published as:

Lukole EA, Cook J, Mosha JF, Mallya E, Aziz T, Kulkarni MA, Matowo NS, Martin J, Rowland M, Kleinschmidt I, Manjurano A, Mosha FW, Protopopoff N. Will a lack of fabric durability be their downfall? Impact of textile durability on the efficacy of three types of dual-active-ingredient long-lasting insecticidal nets: a secondary analysis on malaria prevalence and incidence from a cluster-randomized trial in north-west Tanzania. *Malar J* 2024; 23(1): 199.

5.1 Abstract

The Dual-Active Ingredient long-lasting insecticidal nets (Dual-AI LLIN) have been developed to counteract the reduced efficacy of pyrethroid (PY)-only nets due to widespread pyrethroid insecticide resistance in malaria vector mosquitoes. They constitute half of the nets distributed in sub-Saharan Africa in the past two years. However, their effectiveness once they develop holes is unclear, particularly in pyrethroid-resistant settings. This study evaluates the textile integrity of three dual- AI LLINs compared to standard PY LLN, over 3 years of use in a community in Tanzania and the associated impact on malaria prevalence and incidence. We conducted a secondary analysis of data from a randomized controlled trial (RCT) in North-western Tanzania to evaluate the effectiveness of α-cypermethrin only; pyriproxyfen and α-cypermethrin (PPF-PY); chlorfenapyr and α-cypermethrin (chlorfenapyr-PY); and the synergist piperonyl butoxide and permethrin (PBO-PY) LLINs on malaria infection prevalence and case incidence. We assessed the association between the net textile condition and 1/malaria prevalence over 3 years of use between 2019 and 2022, and 2/malaria case incidence in a cohort of children over 2 years of follow-up between 2019 and 2021. There was no significant association between damaged (OR: 0.98, 95%CI: 0.71-1.37, p-value=0.6550) and too-torn (OR: 1.07, 95%CI: 0.77-1.47, p-value=0.6940) compared to intact nets on malaria prevalence for all net types. However, there were reduced rates of malaria case incidence in children sleeping under a net in good condition compared to too-torn nets (incidence rate ratio (IRR) 0.76 [95%CI: 0.63-0.92], p=0.0047). Malaria incidence was also consistently lower in too-torn PBO-PY LLIN (IRR= 0.37 [95%CI: 0.19-0.72], p= 0.0033) and Chlorfenapyr-PY LLIN (IRR= 0.45 [95%CI: 0.33-0.97], p= 0.0525) compared to an intact PY-only LLIN during the first year of follow up. In year 2, the incidence was only significantly lower in intact Chlorfenapyr-PY LLIN (IRR= 0.49 [95%CI: 0.29-0.81], p= 0.0059) compared to intact PY LLIN. Our study confirmed that sleeping under a Chlorfenapyr-PY LLIN or PBO-PY LLIN offered superior protection to pyrethroid-only nets even when torn. Preventing the development of holes is essential as they impact the level of protection offered against malaria infection.

Trial registration

ClinicalTrials.gov, number (NCT03554616)

5.2 Background

Malaria prevention has relied on mosquito nets treated with pyrethroid insecticides for decades [207]. Scaling up of long-lasting insecticidal nets (LLINs) has averted an estimated 2 billion cases and 12 million deaths between 2000 and 2021 [207]. Due to the emergence and spread of pyrethroid insecticide resistance in malaria vector mosquitoes, new classes of LLINs combining a pyrethroid and a second insecticide with a different mode of action [208, 209], or a pyrethroid and synergist piperonyl butoxide (PBO) [210] have been developed as alternatives to pyrethroid-only LLINs [3]. The addition of these classes of LLINs to the market of malaria vector control products is vital to help mitigate further development of insecticide resistance [207]. Several randomized controlled trials (RCT) have shown the superior efficacy of the combination of pyrethroid and PBO (trade names: Olyset Plus and Permanet 3) [148, 211], pyrethroid-chlorfenapyr insecticides (Interceptor G2) [147, 152], and some nets treated with pyrethroid-pyriproxyfen insecticides (Olyset Duo[146]), compared to standard pyrethroid-only LLIN.

Based on the evidence generated by these RCTs, the new net classes are now being rolled out on a large scale. In sub-Saharan Africa, around 350 million have already been distributed since 2018, and half of the nets distributed in the past two years were PBO-pyrethroid nets or dual active ingredient nets, with these numbers set to increase [69] as these nets are gradually replacing pyrethroid-only nets in areas of pyrethroid resistance. However, the longer-term effectiveness of a net is impacted by its functional survival in field conditions [87]. Several studies have reported reduced effectiveness of holed LLINs in areas with pyrethroid resistance; however, these studies tend to be laboratory-based [204], experimental-hut trials [64, 93, 212], and assessing pyrethroid-only treated LLINs [94, 104, 213].

The present study reports on a secondary analysis of a large randomized controlled trial in Tanzania that evaluated the effectiveness of dual-AI ITNs. In our first report, we showed that combining pyrethroids with either chlorfenapyr or PBO provides further protection against malaria infection prevalence, malaria incidence, and entomological indices over one or two years of use compared to standard LLIN [147] Here, we report on the textile integrity of three dual-AI ITNs; Chlorfenapyr-PY LLIN, Pyriproxyfen-PY LLIN, and PBO-PY LLIN, over time and examine the relationship of net fabric quality with malaria prevalence and incidence.

5.3 Methods

5.3.1 Study design and settings

The study took place in 17 wards (72 villages) on the southern border of Lake Victoria, Misungwi district (latitude 2°51'00.0" S, longitude 33°04'60.0" E), Mwanza region, in north-western Tanzania. This is a secondary analysis of a four-arm, double-blinded cluster randomized trial (CRT) that assessed the effectiveness of dual-AI ITNs on malaria outcomes [147, 151, 214]. The following treatments were randomly allocated to 21 clusters each (Appendix 5.1): Interceptor (alpha-cypermethrin, [control] arm), Interceptor G2 (Chlorfenapyr-PY LLIN (alpha-cypermethrin + chlorfenapyr), Royal Guard (Pyriproxyfen-PY LLIN (alpha-cypermethrin + pyriproxyfen), and Olyset Plus (permethrin + piperonyl butoxide (PBO)). In September/October 2021 (33 months post net distribution), the Tanzanian National Malaria Control Program (NMCP) distributed 40,000 Olyset Plus in the study area via the school net program (SNP).

5.3.2 Participants

Malaria infection prevalence was measured during repeated cross-sectional surveys at 12 months (t12; January/February 2020), 18 months (t18; July/August 2020), 24 months (t24; January/February 2021), 30 months (t30; July/August 2021), and 36 months (t36; January/February 2022) post-intervention. Two children aged between 6 months and 14 years from each of 45 randomly selected households per cluster were tested for malaria infection using rapid diagnostic tests (RDT) (CareStart malaria HRP2 [pf], DiaSys, Wokingham, UK). In each cluster, the textile integrity of study LLINs was assessed in at least 13 randomly selected houses (out of 45) at t12, t24, and t30 and in at least 16 households (to account for fewer nets remaining in the households) at the t36 months survey. Due to the COVID-19 pandemic, holes in the nets were not assessed at t18 to adhere to safety protocols. This secondary analysis is restricted to children in households selected for net textile integrity assessment.

A cohort of 2940 children (35 children per cluster on average) aged 6 months to 10 years was recruited after net distribution in February 2019 and followed for one year until January 2020 to assess malaria case incidence. A second cohort of 3360 (40 children per cluster) was recruited one year after net distribution in February 2020 and followed up for one year until January 2021.

5.3.3 Procedures

Between January 26th and 28th, 2019, the four types of nets were distributed to study arms as allocated and detailed elsewhere [147]. In each household selected for fabric integrity, a maximum of 3 study LLINs used the previous night were randomly selected for hole assessment, with priority given to the net used by the selected children for malaria infection prevalence testing.

In the cohort, malaria parasitaemia was measured bi-weekly at a central meeting point. Children with fever (tympanic temperature ≥37.5°C) or a history of fever in the past 48 h were tested for malaria parasites by rapid diagnostic test (CareStart malaria HRP2/pLDH [pf/ pan] combo, DiaSys, Wokingham, UK). Children with a positive rapid diagnostic test or minor illness were treated by trained study nurses/clinicians as per national guidelines.

At the end of each cohort year (last follow-up visit) from 12th December 2019 to 28th January 2020 for year 1, and from 1st December 2020 to 27th January 2021 for year 2, guardians/child caretakers of all cohort children were asked to bring the nets that a cohort child had been using for textile integrity assessment by trained field-workers. During these last cohort visits, alongside the net integrity assessment, malaria parasitaemia was measured in all children regardless of whether they had a fever or a history of fever. These data provide a direct link between the net condition and malaria infection status of the net's user since the cohort child slept under the same net throughout the previous year. Every three months, the community health workers (CHWs) visited the cohort children to monitor and record net usage, and to monitor that the appropriate net was in use. These nets were labelled with the child names at the beginning of the cohort.

The number and size of holes, hole location on the net, and type of holes were recorded for each selected study net. The size was classified into four categories per WHO guidelines: size 1 = 0.5–1.99 cm, size 2 = 2–9.99 cm, size 3 = 10–25 cm, and size 4= > 25 cm. The size of the holes was estimated by superimposing transparent plastic with illustrations of hole sizes. The hole surface area (HSA) for each net was then calculated as the number of holes counted multiplied by the size category weights as per WHO guidelines[120] as follows: HSA= (1.23 x no. of size1 holes) + (28.28 x no. of size2 holes) + (240.56 x no. of size3 holes) + (706.95 x no. size4 holes). Based on the HSA, each net was then categorized as good (HSA: ≤ 79 cm^2), damaged (HSA: 80-789 cm^2), or too-torn (HSA: > 789 cm^2). Hole types included: holes at the hanging points, holes caused by tears, holes caused by burns, holes caused by rodents, and holes caused by sharp objects.

Data collection was done on smartphones using the Open-Data-Kit (ODK) software. Data from each field team was directly uploaded to a secure database at the London School of Hygiene and Tropical Medicine (LSHTM). After completion of the surveys, datasets were transferred to STATA release 15 (StataCorp, College Station, TX, USA) for further aggregation, cleaning, and preparation for analysis.

5.3.4 Outcomes

The study outcomes were malaria prevalence in children aged 6 months -14 years and incidence in children aged 6 months -10 years using dual-AI ITNs compared with standard PY LLIN with different textile conditions.

5.3.5 Statistical analysis

The analysis for this study was restricted to study nets distributed in January 2019. The cross-sectional data collected at t30 were excluded from analysis due to seasonality (collected during the dry season while the rest of the surveys were in the rainy season).

Household social and economic wealth indices were constructed and analysed by Principal component analysis (PCA) and were subdivided into wealth tertiles. HAS values log-transformed to normalise the distribution. Wald tests of multiple comparisons of means were used to compare hole surface area differences between study LLINs. For cross-sectional data, the association between net physical condition and malaria infection prevalence was assessed using mixed-effects logistic regression. The association between net physical condition and cumulative incidence of malaria infection in cohort study was analysed by mixed-effects Poisson regression with individual follow-up time specified as an offset and cluster set as a random effect. Furthermore, each dual-AI LLIN in different physical conditions was compared to good PY LLIN on malaria infection to assess their superiority in protection. Interactions between net type and net physical condition, survey timepoint, and net type were examined.

5.4 Results

A total of 4876 households were selected for physical integrity assessment over the four cross-sectional surveys between 07th January 2020 and 10th February 2022. Of these, 67% (n=3284) consented to participate in the study. From the consenting households, 5817 children were tested for malaria infection, and 5060 study LLINs (1464 PY-LLIN, 1500 chlorfenapyr-PY LLIN, 1181 pyriproxyfen-PY LLIN, and 915 PBO-PY LLIN) were assessed for fabric integrity (Figure 4.1). A total of 1146 children surveys at t30 were not included in the analysis. At baseline (October 2018), malaria prevalence measured in children aged 6 months to 14 years was 44% (1948/4403), balanced across study arms [214].

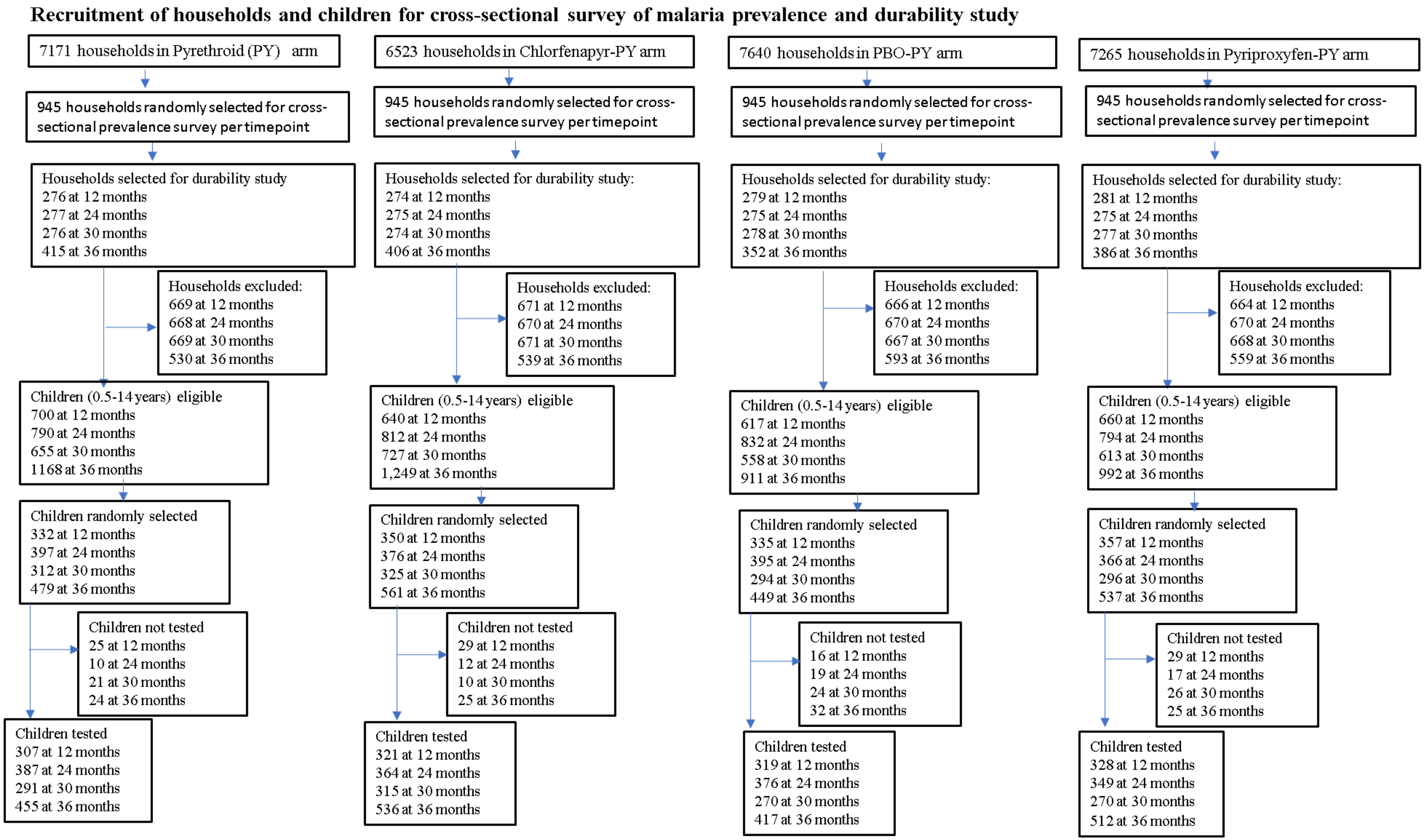


Figure 5.1: Trial profile

Ownership of any net (at least 1 net per household) remained high over the study period, from 99.7% 3 months after net distribution to 96.2% (1036/1077) at t36. Ownership of study net (≥ 1 study LLIN per house) declined over the 3 years of the study from a high of 92% (697/755 houses) after t12 in January 2020 to 62% (672/1077) in January 2022 (***Table 5.1***). Reported study net use recorded during cross-sectional surveys also declined over the 3 years of the study from 72% (3155/4373) three months after the mass distribution in January 2019 [8] to 23% (2068/9044) in January 2022 at t36 (***Table 5.1***). The lowest study net use was in the PBO-PY group at the t36 timepoint [19% (286/1469)]. Between t30 and t36, the ownership of other PBO-PY LLIN in the study area (in all arms) increased from 13% (229/1723) to 33% (1076/3263) due to the local government-led top-up campaigns through the School Net programme (SNP) (Appendix 5.6).

Table 5.1: Household, socioeconomic, and net characteristics of participating households in the cross-sectional surveys

Covariates	**Cross-sectional survey**			
Cross-sectional survey timepoint	**12 months**	**24 months**	**30 months**	**36 months**
Mean number of people per household (sd), N	7.1 (3.0), 755	8.1 (3.4), 803	8.0 (3.6), 649	8.4 (3.8), 1077
Mean number of children (< 15 years) per household (sd), N *	3.6 (1.9), 755	4.2 (2.1), 803	4.0 (2.2), 649	4.2 (2.2), 1077
Mean number of sleeping spaces used last night (sd), N	2.9 (1.4), 755	3.2 (1.6), 803	3.2 (1.5), 649	3.5 (1.7), 1077
Households characteristics:				
% electricity (95%CI), N	24.5 (21.6-27.7)	27.3 (24.3-30.5)	36.1 (32.5-39.8)	37.7 (34.9-40.6)
% open eaves: (95%CI), N	32.1 (28.8-35.5)	34.1 (30.9-37.5)	34.9 (31.4-38.7)	31.57 (28.9-34.4)
Main housing materials				
% floor: earth/sand (95%CI), N	64.8 (61.3-68.1)	67.6 (64.3-70.8)	58.6 (54.7-62.3)	61.8 (58.8-64.6)
% roof: tin (95%CI), N	71.9 (68.6-75.0)	72.7 (69.5-75.7)	77.2 (73.8-80.3)	74.5 (71.8-77.0)
% walls: unburnt bricks or mud (95%CI), N	79.3 (76.3-82.1)	78.5 (75.5-81.2)	76.4 (73.0-79.5)	76.9 (74.3-79.3)
% no ceiling (95%CI), N	97.5 (96.1-98.4)	97.9 (96.6-98.7)	96.5 (94.7-97.6)	96.8 (95.5-97.7)
% plastered walls	67.3 (63.9-70.5)	63.0 (59.6-66.3)	67.5 (63.8-71.0)	67.3 (64.5-70.1)
Mean number of mosquito nets owned per household (sd), N				
Any LLIN	3.8 (1.8), 755	3.1 (1.6), 803	2.7 (1.6), 649	3.0 (1.8), 1077
Study LLIN	2.5 (1.5), 755	1.6 (1.2), 803	1.2 (1.1), 649	1.1 (1.1), 1077
Mean number of nets used last night per household (sd), N				
Any LLIN	2.4 (1.3), 755	2.4 (1.3), 803	2.0 (1.3), 649	2.3 (1.5), 1077
Study LLIN	1.6 (1.2), 755	1.2 (1.1), 803	0.9 (1.0), 649	0.8 (0.9), 1077
Proportion of households with at least 1 net % (n/N)				
Any LLIN	100 (755/755)	98.5 (791/803)	95.5 (620/649)	96.2 (1036/1077)
Study LLIN	92.3 (697/755)	78.3 (629/803)	66.6 (432/649)	62.4 (672/1077)

Proportion of participants reporting using a net the night before % (n/N)				
Any LLIN	81.3 (4306/5294)	75.4 (4899/6502)	62.3 (3220/5170)	67.8 (6128/9041)
Study LLIN	51.6 (2805/5435)	37.4 (2432/6502)	27.8 (1439/5171)	22.9 (2068/9044)
Varieties of nets available in the household	N=3010	N=2513	N=1723	N=3263
% Pyrethroid (PY) LLIN (n)	16.5 (498)	14.0 (352)	14.2 (245)	11.3 (369)
% Chlorfenapyr-PY LLIN (n)	18.5 (557)	13.7 (345)	12.6 (217)	11.7 (318)
% PBO-PY LLIN (n)	13.4 (403)	8.6 (215)	8.1 (139)	4.8 (158)
% Pyriproxyfen-PY LLIN (n)	15.2 (458)	13.0 (327)	10.3 (178)	6.7 (218)
% Permanet 2.0 (n)	17.4 (525)	19.2 (482)	20.3 (349)	16.6 (543)
% Olyset net (n)	13.9 (419)	23.8 (597)	18.6 (312)	13.7 (447)
% Olyset Plus (distributed by NMCP) (n)	0.3 (9)	6.37 (160)	13.3 (229)	33.0 (1076)
% Others nets (n)	1.3 (141)	0.6 (35)	1.2 (45)	1.0 (71)
Number of children tested for malaria (n/N)	1275/1374	1476/1534	1146/1227	1920/2026
% Malaria infection in 6 months-14 years children (95%CI)	18.7 (16.6-20.9)	38.4 (35.9-40.9)	45.6 (42.7-48.5)	31.1 (29.1-33.2)
sd: standard deviation; CI: confidence intervals; N: total number of observations, NMCP: National Malaria Control Program				

In the nets collected during cross-sectional surveys, overall, at t12, 54% (597/1110) of the study nets had at least one hole of any size (53% (152/285)-pyrethroid (PY) LLIN, 48% (162/341)-chlorfenapyr-PY LLIN, 52% (125/241)-pyriproxyfen-PY LLIN, and 65% (158/243)-PBO-PY LLIN), and this increased to 82% (830/1007) (84% (274/326)-PY-LLIN, 81% (280/346)-chlorfenapyr-PY LLIN, 81% (162/200)-pyriproxyfen-PY LLIN and 84% (114/135)-PBO-PY LLIN) at t36. Similarly, the mean Hole Surface Area (HSA) increased from 340 cm^2 to 1242 cm^2 in Pyrethroid-PY, 355 cm^2 to 1325 cm^2 in Chlorfenapyr-PY LLIN, 526 cm^2 to 1301 cm^2 in Pyriproxyfen-PY LLIN and 990 cm^2 to 2060 cm^2 in PBO-PY LLIN between t12 and t36 (Appendix 5.3). There were no significant differences in mean HSA between PY-LLIN, Chlorfenapyr-PY LLIN, and Pyriproxyfen-PY LLIN at any survey timepoint, while PBO-PY LLIN had substantially higher HSA at each timepoint than any other study net (Appendix 5.3). The overall percentage of too—torn nets in the cross-sectional surveys increased from 17% (189/1110) at t12 to 35% (372/1055) at t24 and stabilised between 44% (311/700) at t30 and 43% (432/575) at t36 (Appendix 5.2). In all net brands, the lower part of the nets (bottom zone) was more damaged than the rest of the zones (Appendix 5.4). All new nets had similar dimensions (height: 180 cm); however, after 3 years of field use, the height decreased disproportionately between net brands to 170 cm for PY-LLIN, 174 cm for Chlorfenapyr-PY LLIN, 157 cm for Pyriproxyfen-PY LLIN, and 158 cm for PBO-PY LLIN (Appendix 5.5).

There was no significant association between malaria prevalence and net condition: damaged (OR 0.98, 95% CI 0.71–1.37, p-value = 0.655) and too-torn (OR: 1.07, 95% CI 0.77–1.47, p-value = 0.694) compared to good nets (Table 5.2 ; Appendix 5.10). Malaria infection was significantly lower for children living in clusters that received Chlorfenapyr-PY LLINs compared to those living in clusters that received PY LLINs regardless of the physical condition (Table 5.2). Children from houses with more than 50% of the sleeping spaces covered by the study nets had lower odds of malaria (OR 0.61, 95% CI 0.42–0.87, p-value = 0.006) than households with fewer sleeping spaces covered. The odds of malaria in all arms increased with time since the net distribution, however, was lower at t36 compared to t24, and this was likely related to the distribution of PBO-PY LLIN in all arms in October 2021 (4 months before the t36 survey) in the study area via the school-net programme (SNP). Moreover, children sleeping under nets had lower odds of malaria infection than children not using any nets (Appendix 5.7). In the surveyed households, the majority of households reported closing the main doors at night, getting inside houses, and sleeping time between 21 and 22 hours (Appendix 5.9).

Table 5.2: Association between net physical condition and malaria prevalence in children aged 6 months to 14 years in cross-sectional surveys

Covariate	%Infection (n/N)	Adjusted OR	95% CI	p-value
Net condition				
Good	24.6 (180/733)	1 (Ref)		
Damaged	25.4 (109/429)	0.98	0.71-1.37	0.6550
Too-torn	28.3 (146/516)	1.07	0.77-1.47	0.6940
Study arm				
Pyrethroid (PY) LLIN	33.7 (168/498)	1 (Ref)		
Chlorfenapyr-PY LLIN	17.7 (90/508)	0.40	0.23-0.69	0.001
Pyriproxyfen-PY LLIN	28.4 (101/356)	0.80	0.47-1.38	0.430
PBO-PY LLIN	24.1 (76/316)	0.62	0.35-1.08	0.091
Cross-sectional survey				
12 months post-intervention	17.0 (103/607)	1 (Ref)		
24 months post-intervention	36.1 (210/582)	2.93	2.15-3.99	<0.001
36 months post-intervention	25.0 (122/489)	1.72	1.22-2.43	0.002
Children age				
0-4 years	14.8 (97/656)	1 (Ref)		
5-10 years	30.5 (213/699)	2.86	2.13-3.85	<0.001
11-14 years	38.7 (125/323)	4.62	3.25-6.58	<0.001
SES				
Lowest	27.5 (156/567)	1 (Ref)		
Middle	27.2 (155/571)	0.92	0.68-1.26	0.622
Highest	23.0 (124/540)	0.66	0.46-0.93	0.018
Eaves				
Yes	30.7 (167/544)	1 (Ref)		
No	23.6 (268/1134)	0.78	0.59-1.04	0.096
Household study net coverage*				
Too few (<50%)	33.5 (218)	1 (Ref)		
Moderate/high (>=50%)	24.9 (1460)	0.61	0.42-0.87	0.006

**Household study net coverage: is the proportion of sleeping spaces in the household used last night covered by study net; There was no evidence of interaction between net physical condition and net type, p-value = 0.9814. There was no evidence of interaction between net type and survey timepoint, p-value = 0.5010.*

In the cohort, 5019 children (2256 in year 1 and 2763 in year 2) were assessed alongside the nets they used at the last cohort visit. Of these children, 2239 (99%) in year 1 and 2403 (87%) in year 2 declared to own study nets. These nets were brought for physical condition assessment. Overall, the mean HSA in the cohort nets was 786 cm^2 for year 1 and 1047 cm^2 for year 2. Over the two years of cohort study, the overall percentage of too-torn nets increased from 19% (430/2234) at t12 to 33% (801/2397) at t24 (Appendix 5.2).

There were increased rates of malaria cases in children sleeping under too-torn nets (IRR 1.33 [95% CI 1.12–1.57], p = 0.001) compared to sleeping under good nets (Table 5.3). Lower rates of malaria cases were associated with living in houses with the highest socioeconomic status (IRR 0.77 [95% CI 0.63–0.96], p = 0.019), using chlorfenapyr-PY LLIN (IRR 0.49 [95% CI 0.31–0.79], p = 0.004). Higher rates were associated with using study nets that were 2 years old (IRR 1.41 [95% CI 1.19–1.67], p < 0.000). Older children and living in a house with open eaves were not associated with a higher incidence of malaria

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Table 5.3: Association between net physical condition and malaria case incidence in children aged 6 months to 10 years in the cohort study

Covariate	Number of clinical episodes	Follow-up time child years	Incidence per child per year	Adjusted Rate ratio	95%CI	p-value
Study net condition						
Good	573	2268.4	0.25	1 (ref)		
Damaged	258	902.9	0.29	1.18	0.97-.43	0.0936
Too-torn	379	1184.7	0.32	1.33	1.12-1.57	0.0012
Study arm						
Pyrethroid (PY) LLIN	392	1114.7	0.35	1 (ref)		
Chlorfenapyr-PY LLIN	174	1120.4	0.16	0.49	0.31- 0.79	0.0035
Pyriproxyfen-PY LLIN	363	1124.4	0.32	1.00	0.64-1.58	0.9844
PBO-PY LLIN	281	996.5	0.28	0.83	0.52-1.32	0.4334
Cohort year						
year1	434	1931.4	0.22	1 (ref)		
year2	776	2424.6	0.32	1.41	1.19-1.67	0.0001
Children age group						
0-4 years	512	1879.7	0.27	1 (ref)		
5-10 years	698	2476.3	0.28	1.08	0.92-1.27	0.3604
Socio-economic status						
Lowest	296	899.6	0.33	1 (ref)		
Middle	273	897.2	0.30	0.96	0.79-1.16	0.6542
Highest	217	874.0	0.25	0.77	0.63-0.96	0.0189
Eaves						
No	829	3061.1	0.27	1 (ref)		
Yes	381	1294.8	0.29	1.00	0.82-1.21	0.9768

In order to assess if the dual-AI ITNs were superior to PY LLINs against incidence regardless of their textile conditions, each net type and condition was compared to a PY LLIN in good condition. During the first year of follow-up, the protective effect of too-torn dual-AI ITNs compared to good PY LLIN against malaria case incidence was strongest for too-torn PBO-PY LLIN (IRR 0.37 [95% CI 0.19–0.72], p = 0.003), borderline for chlorfenapyr-PY LLIN (IRR: 0.45 [95% CI 0.33–0.97], p = 0.053) and no additional protection was given by pyriproxyfen-PY LLIN (IRR 1.15 [95% CI 0.61–2.17], p = 0.660). Sleeping under a good PBO-PY LLIN or a good chlorfenapyr-PY LLIN was more protective than sleeping under a good PY LLIN against malaria case incidence. For children using pyriproxyfen-PY LLIN, however, there was a slight decrease in malaria incidence in children sleeping under damaged nets (0.26 cases per child/year) or good nets (0.24 cases per child/year); however, those differences were not significant compared to good PY LLIN (IRR = 0.74 [95% 0.38–1.46], p = 0.386) for damaged pyriproxyfen-PY LLIN and (IRR = 0.78 [95% 0.46–1.34], p = 0.369) for good pyriproxyfen-PY LLIN (Table 5.4).

Table 5.4: Association between net physical condition and malaria case incidence in children aged 6 months to 10 years in year 1 in the cohort study

LLIN type and condition	Number of clinical episodes	Follow-up time child years	Incidence per child per year	Adjusted Rate ratio	95%CI	p-value
Pyrethroid (PY) LLIN						
Good	104	313.48	0.33	1		
Damaged	36	95.67	0.38	0.90	0.57-1.43	0.6538
Too-torn	30	81.17	0.37	0.93	0.56-1.54	0.7778
Pyriproxyfen-PY LLIN						
Good	83	342.67	0.24	0.78	0.46-1.34	0.3688
Damaged	22	84.64	0.26	0.74	0.38-1.46	0.3856
Too-torn	34	84.41	0.40	1.15	0.61-2.17	0.6596
PBO-PY LLIN						
Good	33	199.70	0.17	0.43	0.23-0.80	0.0076
Damaged	12	70.58	0.17	0.61	0.28-1.33	0.2128
Too-torn	22	174.74	0.13	0.37	0.19-0.72	0.0033
Chlorfenapyr-PY LLIN						
Good	34	309.56	0.11	0.35	0.28-0.71	0.0006
Damaged	15	96.40	0.16	0.51	0.38-1.03	0.0675
Too-torn	10	79.30	0.13	0.45	0.33-0.97	0.0525

In year 2, compared to those sleeping under good PY-LLIN, only children sleeping under chlorfenapyr-PY LLIN in good condition had a significant and more substantial protective effect (IRR = 0.49 [95% CI 0.52–1.37], p = 0.006) against malaria case incidence (Table 5.5). There was no reduced risk of infection associated with sleeping under too-torn PBO-PY LLIN (IRR = 1.36 [95% 0.86–2.16], p = 0.186) and too-torn Pyriproxyfen-PY LLIN (IRR = 1.46 [95% CI 0.89–2.37], p = 0.131) in year 2 compared to PY-LLIN in good condition.

Table 5.5: Association between net physical condition and malaria case incidence in children aged 6 months to 10 years in year 2 in the cohort study

LLIN type and condition	Number of clinical episodes	Follow-up time child years	Incidence per child per year	Rate ratio	95%CI	p-value
Pyrethroid (PY) LLIN						
Good	107	312.18	0.34	1		
Damaged	62	159.22	0.39	1.19	0.83-1.70	0.3533
Too-torn	53	152.95	0.35	1.07	0.74-1.56	0.7125
Pyriproxyfen-PY LLIN						
Good	80	299.81	0.27	0.85	0.52-1.37	0.4949
Damaged	57	133.47	0.43	1.37	0.82-2.28	0.2308
Too-torn	87	179.46	0.48	1.46	0.89-2.37	0.1305
PBO-PY LLIN						
Good	67	181.21	0.37	1.18	0.72-1.94	0.5144
Damaged	26	91.19	0.29	0.98	0.54-1.77	0.9505
Too-torn	119	275.63	0.43	1.36	0.86-2.16	0.1858
Chlorfenapyr-PY LIN						
Good	50	317.19	0.16	0.49	0.29-0.81	0.0059
Damaged	36	169.21	0.21	0.67	0.39-1.16	0.1499
Too-torn	30	153.87	0.19	0.64	0.36-1.14	0.1286

5.5 Discussion

As part of a cluster randomised trial of dual-active ingredient malaria vector control interventions, we assessed the textile conditions of the dual-AI ITNs [namely: Chlorfenapyr-PY LLIN, Pyriproxyfen-PY LLIN, and a PBO-PY LLIN] after three years of use in the community [147]. We then explored the associations between net conditions and malaria prevalence (from repeated cross-sectional surveys) and incidence (from a cohort study) in the Mwanza region, Tanzania.

In this study, based on the cross-sectional survey data, there was no evidence indicating that the condition of the net was associated with malaria prevalence. Indeed, it was observed that good, damaged, and too-torn study nets appeared to offer similar levels of personal protection against malaria infection prevalence after adjusting for several covariates, such as net age, child age, presence of eaves in the house, socioeconomic status, and household level net coverage. The strong protection provided by high household coverage of nets (>50%) highlights the importance of promoting high levels of ownership and retention of nets in all households. Additionally, from the repeated cross-sectional measures, we also observed that study nets offered protection to those sleeping under them against malaria infection, and that sleeping under any net with holes provided more protection than sleeping without a net at all, similar to studies undertaken in Malawi[94, 104, 184], and Equatorial Guinea [94].

LLINs, as the principal form of malaria vector control in the study area, reduced malaria infection by 54%, regardless of the net type and whether they had holes, compared to not using a net at all. Notably, even users of pyrethroid-only LLINs were far more protected than non-net users. This finding supports existing evidence that pyrethroid-only LLINs still provide protection compared to sleeping without a net, even in insecticide-resistant settings [103, 104, 184]. Several studies have reported an association between increased levels of damage of pyrethroid-only nets and increased malaria infection [94, 199], while others reported no association [205, 215]. However, while we saw no impact of differences in net integrity on malaria prevalence, in our cohort study, there was a strong association between net textile condition and malaria case incidence, unlike the cohort study in Malawi [184]. In the first year, when the insecticides were in suitable concentrations [147], torn chlorfenapyr-PY LLIN and PBO-PY LLIN were better than good PY-LLIN. However, in the second year, only good chlorfenapyr-PY LLIN was better than good PY-LLIN; this may be explained by waning insecticide concentrations in the dual-AI ITNs over time [147].

Torn pyriproxyfen-PY LLINs (in the first and second year) and torn PBO-PY LLINs (during the second year) of use did not provide superior protection against malaria case incidence compared to good PY LLINs. This is consistent with results generated recently in Tanzania[147] and Benin[152], where Pyriproxyfen-PY LLINs and PBO-PY LLIN did not perform well during the second year. This study supports and adds weight to previous studies that suggested the impaired effectiveness of these two products is likely related to poor fabric integrity, leading to an unexpected decline in community coverage. However, a systematic review of 22 published studies reported that wear and tear were not identified as a reason for not using mosquito nets when they are the only nets available [75].

In this study, ownership, usage, and textile conditions of all nets declined swiftly over the three years after net distribution. The decline was more marked in the PBO-PY LLIN arm followed by pyriproxyfen-PY LLINs, consistent with the results generated recently in Tanzania in a prospective net cohort study done with the same net [88]. It is fairly standard to see a reduction in usage and ownership in places with no continuous distribution of nets [94]. However, through the continuous distribution of nets in antenatal care (ANC) clinics and the expanded program on immunization (EPI) and school-based programs coverage of other nets in the study area was kept high [216].

In all nets, the damage was most severe in the bottom part [86, 87]. This part has been reported to have limited contribution to mosquito prevention[86] as it is always tucked under a mat or mattress. However, improper tucking of the bed net is not an uncommon practice [217-219], and net tucking is very challenging without a proper bed frame; in this study, more than half of the sleeping spaces in surveyed households had no bed frames. For untucked or partially tucked nets, the bottom part will still allow mosquitoes to penetrate. Enhancing the bottom part might remedy nets from early hole development and further enlargement, although other studies have shown that dense knitting in this part of the net did not necessarily make it more durable[220].

Similarities in physical properties (fibers, denier, and integrity) between some nets [chlorfenapyr-PY ITN and PY-ITNs], but differences in protective efficacy against malaria infection emphasize the pivotal role of non-pyrethroid insecticide and synergists (chlorfenapyr and PBO[197]) in malaria vector control. In addition, this study demonstrates that the resilient physical integrity of the nets on its own is not enough to provide sustained protection to users of nets in good conditions against bites of malaria-transmitting mosquitoes even in settings of high net coverage, as it was in the cohort children where we observed >80% use. This is contrary to the review by Okumu (2020) [54] which suggested that physically durable nets could serve a similar purpose as insecticidal nets.

5.6 Limitations

In this study, we only assessed net condition at the end of the year against cumulative incidence over the year; likely, the nets were not in that condition for the whole year. However, when we assessed the association between malaria prevalence and net condition in cohort children during their last visits, it showed similar results. The number of study nets to be assessed decreased over time due decline in coverage, leading to a small number of cross-sectional nets assessed towards the end. However, a better and direct comparison of the protective effect of nets was attained with the cohort nets, which had high access and usage across the study. Net users in the cross-sectional survey tend to replace lightly damaged nets with readily available new ones, likely, the majority of the nets evaluated towards the end of the study were still new or put into usage a few months previously.

5.7 Conclusion

Our results provide evidence that sleeping under too-torn chlorfenapyr-PY LLIN and PBO-PY LLIN offered superior protection compared to sleeping under good standard net and, the protective effect of PBO-PY LLIN diminishes as PBO-treated nets age. The results also show that, when assessing net integrity independent of the protective effect against malaria, chlorfenapyr-PY LLIN (Interceptor G2, polyester net) nets are physically more durable than PBO-PY LLIN (Olyset Plus, polyethylene net) but relatively similar to pyriproxyfen-PY LLIN (Royal Guard, polyethylene net). The future strategies for control programs reliant on the dual-AI ITNs, therefore, not only seek to provide new nets to households that do not have nets but also instigate strategies to inform the population that even a torn dual-AI LLIN is better than an intact standard net or sleeping without a net; small holes in nets should not motivate households to discard the nets. Furthermore, there should be an emphasis on proper net care and repair practices among users, as well as the necessity for manufacturers to develop physically durable LLINs.

6 Chapter 6: Community benefits of Dual-AI ITNs over 3 years of use

This paper covers the PhD objective 3. This chapter describes the secondary analysis of the large cluster randomised controlled trial (RCT) in the Misungwi district. A multivariate analysis was used to determine the association between LLIN usage levels and community benefits given by LLIN at different levels of usage and compare this parameter between dual-AI ITNs and the standard LLIN. These results build on the previous findings on what coverage levels of LLINs can elicit community protection to benefit even those people who are not using nets. The author of this thesis supervised the data collection of all 5 cross-sectional surveys, cleaned the data, analysed the data, and wrote the first draft of the manuscript.

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6.1 Abstract

Long-lasting insecticidal nets (LLINs) were once fully effective for the prevention of malaria; however, mosquitoes have developed resistance to pyrethroids, the main class of insecticides used on nets. Dual active ingredient LLINs (dual-AI ITNs) have been rolled out as an alternative to pyrethroid (PY)-only LLINs to counteract this. Understanding the minimum community usage at which these LLINs elicit an effect that also benefits non-users against malaria infection is important. We conducted a secondary analysis of a 3-year randomized controlled trial (RCT) in 84 clusters in North-western Tanzania to evaluate the effectiveness of three dual-AI ITNs: pyriproxyfen and alpha(α)-cypermethrin, chlorfenapyr and α-cypermethrin, and the piperonyl-butoxide (PBO) and permethrin compared to α-cypermethrin only LLINs. We measured malaria infection prevalence using 5 cross-sectional surveys between 2020 and 2022. We assessed net usage at the cluster level and malaria infection in children aged from 6 months to 14 years in 45 households per cluster. A total of 22,479 children from 12,654 households were tested for malaria using rapid diagnostic tests in January 2020, 2021, & 2022 and July 2020 & 2021. Among non-users, community-level usage of >40% of dual-AI LLIN was significantly associated with protection against malaria infection: chlorfenapyr arm (OR: 0.44 (95% CI: 0.27-0.71), p=0.0009), PBO arm (OR: 0.55 (95% CI: 0.33-0.94), p=0.0277) and pyriproxyfen arm (OR: 0.61 (95% CI: 0.37-0.99), p=0.0470) compared with non-users in clusters with >40% usage of pyrethroid-only LLINs. There were indications of some protection against malaria infection to non-users in the chlorfenapyr arm when community-level usage was ≤40% (OR: 0.65 (95% CI: 0.42-1.01), p=0.0528) compared to those living in clusters with >40% usage of pyrethroid-only LLINs. Our study demonstrated that at a community usage of 40% or more of dual-AI ITNs, non-users benefited from the presence of these nets. Noticeably, even when usage was ≤40% in the chlorfenapyr arm, non-users were better protected than non-users in the higher coverage pyrethroid-only arm. The greater difference in malaria risk observed between users and non-users indicates that LLINs play a crucial role in providing personal protection against malaria infection for the people using the net.

The trial was registered as a clinical trial on www.clinicaltrials.gov: ClinicalTrials.gov (NCT03554616) on 2018-06-13.

6.2 Background

Long-lasting insecticidal nets (LLINs) have been the core intervention for malaria control for many years and have contributed to a decline of >25% global cases and >66% global deaths since 2000 [1, 67, 207, 221]. LLINs work by providing a barrier preventing mosquitoes from taking bloodmeals from people sleeping under them and by killing or reducing a mosquito's lifespan via the insecticide on the netting. The latter can result in a 'community effect' via which even non-users of nets benefit due to the reduced population of infectious mosquitoes [25, 49, 57, 58, 222]. This has been demonstrated in experimental hut trials [105, 223, 224], modelling [49, 225], and community trials [57, 226] where a higher level of community coverage of pyrethroid-only nets was associated with a decrease in malaria risk in those not using nets. Previous findings with pyrethroid-only nets have suggested that community coverage needs to be at least 15% and up to 85% before the community effect is realised [60]. However, this is a wide range to rely on for program implementation.

The community effect will depend on the insecticidal properties of the LLIN [227], as well as LLIN characteristics (coverage, netting integrity), and vector species behaviour (anthropophilic and zoophilic nature) [228]. Moreover, the presence of non-human alternative hosts, time spent indoors, under a net, and outdoors during peak biting hours, and insecticide resistance are also determinants of mass effect.

Insecticide resistance continues to be a threat to the effectiveness of pyrethroid-only LLINs [61, 98, 100, 105-107, 227, 229]. In high insecticide resistance settings, the main mechanism of protection for people using pyrethroid-only nets is likely to be via the physical barrier of the net preventing mosquito bites- meaning non-users do not benefit to the same extent. Switching to novel malaria control tools, such as dual-active ingredient long-lasting insecticidal nets (dual-AI ITNs) will likely restore community effects in areas where the majority of vectors are resistant to pyrethroids [106].

Dual-AI ITNs are more effective than pyrethroid-only nets, where mosquitoes are resistant to pyrethroids [146-149, 151, 152]. This is due to their unique modes of action, ranging from inhibiting the activity of the enzymes that breakdown pyrethroids in resistant vectors (piperonyl butoxide), sterilizing vectors that survive exposure to pyrethroids (pyriproxyfen), to disrupting the vectors' ability to produce energy (chlorfenapyr), thereby restoring the effectiveness of LLINs against resistant mosquitoes [146-149, 151, 152]. Therefore, understanding the coverage levels required for community-wide effects is vital to help determine net coverage targets and plans for future campaigns.

Tanzania has one of the highest burdens of malaria cases and deaths [1]. In Tanzania, malaria is highest in the Lake Victoria zone. The core malaria intervention in the country is LLINs, which have been distributed widely in the country since 2007 [230]. Although Tanzania has made great efforts to implement LLINs in the general population, gaps in use, access, coverage, and ownership remain. As such, several distribution channels including mass campaigns, annual school net program (SNP), antenatal care (ANC), and the Expanded Programme on Immunization, and Targeted Replacement Campaign (TRC) are being implemented across the country. Dual-AI ITNs, particularly piperonyl-butoxide (PBO) LLINs, have been distributed in Tanzania through the SNP and ANC since 2018 in areas with high malaria burden, however, achieving high coverage of the population at risk remains a challenge. An understanding of the required level of coverage to achieve community benefits is key to the proper allocation of limited malaria control resources.

In this study, we assess malaria risk among users and non-users of nets living in areas with different community coverage of dual-AI ITNs as part of a secondary analysis of a large RCT assessing the impact of dual-AI LLIN in Misungwi, Tanzania [147].

6.3 Methods

6.3.1 Study site, design, and participants

Data used for this secondary analysis were collected in a 3-year, four parallel-arm cluster randomized controlled trial (RCT) conducted on the southern border of Lake Victoria, Misungwi district (latitude 2°51'00.0"S, longitude 33°04'60.0" E), Mwanza region, in North-western Tanzania. The RCT evaluated the effectiveness of three types of dual-active-ingredient long-lasting insecticidal nets compared to pyrethroid-only LLINs for reducing malaria. A total of 84 clusters were allocated to one of the four study arms (21 clusters per arm) using restricted randomisation (arms balanced on population size, baseline malaria prevalence, socioeconomic status, LLIN usage, and species composition). The four arms of the trial consisted of: Interceptor® with only pyrethroid (PY) insecticide (alpha-cypermethrin, [control] arm), Interceptor® G2 (chlorfenapyr LLIN (alpha-cypermethrin + chlorfenapyr), Royal Guard® (pyriproxyfen LLIN (alpha-cypermethrin + pyriproxyfen), and Olyset Plus (permethrin + piperonyl-butoxide (PBO)). The main results from the RCT have been previously published and showed that: chlorfenapyr LLINs showed superior efficacy over three years and piperonyl-butoxide LLINs over one year while pyriproxyfen LLINs did not seem to provide significant additional protection compared to pyrethroid-only LLIN [147, 151].

6.3.2 Procedures

A total of 147, 230 study LLINs were distributed (1 net for 2 persons) in 42,394 households as part of the trial between January 26 and January 28, 2019. In addition, there was continuous distribution of pyrethroid-only LLINs and PBO LLINs in the study area through ANC, and in September 2021 (33 months post-trial net distribution), 40,000 PBO LLINs were distributed by the local government across all study arms via SNP.

Malaria infection prevalence was measured during cross-sectional surveys at 12 months (January/February 2020), 18 months (July/August 2020), 24 months (January/February 2021), 30 months (July/August 2021), and 36 months (January/February 2022) post-intervention. At each survey timepoint, a random sample of 45 households in each cluster was selected. Up to two children aged between 6 months and 14 years in consenting households were randomly selected and tested for malaria infection using rapid diagnostic tests (RDT) (CareStart malaria HRP2 [pf], DiaSys, Wokingham, UK). Written informed consent was obtained from an adult guardian in each household interviewed and for selected children. In all consenting households, information (age, and sex) for all residents was recorded and all nets were visually examined by a trained interviewer, and the information about who (age and sex) used the net last night was recorded. Study net usage was then calculated as the percentage of all people (adults and children) who reported using study nets (i.e. chlorfenapyr, pyriproxyfen, and PBO LLINs) the previous night. Community/cluster study LLIN usage was calculated as the percentage of people (adults and children) within a given cluster at each survey point who reported using a dual-AI LLIN the previous night divided by the total number of observations in that cluster.

Data collection took place on smartphones using the Open-Data-Kit (ODK) software. Data from each field team was directly uploaded to a secure online database at the London School of Hygiene and Tropical Medicine (LSHTM) and the copy was retained in Tanzania. After completion of the surveys, datasets were transferred to STATA release 15 (StataCorp, College Station, TX, USA) for further aggregation, cleaning, and analysis.

6.3.3 Statistical analysis

The main outcome of interest was the prevalence of malaria in non-users of nets comparing each of the dual-active-ingredient LLIN arms to the pyrethroid-only LLIN arm at 12 months, 18 months, 24 months, 30 months, and 36 months post net distribution. The secondary outcomes were 1/determine at which level of community net usage can benefit non-users (elicit community effect), 2/ malaria risk differences between users and non-users at each survey timepoint.

Household socio-economic status (SES) indices were constructed based on self-reported ownership of certain goods (animals, poultry, phone, radio, bicycle, motorbike) and household possessions (including electricity, source of drinking water, toilet, number of sleeping rooms, type of cooking fuel). Principal component analysis (PCA) was used to develop a score which was then subdivided into wealth tertiles (lowest, middle, and highest) at the household level. Initially, the malaria prevalence of users and non-users of nets was compared between study arms. House characteristics and structures (including roof, floor, eaves, walls, ceiling, and plastering,) were not included in the construction of SES, instead, they were used to create a household design index and subdivided into tertiles (low-quality, medium-quality, and high quality).

To assess the community effect of the dual-AI ITNs (chlorfenapyr, pyriproxyfen, and PBO LLINs) relative to pyrethroid-only LLIN, the following analyses were conducted: 1/ comparison of malaria prevalence between users and non-users, and 2/ comparison of malaria prevalence in non-users in each dual-AI. LLIN arm and non-users in the pyrethroid-only arm, Analyses 1 and 2 were done regardless of the cluster-level net usage. 3/ To assess whether the level of dual-AI LLIN cluster level usage had an impact on the non-users of the net, a cut-off was selected based on findings of other studies, which indicated that usage levels of pyrethroid-only nets of between 30% and 50% were associated with community protection [49, 231], and to ensure a sufficient number of clusters in each category over time. Other thresholds such like 60%, 50% 30% and 20% were explored but clusters could not be balanced. We compared malaria prevalence in non-users living in clusters with >40% or ≤ 40% community usage of dual-AI LLIN arms *versus* non-users living in pyrethroid-only clusters with community usage >40% (Box 6.1). All malaria prevalence analyses used mixed-effect logistic regression. Models 1 and 2, included cluster as a random effect and fixed effects for survey timepoint, study arm, and adjusted for age group, sex, housing quality, and socio-economic status (SES) and the baseline cluster-level variables used in restricted randomisation. Model 3, which combined all data, cluster, and survey timepoint was included as a random effect, while the study arms categorised ≤40% and >40% cluster level usage as the fixed effect and adjusted for the same variables included in models 1&2. The intervention effect was expressed as adjusted odds ratios (aORs). Differences in characteristics between users and non-users were compared using Chi-square ($\chi 2$), accounting for the clustered design. An interaction test between cluster-level usage (>40% and ≤40%) and survey timepoints was performed to test for the presence of effect modification.

Box 6.1: comparison groups

Comparisons	Group1	Group 2
Analysis 1	Users	Non-users
Analysis 2	Non-users in dual-AI arms	Non-users in pyrethroid-only arm
Analysis 3	Non-users living in clusters with ≤ 40% community usage of study nets in dual-AI arms	Non-users living in clusters with >40% community usage of study nets in pyrethroid-only arm

6.3.4 Ethical considerations

Ethical approval for the RCT was obtained from the institutional review boards of the Tanzanian National Institute for Medical Research (reference number: NIMR/HQ/R.8a/Vol.IX/2743), Kilimanjaro Christian Medical University College (2267), London School of Hygiene and Tropical Medicine (14952), and University of Ottawa (H-05-19-4411).

6.3.5 Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

6.4 Results

Between 07 January 2020 and 10 February 2022, five cross-sectional surveys were conducted in 12,654 randomly selected consenting households post-net distribution. Overall, 23% (N=22479) [(14% (N=4380) at 12 months, 22% (N=4785) at 18 months, 20% (N=4988) at 24 months, 30% (N=3997) at 30 months, and 28% (N=4329) at 36 months)] of children tested for malaria did not sleep under a net the previous night. Residents (all age groups) not sleeping under study LLINs the previous night were more than doubled at 36 months (n=14,940) compared to 5,975 at 12 months. Net use was highest in children under 5 years old (Table 6.1). In houses with not enough nets for every member (i.e. less than 1 net for every 2 people), boys over the age of 5 years were least likely to sleep under a net. People classified as highest SES were least likely to use nets. Net usage between girls and boys when under 5 years of age was similar (Table 6.1).

Table 6.1: Characteristics of non-users and users of the nets

Covariates	n	n
Population (all age groups) in each study arms		
Pyrethroid-only group	18498	5361
Piperonyl butoxide group	16854	6012
Pyriproxyfen group	17462	6128
Chlorfenapyr group	19081	6715
	% users [95% CI] (n)	% non-users[95% CI] (n)
Child age and sex		
0-4 years: Girls	85.8% [84.22,87.26], 3489	14.2% [12.74,15.78], 577
0-4 years: Boys	84.8% [83.38,86.16], 3538	15.2% [13.84,16.62], 633
5-9 years: Girls	77.5% [75.26,79.62], 3141	22.5% [20.38,24.74], 911
5-9 years: Boys	73.9% [71.81,75.84], 2983	26.1% [24.16,28.19], 1055
10-14 years: Girls	72.8% [70.40,75.11], 2218	27.2% [24.89,29.60], 828
10-14 years: Boys	66.0% [63.34,68.45], 2048	34.1% [31.55,36.66], 1058
Household structure quality		
Low quality	76.1% [74.46,77.72], 5814	23.9% [22.28,25.54], 1823
Medium quality	76.5% [74.31,78.49], 5783	23.5% [21.51,25.69], 1780
High quality	80.0% [78.07,81.72], 5820	20.0% [18.28,21.93], 1459
Socio-economic status (SES)		
Lowest	81.0% [79.22,82.71], 5995	19.0% [17.29,20.78], 1404
Middle	78.3% [76.51,80.07], 5730	21.7% [19.93,23.49], 1584
Highest	73.3% [71.25,75.25], 5692	26.7% [24.75,28.75], 2074
Households with not enough coverage of study nets (1 net for 2 people) by child age group and sex		
0-4 years: Girls	89.1% [87.33,90.71], 1764	10.9% [9.288,12.67], 215
0-4 years: Boys	88.4% [86.84,89.71], 1790	11.7% [10.29,13.16], 236
5-9 years: Girls	81.5% [79.25,83.53], 1721	18.5% [16.47,20.75], 391
5-9 years: Boys	77.1% [74.76,79.24], 1651	22.9% [20.76,25.24], 491
10-14 years: Girls	78.2% [75.79,80.48], 1286	21.8% [19.52,24.21], 358
10-14 years: Boys	70.4% [67.47,73.08], 1253	29.7% [26.92,32.53], 528

Following net distribution, overall mean malaria prevalence was 52% (N=5062) in non-users [34% (N=619) at 12 months, 58% (N=1049) at 18 months, 52% (N=998) at 24 months, 63% (N=1202) at 30 months, and 46% (N=1194) at 36 months]; and the mean malaria prevalence in users was 32% (N=11845) [20% (N=3761) at 12 months, 43% (N=3736) at 18 months, 34% (N=3990) at 24 months, 39% (N=2795) at 30 months, 24% (N=3135) at 36 months]. A summary of malaria prevalence among users of study nets, users of other nets, and non-users by study arm and survey timepoint is presented in Appendix 6.1.

In non-users, at 12 months, there was no evidence for lower malaria prevalence in any of the dual AI-LLIN arms compared to the pyrethroid-only arm. At 18 months, 24 months, and 30 months no difference in malaria prevalence was observed in the piperonyl-butoxide arm or pyriproxyfen arm compared with the pyrethroid-only arm (Table 6.2). In non-users living in the chlorfenapyr arm, there appeared to consistently be a reduction in prevalence compared to non-users in the pyrethroid-only arm. The odds of malaria infection were at least 40% lower for non-users living in the chlorfenapyr arm at 18 months [adjusted odds ratio (aOR) 0.56 (95% CI 0.35-0.90), p=0.0166], 24 months [aOR 0.55 (95% CI 0.29-1.03), p=0.0621] and 30 months [aOR 0.57 (95% CI 0.33-0.96), p=0.0353] compared to living in pyrethroid-only arm (Table 6.2). At 36 months, non-users in every dual-AI LLIN arm had lower malaria prevalence than non-users living in the pyrethroid-only arm (Table 6.2).

Table 6.2: Malaria prevalence in children (aged 6 months to 14 years) who are users and non-users at 12, 18, 24, 30, and 36 months surveys

	Non-users analysis: n=5,062			Users analysis: n=17,417			
	n/N (%Prevalence)	aOR (95% CI)*	p-value	n/N (%Prevalence)	aOR (95% CI)*	p-value	p-value‡ for comparison between non-users and users
Pyrethroid-only group							
12 months	64/156 (41.0%)	1 (ref)		286/964 (29.7%)	1 (ref)		0.1444
18 months	154/236 (65.3%)	1 (ref)		488/992 (49.2%)	1 (ref)		0.0165
24 months	127/206 (61.7%)	1 (ref)		422/993 (42.5%)	1 (ref)		0.0208
30 months	174/258 (67.4%)	1 (ref)		333/698 (47.7%)	1 (ref)		0.0005
36 months	164/265 (61.9%)	1 (ref)		243/823 (29.5%)	1 (ref)		<0.0001
Pyriproxyfen group							
12 months	63/178 (35.4%)	0.82 (0.41-1.60)	0.5584	169/891 (19.0%)	0.55 (0.36-0.83)	0.0046	0.0003
18 months	159/257 (61.9%)	0.91 (0.56-1.49)	0.7133	424/895 (47.4%)	0.87 (0.62-1.21)	0.3929	0.0012
24 months	128/261 (49.0%)	0.81 (0.43-1.51)	0.5004	344/997 (34.5%)	0.85 (0.59-1.24)	0.4045	0.0035
30 months	177/300 (59.0%)	0.71 (0.42-1.21)	0.2109	249/704 (35.4%)	0.63 (0.44-0.91)	0.0144	<0.0001
36 months	131/293 (44.7%)	0.59 (0.37-0.95)	0.0284	171/757 (22.6%)	0.75 (0.49-1.13)	0.1628	<0.0001
Piperonyl butoxide group							
12 months	38/127 (29.9%)	0.93 (0.46-1.89)	0.8431	168/941 (17.9%)	0.68 (0.45-1.01)	0.0580	0.0769
18 months	155/269 (57.6%)	0.85 (0.53-1.38)	0.5188	347/891 (39.0%)	0.67 (0.48-0.94)	0.0190	0.0001
24 months	146/260 (56.2%)	0.99 (0.54-1.84)	0.9811	366/999 (36.6%)	0.92 (0.64-1.33)	0.6625	<0.0001
30 months	213/305 (69.8%)	1.40 (0.82-2.38)	0.2117	275/687 (40.0%)	0.80 (0.56-1.16)	0.2410	<0.0001
36 months	154/320 (48.1%)	0.62 (0.40-0.97)	0.0361	182/727 (25.0%)	0.93 (0.62-1.40)	0.7413	<0.0001
Chlorfenapyr group							
12 months	45/158 (28.5%)	0.66 (0.33-1.34)	0.2521	131/965 (13.6%)	0.39 (0.25-0.59)	<0.0001	0.0005
18 months	144/287 (50.2%)	0.56 (0.35-0.90)	0.0166	364/958 (38.0%)	0.57 (0.41-0.80)	0.0009	0.0142
24 months	117/271 (43.2%)	0.55 (0.29-1.03)	0.0621	209/1001 (20.9%)	0.39 (0.27-0.58)	<0.0001	<0.0001
30 months	191/339 (56.3%)	0.57 (0.33-0.96)	0.0353	245/706 (34.7%)	0.64 (0.44-0.93)	0.0187	0.0001
36 months	105/316 (33.2%)	0.33 (0.20-0.53)	<0.0001	156/828 (18.8%)	0.52 (0.35-0.79)	0.0020	0.0018

**aOR=adjusted odds ratio. Each intervention group is compared against the pyrethroid-only group for the same timepoint. Comparison p-value‡=p-value for comparison between users and non-net users by net type at each timepoint after adjusting for age, sex, socio-economic status (SES), and baseline cluster-level variables used in the restricted. Interaction between net type and survey: p=0.3001 in non-net users*

Across all study arms and survey timepoints, malaria infection was generally lower in users compared to non-users (Table 6.2). Malaria prevalence in non-users in the chlorfenapyr arm at each survey timepoint was similar or slightly higher than amongst users in the pyrethroid-only arm, i.e. the personal protection provided by users of pyrethroid-only nets was relatively similar to the protection provided by the community effect in the chlorfenapyr arm as shown in Appendix 6.3.

Table ***6.3*** presents the results of the impact of community usage on community effect by assessing malaria prevalence in non-users in clusters with above and below 40% study net usage. Cluster usage of dual-AI LLIN higher than 40% was associated with reduced odds of malaria infection in non-users living in the pyriproxyfen arm (aOR: 0.61 [95% CI: 0.37-0.99], p=0.0470), PBO arm (aOR 0.55 [95% CI: 0.33-0.94], p=0.0277); and chlorfenapyr arm (aOR 0.44 [95% CI: 0.27-0.71], p=0.0009) compared with their counterparts living in clusters over 40% usage in the pyrethroid-only arm. There was also weak evidence of reduced odds of malaria infection in non-users living in the chlorfenapyr arm 55.1% (157/285) when community-level usage was ≤ 40% compared to those living in the pyrethroid-only arm 45.7% (495/1083) when community usage was > 40%; aOR 0.65 [95% CI: 0.42-1.01]], p= 0.0528).

Table 6.3: Mean malaria prevalence in children (aged 6 months to 14 years) not using nets over three years in ≤ 40 and > 40% cluster level net usage

Covariates	Number of clusters[++]	%Prevalence in non-users (n/N)	aOR (95% CI), p-value*
>40 coverage-pyrethroid-only group	69	55.1 (157/285)	1 (Ref)
≤40 coverage- pyrethroid-only group	36	62.9 (526/836)	1.24 (0.90-1.72), 0.1910
>40 coverage- chlorfenapyr group	59	37.2 (107/288)	0.44 (0.27-0.71), 0.0009
≤40 coverage- chlorfenapyr group	46	45.7 (495/1083)	0.65 (0.42-1.01), 0.0528
>40 coverage- piperonyl butoxide group	33	41.2 (77/187)	0.55 (0.33-0.94), 0.0277
≤40 coverage- piperonyl butoxide group	72	57.5 (629/1094)	1.26 (0.82-1.92), 0.2904
>40 coverage- pyriproxyfen group	44	40.5 (106/262)	0.61 (0.37-0.99), 0.0470
≤40 coverage- pyriproxyfen group	61	53.8 (552/1027)	1.01 (0.66-1.54), 0.9726

**aOR=adjusted odds ratio. Adjusted for age, sex, SES, survey timepoint, and baseline cluster-level variables (net usage, malaria prevalence).* [++] *Number of clusters: total number of clusters contributing to the category over the study period*

A sensitivity analysis was conducted using the same dataset, excluding the 36-month survey (as new PBO LLINs were distributed in all the clusters through SNP a few months before the survey). In this analysis, non-users in chlorfenapyr arm (aOR 0.48 [95% CI: 0.29-0.79], p=0.0040) when cluster level usage was >40% were significantly protected against malaria compared to non-users in >40% cluster usage in pyrethroid-only arm; and very weak evidence with piperonyl-butoxide arm (aOR 0.63 [95% CI: 0.37-1.07], p=0.0895) and pyriproxyfen arm (aOR 0.63 [95% CI: 0.38-1.04], p=0.0711). No statistically significant protection was observed in all three dual-AI ITNs when coverage was below or equal to 40% after excluding 36 months in the analysis. Overall 83 out of the 84 (99%) clusters had >40% usage at 12 months, this reduced to 75% (n=63) at 18 months, 50% (n=42) at 24 months, 18% (n=15) at 30 months and only 2% (n=2) at 36 months (Appendix 6.2).

To see if the effect of community usage was modified by the survey period, we examined for an interaction between cluster-level usage and survey timepoint. The test for interaction between levels of community dual-AI LLIN usage and survey timepoints showed no difference in the effect of community dual-AI LLIN usage on the odds of malaria infection among children who did not use nets (p=0.3092).

6.5 Discussion

This is a secondary analysis of a cluster randomised trial of dual-AI ITNs assessing the community effect of three dual-AI ITNs (chlorfenapyr LLINs, pyriproxyfen LLINs, and PBO LLINs) compared with pyrethroid-only LLIN. Users were always more protected than non-users regardless of the net type and survey timepoint, underscoring the importance of personal protection provided by nets, even in areas of resistant mosquitoes. In addition, regardless of community usage levels, non-users living in the chlorfenapyr arm were more protected than non-users in the pyrethroid-only arm. We also found that cluster usage of dual-AI LLIN above 40% provided significantly better protection against malaria infection to non-users compared to non-users living in the pyrethroid-only arm, suggesting there was less of a community effect in the pyrethroid-only arm. There was borderline evidence of chlorfenapyr LLINs still providing better community protection to non-users, even when community usage was ≤ 40% compared to pyrethroid-only LLINs when cluster usage is >40%. After excluding 36-month survey data, non-users in all dual-AI ITNs when cluster level usage was >40% benefited from their presence, but this was more pronounced in the chlorfenapyr arm than the rest.

An early review by Lines et al [60] identified 21 studies assessing the community effect of pyrethroid-only LLINs and reported a wide range of minimum community coverage levels (from 15% to 85%) which lead to community effects protecting people sleeping without nets. Consistent with this, a study conducted in Kenya [232] concluded that at least 35% community coverage of nets is required to protect people sleeping without nets, while another study[57] reported that residents not using nets and living within 300 meters from a community using insecticide-treated nets (usage greater than 50%) were protected against malaria compared to those further away. Meanwhile, Lindblade et al. [233] found that community net usage (>82%) protected both users and non-users equally. Models have also been used to estimate the threshold of community net usage necessary to elicit a community effect. For example, Killeen et al. [49] modelled that coverage of 35%-65% would be needed to achieve community-wide benefits. Another model [61] suggested that as soon as one person uses an LLIN, there is a small indirect impact on non-users (even if marginal) compared to a hypothetical scenario where nobody is using an LLIN. All models suggest that the benefits for both users and non-users increase with net usage.

The present study adds to the body of existing evidence and demonstrates that when a net is very effective, as observed for chlorfenapyr LLINs, both users and non-users are protected even at moderate to low levels of community coverage. With less effective nets such as piperonyl-butoxide LLIN and pyriproxyfen LLINs, the impact on non-users was not as evident. Up to 30 months, there was no difference in malaria prevalence between non-users in PBO arm and pyrethroid-only arm suggesting limited community protection from PBO LLINs except when PBO LLIN cluster level usage was above 40%. Greater and longer-lasting efficacy has been observed with this class of nets in two other RCTs[148, 149]. Although neither of these trials specifically examined the impact of the net on non-users, in Uganda, the effect of PBO LLIN on malaria prevalence was more pronounced when only clusters receiving PBO LLINs as the dominant net (>75%) were included in the 'as treated' or 'per protocol' analysis [149]. In another study in Tanzania, during the first two years of follow-up, a similar reduction in malaria prevalence was observed for intention-to-treat and per protocol analyses, indicating that both non-users and users may have been protected equally by PBO LLIN [148]. In the third year, however, PBO LLIN showed reduced prevalence among users, as net usage and efficacy declined [234]. However, in this trial, usage of PBO LLIN during the first two years of the study was higher (from 79% in the first year and 54% in the second year) than reported in the present study (74% in the first year and 30% in the second year) and could explain the difference of impact. Pyriproxyfen LLINs were designed to provide a community effect through sterilizing vectors as well as reducing the lifespan of female vectors after they have blood-fed [235] and survived exposure to the insecticide on the net. Pyriproxyfen LLINs seem to have had some impact on malaria indicators in another trial conducted in Burkina Faso [146]. In the present trial, malaria prevalence was reduced in users only at 12 months compared to people using pyrethroid-only LLINs. Consistent with PBO LLIN results, low coverage in the pyriproxyfen arm did not benefit non-users.

It is worth noting in the present study that 36 months after distribution, individuals not using nets in all the dual-AI arms had lower odds of infection compared to those not using nets in the pyrethroid-only arm. This impact was likely associated with the distribution of new PBO LLINs across all arms four months before the 36-month survey, which increased the usage of new nets and effective nets. In addition, PBO may enhance the efficacy of pyriproxyfen as it does for pyrethroid as these two insecticides may have similar mechanisms of resistance [236]. However, even after we excluded 36-month data from the analysis, non-users in all dual-AI arms had reduced malaria infection and the effect was more evident in the chlorfenapyr arm. This provided evidence that an increase in cluster-level usage in the dual-AI arms above 40% will likely elicit stronger community protection.

Regardless of the impact of the nets on non-users, using any net was always more protective against malaria prevalence than not using one. This was observed for all net types including the pyrethroid-only LLIN. This result is consistent with other studies that reported higher malaria prevalence or incidence in non-users compared to users sleeping under standard pyrethroid-only LLINs even in areas with pyrethroid resistance and highlights the importance of the barrier effect of the nets [103, 104, 213, 237, 238]. Furthermore, high usage (> 40%) clusters were unsurprisingly concentrated in the timepoints closest to the distribution of nets implying that the majority of the nets in this category were new nets (with fresh insecticide), whilst, in the later years, the majority of the clusters were concentrated in ≤40% category and likely to be older nets.

This study has several limitations. The study was not designed for this secondary analysis and may have insufficient power to adequately assess separately the impact on users and non-users using multiple tests that would lead to other results occurring by chance. Net usage was estimated on information provided by households' members which might not always be reliable. Finally, non-users were defined as people (children and adults) who did not sleep under any net the night before and might not capture occasional net usage during the week which also may influence the conclusions.

Regardless, this secondary analysis provides insight into the efficacy of these novel dual-AI LLINs within a region characterized by moderate to high malaria transmission and high resistance to pyrethroids. In settings with limited resources and the presence of insecticide resistance, the deployment of an effective net, such as chlorfenapyr LLINs, even at suboptimal coverage, could be considered as it would be more effective and even more cost-effective[147] than high coverage of pyrethroid-only LLINs. This aligns with previous modelling work[239] which emphasized that a massive reduction in mosquitoes would be more important than coverage alone. However, even the most effective net in this study did not produce a sufficient reduction in mosquitoes to prevent users of these nets from being exposed to high levels of malaria infection. A key message was that users were always better protected than non-users and therefore after providing the most effective nets, national malaria control program could consider maximizing usage for better impact. Finally, as observed by other studies[11, 43, 240-243] pyrethroid-only nets still provided some protection in this area of pyrethroid resistance. Non-users of nets in clusters where chlorfenapyr nets were used were similarly protected as users in clusters where pyrethroid only nets were used. As malaria was still high even amongst users of dual-AI ITNs, meaning that these nets did not adequately control malaria and infection prevalence. New, more effective vector control tools are therefore urgently needed to provide better protection against malaria than the protection provided by nets.

6.6 Conclusion

In areas where resistance to pyrethroids is prevalent in malaria vectors, chlorfenapyr LLINs offer enhanced protection to individuals who use them as well as those who do not, even at lower coverage levels. This added protection for non-users could also be attained with nets containing piperonyl-butoxide (PBO) and pyriproxyfen when the overall cluster usage exceeds 40%. Users were more protected than non-users and emphasized the necessity to optimize net usage to benefit from their full potential. Nonetheless, in regions facing constrained financial resources and insecticide resistance, the distribution of the most effective net could be considered over the high-population coverage of conventional nets. This strategic allocation would ensure maximal impact in the control of malaria despite limitations in resources and resistance challenges.

7 Chapter 7: Acceptability of and preferences for dual-AI ITNs compared to standard LLINs

This fourth results chapter covers PhD objective 4.

The author of this thesis supervised KAP data collection, and cross-sectional surveys, and together with other field assistants conducted qualitative data collection, cleaned the data, analysed both qualitative and quantitative data, and wrote the first draft of the manuscript.

The article will be submitted to PLOS One Journal with the following title "**Acceptability of and preferences for different types of dual active ingredient long-lasting insecticidal nets in the rural districts of Tanzania: a mixed method study** " and I am the main author.

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7.1 Abstract

New dual-active ingredient long-lasting insecticidal nets (dual-AI ITNs) have been recommended by WHO in areas with insecticide resistance. User compliance with these novel interventions is likely to be dependent on their acceptability within the socio-cultural context of the population. These factors need to be investigated in the community before wide-scale implementation programs. This study was embedded within two cluster-randomised controlled trials (RCT) aiming to evaluate the efficacy of dual-AI ITNs on malaria indicators in the Muleba, and Misungwi districts in Tanzania. Polyethylene (Olyset Plus, Olyset Net, and Royal Guard), and polyester (Interceptor G2, and Interceptor) rectangular, blue LLINs, with similar dimensions, were distributed in Muleba (Olyset Plus, and Olyset Net), and Misungwi (Olyset Plus, Royal Guard, Interceptor G2, and Interceptor). A mixed method design was used to collect data in each study site, from 2014 to 2017 in Muleba, and from 2018 to 2022 in Misungwi. Knowledge, Attitude, and Practice (KAP) data were collected from 14,475 households (1153 Muleba, and 13,322 Misungwi). Qualitative data were collected in 36 focus group discussions (17 Muleba, and 19 Misungwi), and 44 in-depth interviews (14 in Muleba, and 30 in Misungwi). These discussions and interviews explored users' acceptability, preferences, and perceptions of three brands of dual-AI ITNs (Interceptor G2, Olyset Plus, and Royal Guard), and identified several barriers to their appropriate, and consistent use. Thematic analysis was used to analyse the qualitative data. Quantitative data analysis used descriptive statistics, means, and proportions using STATA version 15. The nuisance of mosquito bites and the perceived risk of malaria influenced overall LLIN usage. Perceived effectiveness and physical attributes of LLINs such as durability, fibre, and texture affected their acceptability. In Misungwi, Interceptor G2 was favoured over the other three brands. In Muleba, where only Olyset Net and Olyset Plus were available, the latter was preferred due to its stronger insecticide. Users in Misungwi reported challenges with LLINs that caused physical side effects, such as skin irritation (Royal Guard and Interceptor), and those that tore easily (Olyset Plus). Both communities preferred blue, polyester, and rectangular LLINs. In Misungwi, polyethylene LLINs (Olyset Plus and Royal Guard) were more prone to misuse than polyester LLINs (Interceptor and Interceptor G2). High bedbug infestations also negatively impacted consistent LLIN usage. Providing LLINs that are acceptable and preferable, drives compliance and use. When making decisions about which LLIN to use, respondents

considered a wide variety of factors including effectiveness (always associated with 'less malaria'), durability, fewer side effects, and those that impede bedbug infestations. Policymakers and programs could recognize, and accommodate the range, and complexity of factors that influence LLIN users. New tools that simultaneously kill bedbugs may help increase LLIN use. Educational programs should reinforce messages that are contextual, and embedded in local cultural practices.

7.2 Background

Malaria caused 608,000 deaths globally in 2022[1]. Tanzania is among the four countries with the highest malaria deaths accounting for 4.4% of the global deaths in 2023[1]. Malaria is highest in the Lake Victoria zone, with the districts of Muleba, and Misungwi having an annual incidence rate of 130.9, and 24.3 per 1,000 population in 2023, respectively [244, 245].

Malaria has a large impact on the economy, causes significant human suffering, and impacts on social development[246, 247]. Countries with intensive malaria have been shown to have income levels of one-third less than countries without malaria, however, those countries that have eliminated malaria have usually had substantially higher economic growth in the subsequent five years[19]. Malaria can be prevented, and cured[26]. The core malaria preventive tools are long-lasting insecticidal nets (LLINs)[185, 198] with the pyrethroid class of insecticides the most commonly used. Acceptability of pyrethroid-only LLIN has been generally high for years since their first distribution, and studies suggest this is related to their perceived effectiveness[71, 248, 249]. However, insecticide resistance to pyrethroid insecticides has reduced the effectiveness of these products, which may impact their acceptability.

In 2017, the World Health Organisation (WHO) recommended the deployment of novel LLINs with a synergist (PBO)[150], and in 2023 dual active ingredient treated LLINs combining pyrethroid, and either pyriproxyfen or chlorfenapyr[3] to control malaria-transmitted by insecticide-resistant vectors. In 2023, 360 million pyrethroid–piperonyl butoxide (PBO) LLINs, and 74 million dual active ingredient LLINs were sent to sub-Saharan Africa (SSA) including Tanzania [69, 207], and the number is expected to increase in 2024. Between 2018 and 2023, the proportion of novel LLINs distributed in SSA increased from 3% to 84% of all LLINs distributed[69]. Following WHO recommendations, the Tanzanian National Malaria Control

Programme (NMCP) under the support of the President's Malaria Initiative (PMI), and Global Fund (FG), has distributed over 2,945,181 PBO LLINs by 2024 [169, 170], and they are considering deployment of dual-AI. LLIN (interceptor G2) for future LLIN campaigns[170].

Previous studies in Tanzania have examined the attitudes of LLIN users, and their adherence to using them [250-253]. Mosquito biting nuisance was identified as the main determinant influencing adherence and consistent use. Those who used LLINs for protection against malaria were more likely to use LLINs than those who used them to prevent mosquito-biting nuisance alone when mosquito density was low. Hot weather was found to be the primary deterrent to LLIN use[75]. Manufacturers of LLINs have attempted to address the problems associated with LLIN ventilation by increasing their mesh size using durable materials. Characteristics of the various brands of LLINs such as durability, texture, mesh size, LLIN size, shape, colour, and insecticide effectiveness have also been found to affect LLIN acceptability and use[71]. Despite that laboratory tests have reported polyethylene LLINs to be stronger than polyester ones [220], studies in Kenya [254], India, and Nepal [255] suggest that polyethylene LLINs are less preferred by users than polyesters because the former have stiff texture, wrinkles after washing (so that they become shortened, and difficult to tuck under bedding), and a large mesh size that allows mosquitoes to penetrate the LLIN to feed on sleepers. However, a study in Uganda [256] found that polyethylene LLINs were preferred, although the difference was small at 4% it was statistically significant. It is unclear why there is a disparity in the preference of LLINs of similar fabric between regions, and countries.

A challenge for malaria control using LLINs in SSA is not only achieving sufficient coverage but also identifying, and addressing the behavioural factors that impact their adherence and consistent use. Providing populations with LLINs they find acceptable could improve user adherence (the extent to which individuals or households follow the recommended practices for using LLINs), and contribute to the overall success of the programme. To address this important operational priority, two studies, embedded in two separate clusters randomised controlled trials (cRCTs), were used to assess acceptability, and preferences for five types of LLINs with different textures, and insecticidal characteristics and to identify barriers to their compliance to proper use, and adherence to consistent use.

7.3 Methods

7.3.1 Study Design

A mixed-method study embedded within two large cluster-randomised controlled trials (RCTs) (Table 7.1) in two districts, Muleba and Misungwi, that aimed to assess the epidemiological and entomological efficacy of different types of dual-AI ITNs against malaria transmitted by resistant mosquitoes [147, 148, 214]. The districts were chosen for the RCTs, based on areas of high malaria prevalence and high insecticide resistance in malaria vectors. The mixed methods study quantitative component included knowledge, attitude, and practice (KAP) surveys pre- and post-intervention and only pre-intervention qualitative study in Muleba RCT (Figure 7.1). In Misungwi 20 KAP questions were included in post-intervention LLIN coverage surveys and malaria prevalence surveys at 3, 12, 18, 24, 30, and 36 months post-intervention; and the qualitative only post-intervention qualitative study (Figure 7.2).

Table 7.1: RCTs in Muleba and Misungwi districts

RCTs	**Muleba RCT: March 2014-December 2017**	**Misungwi RCT: April 2018-March 2022**
Study design	A four-arm, factorial design, cluster-randomised trial (CRT) with 48 clusters and village/hamlet as the unit of randomization. Each cluster was comprised of 1-6 hamlets (sub-village) with up to 450 houses per cluster The four arms/interventions were: 1. Olyset Plus (permethrin + piperonyl butoxide (PBO) LLIN) 2. Olyset Plus + Indoor Residual Spraying (IRS) with pirimiphos-methyl 3. Olyset net + IRS 4. Olyset net [control/reference arm]: (permethrin only LLIN)	A four-arm, superiority design, single-blinded, cluster-randomised trial with 84 clusters with village/hamlet as the unit of randomization. Each cluster was comprised of 1-8 hamlets (sub-village) of between 150-450 houses in the core area of a cluster. The four arms/interventions were: 1. Interceptor G2 (alpha-cypermethrin + chlorfenapyr) 2. Royal Guard (alpha-cypermethrin + pyriproxyfen) 3. Olyset Plus (permethrin + piperonyl-butoxide (PBO)). 4. Interceptor [control/reference arm]: (alpha-cypermethrin)
LLIN distribution	LLINs were distributed from 6th-8th February 2015, and Indoor residual spraying (IRS) took place once in February/March 2015. A total of 90,000 LLINs were distributed in 30,000 households	LLINs were distributed from 26th-28th January 2019, and Indoor residual spraying (IRS) was not implemented in Misungwi. A total of 147,230 LLINs were distributed in 42,394 households
LLIN fabric, colour, shape, and size	Polyethylene, blue, and rectangular (length 180cm, Height 180cm, and Width 160cm)	Polyethylene (Olyset Plus and Royal Guard), polyester (Interceptor G2 and Interceptor), blue, and rectangular (length 180cm, Height 180cm, and Width 160cm)

7.3.2 Study sites, and setting

Muleba is the largest district of the Kagera region, located in the northwest of Tanzania on the Western shore of Lake Victoria. The population in Muleba is predominately *Wahaya* (Haya-speaking people) who depend on bananas as the staple food and coffee as a cash crop. The district covers an area of approximately 3500 km^2 at an altitude ranging from 1,100m-1,600m above sea level, with a population of 637,659 people (2022 population census)[257]. Misungwi is one of the eight districts of the Mwanza region located on the Southern border of Lake Victoria, North-western Tanzania. The population in Misungwi is predominately *Wasukuma* (Sukuma-speaking) who mainly depend on maize, rice, and cassava as the staple food and cotton as a cash crop. The study areas are described in detail elsewhere [147, 148, 258]. Both districts have year-round malaria transmission with two transmission peaks in June/July and December/January. Rain in both districts falls from September to December (low rainy season), and from March to May (high rainy season). At baseline (before study LLINs were distributed), malaria prevalence in the study area in Muleba was 65% and LLIN use was 27.5% compared with malaria prevalence of 44.2% and LLIN use of 61% in the Misungwi site

Misungwi and Muleba exhibited different levels of malaria prevalence and usage of LLIN during the period when the RCTs were conducted (

Table 7.2).

Table 7.2: LLIN use, malaria prevalence, population access and use:access ratio in the study sites from RCT per survey timepoint

District	Survey timepoint	Transmission season	Malaria prevalence	Net use	Population access	Use:access ratio	net type
Muleba	Baseline	High	65	27.5	37	0.74	any net
Muleba	4 months	High	40	76	77.45	0.98	any net
Muleba	9 months	Low	32	76	70.44	1.08	any net
Muleba	16 months	High	38	49	55.41	0.88	any net
Muleba	22 months	Low	49	53	57.62	0.92	any net
Muleba	28 months	High	72	59	53.58	1.10	any net
Muleba	33 months	Low	50	48	58.06	0.83	any net
District	**Survey timepoint**	**Transmission season**	**Malaria prevalence**	**Net use**	**Population access**	**Use:access ratio**	**net type**
Muleba	Baseline	High	65				study net
Muleba	4 months	High	40	70.5	75.09	0.94	study net
Muleba	9 months	Low	32	70.5	67.98	1.04	study net
Muleba	16 months	High	38	44	53.87	0.82	study net
Muleba	22 months	Low	49	41	45.88	0.89	study net
Muleba	28 months	High	72	41	42.5	0.96	study net
Muleba	33 months	Low	50	21.5	35.39	0.61	study net
District	**Survey timepoint**	**Transmission season**	**Malaria prevalence**	**Net use**	**Population access**	**Use:access ratio**	**net type**
Misungwi	Baseline	High	44.2	61.0	65.8	0.93	any net
Misungwi	3 months	High	Not assessed	81.8	93.8	0.87	any net
Misungwi	12 months	Low	18.7	83.0	84.3	0.98	any net
Misungwi	18 months	High	46.7	72.2	80.8	0.89	any net
Misungwi	24 months	Low	38.4	72.2	75.2	0.96	any net
Misungwi	30 months	High	45.6	62.6	75.5	0.83	any net
Misungwi	36 months	Low	31.1	66.7	76.1	0.88	any net
District	**Survey timepoint**	**Transmission season**	**Malaria prevalence**	**Net use**	**Population access**	**Use:access ratio**	**net type**
Misungwi	Baseline	High	44.2				study net
Misungwi	3 months	High	Not assessed	72.1	76.5	0.94	study net
Misungwi	12 months	Low	18.7	61.8	55.8	1.11	study net
Misungwi	18 months	High	46.7	47.8	41.2	1.16	study net
Misungwi	24 months	Low	38.4	40.9	30.8	1.33	study net
Misungwi	30 months	High	45.6	29.6	30.8	0.96	study net
Misungwi	36 months	Low	31.1	20.8	15.4	1.35	study net

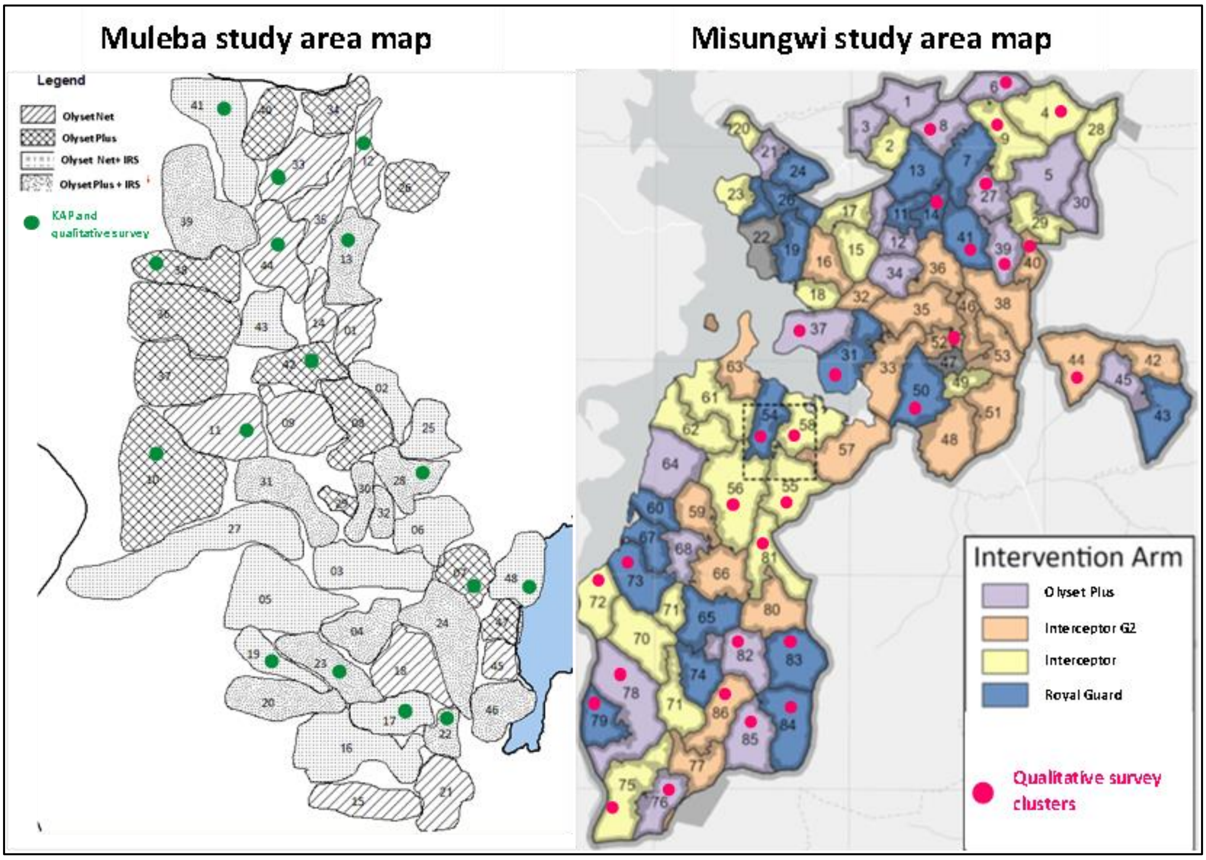

Figure 7.1: Distribution of clusters where qualitative and quantitative studies were conducted

7.3.3 Sampling

In Muleba, in both pre and post-intervention KAP phases, 16 clusters (4 clusters per study arm) were purposively selected based on study arm, LLIN use status [high (>40%) or low (≤40%)] and malaria risk [high (>30%) or low (≤30%)]. From each cluster, 40 households were randomly selected (using STATA version 13) from a household master list generated during household census and renumeration. In Misungwi, in each of the 84 clusters, at each survey timepoint (12, 18, 24, 30, and 36 post-intervention), 45 households were randomly selected (using STATA version 15) from a household master list, except at 3 months LLIN (Figure 7.2) coverage survey where 10 households were selected in each cluster.

In each of the two sites, participants for IDI and FGD were purposively sampled by study arm, LLIN usage (high/low), and malaria risk (high/low), age, sex. Sixteen clusters (same clusters involved in pre and post-KAP surveys) from the RCT in Muleba and 20 (5 clusters per arm) were purposively sampled from Misungwi RCT, to include those with high/low LLIN usage and high/low malaria prevalence (Appendix 7.1). The selection of respondents for quantitative and qualitative interviews and FGDs included a mix of random and purposive sampling.

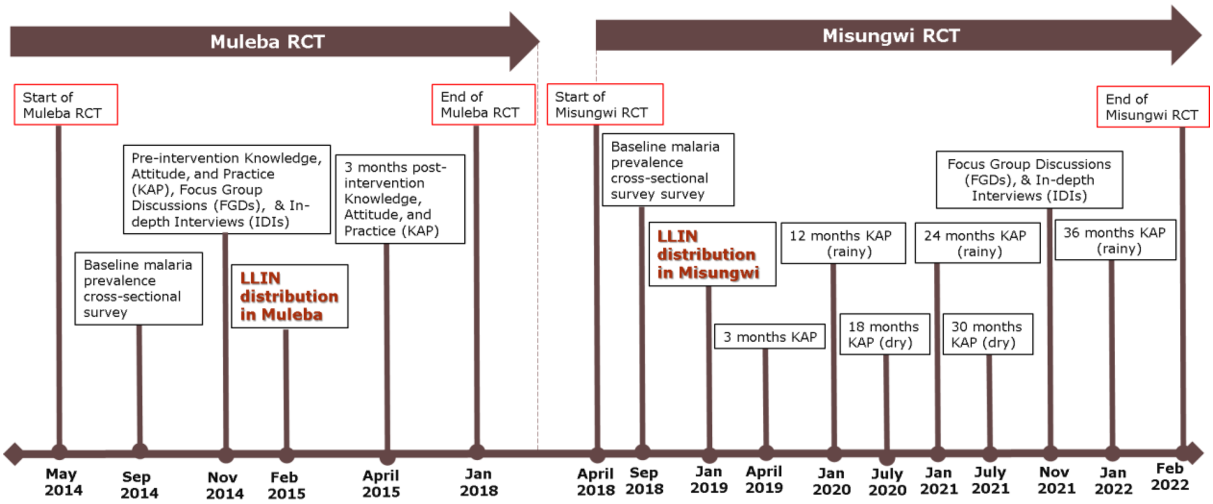

Figure 7.2: KAP, FGDs, and IDIs data collection timeline

7.4 Data collection

7.4.1 Knowledge, Attitude, and Practice (KAP)

In Muleba, baseline and post-intervention KAP surveys were conducted across four clusters in each intervention arm. The baseline survey randomly selected 701 households with children under 15 from 16 clusters, collecting data on household demographics, economic status, LLIN ownership and usage, malaria knowledge, sources of malaria information, personal agency, perceived norms, perception of failed LLINs, and treatment-seeking behaviour. For the post-intervention survey, conducted three months later, 960 households were randomly selected, including those without children under 15, to capture broader experiences with Behaviour communication (BCC)/Information Education Communication (IEC) messages and preventive campaigns. This survey added questions on the reception of intervention materials and household involvement in LLIN and IRS campaigns. Pendragon Forms (Pendragon Corporation Software, Libertyville, USA) on Personal Digital Assistants (PDAs) was used to collect data.

In Misungwi, 10 to 20 KAP questions were embedded within LLIN usage and malaria prevalence surveys conducted at various intervals up to 36 months post-intervention. These questions, adjusted for each survey, covered similar topics as in Muleba but were shorter. The surveys were conducted using Android Tablets with Open Data Kit (ODK) software.

In both Muleba and Misungwi, respondents were primarily household heads, their spouses, or other adults over 18. Data collection was conducted by field workers who had completed secondary education. These field workers underwent one week of training covering the study design, field procedures, and questionnaires

7.4.2 General procedures for FGDs and IDIs

The FGDs and IDIs were conducted in Muleba between November and December 2014 and in Misungwi in November 2021. At each site, there were two teams of researchers in Muleba and three in Misungwi, all holding at least a bachelor's degree and fluent in local languages. Each team included one experienced researcher and one or two note-takers. Interviews were led by the experienced researchers, while the note-takers, after receiving two weeks of intensive training, were responsible for taking notes and summarizing the interviews. This training covered the study design, ethical treatment of participants, use of semi-structured guides, operation of tape recorders, and note-taking techniques. During interviews, one researcher facilitated the discussion and controlled the tape recorder, while the other(s) focused on note-taking. A semi-structured guide was used to steer the discussions, focusing on malaria knowledge, the purpose of using LLINs, rumours or misconceptions about LLINs, factors influencing their use, non-use, and misuse, as well as participants' perceptions of LLIN characteristics and other preventive practices.

In Misungwi, due to the presence of four distinct LLIN brands, participants were given the opportunity to examine each brand during discussions, enabling them to accurately identify the specific LLIN they received and provide informed feedback. All FGDs and interviews in both sites were conducted in *Kiswahili, Kihaya,* and *Kisukuma*. These sessions were digitally recorded and later transferred to a computer, where they were saved as sound files.

7.4.3 In-depth Interviews (IDIs)

In both sites, IDIs were held with purposively selected district, ward, and village leaders, health facility in-charges, Environmental Health Officers (EHOs), influential community members, and heads or spouses of households. These interviews explored perceptions of malaria control and prevention interventions, their expected and unexpected impacts, and contextual factors affecting the uptake of these interventions. Key informants were contacted directly and asked to choose a convenient time for their interview. The interviews were conducted in locations that ensured privacy and confidentiality for the participants.

7.4.4 Focus Group Discussions (FGD)

In each site, focus group discussions (FGDs) were conducted within the same demographic strata, (i.e. homogenous FGDs). These groups included married men (26+ years), married women (26+ years), young men (16-25 years), and young women (16-25 years). In Misungwi, an additional group of primary school children (9-15 years) was included. The discussions focused on topics such as knowledge, attitudes, and practices related to malaria prevention and treatment, drivers and barriers to LLIN use, misconceptions about malaria and LLINs, and the acceptability and preferences for LLINs.

Table 7.3: FGDs, IDI, and KAP done in Muleba, and Misungwi

Study sites (districts)	**Muleba**	**Misungwi**
KAP Survey: Households Visited (Consented and Interviewed)		
Pre-intervention	593 (560)	Not conducted
3 months post-intervention	760 (593)	840 (668)
12 months	Not conducted	3780 (2540)
18 months	Not conducted	3780 (2656)
24 months	Not conducted	3780 (2736)
30 months	Not conducted	3780 (2289)
36 months	Not conducted	3780 (2433)
IDI: number (total interviewees)		
District Health workers	2 (2)	1 (1)
In-charges of dispensaries	Not conducted	5 (5)
Villages and ward leaders	8 (8)	5 (5)
Community health workers	4 (4)	6 (6)
Household Heads and Spouses	Not conducted	2 (9)
FGD: Number (total participants)		
Married men (26+ years)	5 (41)	3 (34)
Married women (26+ years)	5 (45)	4 (46)
Young men (16-25 years)	4 (30)	4 (40)
Young women (16-25 years)	3 (27)	2 (24)
Primary school boys (9-15years)	Not conducted	2 (24)
Primary school girls (9-15years)	Not conducted	4 (48)

7.4.5 Data management and analysis

In Muleba, quantitative data were transferred from the PDAs to a Microsoft Access database. In Misungwi, data were uploaded to a server and downloaded as comma-delimited files. All data were then imported into STATA version 15 (Stata Corporation, College Station, Texas, USA). Data from all survey points were combined for analysis at each study site. Quantitative data analysis involved using descriptive statistics means, and proportions in STATA, with graphs created in Excel.

Interviews and discussions were primarily conducted in the mornings, lasting between 30 and 60 minutes. Each afternoon, a thorough summary of the main points for each theme and study arm was created using the notes taken. In cases of discrepancies, the digital recordings were reviewed. In Muleba, digital recordings of FGDs and IDIs were transcribed verbatim from Kihaya/Kiswahili and then translated into English. In Misungwi, recordings were directly transcribed and translated into English by an experienced translator. A sample of IDIs and FGDs was double-checked by the lead researcher (EL) before analysis.

Data were imported into NVivo version 14.0. for coding, text searching, and data merging. Thematic analysis was conducted to systematically examine themes and sub-themes, considering the frequency, intensity, and diversity of views expressed within each theme. This flexible approach allowed for the identification, analysis, and reporting of patterns within the data. Matrices were constructed for each main theme, incorporating relevant data from in-depth interviews (IDIs) and focus group discussions (FGDs). These matrices facilitated cross-comparisons across interviews and groups to assess the strength of acceptability. All data were analysed using this method. Relevant quotes were selected to support and illustrate the findings.

To maintain anonymity, FGD participants were assigned random numbers, and IDI participants were coded. Quotes. were reported using participant numbers or IDI codes, along with the district and cluster category, whether from areas of low or high malaria prevalence or low or high LLIN usage

Table 7.4: Definition of terms

Term	Definition
Adherence to LLINs/ITNs use	The extent to which individuals or households follow the recommended practices for using LLINs. It specifically involves: consistent use (using the LLIN even during non-peak malaria seasons), proper installation, care and repair and avoiding misuse
Consistent LLINs/ITNs use	Regular and sustained use of LLINs by individuals or households for protection against malaria mosquitoes. Specifically, it means that people sleep under the net every night. High adherence ensures consistent use, but consistent use alone does not guarantee adherence if the net is not properly maintained or used effectively.
Acceptability	The degree to which an LLINs is considered suitable, desirable, and appropriate by the target population, and whether individuals and communities are willing to use and sustain the proper use of nets.

7.4.6 Ethics

Muleba trial: The trial was approved by the ethics review committees of the Kilimanjaro Christian Medical University College, the London School of Hygiene & Tropical Medicine, and the Tanzanian Medical Research Coordinating Committee (NIMR/HQ/R.8a/VolIX/1803). A trial steering committee reviewed progress. Written informed consent was provided by adult participants.

Misungwi trial: Ethical approval for the RCT was secured from the institutional review boards of the Tanzanian National Institute for Medical Research (NIMR/HQ/R.8a/Vol.IX/2743), Kilimanjaro Christian Medical University College (2267), London School of Hygiene & Tropical Medicine (14952; 14952-1), and the University of Ottawa (H-05-19-4411). Written informed consent was obtained from adult participants. For children, written assent forms were completed by both the children and their parents or guardians prior to the discussions.

7.5 Results

A total of 1,153 households participated in the Knowledge, Attitudes, and Practices (KAP) surveys in Muleba, with 560 households surveyed pre-intervention and 593 surveyed post-intervention. In Misungwi, KAP which was embedded in prevalence surveys included responses from 13,322 households across 6 surveys. Additionally, qualitative data were collected through 44 in-depth interviews (IDIs) (14 conducted in Muleba and 30 in Misungwi), and 36 focus group discussions (FGDs), with 17 in Muleba and 19 in Misungwi, as detailed in (Table 7.3).

Knowledge, and awareness about malaria

In both districts, the majority of respondents correctly identified mosquito bites as the cause of malaria (92.5% in Muleba, 86.6% in Misungwi) (Table 7.5). However, a small percentage of respondents still hold misconceptions such as attributing malaria to superstitions (11.4%), sun exposure (7.1% at baseline, increasing to 16.9% post-intervention), or contaminated air and water. Most respondents were aware that using LLINs helps prevent malaria, with 90.5% in Muleba recognizing their importance. However, the practical application of this knowledge varied, especially during hot seasons (Box 7.1).

Table 7.5: Knowledge of malaria and preventive practices in Muleba and Misungwi (data from KAP surveys)

	Muleba			Misungwi
	Baseline (Dec 2014): % (n)	**Post (April 2015): % (n)**		**Post-intervention (2020-2022): % (n)**
How is malaria transmitted to humans?**				
Bite of an infected mosquito	92.5 (518)	91.7 (544)		86.6 (2,106)
Too much exposure to the sun	7.1 (40)	16.9 (100)		0
Eating contaminated food	7.0 (39)	7.1 (42)		0.3 (8)
Drinking contaminated water	8.6 (48)	9.3 (55)		1.0 (24)
Coming into close contact with a malaria patient	0.5 (3)	0.2 (1)		0.2 (4)
Don't know	4.7 (26)	5.4 (32)		0
Superstitions	NA	NA		11.4 (277)
Breathing contaminated air	NA	NA		2.8 (69)
Ways to prevent malaria**				
Sleeping under LLINs	90.5 (507)	90.6 (537)		NA
Spraying insecticide inside the house	30.7 (172)	43.5 (258)		NA
Trimming bushes around the house	15.7 (88)	11.5 (68)		NA
Cleaning dark corners of the house	13.6 (76)	14.7 (87)		NA

Wearing long-sleeved clothes	5.2 (29)	1.7 (10)		NA
Making fire/smoke	0.5 (3)	0.7 (4)		NA
Don't know	8.2 (46)	6.9 (41)		NA
Personal protection measures against malaria				
Mosquito nets	87.7 (490)	NA		NA
Closing windows and doors	15.9 (89)	NA		NA
Gauze wire in windows	7.9 (44)	NA		NA
Mosquito coil	4.3 (24)	NA		NA
Where do you first seek malaria treatment?				
Hospital, health center, or dispensary	57.1 (320)	76.1 (451)		45.4 (1,535)
Drug shop/pharmacy/general shop	35.9 (201)	18.7 (111)		54.0 (1,823)
Traditional healer (herbalist)	5.5 (31)	3.9 (23)		0.3 (9)
Community Health Worker	0.5 (3)	0.3 (2)		0.21 (7)
Traditional healer (spiritual)	0.4 (2)	0		0 (0)
Other	0.5 (3)	1.0 (6)		0.15 (5)

Table 7.6: Attitudes towards LLIN practices in Muleba and Misungwi

	Muleba			**Misungwi**
	Baseline (Dec 2014): % (n)	**Post (April 2015): % (n)**		**Post-intervention (2020-2022): % (n)**
Mosquito net colour preference				
Blue	42.0 (235)	NA		88.0 (2408)
Any colour	33.8 (189)	NA		1.2 (41)
Green	16.4 (92)	NA		3.6 (98)
White	4.1 (23)	NA		4.5 (123)
Black	1.8 (10)	NA		0.3 (8)
Yellow	0.7 (4)	NA		0
Red	0.5 (3)	NA		0.9 (25)
Other	0.7 (4)	NA		1.5 (41)
Net shape preference				
Rectangular/Square	61.4 (344)	NA		90.7 (2482)
Any shape	21.8 (122)	NA		2.4 (66)
Conical (round)	16.3 (91)	NA		6.7 (184)
Other	0.5 (3)	NA		0.2 (4)
Net material preference				
Soft (polyesters)	NA	NA		85.5 (2338)
Hard (polyethylene)	NA	NA		11.4 (312)
Any material	NA	NA		3.0 (83)
Don't know	NA	NA		0.1 (3)
How often do you sleep under the LLIN?				
Always	47.8 (266)	94 (548)		NA
Never	27.1 (151)	3.6 (21)		NA
Sometimes	25.1 (140)	2.4 (14)		NA
Why do you sometimes or never sleep under the LLIN?				

Not enough nets	56.5 (144)	20 (5)		NA
Net worn out/too torn	29.4 (75)	8 (2)		NA
Other reasons	14.1 (36)	32 (8)		NA
Net not hung	0 (0)	24 (6)		NA
House sprayed	0 (0)	16 (4)		NA
What do you do when a LLIN has failed?				
Discard the net	49.1 (273)	NA		37.7 (1778)
Repair and reuse the net	31.5 (175)	NA		27.4 (1293)
use for alternative purposes	19.4 (108)	NA		34.9 (1651)
What alternative purposes do you use the net for?				
Ropes	0 (0)	NA		54.5 (1034)
Used as bedsheet/mattress	47.7 (51)	NA		28.6 (543)
Cover holes in the wall(s)	37.4 (40)	NA		2.8 (53)
Enclosing poultry	11.2 (12)	NA		6.3 (120)
Curtains	2.8 (3)	NA		3.6 (68)
Cover eaves	8.4 (9)	NA		1.0 (19)
Collect edible flying ants	3.7 (4)	NA		0 (0)
** Multiple answers allowed		NA = not assessed		

In the qualitative studies (Box 7.1) despite high awareness about malaria, some respondents, especially in Misungwi, believed that malaria could be spread through the air or from a breastfeeding mother to her child. Additionally, there was skepticism about whether malaria could be a direct cause of death, with some attributing deaths labeled as malaria to other causes, including witchcraft.

"…At funerals, if the cause of death is stated as malaria, there is often a collective murmur of disbelief, with people questioning, "Has only malaria killed him/her?" They struggle to accept that malaria alone could be the cause of death". (P9: FGD Misungwi, low malaria, and low net use cluster)

Box 7.1: Malaria knowledge, and awareness (quotes)

<u>KII 1: Muleba, district level</u>

Respondent (R): Many people do not use LLINs during hot seasons, and it is that time when mosquitoes and malaria are peaking…Sensitization messages need to be intensified for a change of behaviour to use LLINs all the time.

<u>IDI 1: Misungwi, high malaria & high net use cluster</u>

R: I own a restaurant (many people gather here), I overhear them saying that my child is suffering from malaria, so malaria is high in our community, and the children suffer most.

R: We usually use nets during the heavy rain season, but we were advised to use them year-round since mosquitoes are still present. Some people, when summer begins, take down their mosquito nets and store them away.

R: I have learned that using mosquito nets helps you a lot not to catch malaria, and if you don't use it you will suffer from malaria since there are a lot of mosquitoes in here….it also can protect you from snakes, and other dangerous insects like spiders.

<u>FGD 1: Muleba, high malaria & high net use cluster</u>

P5: Malaria is caused by mosquitoes. Not all mosquitoes cause malaria, the most known to cause malaria are the female anopheles…Mosquito bites all the time, but the ones that transmit malaria bite late at night.
FGD 2: Misungwi, high malaria & high net use cluster P6: The first protection we take is using the best quality mosquito net not like the mosquito nets that we received before which had big holes. Also, the family must drink clean water. Facilitator: How is malaria transmitted from one person to the other? P7: Malaria spread through the air from one person to the other. Yes, when one sneezes while he/she has malaria fever, when that air reaches you it means you will also catch malaria, yes it spreads through the air.
FGD 3: Misungwi, low malaria & high net use cluster P2: There is one family in our neighborhood whose child had malaria, and they delayed taking him to the hospital after three days of taking antibiotics he did not recover so they took him to Mitindo Hospital [district hospital] where tests revealed he had chronic malaria. Unfortunately, despite the hospital's efforts, the child passed away shortly after. So, we can say it is a big problem that's the reason we are losing many children because of malaria. P7: It's not possible to recognize that I have malaria, but I might have symptoms like fever, headache, joint pains, chills, and tiredness. Another symptom that I never understand is stomach ache because once I go for the test it is confirmed that I have malaria.
FGD 4: Misungwi, low malaria & low net use cluster P5: What I know is if the mosquito bites a breastfeeding woman if that woman gets malaria, she will be able to infect the child

P = participant in FGD; R = Respondent in IDI

Drivers for LLIN use

During the baseline survey in Muleba, only 47.8% of households reported always sleeping under LLINs. However, this figure increased to 94% three months after LLIN distribution. The main drivers of consistent LLIN use included the perception that LLINs decrease malaria risk, avoid mosquito bites, and protect from other pests like spiders, snakes, and rodents.

"….Many households lack enough nets, and the ones they have are badly torn. You know, in the last campaign, only two-bed nets were provided per household, regardless of the number of sleeping places or people". (ID! 2, Muleba, low malaria & low net usage cluster).

"One day, my wife and I visited a relative in a distant village. At night, they set up a net where we were allocated to sleep. To our surprise, when we woke up in the morning, we saw a tiny snake on the roof of the net. Since then, I always use a net when I sleep, whether there are mosquitoes or not, you never know what might show up." (P5: Misungwi, high malaria & high net usage cluster)

"They are using nets to prevent mosquitoes; it has become their habit nowadays. Also, the level of disease has decreased because mosquito nets protect them. Even in my family, there was frequent fever but once we started using mosquito nets fever has decreased" (IDI 3: Misungwi, high malaria & high net usage cluster)

"There are so many mosquitoes in our environment, you cannot sleep without using a mosquito net, and these mosquitoes are very tiny, but their bites are intense, often leading to skin rashes" (P6: FGD 2: Misungwi, high malaria & high net usage cluster)

Moreover, the primary reason for using LLINs was to avoid mosquito bites and malaria, but a key motivator was also the financial burden of recurrent malaria cases. LLINs are seen as a way to reduce these costs and protect family finances.

"…I'm their sister, and I encourage them to use mosquito nets because whenever they fall sick, they come to me right away asking for money for medication." (P9: FGD 5: Misungwi, low malaria & high net usage cluster)

Barriers to consistent use of LLINs, acceptability, favourable, and unfavourable characteristics of LLINs

Adverse effects and bed bugs

Itching and facial burning sensations were cited as significant factors contributing to the inconsistent utilization of LLINs, particularly during the initial period of use across all LLIN types. These adverse reactions were most frequently reported in the IDIs and FGDs by participants who received the Interceptor and Royal Guard brands in Misungwi (Appendix 7.3).

Another frequently identified barrier in discussions and interviews was the widespread belief that LLINs contribute to bedbug infestations. Respondents commonly reported that bedbugs tend to congregate at the hanging points of the LLINs and descend during the night to feed on sleepers. Consequently, to mitigate the risk of bedbug bites, many participants opted to avoid hanging the nets altogether. The discomfort and intense itching associated with bedbug bites compelled some users to either discard their infested LLINs or subject them to washing with hot water, a practice that likely contributed to net shrinkage. While bedbugs are not disease vectors, their bites cause significant discomfort and distress.

"…In our community, when people first began using mosquito nets, they encountered significant infestations of bedbugs. As a result, many individuals discontinued the use of the nets". (IDI 5: Misungwi, high malaria & high net use cluster)

"The belief that mosquito nets are responsible for the emergence of bedbugs has become widespread within the community, leading to numerous complaints. For instance, there was a woman who hung her mosquito nets elsewhere without using them, and when we inquired why, she responded that the nets had caused bedbugs. We took the opportunity to educate her about the environmental factors that contribute to bedbug infestations and provided her with guidance on effective measures to eliminate them. It is important to approach this issue

sensitively; directly attributing bedbugs to poor hygiene can be upsetting for community members. Instead, the focus should be on educating them about practical steps to prevent bedbugs from thriving in their homes". (KII 2: Misungwi, district level)

However, across both the IDIs and FGDs, participants consistently avoided directly admitting to having bedbugs in their own homes, as the presence of bedbugs is culturally stigmatized and often associated with poor hygiene. Acknowledging such an infestation is seen as shameful, leading individuals to speak about the issue in a detached, third-person manner, rather than personalizing their accounts. This tendency to disassociate from the problem underscores the social stigma attached to bedbugs. Despite this reluctance to admit personal experiences, the KAP study revealed significant infestation with an average of more than 14 bedbugs per net, indicating a significant infestation within the nets.

"What my colleague mentioned is true, many individuals in our community are reluctant to use mosquito nets due to the fear of bedbug infestations. For them, the discomfort of bedbug bites outweighs the risk of contracting malaria. Consequently, we are requesting that the project consider providing nets that offer protection against both mosquitoes and bedbugs. If such nets were available, no one would hesitate to use them, as it would address both concerns simultaneously, effectively eliminating the incentive to remove the nets". (P3: FGD 3: Misungwi, low malaria & high net use cluster)

<u>LLIN supply and perceived physical integrity</u>

In a quantitative survey, barriers to consistent LLIN use included insufficient supply (56.5% in Muleba), LLINs being too torn (29.4%). Across all IDIs and FGDs, respondents consistently cited excessive damage to the study LLINs as a primary constraint to LLIN use. In Misungwi, where both polyethylene and polyester LLINs were distributed, polyethylene LLINs (specifically Olyset Plus) were most frequently mentioned as being prone to damage.

"I noticed that this net [Olyset Plus] tears easily, which is why it's no longer available in many households. Even a slight squeeze on the bed can cause it to rip. (P1: FGD Misungwi, low net usage cluster)

Perceptions (infertility, impotence and poor sexual drive)

Cultural beliefs also played a role, with some respondents citing that LLINs could cause impotence or infertility.

"You know, some people spread misinformation, claiming in front of others that mosquito nets negatively affect male sexual performance… (laughter)". (IDI 3: Misungwi, high malaria village).

"…They believe that using mosquito nets can diminish sexual drive in men, and they also claim that women who sleep under them may struggle to conceive". (IDI 4: Misungwi, low malaria & low net usage cluster)

However, other participants in the same focus group discussions dismissed this belief, arguing that a lack of male sexual drive is a personal issue and unrelated to the use of LLINs, seeing it instead as an individual problem.

"…(amidst loud group laughter)… There is no such thing; that is a personal problem. If the drive isn't there, it simply isn't there. If you're unwell, that's your individual issue. The nation will not succumb to malaria because of such a misguided belief" (P4: FGD 6: Muleba, high malaria & low net usage cluster).

"…that is a personal issue. I've been using mosquito nets for years, yet my performance [referring to sexual drive] remains strong, and my wife continues to bear children. In fact, we just welcomed a baby girl two months ago (followed by loud and hearty group laughter)" (P2: FGD 3, Misungwi, low malaria & high net use cluster).

Conversely, in Muleba, where IDIs and FGDs were conducted only pre-intervention, and KAP when LLINs were still new, perceptions of damage to the study LLINs were not assessed.

<u>Low mosquito density during dry season</u>

Some respondents indicated a belief that the mosquito population had declined, reducing the perceived necessity of using LLINs. This belief is consistent with the common perception that mosquitoes are absent during the dry season.

"…For instance, I once visited my uncle and noticed that they weren't using nets. I asked them, 'Why aren't you using nets?' They responded that there are no mosquitoes anymore. I explained that this isn't true because mosquitoes come and bite you at midnight while you're asleep, making it difficult to notice. I then asked whether they had recently been sick with malaria, and he replied, 'We're puzzled about why we're getting malaria when there are no mosquitoes.' I told them that mosquitoes are indeed present, and therefore, they need to use nets." (P9: FGD 5: low malaria & high net usage cluster)

Additional barriers to LLIN use were also identified during the IDIs and FGDs in the Misungwi district. Among married women, there were reports of difficulties in using LLINs, particularly when their husbands returned home late from drinking.

<u>Acceptability, favourable, and unfavourable characteristics of LLINs</u>

In both districts, blue LLINs were the most preferred colour in Misungwi (88%), while in Muleba, the preference for blue was lower at 42%, with 16.4% preferring green and 33.8% being colour indifferent. Rectangular nets were preferred by 61.4% of respondents in Muleba and 90.7% in Misungwi (Table 7.6).

"I prefer the blue mosquito net because it holds up well against dust. With our red soil and uncemented floors, it's a relief to have a net that stays clean and doesn't show dirt easily.". (P8: FGD 2, Misungwi, high malaria & high net use cluster).

Preferences of dual-AI ITNs compared to standard LLINs

The Interceptor G2 (which has similar physical features as Interceptor)was particularly preferred to Interceptor for its effectiveness in reducing malaria among children, that was always referred to 'less malaria'.

"This mosquito net [Interceptor G2] does not get damaged easily, it last up to four years before showing wear. It's soft, easy to wash, and effectively prevents mosquito penetration." (P3: FGD 7, high malaria & high net use cluster).

"These were the best [Interceptor G2], they do not bring bedbugs like the previous ones [referring to other nets non-study nets-standard nets], which caused a lot of problems with infestations." (P4: FGD 7, high malaria & high net use cluster).

Olyset Plus was distributed across both districts, with user perceptions differing by location. In Muleba, Olyset Plus was preferred over Olyset Net due to its stronger insecticidal properties, despite both nets being made of the same fabric (*Table 7.7*). In Misungwi, LLINs were evaluated based on physical characteristics and efficacy, with the KAP survey indicating a strong preference for polyester nets (Interceptor and Interceptor G2) over polyethylene alternatives (Olyset Plus and Royal Guard) (*Table 7.7*).

Olyset Plus was preferred to Olyset net in Muleba due to its favourable qualities (strong insecticidal properties) (Table 7.6), but in Misungwi, participants expressed concerns about its physical integrity compared to the standard LLIN, noting that the Olyset Plus did not last as long as expected.

"This net [Olyset plus], is good, but it tears easily." (P7: FGD Misungwi, low net use cluster)

There was no difference in the preference between Royal Guard and Interceptor nets as both nets caused adverse effects like skin irritation and facial burning. These negative experiences led to a decrease in LLIN use among some respondents especially in few days after distribution.

"Due to the negative impact of the net [in Royal Guard arm], such as causing rashes when first used, people stopped using them entirely" (P11: FGD 7, high malaria & high net use cluster).

Table 7.7: Comparing study LLIN and IRS in the Muleba district (data from KAP survey)

	Muleba	
How do you compare project LLINs to your previous LLINs?	**Olyset net: % (n)**	**Olyset Plus: % (n)**
This LLIN is better	62.2 (166)	72.7 (216)
The LLIN is the same as previous nets	26.6 (71)	17.5 (52)
This LLIN is worse	3.8 (10)	2.7 (8)
Don't know	7.5 (20)	7.1 (21)
Why is this project LLINs better than your previous LLINs?	**Olyset net**	**Olyset Plus**
This round the nets have strong insecticide	58.1 (97)	61.8 (134)
This round the nets are larger	18.6 (31)	16.1 (35)
This round premade holes in the net are smaller	13.2 (22)	14.8 (32)
This round the nets are durable	9.6 (16)	5.5 (12)
Other	0	1.8 (3)
How long does the insecticide last on IRS and LLINs?	**IRS**	**LLINs**
Don't know	67.5 (400)	60.7 (360)
One year or less	22.9 (136)	19.7 (117)
Two years	1.7 (10)	2.5 (15)
Three years	1.7 (10)	2.2 (13)
Four years	0.3 (2)	0.5 (3)
Five years or more	5.9 (35)	14.3 (85)
Do you prefer LLINs or IRS?		
LLINs	46.1 (269)	Not Assessed (NA)
IRS	20.6 (120)	NA
Equally preferred	33.2 (194)	NA
Don't know	0.2 (1)	NA
Why do you prefer LLINs or Indoor residual spraying (IRS)		
LLIN: can easily purchase insecticide to retreat nets	4.6 (23)	NA
LLIN: easy to get a good night's sleep	12.3 (62)	NA
LLIN: have insecticide on them so mosquitoes can't penetrate	9.1 (46)	NA
LLIN: help avoid other pests and rodents	4.2 (21)	NA
LLIN: lasts longer and protects sleepers longer than IRS	18.4 (93)	NA
LLIN: protect you even if the household has open eaves	3.4 (17)	NA
LLIN: allow for changes in the walls of houses (ex. Plastering)	3.2 (16)	NA
IRS: because if nets are not enough, IRS kills all mosquitoes	7.9 (40)	NA

IRS: spraying is more effective than nets	1.8 (9)	NA
IRS: spraying is safer than nets	0.8 (4)	NA
IRS: spraying kills more mosquitoes than nets	3.4 (17)	NA
IRS: spraying kills other pests	5.8 (29)	NA
IRS: spraying lasts longer than nets	0.8 (4)	NA
Don't know	0.6 (3)	NA
Other	23.8 (120)	NA

Table 7.8: Comparing study nets in Misungwi district (data from KAP survey)

How do you compare project nets to your previous nets?	**Royal Guard: % (n)**	**Olyset Plus: % (n)**	**Interceptor: % (n)**	**Interceptor G2: % (n)**
This net is better	59.4 (590)	48.4 (366)	69.3 (802)	66.1 (869)
The net is the same as previous nets	28.4 (282)	38.4 (291)	23.6 (273)	28.7 (377)
This net is worse	6.8 (68)	7.5 (57)	2.2 (25)	1.5 (20)
Don't know	5.4 (54)	5.7 (43)	4.9 (57)	3.7 (49)
Why is this net better?	**Royal Guard**	**Olyset Plus**	**Interceptor**	**Interceptor G2**
Does not allow mosquitoes to penetrate	80.5 (494)	74.8 (294)	71.7 (616)	69.2 (659)
The net is soft	7.3 (45)	9.2 (36)	12.0 (103)	10.2 (97)
Does not acquire holes easily	5.2 (32)	4.8 (19)	4.9 (42)	8.2 (78)
It is new	1.1 (7)	0.8 (3)	0 (0)	0 (0)
Don't know/other	4.7 (29)	8.4 (33)	9.5 (82)	11.8 (112)
Does not itch	0.2 (1)	0.3 (1)	0.6 (5)	0.2 (2)
Children don't get malaria	1.0 (6)	1.8 (7)	0.1 (1)	0.4 (4)
Proportion of nets with bedbugs: % (n/N)	19.4 (87/448)	19.0 (58/305)	28.7 (218/761)	22.2 (157/707)
Mean number of bedbugs per LLIN observed by field worker (95% CI)*	**14.2 [12.1-16.3]**	**14.3 [11.7-16.9]**	**14.8 [13.4-16.1]**	**15.4 [13.7-16.9]**
** We capped bedbug counts at 30*				

Repurposing of long-lasting insecticidal nets (LLINs)

In both Muleba and Misungwi, participants reported repurposing LLINs for various tasks, including making ropes, protecting crops, and screening windows (Box 7.2). For example, 54.5% of respondents in Misungwi used LLINs as ropes, and 28.6% used them as bed sheets or mattresses (Table 7.6).

Figure 7.3: Repurposing LLINs: A) window screening, B) protecting seedlings, C) fishing traps, D) poultry enclosures, E) drying crops, F) ropes for tying goats

Box 7.2: Repurposing of long-lasting insecticidal nets (LLINs) (quotes)

<u>FGD 2: Misungwi, high malaria, & high net use cluster</u>
P3: They use nets to protect seedlings
P4: I've seen others use them as poultry fences
P7: They prefer mosquito nets for ropes because they don't cut easily
<u>FGD 4: Misungwi, low malaria & low net use cluster</u>
P1: They use them to store plastic bottles collected for recycling.
<u>FGD 3: Misungwi, low malaria & high net use cluster</u>
P2: They use nets to protect harvested crops.
P4: Some use them as mattresses for children, regardless of whether they are new or old.
P5: The preferred nets are hard and strong ones [polyethylene ones].
P7: I was fined for tying six bags of scarlet eggplant with net strings at Buhongwa market.
<u>Facilitator:</u> Who fined you, and how much did you pay?
P7: An environmental health officer came by and questioned who permitted me to use a net for tying my bags. I ended up having to pay two thousand per bag as a fine, so I've decided never to use those nets again, except for sleeping under them

Increasing LLIN use: participant recommendations

The study identified key recommendations for maintaining LLIN use. Regular awareness programs should be tailored to local languages to reach both literate and illiterate populations, addressing misconceptions and educating on malaria, LLIN benefits, and proper use. Training and incentives for community health workers (CHW) to conduct door-to-door education were also recommended to boost program coverage, while village meetings were discouraged due to low attendance.

"They [CHW] are volunteers, but their work is challenging, and they need some motivation. Please support them during activities, as they often work in harsh environments. For instance, during a COVID-19 project, the DMO offered ten thousand shillings to CHWs for vaccinating ten people". (KII 2, Misungwi)

A shift in LLIN colour was also identified as a potential factor that could positively influence uptake

"… Given that blue nets have been widely used, it would be beneficial to switch to green ones, as people are familiar with and more accepting of them. The color change could serve as a motivator, encouraging greater use, since many are weary of the blue nets and have started to associate them with negative beliefs. Introducing a different colour could help dispel these associations and persuade more people to use the nets". (P7: FGD 3: Misungwi, low malaria & high net use cluster)

7.6 Discussion

This study evaluated community perceptions of malaria prevention and the acceptability of dual-AI ITNs compared to standard LLINs in Muleba and Misungwi districts, Tanzania, where these novel nets were introduced. Acceptability, as defined in this study, the extent to which nets are perceived as suitable and effective by users, was high for dual-AI ITNs but varied across net types. Interceptor G2 was most preferred in Misungwi due to its perceived physical integrity, soft texture, and superior effectiveness in reducing malaria, particularly among children. By contrast, Olyset Plus, though effective, was less favoured in Misungwi due to frequent tearing, whereas it was well-received in Muleba for its strong insecticide properties. Differences between dual-AI and standard LLINs were noted, particularly in side effects, with participants reporting skin irritation and itching associated with Royal Guard and Interceptor, likely due to higher concentrations of alpha-cypermethrin. Preferences were primarily driven by net durability, comfort, and efficacy, underscoring the need for user centric designs to optimize acceptability and consistent usage.

Participants in Misungwi reported that mosquitoes could penetrate the Royal Guard and Olyset Plus nets, likely due to wear and tear or shrinking over time [259]. Participants frequently reported side effects associated with dual-AI LLINs, including skin irritation, facial burning, and rashes. These effects were particularly noted with Royal Guard and Interceptor, aligning with concerns from other studies that evaluated nets containing alphacypermethrin [152, 208, 209, 260], known to cause more side effects than permethrin [190, 260, 261]. For example, research from Benin [152] reported similar transient adverse events, such as itching and mild skin irritation, associated with these insecticides. However, adverse reactions tended to diminish within a few hours or days of consistent use, suggesting they were primarily short-term adaptations to the chemical agents rather than prolonged issues. In Misungwi and Benin, (55% and 63.8%) of Interceptor users and (48% and 52.5%) of Royal Guard users reported side effects respectively [147, 152], higher than that reported in Liberia, where only 17.5% reported issues with Interceptor LLINs [262]. The qualitative findings in this study showed that while side effects were commonly cited, they did not significantly deter LLIN usage in most households. Participants often emphasized the protective benefits of the nets against malaria, which outweighed their discomfort. However, in a small subset of users, these effects did lead to reduced adherence or even discontinuation of net use. Such reactions were particularly prevalent individuals with sensitive skin. Given these findings, there is a strong case for more active monitoring of adverse events associated with LLIN/ITNs use during distribution campaigns. This could include community reporting mechanisms and periodic follow-ups with households to address concerns and reinforce the health benefits of LLINs/ITNs. Clear communication about the transient nature of most side effects and strategies to mitigate discomfort (e.g., washing nets before use as it was done in this study) may further enhance user compliance and satisfaction.

The study also highlighted significant knowledge gaps regarding malaria transmission, particularly during dry seasons when mosquito-biting nuisance is lower [75, 263, 264]. LLINs were primarily used when mosquito nuisance was high or malaria was perceived as a significant threat. Previous studies have similarly linked high mosquito densities and perceived malaria risk with LLIN compliance [219, 265]. A common reason for non-use was the lack of net ownership, often due to excessive damage to nets, particularly Olyset Plus and Royal Guard [88, 259, 266].

LLIN acceptability varied with the degree of mosquito nuisance and perceived malaria threat. In high-nuisance areas, participants used LLINs despite unfavourable characteristics, whereas in low-nuisance areas like Misungwi, these characteristics led to intermittent or non-use. Control programs in Tanzania and SSA should address issues of intermittent use, misuse, and poor fabric integrity of LLINs, which compromise malaria prevention efforts.

Educational programs should emphasize that the risk of malaria persists even when mosquito numbers are low and that while LLINs reduce malaria risk, they do not eliminate it [217]. This may help manage expectations and increase acceptability by encouraging the use of complementary prevention tools. While knowledge about malaria prevention was high among participants, with over 90% demonstrating a clear understanding of key practices, the practical application of this knowledge often varied, particularly during the hot season. This discrepancy underscores the complex relationship between knowledge and behaviour. Evidence suggests that knowledge alone is rarely sufficient to drive or sustain behavioural change [267]; contextual factors such as environmental conditions, cultural norms, and competing priorities significantly influence decision-making. For example, despite high levels of knowledge, some participants reported reduced ITN/LLIN use during the hot season, citing discomfort as a barrier. These findings highlight the need for interventions that move beyond knowledge dissemination to address the broader determinants of behaviour, such as convenience, accessibility, and reinforcement through community-based programs.

Preferences for LLINs varied by region and culture, with a preference for blue, rectangular LLINs in this study, similar to findings in Kenya [254, 255]. However, in other regions, preferences for LLIN shape differ, as seen in Ethiopia and Senegal who preferred conical LLINs (as they are easy to install) [85, 268, 269].

The study also highlighted misconceptions about malaria transmission, such as beliefs that it could be contracted from contaminated water, bad air, sun exposure, or superstition. Additionally, some participants believed LLINs caused bedbugs [254], which needs to be addressed in control efforts [270-273]. Although LLINs are not designed to kill bedbugs, new nets with fresh insecticide can temporarily reduce bedbug populations, but resistance to pyrethroids has reduced their effectiveness [248, 268, 273-277]. Non-pyrethroid insecticides like chlorfenapyr, found in Interceptor G2, may be more effective against resistant bedbugs [278].

This study aimed to explore the acceptability and use of dual-AI ITNs and to inform future campaigns. Potential biases in reporting these adverse events especially arising from qualitative study may arise. The convenience sampling method and lack of randomization may have overrepresented households experiencing side effects. Furthermore, the absence of a control group precluded direct comparisons of side effect prevalence between dual-AI LLINs and standard pyrethroid-only LLINs. This limitation highlights the importance of incorporating more rigorous, comparative frameworks in future studies. While the qualitative nature of the research limits representativeness within the population, the inclusion of quantitative data from two districts provides a broader perspective. The variation in desirability of Olyset Plus between sites suggests a need for further studies where it is deployed on a larger scale. The study acknowledges potential biases in self-reported data on LLIN ownership and usage frequency

7.7 Conclusion

This study demonstrates that the acceptability and consistent use of dual-AI ITNs are strongly influenced by their perceived effectiveness, fabric characteristics, and comfort. While Interceptor G2 and Olyset Plus were the most preferred nets in their respective districts, challenges such as durability issues and adverse reactions must be addressed to optimize compliance. For the NMCPs, these findings reinforce the importance of engaging communities in the selection and evaluation of LLINs to ensure their needs are met. Future campaigns should prioritize distributing LLINs with high acceptability while incorporating educational initiatives to dispel misconceptions and promote consistent usage. These efforts are essential for enhancing the success and sustainability of malaria control programs in Tanzania and similar high-transmission settings.

7.8 Other results

7.8.1 Distinct differences between Misungwi and Muleba that might influence misuse of nets

Both districts had similar exposure to SBCC messages and comparable knowledge of malaria prevention. However, Misungwi households had more LLINs at baseline (62.3% access of 1 LLIN per sleeping space used last night, and 61% usage) [159] than Muleba (16.9% access and 27.1% usage) [279], and cultural, lifestyle, and SES differences between the districts may have influenced LLIN use (Table 7.9).

Table 7.9: Differences between Muleba and Misungwi

Category	Muleba	Misungwi
Ethnic Composition	Predominantly Haya, with a rich history in coffee and banana farming, and traditional iron smelting.	Predominantly Sukuma, strong agricultural and pastoral culture, focus on cotton and cattle herding.
Crop Types	Annual, less frequent farming needed	Perennial crops like rice, sweet potatoes, cassava, and sorghum, require seasonal farming and vegetation clearance.
Climate & Landscape	Greener, cooler, wetter, tropical rainforest climate, hilly, forested, nucleated settlements.	Hotter, drier, tropical savanna climate, flat landscape, scattered settlements, sparse tree cover.
Soil Fertility	More fertile soils, lush vegetation, farming done by hand hoe.	Less fertile soils, farming with ploughs drawn by cattle.
Housing	Traditional houses are made of bricks/mud and poles, thatched with banana leaves or dry barks, and tin roofs.	Simple houses made of mud bricks, thatched roofs, and increasing use of iron sheets.
Resource Use	Banana dry barks are used as ropes, less need for synthetic ropes.	With limited plant resources, LLINs were repurposed as ropes for various uses, including tying cattle yokes.
Crop Types	Annual, less frequent farming is needed.	Perennial crops like rice, cotton, and sorghum, require seasonal farming and vegetation clearance.
Literacy Rate	Relatively higher, better access to education due to historical missionary activities.	Lower literacy rate, challenges in education access due to economic constraints, and dispersed population.
Economic Development	Moderately developed, income from agriculture and fishing, higher per capita income from coffee.	Lower socio-economic status, income from agriculture and livestock, reliance on subsistence farming.

7.8.2 Eliud Lukole's 6.14 kilometers walk

In November 2021, during the qualitative study conducted in the Misungwi district (comprising Focus Group Discussions [FGDs] and In-Depth Interviews [IDIs]), I took the opportunity to explore various alternative uses of nets in 43 households in one of the hamlets. As I walked through the area (Figure 7.5). I used a handheld GPS to record geolocations and took photographs of repurposed LLINs, especially those where whole or large sections were utilized. I engaged with residents, asking about the reasons behind each specific use. The majority of these LLINs were employed to cover crops or as barriers around areas where crops were planted. At household number 28, I had an extended conversation with a potato farmer who was tending to his tomato nursery (Figure 7.4). The collected images were then superimposed onto a map, corresponding to their geolocations, to illustrate the proximity of these households (Figure 7.5).

At house number 28, I had the pleasure of meeting the head of the household, a dedicated tomato farmer who was carefully tending to his nursery of young tomato seedlings. He shared with me the importance of safeguarding his crop, explaining that these were not just any ordinary seedlings, they were hybrid varieties, precious and costly. With 20 grams of seeds sown, each 10 grams priced at 150,000 Tanzania shillings (about $60) market price, he had invested a total of 300,000 Tanzania shillings ($120) in this fragile nursery. "Imagine letting the chickens loose and other pests destroy everything," he said with a determined smile. "That's simply not an option. I must protect these young plants at all costs".

Figure 7.4: Tomato nursery at house number 28

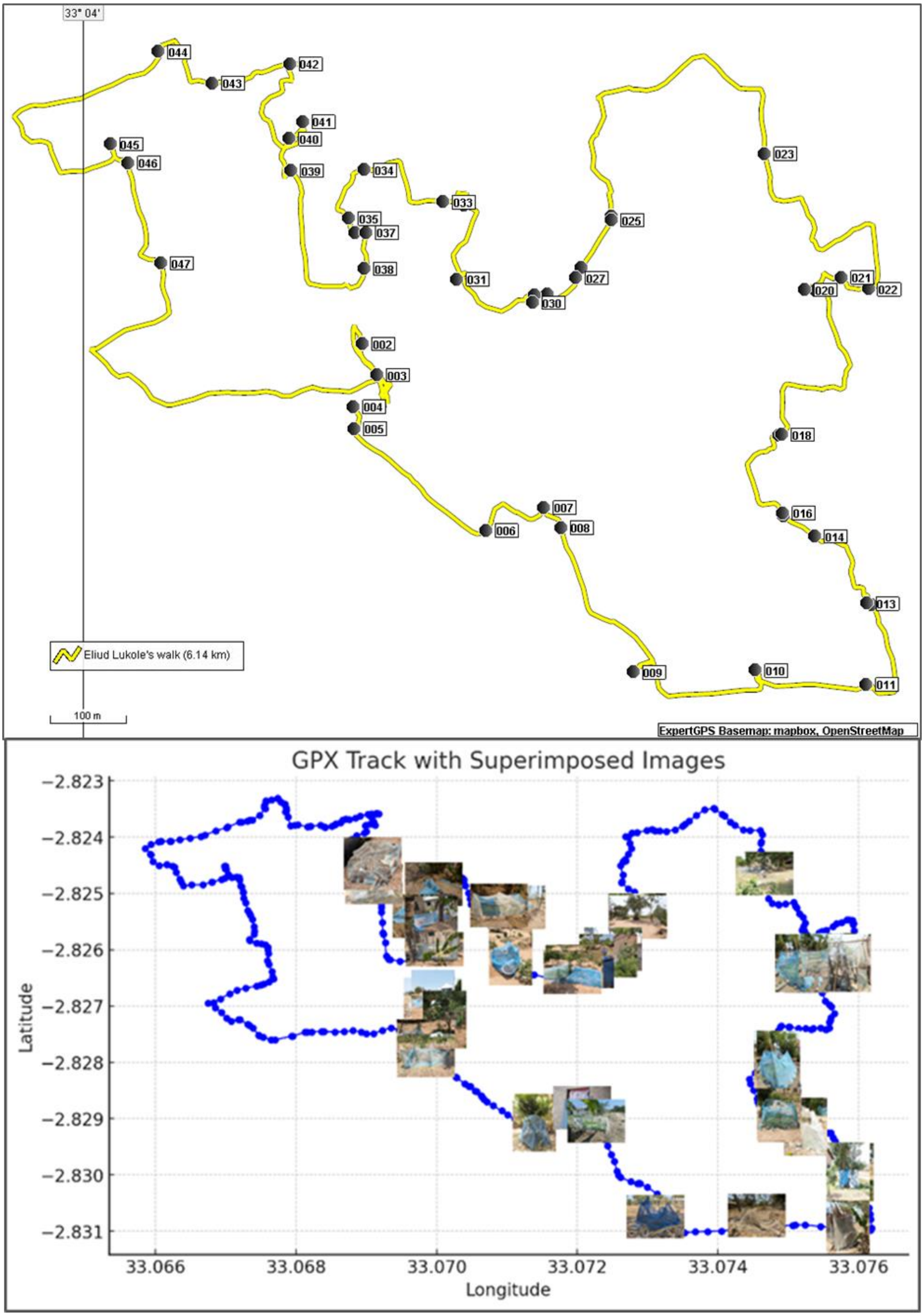

Figure 7.5: Eliud Lukole's 6.14 km track and superimposed pictures

Figure 7.6: Sample of pictures taken. Other pictures are in Appendix 7.1

8 Chapter 8: General Discussion

8.1 Summary of key findings from the thesis

Using an LLIN regardless of type and damage is better than not using an LLIN

Using an LLIN, including a standard pyrethroid-based LLIN, irrespective of its physical condition and age, conferred significantly greater protection against malaria than not using a net at all, even in the context of elevated pyrethroid resistance. Across all study arms and throughout the study period, individuals who did not use nets consistently faced a higher risk of contracting malaria, regardless of net coverage and usage rates.

Non-users residing in Interceptor G2 clusters were more protected than those in pyrethroid-only LLIN clusters and experienced similar levels of protection as users of pyrethroid-only LLINs.

The risk of contracting malaria was 2.2 times higher for non-users residing in clusters with Interceptor (a pyrethroid-only LLIN) compared to those in clusters with Interceptor G2 (chlorfenapyr-pyrethroid LLIN) and 1.7 times higher compared to those with Olyset Plus (pyrethroid-PBO LLIN). However, the malaria risk for non-users in Interceptor G2 clusters was comparable to that of users of pyrethroid-only LLINs. In contrast, neither Olyset Plus nor Royal Guard offered similar protection to non-users. Non-users living in clusters with Royal Guard and Olyset Plus were protected against malaria when more than 40% of the population used LLINs. Conversely, non-users in Interceptor G2 clusters were better protected irrespective of the overall usage levels within the cluster.

Torn dual-active ingredient (AI) LLINs provided greater protection against new malaria cases compared to pyrethroid-only LLINs in good condition.

Among children aged 0.5-14 years, those who slept under torn Olyset Plus and Interceptor G2 nets had a lower incidence of malaria, with 63% and 55% reduced rates respectively, compared to those who slept under good standard LLINs (Interceptor LLIN), after adjusting for the age of the LLIN. Notably, there was no significant association between malaria prevalence and the physical condition of the LLINs at either study site.

Dual-AI ITNs do not last for 3 years under field conditions

The median functional lifespan of all LLINs assessed was less than 3 years. Specifically, Olyset Plus exhibited a survival time of 1.6 years in Muleba [87] and only 0.9 years in Misungwi [88]. In comparison, Royal Guard, Interceptor G2, and Interceptor demonstrated a survival time of 1.9 years in Misungwi [88]. Physical integrity deficits were prevalent across all LLIN types at both study sites, with the most significant degradation observed in Olyset Plus nets in Misungwi [87, 88, 259]. The difference in LLIN misuse between Muleba and Misungwi, likely influenced by cultural, and socioeconomic differences, as well as greater LLIN access in Misungwi, may explain the shorter survival rate observed.

Polyester, rectangular, and blue LLINs are generally more acceptable and preferred by users

In Muleba, Olyset Plus was perceived as more desirable due to its perceived superior insecticidal efficacy compared to the standard Olyset net. In Misungwi, among the four net brands evaluated, Interceptor G2 was preferred because the community associated it with fewer malaria cases in their homes, noted its greater resistance to damage, and appreciated its soft texture. Overall, LLINs were favoured over indoor residual spraying (IRS) in Muleba, as most users reported experiencing better sleep quality and longer-lasting protection with LLINs.

8.2 Summary of all available evidence generated by the two RCTs (Muleba and Misungwi)

Both RCTs demonstrated that dual-active ingredient (AI) LLINs were more effective in controlling malaria than pyrethroid-only LLINs. However, the duration of effectiveness of Olyset Plus varied by study site. In Muleba, Olyset Plus outperformed standard LLINs in reducing malaria infection for up to 33 months[148, 234] and *Anopheles* density for up to 21 months [234]. Conversely, in Misungwi, the reduction in malaria prevalence due to Olyset Plus, compared to standard LLINs, was only observed for the first 18 months [147, 151], while the reduction in *Anopheles* density persisted for up to 36 months [280].

The Misungwi RCT further indicated that parasite prevalence at 12, 24, and 36 months post-intervention was reduced by 53%, 55%, and 43%, respectively, in villages that received chlorfenapyr-pyrethroid LLINs (Interceptor G2) compared to those receiving pyrethroid-only LLINs [147, 151]. Interceptor G2 also demonstrated superior efficacy in reducing *Anopheles* density up to 36 months [280]. Unexpectedly, the Royal Guard did not show superiority over standard LLINs in reducing malaria infections [147, 151] or *Anopheles'* density over the 36 months [280].

Insecticide resistance intensity was assessed in both trials. The results indicated a significant increase in pyrethroid resistance intensity over time in Misungwi, particularly for permethrin in the piperonyl butoxide (PBO) clusters, whereas no change was observed in the clusters which received Interceptor G2. However, caution is warranted in interpreting these findings, as collections were conducted in only two clusters per arm. However, similarly to the first trial, data collection was limited to two clusters per arm [281]. LC50 values for permethrin in Muleba were generally lower than those in Misungwi, with values being three to five times the diagnostic concentration (21.5 µg/mL) for *An. gambiae and An. funestus*, respectively [282]. In Misungwi, the LC50 for *An. funestus* ranged from less than one time in year one to more than 100 times in year three in the PBO arm, depending on the cluster [281]. Resistance to PPF was also observed at the onset of the trial [281].

By the end of 3 years, insecticidal content declined rapidly with only 3% of PBO and 45% of permethrin in Olyset Plus; 8% of chlorfenapyr and 28% of alpha-cypermethrin in Interceptor G2; and 27.8% of pyriproxyfen and 62% of alpha-cypermethrin remaining in the LLINs [147, 151]. In addition to these findings, recent experimental hut trials [283] revealed that the Interceptor G2 LLINs had higher 72-hour mortality (44% vs. 21%) up to 12 months. Olyset Plus showed significant 24-hour mortality initially, which diminished by 12 months (17% vs. 13%). Conversely, the Royal Guard did not significantly impact mosquito fertility during the first 12 months and thereafter. These findings further elucidate why torn Olyset Plus and Interceptor G2 LLINs failed to provide significant protection compared to good-standard LLINs in reducing malaria incidence

8.3 Implications of findings for the deployment of dual-AI ITNs

Given the constrained and competitive nature of malaria control funding, it can be argued that limited resources should be strategically allocated to enhance ownership, access, and utilization of dual-AI ITNs in regions where pyrethroid resistance is prevalent. This approach aligns with WHO malaria control guidelines and would likely maximize the effectiveness of interventions in these high-resistance areas [3]. The data of this study provided significant evidence regarding the durability, effectiveness, acceptability, and preferences for dual-AI ITNs in real-world community settings.

The findings from objectives 1 and 2 clearly indicate that dual-AI ITNs, much like their standard counterparts, failed to maintain their physical integrity over the intended three-year lifespan. These nets are prone to tearing relatively easily, raising concerns about their long-term efficacy in malaria control. These observations are consistent with earlier research, including studies conducted in Tanzania[86], Ethiopia [91], the Democratic Republic of the Congo (DRC) [90], Zanzibar [89], Mozambique [83], and Burkina Faso [84] which similarly reported that both dual-AI ITNs and standard LLINs experience durability issues, thereby limiting their operational lifespan in the field. However, in some other regions, such as Nigeria, the lifespan of LLINs has been reported to exceed three years [81, 82]. This increased durability is strongly associated with extensive exposure to social behaviour change communication (SBCC) messages [84, 97, 201]. To enhance the physical integrity and efficacy of LLINs, the National Malaria Control Program (NMCP) should promote key household practices: washing nets gently with mild soap and air-drying in the shade to preserve insecticidal properties; promptly repairing holes using locally available materials; minimizing strain on fabric by proper hanging and careful handling; and storing nets in clean, dry conditions when not in use. These evidence-based strategies, supported by studies from Tanzania [89, 250, 251], underscore the importance of community education initiatives to reinforce proper net care, thereby extending LLIN lifespan and strengthening malaria control efforts.

Objective 4 of this study provided additional evidence that the acceptability and preference for dual-AI long-lasting insecticidal nets (LLINs) are significantly influenced by specific physical characteristics of the nets, as well as the community's perceptions regarding their efficacy in malaria prevention. This observation is consistent with findings from other regions where similar trends have been documented. Specifically, polyester LLINs were more favoured than their polyethylene counterparts. Such a preference for polyester LLINs has also been documented in previous studies conducted in Benin [73] and Kenya [254]. Additionally, other studies identified a notable preference for conical LLINs over rectangular ones in Kenya[254], and Ethiopia [269], which contrasts with the general preference for rectangular LLINs found in this study. These regional variations highlight the critical need for pilot studies prior to the large-scale distribution of LLINs in any community. Conducting such studies is essential to determine local preferences, which can significantly influence the acceptance, consistent use, and overall effectiveness of LLINs in reducing malaria transmission. However, implementing pilot studies and results from thereof can be challenging. These approaches require the production and supply of multiple distinct batches of LLINs with varying designs, colours, and features. This logistical complexity often leads to a "one-size-fits-all" approach, which prioritizes ease of manufacturing and distribution over community preferences. Yet, such an approach may result in providing tools that the community does not prefer or fully utilize, thereby compromising the effectiveness of malaria control efforts. Reassessing this strategy to incorporate community-specific preferences could enhance LLIN adoption and sustained usage.

The study also yielded crucial insights into the long-term performance of dual-AI ITNs. It was observed that, even when torn, dual-AI ITNs initially provided a level of protection against malaria that surpassed that of good-standard LLINs. This suggests that dual-AI ITNs may have the potential to restore protection that is typically lost with aged and damaged pyrethroid-only LLINs [94]. However, as the dual-AI ITNs continued to degrade [88] and the active insecticidal ingredients diminished [147, 151], their effectiveness significantly declined [151]. By the end of the second year, torn dual-AI ITNs no longer outperformed good-standard LLINs in preventing malaria.

This decline in efficacy was paralleled by a sharp drop in usage rates, which fell to below 40% within 12 months post-intervention. Although these LLINs have the potential to remain effective despite damage, the perception of reduced efficacy due to large holes led to decreased usage. This observation is corroborated by qualitative findings from participants, who reported discontinuing the use of their nets once substantial holes developed. This behaviour is particularly concerning, as it may compromise community-wide protection, which, as indicated by this study, is only achieved when the usage rate of dual-AI ITNs exceeds 40%, thereby diminishing the potential public health benefits of the intervention [284]. Moreover, results from experimental hut trials demonstrated that the bio-efficacy advantage of dual-AI ITNs over standard LLINs did not extend beyond 12 months[283], likely exacerbated by the premature and pronounced decline in insecticide bioavailability within the dual-AI ITNs [147, 151].

These findings underscore a critical challenge in malaria control: the necessity for LLINs that are not only potent in their insecticidal properties but also robust enough to endure the wear and tear of daily use in community settings [77]. The premature degradation of LLINs presents a significant threat, as it leaves communities vulnerable to increased malaria transmission in the period leading up to the typical 3-year distribution cycle, particularly for households that do not benefit from continuous distribution programs [77].

Despite this, overall net usage in the Misungwi study area remained relatively high, ranging from 85.3% to 62.3% throughout the study period. This trend suggests that if the standard of care were replaced by more effective LLINs, such as the Interceptor G2, sustained malaria reduction could be achieved, preventing the resurgence observed after the first year of use (with malaria prevalence of 15.7% in year one, 33.2% in year two, and 32.3% in year three in the Interceptor G2 group). However, the effectiveness of continuous distribution programs, integrated with antenatal clinics, vaccination initiatives, and school-based distributions, needs careful consideration. These programs can potentially lead to oversupply in some households, while others remain under-protected due to insufficient net top-ups. Although indirect protection may be conferred by high community-wide LLIN usage (>40%), there remains a critical need to maintain high levels of net use, care, and repair through social and behaviour change communication (SBCC) strategies [81, 82].

The deployment of dual-AI ITNs is likely to face similar implementation challenges as standard LLINs especially when there is an oversupply of LLINs. One significant issue is the perception that LLINs, being freely distributed, can be repurposed for other uses, such as protecting poultry and seedlings [285]. Despite widespread awareness of the protective value of LLINs against malaria, immediate needs, such as ropes, safeguarding livestock, and plants, or even making fishing traps, sometimes take precedence [286, 287]. The researcher also documented the sale of LLINs in local markets, particularly in Misungwi, although direct admissions of selling freely distributed LLINs were limited due to the prohibition against the sale of LLINs. A few individuals, however, acknowledged selling nets out of financial necessity. To address these issues, stringent regulatory measures are needed to prevent the unauthorized commercial sale of campaign-distributed LLINs, which are intended solely for malaria prevention.

8.4 Dual-AI ITNs: effective but insufficient alone-the need for integrated malaria control strategies

The decision to discontinue the use of Indoor Residual Spraying (IRS) in many malaria-endemic regions, particularly within sub-Saharan Africa, has largely been influenced by findings from the Muleba trial assessing the efficacy of combining piperonyl-butoxide-treated LLINs (PBO LLINs) with IRS interventions [148]. The Tanzania (Muleba) trial [148] and Sierra Leone, and Ethiopia[288], have demonstrated that the addition of IRS to PBO LLINs does not yield a statistically significant enhancement in malaria control outcomes when compared to the deployment of PBO LLINs alone. A limitation of our Muleba trial is that IRS was conducted only once during the first year when PBO LLIN efficacy was at its peak. The outcomes may have differed if annual spraying had been conducted as the nets aged. Additionally, there is a potential antagonistic interaction between pirimiphos-methyl IRS and pyrethroid-PBO LLINs, which could diminish the combined effectiveness, as observed in Benin [289]. Crucially, these studies did not extend to evaluating the potential synergistic effects of combining IRS with other dual-AI LINs, such as those incorporating chlorfenapyr or pyriproxyfen, which represent newer innovations in malaria vector control.

In trials conducted in various settings, particularly in Benin and Tanzania, the Interceptor G2 LLINs comprising both alpha-cypermethrin and chlorfenapyr have consistently demonstrated superior efficacy in reducing malaria prevalence [147, 151-153]. Despite the marked effectiveness of Interceptor G2 LLINs, malaria transmission persists as a significant public health challenge within these regions. For instance, in the Misungwi RCT, malaria prevalence among individuals using highly effective LLINs like the Interceptor G2 ranged from 13% to 38%. This ongoing transmission underscores the necessity of adopting a multi-faceted approach to malaria control, wherein multiple interventions are strategically used in tandem to achieve greater impact.

The sustained prevalence of malaria in areas where highly effective tools, such as Interceptor G2 LLINs, are deployed, highlights the complexity inherent in malaria control efforts and the imperative for integrating diverse strategies [147, 151-153]. While IRS may no longer be recommended in regions where dual-AI LLIN are prevalent [3], the persistent burden of malaria suggests that supplementary interventions including the targeted IRS in areas of high transmission, enhanced surveillance mechanisms, and increased community engagement remain critical for driving malaria transmission to more manageable levels. This scenario calls for adaptive, evidence-based approaches to ensure that malaria control initiatives are not only effective but also sustainable over the long term.

8.5 Generalizability of the study findings

This study was conducted in rural districts of Tanzania, East Africa, characterized by moderate to high malaria transmission, which intensifies following the short and long rainy seasons, and by moderate to high levels of insecticide resistance. The findings from this study are potentially applicable to other regions in East Africa and beyond, where malaria transmission intensity and vector populations are similar. The study sites exhibited a broad range of malaria transmission intensities, with prevalence rates spanning from 10% to 89% in Muleba and 0% to 79% in Misungwi. Notably, in Misungwi, over 71% of the population typically retires indoors to sleep after 21:00 hours, thereby increasing their risk of exposure to exophilic vectors [290, 291].

The predominant malaria vectors in the study areas were *An. gambiae* sensu lato (s.l.) and *An.* funestus *s.l.,* both of which are highly anthropophilic, endophilic, and endophagic, making them particularly amenable to control [157]. In Muleba, 95.7% (13,106/13,689) of the collected Anopheline specimens were identified as belonging to the *An. gambiae s.*s. complex, with 3.7% (510/13,689) attributed to *An. funestus* [148]. Conversely, in Misungwi, 5.8% (1,515/26,345) of the collected *Anopheles* specimens were classified within the *An. gambiae s.l*. complex, while a significant majority, 94.3% (24,830/26,345), were identified as *An. funestus* [147]. These variations in vector species composition and the differential intensity of insecticide resistance may account for the observed disparities in the efficacy of Olyset Plus (PBO LLIN) between the Muleba and Misungwi study sites. As such, the outcomes of this study may be extrapolated to regions where *An. gambiae* sensu stricto (ss)*,* or *An. funestus* predominate, under conditions similar to those in Muleba or Misungwi

However, the application of these findings to other geographical settings should be approached with caution. This caution is warranted by the observed discrepancies between the results from Misungwi and those from a sister trial conducted in Benin, which utilized comparable methodologies and LLIN types, similar levels of insecticide resistance, but higher malaria transmission levels and different species with *An. coluzzi* being the main vectors followed by *An. gambiae* s.s. In the Benin trial, by the third year, Interceptor G2 did not demonstrate superior efficacy in reducing malaria incidence compared to pyrethroid-only LLINs, despite moderate net usage (52.2%) [153]. This contrasts with the significantly lower net usage observed in Tanzania (Misungwi RCT) during the third year (22.8%) [151]. A potential explanation for the divergence in outcomes between the Tanzania and Benin RCTs in the third year may be the substantially higher vector density (3.7 vectors/house/night in Tanzania versus 27.9 in Benin) and a greater entomological inoculation rate (EIR) (0.14 infectious bites/person/year in Tanzania versus 0.69 in Benin) [147, 152]. The heterogeneity in vector ecology and malaria epidemiology across Africa, and especially in comparison to non-African regions, complicates the direct extrapolation of these findings to settings outside Africa. Nevertheless, the documented short functional lifespan of the LLINs under field conditions is likely to be a relevant similar ecological and epidemiological context worldwide.

8.6 Strengths

This study rigorously assessed the effectiveness, median functional survival, and the impact of physical damage (holes) on the performance of dual-AI ITNs, as well as the community-level effects, acceptability, and user preferences for these LLINs. The study leveraged randomized controlled trials (RCTs) with concurrent controls, alongside physical integrity studies, to explore in greater detail the relationship between net damage (holes) and malaria incidence, as well as the impact on community-wide protection. RCTs are widely recognized as the gold standard for hypothesis testing due to their ability to minimize the influence of confounding variables and bias. Randomization ensures that any imbalances between groups are minimized, thereby reducing the necessity for adjustments, particularly concerning baseline covariates.

The study maintained high data integrity, with minimal missing data, attributable to the stringent standards upheld during data collection and entry. The use of Personal Digital Assistants (PDAs) in Muleba and tablets/smartphones in Misungwi facilitated efficient data collection, contributing to the low levels of missing data. The combination of cross-sectional, cohort, and qualitative study designs provided a comprehensive approach to corroborating the outcomes of interest. Refusal rates among households and children participating in the study remained consistently low across all surveys, attributable to comprehensive community engagement, thereby enhancing the overall reliability of the findings.

A notable strength of this study was the inclusion of both epidemiological and entomological outcomes. The entomological analysis, which is detailed in Appendix 9, adds depth to the understanding of the results, even though it was not conducted by the author of this thesis. The lower Entomological Inoculation Rate (EIR) and reduced mosquito density observed in the dual-AI ITNs, particularly in the Interceptor G2 arms, align with the findings from the epidemiological analysis, thereby reinforcing the study's conclusions.

8.7 Limitations

The study encountered several limitations that may have influenced the overall findings. One of the most significant challenges was the observed decline in net usage by the third year across both study sites, which not only potentially confounded some of the results but also underscored the rapid rate at which nets are discarded within the community. The reduction in net usage led to a decreased sample size over time due to declining coverage. Nevertheless, a more precise comparison of protective effects was achieved through the cohort of children who maintained higher levels of access to and use of LLINs.

The longitudinal study in Chapter 4 classified only 3% of nets as repurposed, a stark contrast to Chapter 7 findings, where qualitative methods revealed frequent misuse, including agricultural and domestic repurposing. Photographic evidence, such as nets used as crop covers and window screens, further highlights this discrepancy, which likely arises from methodological differences. Systematic surveys in Chapter 4 may have underrepresented nuanced repurposing behaviours, whereas qualitative approaches in Chapter 7 captured broader community practices, often linked to net oversupply or expectations of replacements during campaigns. To reconcile these gaps, future studies should combine quantitative and qualitative methods to yield a holistic understanding of LLIN use and misuse.

Methodological limitations further constrain the findings. Chapter 4's longitudinal durability analysis sampled only accessible clusters near main roads, limiting representativeness, while the exclusion of missing or untagged nets at baseline may have introduced selection bias. Seasonal biases in Chapter 5, stemming from the exclusion of t30 timepoints (dry and high transmission season), restricted generalizability, as did potential crossarm contamination from PBO LLIN distributions between 30 and 36 months in Chapter 6. Adverse event reporting in Chapter 7, based on convenience sampling, may have introduced reporting bias. However, a randomized net usage survey conducted three months post-intervention reported side effects in 44.1% of participants using pyrethroid-only LLINs (Interceptor) and 38.8% using Royal Guard ITNs, substantiating the occurrence of side effects across net types.

Additionally, the rationale for adopting a 40% threshold for community protection in Chapter 6, based on balancing clusters rather than epidemiological factors, highlights the need for further exploration of alternative thresholds, such as 20%, 30% 50% or 60%, to optimize interventions and ensure robust malaria control outcomes.

8.8 Recommendations for further research

Enhancing manufacturing quality and developing stronger LLINs (more resilient materials that withstand environmental stressors), alongside alternative insecticide impregnation methods (that are less prone to wash-off), which can last with adequate concentrations up to three years can extend LLIN functional survival. Continuous field monitoring of LLINs that identify issues early, and guide adaptive improvements are crucial. These efforts support sustainable, evidence-based malaria control strategies, ensuring the long-term effectiveness of LLINs under various environmental conditions.

If adequate funding is secured, combining IRS with chlorfenapyr-based LLINs (which have demonstrated efficacy) could offer a higher level of protection than relying on dual-AI ITNs alone. Although the IRS is often considered expensive due to its bi-annual application requirements, implementing a 2-3-year cycle of the IRS after the deployment of dual-AI ITNs could help lower intervention costs and prevent malaria resurgence. This approach is supported by evidence from Uganda and Tanzania, where malaria rebounded three years after IRS withdrawal [292-294].

Another advantage of layering IRS onto dual-AI ITNs is that IRS has greater flexibility in terms of the insecticides used, unlike LLINs, which have limited options and are therefore more vulnerable to resistance. Moreover, once dual-AI ITNs are distributed en masse, there is a significant risk of resistance as has already been observed [281], which could undermine malaria control efforts relying solely on dual-AI LLIN. Given the widespread pyrethroid resistance in 43 malaria-endemic sub-Saharan African countries by 2024, relying exclusively on LLINs could pose serious threats to malaria control [101].

8.9 Conclusion

This thesis provides compelling evidence that dual-AI ITNs exhibit a functional lifespan of less than three years under field conditions. The data indicate that the community-wide impact of dual-AI ITNs becomes particularly pronounced when net usage exceeds 40% within a given population. Notably, protective effects were observed even at lower coverage levels with Interceptor G2, highlighting its efficacy. Users of Olyset Plus and Interceptor G2 nets experienced protection even when the nets were damaged, which stands in contrast to the diminished efficacy observed among users of well-maintained pyrethroid-only LLINs. The perceived efficacy and physical integrity of these dual-AI ITNs were crucial factors driving their acceptability and user preferences.

Given the observed discrepancies in the effectiveness and acceptability of Olyset Plus between Muleba and Misungwi in Tanzania, as well as the differing three-year effectiveness outcomes for Interceptor G2 between Tanzania and Benin, further investigation is necessary to uncover the underlying causes of these variations. Nonetheless, based on the current body of evidence, it is advisable for national malaria control programs to consider the adoption of Interceptor G2 as the new standard of care.

References

1. WHO, *World Malaria report* 2023.
2. Reyburn, H., *New WHO guidelines for the treatment of malaria.* BMJ, 2010. **340**: p. c2637.
3. WHO, *WHO Guidelines for malaria*. 2024.
4. Saili, K., et al., *House Screening Reduces Exposure to Indoor Host-Seeking and Biting Malaria Vectors: Evidence from Rural South-East Zambia.* Trop Med Infect Dis, 2024. **9**(1).
5. Lindsay, S.W., et al., *Recommendations for building out mosquito-transmitted diseases in sub-Saharan Africa: the DELIVER mnemonic.* Philos Trans R Soc Lond B Biol Sci, 2021. **376**(1818): p. 20190814.
6. Abong'o, B., et al., *Screening eaves of houses reduces indoor mosquito density in rural, western Kenya.* Malar J, 2022. **21**(1): p. 377.
7. Kirby, M.J., et al., *Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial.* Lancet, 2009. **374**(9694): p. 998-1009.
8. Getawen, S.K., et al., *Exploring the impact of house screening intervention on entomological indices and incidence of malaria in Arba Minch town, southwest Ethiopia: A randomized control trial.* Acta Trop, 2018. **181**: p. 84-94.
9. Fox, T., et al., *House modifications for preventing malaria.* Cochrane Database Syst Rev, 2022. **10**(10): p. CD013398.
10. Furnival-Adams, J., et al., *House modifications for preventing malaria.* Cochrane Database Syst Rev, 2021. **1**(1): p. CD013398.
11. Pryce, J., M. Richardson, and C. Lengeler, *Insecticide-treated nets for preventing malaria.* Cochrane Database Syst Rev, 2018. **11**: p. CD000363.
12. Maheu-Giroux, M. and M.C. Castro, *Cost-effectiveness of larviciding for urban malaria control in Tanzania.* Malar J, 2014. **13**: p. 477.
13. Choi, L., S. Majambere, and A.L. Wilson, *Larviciding to prevent malaria transmission.* Cochrane Database Syst Rev, 2019. **8**(8): p. CD012736.
14. Bhatt, S., et al., *The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015.* Nature, 2015. **526**(7572): p. 207-211.
15. WHO, *Global technical strategy for malaria 2016-2030.* 2015.
16. WHO, *Global technical strategy for malaria 2016–2030, 2021 update*. 2021.
17. Wafula, S.T., et al., *What are the pathways between poverty and malaria in sub-Saharan Africa? A systematic review of mediation studies.* Infect Dis Poverty, 2023. **12**(1): p. 58.
18. Degarege, A., et al., *Improving socioeconomic status may reduce the burden of malaria in sub Saharan Africa: A systematic review and meta-analysis.* PLoS One, 2019. **14**(1): p. e0211205.
19. Breman, J.G., A. Egan, and G.T. Keusch, *The intolerable burden of malaria: a new look at the numbers.* Am J Trop Med Hyg, 2001. **64**(1-2 Suppl): p. iv-vii.
20. Andrade, M.V., et al., *The economic burden of malaria: a systematic review.* Malar J, 2022. **21**(1): p. 283.
21. Sicuri, E., et al., *The economic costs of malaria in children in three sub-Saharan countries: Ghana, Tanzania and Kenya.* Malar J, 2013. **12**: p. 307.
22. Li, J., et al., *Current Status of Malaria Control and Elimination in Africa: Epidemiology, Diagnosis, Treatment, Progress and Challenges.* J Epidemiol Glob Health, 2024.
23. Gboeloh, L.B.E., Kingsley, *Effects of malaria on school attendance among primary school pupils in Khana Local Government Area, Rivers State, Nigeria.* International Journal of Life Sciences and Technology, 2016. **Vol. 9**.
24. Lukwa, A.T., et al., *Effect of malaria on productivity in a workplace: the case of a banana plantation in Zimbabwe.* Malar J, 2019. **18**(1): p. 390.

25. Beier, J.C., *Malaria parasite development in mosquitoes.* Annu Rev Entomol, 1998. **43**: p. 519-43.
26. (RBM), R.B.M. *What is Malaria?* 2024; Available from: https://endmalaria.org/about-malaria/what-malaria.
27. Paul, R.E., M. Diallo, and P.T. Brey, *Mosquitoes and transmission of malaria parasites - not just vectors.* Malar J, 2004. **3**: p. 39.
28. Liu, Z., J. Miao, and L. Cui, *Gametocytogenesis in malaria parasite: commitment, development and regulation.* Future Microbiol, 2011. **6**(11): p. 1351-69.
29. Collins, W.E. and G.M. Jeffery, *Plasmodium malariae: parasite and disease.* Clin Microbiol Rev, 2007. **20**(4): p. 579-92.
30. Ghosh, A., M.J. Edwards, and M. Jacobs-Lorena, *The journey of the malaria parasite in the mosquito: hopes for the new century.* Parasitol Today, 2000. **16**(5): p. 196-201.
31. Menard, R., *The journey of the malaria sporozoite through its hosts: two parasite proteins lead the way.* Microbes Infect, 2000. **2**(6): p. 633-42.
32. Pimenta, P.F., M. Touray, and L. Miller, *The journey of malaria sporozoites in the mosquito salivary gland.* J Eukaryot Microbiol, 1994. **41**(6): p. 608-24.
33. Sinnis, P., *The malaria sporozoite's journey into the liver.* Infect Agents Dis, 1996. **5**(3): p. 182-9.
34. Mawson, A.R., *The pathogenesis of malaria: a new perspective.* Pathog Glob Health, 2013. **107**(3): p. 122-9.
35. Hoffman, J.M.C.a.S.L., *Chapter 83: Malaria*, in *Medical Microbiology. 4th edition.* 1996, The University of Texas Medical Branch at Galveston.
36. Lindblade, K.A., et al., *The silent threat: asymptomatic parasitemia and malaria transmission.* Expert Rev Anti Infect Ther, 2013. **11**(6): p. 623-39.
37. Sumner, K.M., et al., *Genotyping cognate Plasmodium falciparum in humans and mosquitoes to estimate onward transmission of asymptomatic infections.* Nat Commun, 2021. **12**(1): p. 909.
38. Bousema, J.T., et al., *Plasmodium falciparum gametocyte carriage in asymptomatic children in western Kenya.* Malar J, 2004. **3**: p. 18.
39. Tiono, A.B., et al., *Lessons learned from the use of HRP-2 based rapid diagnostic test in community-wide screening and treatment of asymptomatic carriers of Plasmodium falciparum in Burkina Faso.* Malar J, 2014. **13**: p. 30.
40. Rodriguez-Barraquer, I., et al., *Quantification of anti-parasite and anti-disease immunity to malaria as a function of age and exposure.* Elife, 2018. **7**.
41. Sinka, M.E., et al., *The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic precis.* Parasit Vectors, 2010. **3**: p. 117.
42. Lengeler, C. and R.W. Snow, *From efficacy to effectiveness: insecticide-treated bednets in Africa.* Bull World Health Organ, 1996. **74**(3): p. 325-32.
43. Lengeler, C., *Insecticide-treated bed nets and curtains for preventing malaria.* Cochrane Database Syst Rev, 2004(2): p. CD000363.
44. Lengeler, C., et al., *Relative versus absolute risk of dying reduction after using insecticide-treated nets for malaria control in Africa.* Trop Med Int Health, 1998. **3**(4): p. 286-90.
45. Casida, J.E., *Pyrethrum flowers and pyrethroid insecticides.* Environ Health Perspect, 1980. **34**: p. 189-202.
46. Service, T.A.M.A.E. *What is a pyrethroid insecticide?* 2018 [cited 2024; Available from: https://citybugs.tamu.edu/factsheets/ipm/ent-6003/.
47. WHO-WHOPES, *Safety of Pyrethroids for Public Health Use*, D.M. Zaim/WHOPES, Editor. 2005, World Health Organization: Geneva. p. 5.
48. Kilian, A., *Balancing the roles of the commercial and public sectors. Case study of three countries in sub-Sahara Africa, Malaria Consortium Learning Paper Series: www.malariaconsortium.org/pages/learning-papers.htm.* 2013.

49. Killeen, G.F., et al., *Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets.* PLoS Med, 2007. **4**(7): p. e229.
50. WHO-WHOPES, *Report of the 5th WHOPES working group meeting. Review of Olyset Net and bifenthrin 10 percent WP*. 2001: Geneva, World Health Organization, 2001.
51. WHO-WHOPES, *Report of the seventh WHOPES working group meeting WHO/HQ, Geneva 2–4 December 2003: Review of VectoBac® WG, PermaNet®, Gokilaht®-S 5EC*. 2003, World Health Organisation: WHO/HQ, Geneva.
52. Renggli, S., et al., *Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania.* Malar J, 2013. **12**: p. 85.
53. Lindblom, G., *Mosquito ≪Nets≫ etc. in Africa.* Ethnos, 1948. **13**(3-4): p. 124-140.
54. Okumu, F., *The fabric of life: what if mosquito nets were durable and widely available but insecticide-free?* Malar J, 2020. **19**(1): p. 260.
55. Killeen, G.F., et al., *Quantifying behavioural interactions between humans and mosquitoes: evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania.* BMC Infect Dis, 2006. **6**: p. 161.
56. Spitzen, J., et al., *Absence of close-range excitorepellent effects in malaria mosquitoes exposed to deltamethrin-treated bed nets.* Am J Trop Med Hyg, 2014. **90**(6): p. 1124-32.
57. Hawley, W.A., et al., *Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya.* Am J Trop Med Hyg, 2003. **68**(4 Suppl): p. 121-7.
58. Hambisa, M.T., et al., *Long lasting insecticidal net use and its associated factors in Limmu Seka District, South West Ethiopia.* BMC Public Health, 2018. **18**(1): p. 124.
59. Smith, D.L., et al., *Ross, macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens.* PLoS Pathog, 2012. **8**(4): p. e1002588.
60. Lines, J.C., N.; Paintain, L., *Systematic reviews, background papers and other unpublished evidence considered in the development of recommendations*, in *WHO Guidelines for malaria*. 2022.
61. Unwin, H.J.T., et al., *Quantifying the direct and indirect protection provided by insecticide treated bed nets against malaria.* Nat Commun, 2023. **14**(1): p. 676.
62. Program, W.-G.M., *WHO Guidance for countries on combining indoor residual spraying and long-lasting insecticidal nets. Geneva: World Health Organization 2014* . 2014.
63. WHO, *Indoor Residual Spraying: Use of Indoor residual spraying for scaling up global malaria control and elimination*. 2006, WHO: Geneva.
64. N'Guessan, R., et al., *Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin.* Emerg Infect Dis, 2007. **13**(2): p. 199-206.
65. WHO, *The African Summit on Roll Back Malaria*. 2000: Abuja, Nigeria.
66. WHO, *World malaria report*. 2010: Geneva
67. WHO, *World malaria report 2019*. 2019: Geneva.
68. WHO, *World malaria report: Years of global progress & challenges*. 2020.
69. (AMP), T.A.f.M.P. *Net mapping project. The elliance for malaria prevention*. 2024 11/03/2024; Available from: https://allianceformalariaprevention.com.
70. change, B.A.f.S.a.B. *ITN Access and Use Report*. 2025 [cited 2024 02 June]; Available from: https://breakthroughactionandresearch.org/resources/itn-use-and-access-report/.
71. Atkinson, J.A., et al., *A qualitative study on the acceptability and preference of three types of long-lasting insecticide-treated bed nets in Solomon Islands: implications for malaria elimination.* Malar J, 2009. **8**: p. 119.
72. Grietens, K.P., et al., *Traditional nets interfere with the uptake of long-lasting insecticidal nets in the Peruvian Amazon: the relevance of net preference for achieving high coverage and use.* PLoS One, 2013. **8**(1): p. e50294.

73. Corine Ngufor, J.F., Augustin Fongnikin, ,Martin Akogbeto, *The physical and insecticidal durability of two dual active ingredient nets (Interceptor® G2 and Royal Guard®) in Benin, West Africa; results for a durability study embedded in a cluster randomised controlled trial-(priprint)*. 2024.
74. Koenker, H. and J.O. Yukich, *Effect of user preferences on ITN use: a review of literature and data.* Malar J, 2017. **16**(1): p. 233.
75. Pulford, J., et al., *Reported reasons for not using a mosquito net when one is available: a review of the published literature.* Malar J, 2011. **10**: p. 83.
76. Brake, S., et al., *Understanding the current state-of-the-art of long-lasting insecticide nets and potential for sustainable alternatives.* Curr Res Parasitol Vector Borne Dis, 2022. **2**: p. 100101.
77. Kilian, A., et al., *Correlation of textile 'resistance to damage' scores with actual physical survival of long-lasting insecticidal nets in the field.* Malar J, 2021. **20**(1): p. 29.
78. Gimnig, J.E., et al., *Letter to the editors.* Trop Med Int Health, 2006. **11**(2): p. 252-3.
79. WHO, *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions*. 2011.
80. WHO-VCTEG, *Estimating functional survival of long-lasting insecticidal nets from field data*, in *Vector Control Technical Expert Group Report to MPAC September 2013*. 2013: Crowne Plaza Hotel, Geneva.
81. Kilian, A., et al., *Field durability of the same type of long-lasting insecticidal net varies between regions in Nigeria due to differences in household behaviour and living conditions.* Malar J, 2015. **14**: p. 123.
82. Obi, E., et al., *Monitoring the physical and insecticidal durability of the long-lasting insecticidal net DawaPlus((R)) 2.0 in three States in Nigeria.* Malar J, 2020. **19**(1): p. 124.
83. Abilio, A.P., et al., *Monitoring the durability of the long-lasting insecticidal nets MAGNet and Royal Sentry in three ecological zones of Mozambique.* Malar J, 2020. **19**(1): p. 209.
84. Raharinjatovo, J., et al., *Physical and insecticidal durability of Interceptor((R)), Interceptor((R)) G2, and PermaNet((R)) 3.0 insecticide-treated nets in Burkina Faso: results of durability monitoring in three sites from 2019 to 2022.* Malar J, 2024. **23**(1): p. 173.
85. Diouf, M., et al., *Survival of eight LLIN brands 6, 12, 24 and 36 months after a mass distribution campaign in rural and urban settings in Senegal.* BMC Public Health, 2022. **22**(1): p. 719.
86. Lorenz, L.M., et al., *Comparative functional survival and equivalent annual cost of 3 long-lasting insecticidal net (LLIN) products in Tanzania: A randomised trial with 3-year follow up.* PLoS Med, 2020. **17**(9): p. e1003248.
87. Lukole, E., et al., *Protective efficacy of holed and aging PBO-pyrethroid synergist-treated nets on malaria infection prevalence in north-western Tanzania.* PLOS Glob Public Health, 2022. **2**(10): p. e0000453.
88. Martin, J., et al., *Monitoring of Fabric Integrity and Attrition Rate of Dual-Active Ingredient Long-Lasting Insecticidal Nets in Tanzania: A Prospective Cohort Study Nested in a Cluster Randomized Controlled Trial.* Insects, 2024. **15**(2).
89. Haji, K.A., et al., *Monitoring the durability of the long-lasting insecticidal nets Olyset((R)) and PermaNet((R)) 2.0 in similar use environments in Zanzibar.* Malar J, 2020. **19**(1): p. 187.
90. Mansiangi, P., et al., *Comparing the durability of the long-lasting insecticidal nets DawaPlus((R)) 2.0 and DuraNet(c) in northwest Democratic Republic of Congo.* Malar J, 2020. **19**(1): p. 189.
91. Hiruy, H.N., et al., *Durability of long-lasting insecticidal nets (LLINs) in Ethiopia.* Malar J, 2023. **22**(1): p. 109.
92. PMI-USAID, *Monitoring of attrition, physical Integrity, and insecticidal activity of long-lasting insecticidal net in Ethiopia: A longitudinal multi-site study*. 2019.

93. Irish, S., et al., *Loss of protection with insecticide-treated nets against pyrethroid-resistant Culex quinquefasciatus mosquitoes once nets become holed: an experimental hut study.* Parasit Vectors, 2008. **1**(1): p. 17.
94. Rehman, A.M., et al., *How much does malaria vector control quality matter: the epidemiological impact of holed nets and inadequate indoor residual spraying.* PLoS One, 2011. **6**(4): p. e19205.
95. Koenker, H., et al., *Annual distributions of insecticide-treated nets to schoolchildren and other key populations to maintain higher ITN access than with mass campaigns: a modelling study for mainland Tanzania.* Malar J, 2022. **21**(1): p. 246.
96. Koenker, H.M., et al., *Analysing and recommending options for maintaining universal coverage with long-lasting insecticidal nets: the case of Tanzania in 2011.* Malar J, 2013. **12**: p. 150.
97. Kilian, A., et al., *Variation of physical durability between LLIN products and net use environments: summary of findings from four African countries.* Malar J, 2021. **20**(1): p. 26.
98. Ranson, H., et al., *Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control?* Trends Parasitol, 2011. **27**(2): p. 91-8.
99. WHO-GMP, *Global plan for insecticide resistance management in malaria vectors*. 2012.
100. Ranson, H. and N. Lissenden, *Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control.* Trends Parasitol, 2016. **32**(3): p. 187-196.
101. WHO, *Malaria threats map*. 2024, WHO.
102. Hemingway, J., et al., *Averting a malaria disaster: will insecticide resistance derail malaria control?* Lancet, 2016. **387**(10029): p. 1785-8.
103. Kleinschmidt, I., et al., *Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study.* Lancet Infect Dis, 2018. **18**(6): p. 640-649.
104. Lindblade, K.A., et al., *A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi.* Malar J, 2015. **14**: p. 31.
105. Strode, C., et al., *The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis.* PLoS Med, 2014. **11**(3): p. e1001619.
106. Churcher, T.S., et al., *The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa.* Elife, 2016. **5**.
107. Agossa, F.R., et al., *Impact of insecticide resistance on the effectiveness of pyrethroid-based malaria vectors control tools in Benin: decreased toxicity and repellent effect.* PLoS One, 2015. **10**(12): p. e0145207.
108. West, P.A., et al., *Indoor residual spraying in combination with insecticide-treated nets compared to insecticide-treated nets alone for protection against malaria: a cluster randomised trial in Tanzania.* PLoS Med, 2014. **11**(4): p. e1001630.
109. Le Menach, A., et al., *An elaborated feeding cycle model for reductions in vectorial capacity of night-biting mosquitoes by insecticide-treated nets.* Malar J, 2007. **6**: p. 10.
110. Mohammed-Awel, J., et al., *Mathematical assessment of the role of vector insecticide resistance and feeding/resting behavior on malaria transmission dynamics: Optimal control analysis.* Infect Dis Model, 2018. **3**: p. 301-321.
111. Gimnig, J.E., et al., *The Effect of Indoor Residual Spraying on the Prevalence of Malaria Parasite Infection, Clinical Malaria and Anemia in an Area of Perennial Transmission and Moderate Coverage of Insecticide Treated Nets in Western Kenya.* PLoS One, 2016. **11**(1): p. e0145282.
112. Stelmach, R., et al., *Cost-Effectiveness of Indoor Residual Spraying of Households with Insecticide for Malaria Prevention and Control in Tanzania.* Am J Trop Med Hyg, 2018. **99**(3): p. 627-637.

113. WHO, *WHO guideline for the prequalification assessment of insecticide-treated nets*. 2023, World Health Organization: Geneva, Switzerland.
114. WHO/HTM/GMP, *The evaluation process for vector control products: Information note.* 2017. **13**.
115. WHO, *WHO Prequalification of Vector Control Products: Overview of the WHO Prequalification Assessment of Vector Control Products*. 2021, World Health Organisation: Geneva. p. 5.
116. WHOPES, *15th WHOPES working group meeting: review of olyset plus, interceptor LN, malathion 440 EW, vectobac GR*

2012: WHO/HQ, Geneva.
117. WHOPES, *Report of the 16th working WHOPES group meeting: Review of: pirimiphos-methyl 300 cs, chlorfenapyr 240 sc, deltamethrin 62.5 sc-pe ,duranet LN, netprotect ln, yahe LN, spinosad 83.3 monolayer DT, spinosad 25 extended release GR.* 2013: WHO/HQ, GENEVA: 22–30 JULY 2013.
118. WHO-WHOPES, *12th WHOPES working group meeting: Review of Bioflash GR, Permanet 2.0, Permanet 3.0, Permanet 2.5, Lambda-cyhalothrin LN*. 2008: WHO/HQ Geneva 2008.
119. WHO-WHOPES, *Report of the thirteenth WHOPES working group meeting: Review of Olyset® LN, Dawaplus® 2.0 LN, Tianjin Yorkool® LN*. 2009, World Health Organisation (WHO): WHO/HQ Geneva.
120. WHO-WHOPES, *Guidelines for laboratory and field-testing of long-lasting insecticidal nets: Control of neglected tropical diseases WHO pesticide evaluation scheme*. 2013, World Health Organisation (WHO): Geneva.
121. WHO, *Norms, standards and processes underpinning development of WHO recommendations on vector control*. 2020, World Health Organisation: Geneva.
122. WHO-WHOPES, *Review of: interceptor g2 ln, dawaplus 3.0 ln, dawaplus 4.0 ln, sumilarv 2 mr, chlorfenapyr 240 sc*. 2017.
123. (USAID), U.S.A.f.I.D., *Integrated Vector Management Programs for Malaria Vector Control (Version 2017): Programmatic Environmental Assessment*. 2017: The Cadmus Group, Inc. 100 Fifth Avenue, Suite 100 Waltham, MA 02451.
124. WHO-EAG, *Design of epidemiological trials for vector control products: Report of a WHO expert advisory group*. 2017, World Health Organisation (WHO): WHO: Geneva, Switzerland.
125. Sumitomo Chemical Co., L., *Olyset-Plus-Technical-Brochure*. 2021, Environmental Health Division: Tokyo Nihombashi Tower, Japan.
126. Cross A, B., C.; Buhl, K.; Jenkins, J. *Piperonyl Butoxide (PBO) general fact sheet; National Pesticide Information Center, Oregon State University Extension Services. npic.orst.edu/factsheets/pbogen.html.* 2017.
127. Pennetier, C., et al., *Efficacy of Olyset(R) Plus, a new long-lasting insecticidal net incorporating permethrin and piperonyl-butoxide against multi-resistant malaria vectors [corrected].* PLoS One, 2013. **8**(10): p. e75134.
128. Tungu, P., et al., *Evaluation of PermaNet 3.0 a deltamethrin-PBO combination net against Anopheles gambiae and pyrethroid resistant Culex quinquefasciatus mosquitoes: an experimental hut trial in Tanzania.* Malar J, 2010. **9**: p. 21.
129. Yewhalaw, D., et al., *Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant Anopheles arabiensis from South-Western Ethiopia.* Parasit Vectors, 2012. **5**: p. 159.
130. Okia, M., et al., *Bioefficacy of long-lasting insecticidal nets against pyrethroid-resistant populations of Anopheles gambiae s.s. from different malaria transmission zones in Uganda.* Parasit Vectors, 2013. **6**: p. 130.
131. Abilio, A.P., et al., *Bio-efficacy of new long-lasting insecticide-treated bed nets against Anopheles funestus and Anopheles gambiae from central and northern Mozambique.* Malar J, 2015. **14**: p. 352.

132. Allossogbe, M., et al., *WHO cone bio-assays of classical and new-generation long-lasting insecticidal nets call for innovative insecticides targeting the knock-down resistance mechanism in Benin.* Malar J, 2017. **16**(1): p. 77.
133. Corbel, V., et al., *Field efficacy of a new mosaic long-lasting mosquito net (PermaNet 3.0) against pyrethroid-resistant malaria vectors: a multi centre study in Western and Central Africa.* Malar J, 2010. **9**: p. 113.
134. Toe, K.H., et al., *Do bednets including piperonyl butoxide offer additional protection against populations of Anopheles gambiae s.l. that are highly resistant to pyrethroids? An experimental hut evaluation in Burkina Fasov.* Med Vet Entomol, 2018. **32**(4): p. 407-416.
135. Ketoh, G.K., et al., *Efficacy of two PBO long lasting insecticidal nets against natural populations of Anopheles gambiae s.l. in experimental huts, Kolokope, Togo.* PLoS One, 2018. **13**(7): p. e0192492.
136. BASF, *Interceptor® G2: the second-generation net to control insecticide resistant mosquitoes,* . Product Information, 2017.
137. Black, B.C., *Insecticidal action and mitochondrial uncoupling activity of AC-303,630 and related halogenated pyrroles. Pestic. Biochem. Physiol, . 50: p. 115–128.* 1994.
138. N'Guessan, R., et al., *A Chlorfenapyr Mixture Net Interceptor(R) G2 Shows High Efficacy and Wash Durability against Resistant Mosquitoes in West Africa.* PLoS One, 2016. **11**(11): p. e0165925.
139. Bayili, K., et al., *Evaluation of efficacy of Interceptor((R)) G2, a long-lasting insecticide net coated with a mixture of chlorfenapyr and alpha-cypermethrin, against pyrethroid resistant Anopheles gambiae s.l. in Burkina Faso.* Malar J, 2017. **16**(1): p. 190.
140. WHO-WHOPES, *Determination of alpha-cypermethrin and chlorfenapyr in net samples from the WHOPES Phase II study on Interceptor® G2 in Tanzania*. 2017, Report of the Walloon Agricultural Research Centre, Gembloux, Belgium for WHO,.
141. Tungu, P.K., et al., *Efficacy of interceptor(R) G2, a long-lasting insecticide mixture net treated with chlorfenapyr and alpha-cypermethrin against Anopheles funestus: experimental hut trials in north-eastern Tanzania.* Malar J, 2021. **20**(1): p. 180.
142. Harris, C., et al., *Sterilising effects of pyriproxyfen on Anopheles arabiensis and its potential use in malaria control.* Parasit Vectors, 2013. **6**: p. 144.
143. Ngufor, C., et al., *Efficacy of the Olyset Duo net against insecticide-resistant mosquito vectors of malaria.* Sci Transl Med, 2016. **8**(356): p. 356ra121.
144. Koffi, A.A., et al., *Efficacy of Olyset(R) Duo, a permethrin and pyriproxyfen mixture net against wild pyrethroid-resistant Anopheles gambiae s.s. from Cote d'Ivoire: an experimental hut trial.* Parasite, 2015. **22**: p. 28.
145. Ngufor, C., et al., *Efficacy of Royal Guard, a new alpha-cypermethrin and pyriproxyfen treated mosquito net, against pyrethroid-resistant malaria vectors.* Sci Rep, 2020. **10**(1): p. 12227.
146. Tiono, A.B., et al., *Efficacy of Olyset Duo, a bednet containing pyriproxyfen and permethrin, versus a permethrin-only net against clinical malaria in an area with highly pyrethroid-resistant vectors in rural Burkina Faso: a cluster-randomised controlled trial.* Lancet, 2018. **392**(10147): p. 569-580.
147. Mosha JF, K.M., Lukole E, Matowo NS, Pitt C, Messenger LA, Mallya E, Jumanne M, Aziz T, Kaaya R, Shirima BA, Isaya G, Taljaard M, Martin J, Hashim R, Thickstun C, Manjurano A, Kleinschmidt I, Mosha FW, Rowland M, Protopopoff N. , *Effectiveness and cost-effectiveness against malaria of three types of dual-active-ingredient long-lasting insecticidal nets (LLINs) compared with pyrethroid-only LLINs in Tanzania: a four-arm, cluster-randomised trial.* Lancet. 2022 Mar 26;399(10331):1227-1241. , 2022.
148. Protopopoff, N., et al., *Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial.* Lancet, 2018. **391**(10130): p. 1577-1588.

149. Staedke, S.G., et al., *Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign.* Lancet, 2020. **395**(10232): p. 1292-1303.
150. WHO-GMP, *Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide: recommendations, September 2017 (revised December 2017)*. 2017.
151. Mosha, J.F., et al., *Effectiveness of long-lasting insecticidal nets with pyriproxyfen-pyrethroid, chlorfenapyr-pyrethroid, or piperonyl butoxide-pyrethroid versus pyrethroid only against malaria in Tanzania: final-year results of a four-arm, single-blind, cluster-randomised trial.* Lancet Infect Dis, 2023.
152. Accrombessi, M., et al., *Efficacy of pyriproxyfen-pyrethroid long-lasting insecticidal nets (LLINs) and chlorfenapyr-pyrethroid LLINs compared with pyrethroid-only LLINs for malaria control in Benin: a cluster-randomised, superiority trial.* Lancet, 2023. **401**(10375): p. 435-446.
153. Accrombessi, M., et al., *Effectiveness of pyriproxyfen-pyrethroid and chlorfenapyr-pyrethroid long-lasting insecticidal nets (LLINs) compared with pyrethroid-only LLINs for malaria control in the third year post-distribution: a secondary analysis of a cluster-randomised controlled trial in Benin.* Lancet Infect Dis, 2024. **24**(6): p. 619-628.
154. (NBS), N.B.o.S., *Census information dissemination platform: census results*. 2022, National Bureau of Statistics: Tanzania.
155. USAID-PMI, *President's malaria initiative FY Tanzania malaria operational plan.* 2020.
156. Shem O. Wandiga, M.O., Daniel Olago, Andrew Githeko, Faith Githui, Michael Marshall, Tim Downs, Alfred Opere, Christopher Oludhe, Gilbert O. Ouma, Pius Z. Yanda, Richard Kangalawe, Robert Kabumbuli, James Kathuri, Eugene Apindi, Lydia Olaka, Laban Ogallo, Paul Mugambi, Rehema Sigalla, Robinah Nanyunja, Timothy Baguma & Pius Achola *Vulnerability to epidemic malaria in the highlands of Lake Victoria basin: the role of climate change/variability, hydrology and socio-economic factors.* 2009.
157. Mwalimu, C.D., et al., *Dynamics of malaria vector composition and Plasmodium falciparum infection in mainland Tanzania: 2017-2021 data from the national malaria vector entomological surveillance.* Malar J, 2024. **23**(1): p. 29.
158. Kabula, B., et al., *Susceptibility status of malaria vectors to insecticides commonly used for malaria control in Tanzania.* Trop Med Int Health, 2012. **17**(6): p. 742-50.
159. Matowo, N.S., et al., *An increasing role of pyrethroid-resistant Anopheles funestus in malaria transmission in the Lake Zone, Tanzania.* Sci Rep, 2021. **11**(1): p. 13457.
160. USAID-PMI, *President's malaria initiative Tanzania malaria operational plan Fy 2019.* 2019.
161. Kakilla, C., et al., *Malaria vector species composition and entomological indices following indoor residual spraying in regions bordering Lake Victoria, Tanzania.* Malar J, 2020. **19**(1): p. 383.
162. Matiya, D.J., et al., *Dynamics and monitoring of insecticide resistance in malaria vectors across mainland Tanzania from 1997 to 2017: a systematic review.* Malar J, 2019. **18**(1): p. 102.
163. NMCP, *National malaria strategic plan 2014–2020 abridged version*. 2014, MINISTRY OF HEALTH AND SOCIAL WELFARE.
164. Caldas de Castro, M., et al., *Integrated urban malaria control: a case study in dar es salaam, Tanzania.* Am J Trop Med Hyg, 2004. **71**(2 Suppl): p. 103-17.
165. Najera, J.A., M. Gonzalez-Silva, and P.L. Alonso, *Some lessons for the future from the Global Malaria Eradication Programme (1955-1969).* PLoS Med, 2011. **8**(1): p. e1000412.
166. Collins, P., *Decentralisation and Local Administration for Development in Tanzania.* Africa Today, 1974. **21**(3): p. 15-25.
167. Mwaluko, G.M.P., *Health and Disease in Tanzania*. 1991: Harper Collins Academic.
168. Program, N.M.C., *NATIONAL MALARIA STRATEGIC PLAN 2021-2025: TRANSITIONING TO MALARIA ELIMINATION IN PHASES*. 2020, National Malaria control Program: Tanzania

169. (Mainland), U.S.P.s.M.I.T., *U.S. PRESIDENT'S MALARIA INITIATIVE Tanzania (Mainland) Malaria Operational Plan FY 2023*. 2023.
170. (Mainland), U.S.P.S.M.I.T., *U.S. PRESIDENT'S MALARIA INITIATIVE Tanzania (Mainland) Malaria Operational Plan FY 2024*. 2024.
171. Gale, E.A., *The Hawthorne studies-a fable for our times?* QJM, 2004. **97**(7): p. 439-49.
172. McCarney, R., et al., *The Hawthorne Effect: a randomised, controlled trial.* BMC Med Res Methodol, 2007. **7**: p. 30.
173. Holden, J.D., *Hawthorne effects and research into professional practice.* J Eval Clin Pract, 2001. **7**(1): p. 65-70.
174. NMCP, *Tanzania malaria programme review 2010: programme review proposal*. 2010.
175. Carlstedt, A., *Malaria catastrophe in east Africa.* Lancet, 1997. **350**(9085): p. 1180.
176. (PMI), P.s.M.I., *Evaluation of the Impact of Malaria Interventions on Mortality in Children in Mainland Tanzania*. 2012.
177. INITIATIVE, P.S.M., *TANZANIA Malaria Operational Plan FY 2019*. 2019.
178. Initiative, P.s.M., *School Net Program (SNP6) Final Report*. 2018.
179. Initiative, P.s.M., *Tanzania Vector Control Scale-up Project: Spray Performance Report: November 2011–May 2012*. 2012.
180. Products, T.B. *Tanzania Biotech Products Ltd Biolarvicides Plant- Kibaha.* 2020; Available from: https://endmalaria.org/sites/default/files/u224/4_Samwel%20Mziray.pdf.
181. IVCC, *Larviciding for malaria control in Africa: A gap analysis and change management proposal*. 2017.
182. AMP, *Net mapping project. The elliance for malaria prevention*. 2021.
183. WHO, *World malaria report*. 2021.
184. Shah, M.P., et al., *The effectiveness of older insecticide-treated bed nets (ITNs) to prevent malaria infection in an area of moderate pyrethroid resistance: results from a cohort study in Malawi.* Malar J, 2020. **19**(1): p. 24.
185. WHO, *Guildeline for malaria vector control.* 2019.
186. Gichuki, P.M., et al., *Bioefficacy and durability of Olyset((R)) Plus, a permethrin and piperonyl butoxide-treated insecticidal net in a 3-year long trial in Kenya.* Infect Dis Poverty, 2021. **10**(1): p. 135.
187. Frank Mechan1*, A.K., Violet Tuhaise2, et al., *LLIN Evaluation in Uganda Project (LLINEUP) – The durability of long-lasting insecticidal nets treated with and without piperonyl butoxide (PBO) in Uganda.* 2022.
188. Massue, D.J., et al., *Durability of Olyset campaign nets distributed between 2009 and 2011 in eight districts of Tanzania.* Malar J, 2016. **15**: p. 176.
189. NBS, *2012 population and housing census: population distribution by administrative areas*. 2013, National Bureau of Statistics, Ministry of Finance & Office of Chief Government Statistician: Dar-es-Salaam.
190. WHO, *WHO specifications and evaluations for public health pesticides: permethrin (40:60 cis:trans isomer ratio) + piperonyl butoxide long-lasting (incorporated into filaments)insecticidal net*. 2013.
191. WHO_CDS_WHOPES, *Report of the Fifth WHOPES working group meeting*, in *Review of: Olyset Net, Bifenthrin 10% WP*. 2001: WHO/HQ, Geneva.
192. Lorenz, L.M., et al., *Investigating mosquito net durability for malaria control in Tanzania - attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): study protocol.* BMC Public Health, 2014. **14**: p. 1266.
193. WHO-GMP, *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes: Second edition*. 2016.
194. Koenker, H., et al., *What happens to lost nets: a multi-country analysis of reasons for LLIN attrition using 14 household surveys in four countries.* Malar J, 2014. **13**: p. 464.
195. Kilian, A., et al., *Long-term field performance of a polyester-based long-lasting insecticidal mosquito net in rural Uganda.* Malar J, 2008. **7**: p. 49.

196. Kilian, A., et al., *Evidence for a useful life of more than three years for a polyester-based long-lasting insecticidal mosquito net in Western Uganda.* Malar J, 2011. **10**: p. 299.
197. Martin, J.L., et al., *Personal protection with PBO-pyrethroid synergist-treated nets after 2 years of household use against pyrethroid-resistant Anopheles in Tanzania.* Parasit Vectors, 2021. **14**(1): p. 150.
198. Bhatt, S., et al., *Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017.* Elife, 2015. **4**.
199. Mejia, P., et al., *Physical condition of Olyset(R) nets after five years of utilization in rural western Kenya.* Malar J, 2013. **12**: p. 158.
200. Helinski, M.H., et al., *Impact of a behaviour change communication programme on net durability in eastern Uganda.* Malar J, 2015. **14**: p. 366.
201. Koenker, H., et al., *Impact of a behaviour change intervention on long-lasting insecticidal net care and repair behaviour and net condition in Nasarawa State, Nigeria.* Malar J, 2015. **14**: p. 18.
202. Zewde, A., et al., *Knowledge and perception towards net care and repair practice in Ethiopia.* Malar J, 2017. **16**(1): p. 396.
203. Santos, E.M., et al., *Malaria education interventions addressing bed net care and repair practices: a systematic review.* Pathog Glob Health, 2020. **114**(1): p. 2-15.
204. Ochomo, E.O., et al., *The efficacy of long-lasting nets with declining physical integrity may be compromised in areas with high levels of pyrethroid resistance.* Malar J, 2013. **12**: p. 368.
205. Minta, A.A., et al., *The effect of holes in long-lasting insecticidal nets on malaria in Malawi: results from a case-control study.* Malar J, 2017. **16**(1): p. 394.
206. Sherrard-Smith, E., et al., *Optimising the deployment of vector control tools against malaria: a data-informed modelling study.* Lancet Planet Health, 2022. **6**(2): p. e100-e109.
207. WHO, *World malaria report 2022.* 2022.
208. WHO-WHOPES, *Specifications and evaluations for public health pesticides: alpha-cypermethrin +chlorfenapyr*. 2019.
209. WHO, *WHO specifications and evaluations for public health pesticides alpha-cypermethrin + pyriproxyfen: long-lasting (incorporated into filaments) insecticidal net*. 2018.
210. WHO, *WHO specifications and evaluations for public health pesticides: deltamethrin + piperonyl butoxide long-lasting (incorporated into filaments) insecticidal net*. 2017.
211. Rugnao, S., et al., *LLIN Evaluation in Uganda Project (LLINEUP): factors associated with childhood parasitaemia and anaemia 3 years after a national long-lasting insecticidal net distribution campaign: a cross-sectional survey.* Malar J, 2019. **18**(1): p. 207.
212. Ngufor, C., et al., *Combining indoor residual spraying with chlorfenapyr and long-lasting insecticidal bed nets for improved control of pyrethroid-resistant Anopheles gambiae: an experimental hut trial in Benin.* Malar J, 2011. **10**: p. 343.
213. Bradley, J., et al., *Insecticide-treated nets provide protection against malaria to children in an area of insecticide resistance in Southern Benin.* Malar J, 2017. **16**(1): p. 225.
214. Mosha, J.F., et al., *Protocol for a four parallel-arm, single-blind, cluster-randomised trial to assess the effectiveness of three types of dual active ingredient treated nets compared to pyrethroid-only long-lasting insecticidal nets to prevent malaria transmitted by pyrethroid insecticide-resistant vector mosquitoes in Tanzania.* BMJ Open, 2021. **11**(3): p. e046664.
215. Obala, A.A., et al., *What Is Threatening the Effectiveness of Insecticide-Treated Bednets? A Case-Control Study of Environmental, Behavioral, and Physical Factors Associated with Prevention Failure.* PLoS One, 2015. **10**(7): p. e0132778.
216. WHO-GMP, *Achieving and maintaining universal coverage with long-lasting insecticidal nets for malaria control: Recommendations.* 2017.
217. Msellemu, D., et al., *The underlying reasons for very high levels of bed net use, and higher malaria infection prevalence among bed net users than non-users in the Tanzanian city of Dar es Salaam: a qualitative study.* Malar J, 2017. **16**(1): p. 423.

218. Teklemariam, Z., et al., *Ownership and utilization of insecticide-treated nets (ITNs) for malaria control in Harari National Regional State, Eastern Ethiopia.* Pan Afr Med J, 2015. **21**: p. 52.
219. Pooseesod, K., et al., *Ownership and utilization of bed nets and reasons for use or non-use of bed nets among community members at risk of malaria along the Thai-Myanmar border.* Malar J, 2021. **20**(1): p. 305.
220. Skovmand, O. and R. Bosselmann, *Strength of bed nets as function of denier, knitting pattern, texturizing and polymer.* Malar J, 2011. **10**: p. 87.
221. Cibulskis, R.E., et al., *Malaria: Global progress 2000 - 2015 and future challenges.* Infect Dis Poverty, 2016. **5**(1): p. 61.
222. Eisele, T.P. and R.W. Steketee, *African malaria control programs deliver ITNs and achieve what the clinical trials predicted.* PLoS Med, 2011. **8**(9): p. e1001088.
223. Bayili, K., et al., *Experimental hut evaluation of DawaPlus 3.0 LN and DawaPlus 4.0 LN treated with deltamethrin and PBO against free-flying populations of Anopheles gambiae s.l. in Vallee du Kou, Burkina Faso.* PLoS One, 2019. **14**(12): p. e0226191.
224. Menze, B.D., et al., *An Experimental Hut Evaluation of PBO-Based and Pyrethroid-Only Nets against the Malaria Vector Anopheles funestus Reveals a Loss of Bed Nets Efficacy Associated with GSTe2 Metabolic Resistance.* Genes (Basel), 2020. **11**(2).
225. Chitnis, N., T. Smith, and R. Steketee, *A mathematical model for the dynamics of malaria in mosquitoes feeding on a heterogeneous host population.* J Biol Dyn, 2008. **2**(3): p. 259-85.
226. Levitz, L., et al., *Effect of individual and community-level bed net usage on malaria prevalence among under-fives in the Democratic Republic of Congo.* Malar J, 2018. **17**(1): p. 39.
227. WHO, *Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: trends in pyrethroid resistance during a WHO-coordinated multi-country prospective study.* Parasit Vectors, 2018. **11**(1): p. 550.
228. Kim, S., et al., *Using a human-centered design approach to determine consumer preferences for long-lasting insecticidal nets in Ghana.* Glob Health Sci Pract, 2019. **7**(2): p. 160-170.
229. Protopopoff, N., et al., *High level of resistance in the mosquito Anopheles gambiae to pyrethroid insecticides and reduced susceptibility to bendiocarb in north-western Tanzania.* Malar J, 2013. **12**: p. 149.
230. Bonner, K., et al., *Design, implementation and evaluation of a national campaign to distribute nine million free LLINs to children under five years of age in Tanzania.* Malar J, 2011. **10**: p. 73.
231. Larsen, D.A., et al., *Community coverage with insecticide-treated mosquito nets and observed associations with all-cause child mortality and malaria parasite infections.* Am J Trop Med Hyg, 2014. **91**(5): p. 950-8.
232. Komazawa, O., et al., *Are long-lasting insecticidal nets effective for preventing childhood deaths among non-net users? A community-based cohort study in western Kenya.* PLoS One, 2012. **7**(11): p. e49604.
233. Lindblade, K.A., et al., *Sustainability of reductions in malaria transmission and infant mortality in western Kenya with use of insecticide-treated bednets: 4 to 6 years of follow-up.* JAMA, 2004. **291**(21): p. 2571-80.
234. Protopopoff, N., et al., *Effectiveness of piperonyl butoxide and pyrethroid-treated long-lasting insecticidal nets (LLINs) versus pyrethroid-only LLINs with and without indoor residual spray against malaria infection: third year results of a cluster, randomised controlled, two-by-two factorial design trial in Tanzania.* Malar J, 2023. **22**(1): p. 294.
235. Kawada, H., et al., *A small-scale field trial of pyriproxyfen-impregnated bed nets against pyrethroid-resistant Anopheles gambiae s.s. in western Kenya.* PLoS One, 2014. **9**(10): p. e111195.
236. Yunta, C., et al., *Pyriproxyfen is metabolized by P450s associated with pyrethroid resistance in An. gambiae.* Insect Biochem Mol Biol, 2016. **78**: p. 50-57.

237. Ochomo, E., et al., *Insecticide-Treated Nets and Protection against Insecticide-Resistant Malaria Vectors in Western Kenya.* Emerg Infect Dis, 2017. **23**(5): p. 758-764.
238. Tokponnon, F.T., et al., *Impact of long-lasting, insecticidal nets on anaemia and prevalence of Plasmodium falciparum among children under five years in areas with highly resistant malaria vectors.* Malar J, 2014. **13**: p. 76.
239. Killeen, G.F., *Control of malaria vectors and management of insecticide resistance through universal coverage with next-generation insecticide-treated nets.* Lancet, 2020. **395**(10233): p. 1394-1400.
240. Gamble, C., J.P. Ekwaru, and F.O. ter Kuile, *Insecticide-treated nets for preventing malaria in pregnancy.* Cochrane Database Syst Rev, 2006. **2006**(2): p. CD003755.
241. Barreaux, P., et al., *Pyrethroid-treated bed nets impair blood feeding performance in insecticide resistant mosquitoes.* Sci Rep, 2023. **13**(1): p. 10055.
242. Lindblade, K.A., et al., *Impact of sustained use of insecticide-treated bednets on malaria vector species distribution and culicine mosquitoes.* J Med Entomol, 2006. **43**(2): p. 428-32.
243. Escamilla, V., et al., *Effects of community-level bed net coverage on malaria morbidity in Lilongwe, Malawi.* Malar J, 2017. **16**(1): p. 142.
244. (MMV), M.f.M.V. *Knowledge sharing for severe malaria: Severe malaria observatory*. 2023; Available from: https://www.severemalaria.org/countries/tanzania.
245. (PMI), U.S.P.s.M.I., *TANZANIA (MAINLAND) MALARIA PROFILE*. 2022.
246. Vitor-Silva, S., et al., *Malaria is associated with poor school performance in an endemic area of the Brazilian Amazon.* Malar J, 2009. **8**: p. 230.
247. Alonso, S., et al., *The economic burden of malaria on households and the health system in a high transmission district of Mozambique.* Malar J, 2019. **18**(1): p. 360.
248. Sood, R.D., et al., *Community awareness, perceptions, acceptability and preferences for using LLIN against malaria in villages of Uttar Pradesh, India.* J Vector Borne Dis, 2010. **47**(4): p. 243-8.
249. Obidimma Ezezika, Y.E.-B., Abitha Nadarajah, Kathryn Barrett, *Implementation of insecticide-treated malaria bed nets in Tanzania: a systematic review.* Journal of Global Health Reports, 2022.
250. Mboma, Z.M., et al., *'For the poor, sleep is leisure': understanding perceptions, barriers and motivators to mosquito net care and repair in southern Tanzania.* Malar J, 2018. **17**(1): p. 375.
251. Dillip, A., et al., *'To be honest, women do everything': understanding roles of men and women in net care and repair in Southern Tanzania.* Malar J, 2018. **17**(1): p. 459.
252. Tsuang, A., J. Lines, and K. Hanson, *Which family members use the best nets? An analysis of the condition of mosquito nets and their distribution within households in Tanzania.* Malar J, 2010. **9**: p. 211.
253. Madumla, E.P., et al., *"In starvation, a bone can also be meat": a mixed methods evaluation of factors associated with discarding of long-lasting insecticidal nets in Bagamoyo, Tanzania.* Malar J, 2022. **21**(1): p. 101.
254. USAID-PMI, *Understanding Users Preferences Of LLIN Length/Height, Colour, Shape and Texture: A Qualitative Study Post Mass LLIN distribution in Kenya*. 2019: Kenya.
255. Das, M.L., et al., *Population preference of net texture prior to bed net trial in Kala-Azar-endemic areas.* PLoS Negl Trop Dis, 2007. **1**(3): p. e100.
256. Kwiringira, A., et al., *Ownership and use of long-lasting insecticidal nets three months after a mass distribution campaign in Uganda, 2021.* Malar J, 2022. **21**(1): p. 367.
257. Zanzibar, N.B.o.S.T.a.t.C.G.S., *Administrative Units Population Distribution Report Ministry of Finance and Planning National Bureau of Statistics Tanzania and Presidents' Office - Finance and Planning Office of the Chief Government Statistician Zanzibar*. 2022.
258. Jacklin F. Mosha, M.A.K., Louisa A. Messenger, Mark Rowland, Nancy Matowo, Catherine Pitt, Eliud Lukole, Monica Taljaard,Charles Thickstun, Alphaxard Manjurano, Franklin W. Mosha, Immo Kleinschmidt, Natacha Protopopoff, *Protocol for a four parallel-arm, singleblind,*

cluster-randomised trial to assess the effectiveness of three types of dual active ingredient treated nets compared to pyrethroid-only long-lasting insecticidal nets to prevent malaria transmitted by pyrethroid insecticideresistant vector mosquitoes in Tanzania. 2021.

259. Lukole, E.A., et al., *Will a lack of fabric durability be their downfall? Impact of textile durability on the efficacy of three types of dual-active-ingredient long-lasting insecticidal nets: a secondary analysis on malaria prevalence and incidence from a cluster-randomized trial in north-west Tanzania.* Malar J, 2024. **23**(1): p. 199.
260. WHO, *Specifications and Evaluations for Public Health Pesticides alpha-Cypermethrin Long-Lasting (Coated Onto Filaments) Insecticidal Net*. 2015.
261. Chrustek, A., et al., *Current Research on the Safety of Pyrethroids Used as Insecticides.* Medicina (Kaunas), 2018. **54**(4).
262. Banek, K., A. Kilian, and R. Allan, *Evaluation of Interceptor long-lasting insecticidal nets in eight communities in Liberia.* Malar J, 2010. **9**: p. 84.
263. Korenromp, E.L., et al., *Monitoring mosquito net coverage for malaria control in Africa: possession vs. use by children under 5 years.* Trop Med Int Health, 2003. **8**(8): p. 693-703.
264. Koenker, H., et al., *Reported reasons for non-use of insecticide-treated nets in large national household surveys, 2009-2021.* Malar J, 2023. **22**(1): p. 61.
265. Akello, A.R., et al., *Factors influencing consistent use of bed nets for the control of malaria among children under 5 years in Soroti District, North Eastern Uganda.* Malar J, 2022. **21**(1): p. 363.
266. Solomon, T., et al., *Low use of long-lasting insecticidal nets for malaria prevention in south-central Ethiopia: A community-based cohort study.* PLoS One, 2019. **14**(1): p. e0210578.
267. (PMI), U.P.s.M.I., *Social and Behavior Change for Insecticide-Treated Nets* 2019.
268. Malede, A., et al., *Barriers of persistent long-lasting insecticidal nets utilization in villages around Lake Tana, Northwest Ethiopia: a qualitative study.* BMC Public Health, 2019. **19**(1): p. 1303.
269. Baume, C.A., R. Reithinger, and S. Woldehanna, *Factors associated with use and non-use of mosquito nets owned in Oromia and Amhara regional states, Ethiopia.* Malar J, 2009. **8**: p. 264.
270. Agbaje, E.O., *Knowledge and Misconceptions about Malaria among Nigeria School Children.* Nigerian Quarterly Journal of Hospital Medicine, 2006.
271. TRAVM, *Common misconceptions about malaria*, in *Thai Travel Clinic Blog*. 2016, Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University.
272. Obol, J., K. David Lagoro, and O. Christopher Garimoi, *Knowledge and Misconceptions about Malaria among Pregnant Women in a Post-Conflict Internally Displaced Persons' Camps in Gulu District, Northern Uganda.* Malar Res Treat, 2011. **2011**: p. 107987.
273. (MSF), M.S.F., *Public Health Engineering: In Precarious Situations*. 2010: Paris, France.
274. Myamba, J., et al., *Pyrethroid resistance in tropical bedbugs, Cimex hemipterus, associated with use of treated bednets.* Med Vet Entomol, 2002. **16**(4): p. 448-51.
275. Davies, T.G., L.M. Field, and M.S. Williamson, *The re-emergence of the bed bug as a nuisance pest: implications of resistance to the pyrethroid insecticides.* Med Vet Entomol, 2012. **26**(3): p. 241-54.
276. Dang, K., et al., *Insecticide resistance and resistance mechanisms in bed bugs, Cimex spp. (Hemiptera: Cimicidae).* Parasit Vectors, 2017. **10**(1): p. 318.
277. Dang, K., et al., *Identification of putative kdr mutations in the tropical bed bug, Cimex hemipterus (Hemiptera: Cimicidae).* Pest Manag Sci, 2015. **71**(7): p. 1015-20.
278. Tawatsin, A., et al., *Insecticide resistance in bedbugs in Thailand and laboratory evaluation of insecticides for the control of Cimex hemipterus and Cimex lectularius (Hemiptera: Cimicidae).* J Med Entomol, 2011. **48**(5): p. 1023-30.
279. Mosha, J.F., et al., *Risk factors for malaria infection prevalence and household vector density between mass distribution campaigns of long-lasting insecticidal nets in North-western Tanzania.* Malar J, 2020. **19**(1): p. 297.

280. Matowo, N.S., et al., *Differential impact of dual-active ingredient long-lasting insecticidal nets on primary malaria vectors: a secondary analysis of a 3-year, single-blind, cluster-randomised controlled trial in rural Tanzania.* Lancet Planet Health, 2023. **7**(5): p. e370-e380.
281. Messenger, L.A., et al., *Effects of next-generation, dual-active-ingredient, long-lasting insecticidal net deployment on insecticide resistance in malaria vectors in Tanzania: an analysis of a 3-year, cluster-randomised controlled trial.* Lancet Planet Health, 2023. **7**(8): p. e673-e683.
282. Matowo, J., et al., *Expression of pyrethroid metabolizing P450 enzymes characterizes highly resistant Anopheles vector species targeted by successful deployment of PBO-treated bednets in Tanzania.* PLoS One, 2022. **17**(1): p. e0249440.
283. Jackline L. Martin, L.A.M., Mark Rowland, Franklin W Mosha, Edmund Bernard, Monica Kisamo, Shaban Limbe, Patric Hape , Charles Thickstun, Crene Steven, Oliva Moshi, Boniface Shirima , Nancy S. Matowo, Jacklin F Mosha , Dominic P Dee, Thomas S Churcher, Manisha A. Kulkarni, Alphaxard Manjurano and Natacha Protopopoff, *Bio-efficacy of field aged novel class of long-lasting insecticidal nets, against pyrethroid resistant malaria vectors in Tanzania: A series of experimental hut trials (preprint).* 2023.
284. Briet, O., et al., *Attrition, physical integrity and insecticidal activity of long-lasting insecticidal nets in sub-Saharan Africa and modelling of their impact on vectorial capacity.* Malar J, 2020. **19**(1): p. 310.
285. Minakawa, N., et al., *Unforeseen misuses of bed nets in fishing villages along Lake Victoria.* Malar J, 2008. **7**: p. 165.
286. Doda, Z., et al., *A qualitative study of use of long-lasting insecticidal nets (LLINs) for intended and unintended purposes in Adami Tullu, East Shewa Zone, Ethiopia.* Malar J, 2018. **17**(1): p. 69.
287. Msangi, S.J., et al., *Challenges of proper disposal of old long-lasting insecticidal nets and its alternative uses in rural south-eastern Tanzania.* PLoS One, 2024. **19**(2): p. e0279143.
288. Hilton, E., *Results from impact evaluations of IRS and PBO ITNs: Ethiopia and Sierra Leone*. 2024.
289. Syme, T., et al., *Pyrethroid-piperonyl butoxide (PBO) nets reduce the efficacy of indoor residual spraying with pirimiphos-methyl against pyrethroid-resistant malaria vectors.* Sci Rep, 2022. **12**(1): p. 6857.
290. Reddy, M.R., et al., *Outdoor host seeking behaviour of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea.* Malar J, 2011. **10**: p. 184.
291. Githu, V., et al., *Comparative evaluation of different versions of exposure-free mosquito electrocuting traps and barrier screen trap for monitoring outdoor densities and biting time phenotypes by malaria and filariasis vectors in Tanzania.* Parasit Vectors, 2022. **15**(1): p. 420.
292. Namuganga, J.F., et al., *The impact of stopping and starting indoor residual spraying on malaria burden in Uganda.* Nat Commun, 2021. **12**(1): p. 2635.
293. Raouf, S., et al., *Resurgence of Malaria Following Discontinuation of Indoor Residual Spraying of Insecticide in an Area of Uganda With Previously High-Transmission Intensity.* Clin Infect Dis, 2017. **65**(3): p. 453-460.
294. Charles Dismas Mwalimu, S.N.L., William Nhandi Kisinza, Stephen M Magesa, Samson Kiware, *The impact of Indoor Residual Spraying (IRS) withdrawal in the Lake Zone Regions in Mainland Tanzania. Should Tanzania withdrawal its IRS program? (preprint).* 2023.