Final Scientific Report for

PMI Activity TZ-1,2: IRS and LLIN: Integration of methods and insecticide mode of actions for control of African malaria vector mosquitoes

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Abstract

Background: Long lasting Insecticidal nets (LLINs) and indoor residual spraying (IRS) are the preferred techniques for malaria vector control in Africa, where their application has a proven contribution to the recent significant reductions in the burden of the disease. Even though both methods are commonly used together in the same households, evidence of improved malaria control due to the use of combinations as opposed to use of either method alone has been minimal and inconclusive.

Objectives and methods: To measure the mode of action of three classes of insecticides used for IRS at the WHO recommended dose: the organochlorine DDT 70 wettable powder (AVIMA, South Africa) at 2g/m^2; the pyrethroid lambda-cyhalothrin capsule suspension ICON CS, (Syngenta, Switzerland), at 0.03g/m^2; and the organophosphate pirimiphos-methyl (PM) emulsified concentrate, also known as actellic (Syngenta, Switzerland), at 2g/m^2 used alone or in combination with three leading LLIN brands: PermaNet 2.0® nets (Vastergaard, Switzerland), Olyset® nets (manufactured by A-Z, Tanzania), and Icon Life® nets (Bestnet Europe ltd, Denmark). All LLINS were used intact and were not subjected to repeated washing to reflect their optimum performance. The control was untreated polyester net. Data were collected from experimental huts developed during the project to measure both behavioral and toxic modes of actions of insecticides in Southern Tanzania. The primary malaria vector is *Anopheles arabiensis* with >90% susceptibility to insecticides of all classes at diagnostic doses in WHO susceptibility assays. Two rounds of data collection were performed: 1) 4 months during the dry season 2) six months during the wet season. Data generated from the experimental hut studies were analysed with Poisson-lognormal generalized linear mixed effects models (GLMM). Data was also simulated using deterministic mathematical model to measure potential impacts of each IRS, LLIN and combination thereof on malaria at a community level.

Results

***Bite prevention (feeding inhibition)*:** During both rounds, all the IRS treatments, LLINs and the controls (which consisted of intact untreated mosquito nets), provided greater than 99% protection from potentially infectious bites by the malaria vector, *An. arabiensis*, for the entire duration of the study. Most of the mosquitoes were caught inside the exit traps as opposed to inside the experimental huts, regardless of whether the huts were had LLINs, IRS or non-insecticidal nets. More than 95% of *An. arabiensis, Culex pipiens quinquefasciatus* and *Mansonia africana / uniformis* mosquitoes were caught inside the exit traps while exiting the huts.

***Toxicity*:** All IRS treatments, all the LLINs and the majority of LLIN/IRS combinations significantly increased proportions of dead *An. arabiensis* mosquitoes, relative to the control huts. The most toxic IRS relative to the controls was PM (RR = 2.21 (1.82 – 2.68), P < 0.001), followed by ICON CS (RR = 1.55 (1.27 – 1.89), P < 0.001) and then DDT (RR = 1.44 (1.18 – 1.77), P < 0.001). The most toxic LLIN relative to the controls was PermaNet 2.0® nets (RR = 1.65 (1.58 – 1.74), P < 0.001), followed by Icon Life® nets (RR = 1.55 (1.42 – 1.69), P < 0.001) and then Olyset® nets (RR = 1.33 (1.12 – 1.47), P < 0.001).

Combinations of IRS and LLINs relative to LLINs alone: In most cases, there was no significant increase in *An. arabiensis* mortality in huts combining LLINs plus IRS, relative to huts having LLINs only, except in cases where the specific IRS treatment was PM. Addition of PM significantly increased proportional mortality of *An. arabiensis* when combined with Olyset® nets (RR = 1.38 (1.14 – 1.65), P = 0.001), PermaNet 2.0® nets (RR = 1.42 (1.18 – 1.71), P <0.001) and Icon Life® (RR = 1.24 (1.03 – 1.49), P = 0.023). Combinations of LLINs and DDT or lambda cyhalothrin resulted in marginal increases in *An. arabiensis* mortality relative to huts with LLINs alone although none of these combinations resulted in a statistically significant increase.

***Combinations of IRS and LLINs relative to IRS alone*:** There was a trend of significant increases in *An. arabiensis* mortality in huts having IRS plus LLINs, relative to huts having just the IRS alone, except for the combinations of 1) Olyset® with ICON CS, 2) DDT with Olyset® or 3) DDT with Icon Life® nets. In the huts that had been sprayed with PM, there was a significant increase in *An. arabiensis* mortality whenever Icon Life® nets (RR = 1.39 (1.18 – 1.63), P < 0.001), Olyset® nets (RR = 1.32 (1.13 – 1.55), P = 0.001) or PermaNet 2.0® nets (RR = 1.26 (1.08 – 1.48), P = 0.004) were added, relative to the huts where PM IRS was used alone. Similarly, in the huts that had been sprayed with ICON CS, there was a significant increase in *An. arabiensis* mortality in combination with Icon Life® nets (RR = 1.43 (1.19 – 1.73), P < 0.001) or PermaNet 2.0® nets (RR = 1.70 (1.35 – 2.13), P < 0.001), but not Olyset® nets (RR = 1.16 (0.92 – 1.45), P = 0.210), relative to the IRS alone.
In huts sprayed with DDT, none of the LLINs significantly improved proportional mortality of the *An. arabiensis* mosquitoes, except PermaNet 2.0® nets (RR = 1.18 (1.06 – 1.32), P = 0.003).

Residual efficacy bioassays of IRS**:** All IRS formulations were highly effective during the first month after spraying and rapidly decayed losing most activity within 1-3 months. In month 1, all *An. arabiensis* exposed to palm ceilings sprayed with either PM or ICON CS died, and 85% were killed by DDT (despite full susceptibility most likely because it flaked away). On mud walls sprayed with the same chemicals, 100%, 90.0% and 97.5% mortality was observed, respectively, during the first month. Activity of the IRS declined significantly so that by the third month, PM on palm and mud killed 42.5% and 55.0% of exposed *An. arabiensis*, respectively. ICON CS killed only 46.3% on palm and 52.5% on mud walls. By month 6, PM had nearly entirely decayed, killing only 7.5% of *An. arabiensis* exposed to sprayed palm ceilings and 27.5% of those exposed to sprayed mud walls; ICON CS killed 30.0% on ceilings and 27.5% on walls. DDT had a longer residual action, killing 42.5% of *An. arabiensis* exposed to sprayed ceilings, and 36.3% of those exposed to sprayed walls after 6 months.

Residual efficacy bioassays of LLINs**:** While all the LLINs generally performed better (i.e. killed more mosquitoes) on wire frame assays than on the cone assays, their activity rapidly deteriorated by the second month of use relative to new nets. Only PermaNet® nets retained mosquitocidal efficacy of >80% by the sixth month of net use (killing 92.7% on wire ball tests and 84% on cone assays). All the LLINs however retained very high knock-down rates (> 90% in wire ball tests and >80% in cone tests) on the exposed mosquitoes, except Olyset® nets whose knock-down activity reduced to 72.7% on wire ball tests and 62% on cone tests by the sixth month.

Conclusions: Both the field studies and the model simulations showed that any synergies or redundancies resulting from LLIN/IRS combinations are primarily a function of modes of action of active ingredients used in the two interventions. None of the IRS or LLINs tested was deterrent so they do not protect by keeping mosquitoes from houses in this setting. Very few mosquitoes were able to obtain a blood meal due to the use of intact LLINs and untreated control nets. Therefore, where households are correctly using and maintaining LLINs there is no added value in the additional application of IRS unless the IRS chemical is highly toxic and non-irritant, as is PM. This compound consistently increased mosquito mortality in combination with any LLIN even though mosquitoes did not rest indoors as they were unable to obtain a blood meal. The average duration of effect of insecticides in this setting was 3 months, far lower than that stated by the manufacturers, so IRS should be carefully timed. Where IRS is the pre-existing intervention, providing households with additional LLINs confers additional protection. Therefore, IRS households should always be supplemented with nets, preferably LLINs, which not only protect house occupants against mosquito bites, but also kill additional mosquitoes. Finally, where resources are limited, priority should be given to providing everybody with LLINs and ensuring that these nets are consistently and appropriately used, rather than trying to implement both LLINs and IRS in the same community at the same time.

Introduction

Long Lasting Insecticidal nets (LLINs) and application of insecticides through Indoor Residual Spraying (IRS) are the preferred techniques for malaria vector control [1-3], resulting in reduced malaria burden in many endemic countries [4]. The two methods are commonly used together and many governments have incorporated both of them in state policies.

Effective combinations of vector control methods require scientific verification for expected added value, which can enable policy makers to select the most appropriate combinations, for example IRS insecticides and types of LLINs, while considering factors such as baseline transmission intensities and the behavior of the local vector populations. In situations where resources are limited, such evidence may also guide resource allocation. For example if it were determined that there is no added value from using IRS alongside LLINs, resources could be diverted to other sectors or strengthen existing LLIN operations.

Today, most of the existing information on benefits of LLINs and IRS is derived from controlled trials where the methods were tested individually. However in operational programs, it is common that the two methods are used together: either concurrently or one after the other. For example, IRS is often performed in response to malaria epidemics while LLINs are continuously distributed through national programs or public-private partnerships [5], resulting in a situation of overlap between IRS and LLIN coverage. Unfortunately, there is not yet sufficient substantive evidence of benefits or failures due to such combined use, or whether the two methods complement or diminish the beneficial effects of each other [6]. The other challenge is the determination of appropriate insecticides to optimize any such combination. These important questions require controlled field experiments, conducted in malaria endemic areas, where vectors are monitored under exposure to different IRS compounds, LLINs or combinations thereof. The aim of this research was therefore to contribute towards generation of this essential evidence, by way of experimental hut studies and mathematical simulations. We investigated whether there would be any added protective advantages when any of three selected LLINs are combined with different IRS chemicals currently approved for malaria control, as opposed to using any of the treatments alone. Data generated from these experimental hut studies was then input into an optimized deterministic mathematical model, simulating a typical malaria endemic village to assess potential benefits and or limitations of LLIN/IRS combinations at community level.

Rationale: Between 2003 and 2010, about US$ 450 million in external funding was allocated to scale up the malaria control program in Tanzania by PMI, reducing malaria by around 20%. Building on this success Tanzania has articulated even more ambitious malaria control goals: 1) maintain universal LLIN coverage, and 2) conduct IRS in half of the country. Therefore, many houses where people are using LLINs will also have IRS applied. It is costly and logistically demanding to conduct IRS and the insecticides used vary in their longevity and mode of action. Therefore, careful selection of the most cost-effective and effective insecticidal tools is essential to maximize health gains from this ambitious program.

Study Objective

To determine whether there is any added advantage in combining LLINs and IRS at household level and to recommend the most appropriate insecticides for combined use if there would be any scientific rationale for such combinations.

Specific objectives:

1. Review existing evidence on the modes of action of insecticides used for IRS and LLINs and potential benefits or limitations of combining LLINs and IRS in the same households

2. Develop and optimize an experimental huts assay based on local housing design for evaluation of different LLINs and IRS insecticides and their combinations for malaria vector control in this setting

3. Characterize and compare the mode of action of the different IRS and LLINs, and to evaluate the relative efficacy of interventions used singly and in combination at the household level

4. Assess the bio-efficacy and residual activity of commonly used LLINs and IRS

5. Develop and test a mathematical simulation that combines modes of action of different insecticides with behavior of target malaria vectors to assess synergies and redundancies in various LLIN-IRS combinations, at the community level

Study Methodology

Study area

The field experiments were conducted in Lupiro village (8.385°S and 36.670°E), Ulanga District, Tanzania. The village is 300m above sea level on the Kilombero river valley, 26 km south of Ifakara town, where Ifakara Health Institute (IHI) is located. It borders many small contiguous and perennially swampy rice fields to the northern and eastern sides. The annual rainfall is 1200-1800mm, while temperatures range between 20°C and 32.6°C.

Composition of malaria vector populations (which previously included a mixture of *Anopheles gambiae sensu stricto, Anopheles arabiensis* and *Anopheles funestus*) has shifted dramatically in recent years, most likely because of high ITN coverage [7], so that today, the most abundant vector is *Anopheles arabiensis*, constituting > 95% of the *An. gambiae* complex species. *An. arabiensis* and *An. funestus* species are now the main contributors to malaria transmission in the area. WHO insecticide susceptibility tests conducted at the time of this study showed that the *An. arabiensis* here are 100% susceptible to DDT, and but have slightly reduced susceptibility to pyrethroids, deltamethrin (95.8%), lambda cyhalothrin (90.2%) and permethrin (95.2%).

Table 1 IRS compounds evaluated

Compound	Trade Name	Dose	Cost /Kg ($)[1]	Cost / building ($)	Duration of action	Note
Lambda-cyhalothrin	ICON CS (Syngenta)	0.03g/m^2	72	0.09	3–6 months	Widely used for IRS in PMI countries Pyrethroid – resistance issues
DDT	DDT 750 WP (Avima)	2.0g/m^2	3	0.25	> 6 months	Widely used for IRS in PMI countries Organochlorine, long lasting
Pyrimiphos methyl	Actellic 50 EC (Syngenta)	2.0g/m^2	18	1.50	2-3 months	Low human toxicity and high toxicity to resistant mosquitoes Organophosphate

Table 2 LLINs evaluated

Compound	Trade Name of LLIN	Dose mg/m2	Denier	Note
Permethrin incorporated into polyethylene filaments	OLYSET® (A to Z)	1000	150	Widely used in PMI countries. Made in Tanzania
Deltamethrin coated on polyester	PERMANET 2.0® (Vestergaard Frandsen)	55-62	100	Widely used globally
Deltamethrin incorporated into polyethylene filaments	NET PROTECT®** (distributed by BestNet)	65	115	Tested as it is a new super strong but soft net

All the IRS compounds and all the LLINs have either full or interim approval from WHO, and represent a diversity of common insecticides currently applicable for vector control in Africa [8].

[1] Data from Sources and prices of selected products for the prevention, diagnosis and treatment of malaria. Geneva: WHO, UNICEF, Population Services International, Management Sciences for Health; 2004.

** **Important Note:** Regarding the LLINs referred to as **Icon Life® nets**, the supplier (Syngenta ltd) informed us at the end of our studies that this net type is the same as the one branded as **NetProtect®**, which has actually been given an interim approval by WHO (http://www.who.int/whopes/quality/en). However, in this report, the brand name **Icon Life®** has been retained, given that this was the label on the actual nets that we actually evaluated in the studies described here.

Design, construction and evaluation of experimental huts for testing LLINs and IRS:
To ensure the accurate comparison of indoor vector control-technologies including LLINs and IRS in this setting we created and evaluated an improved experimental hut design based on local housing construction (dimensions and materials) – now known as The Ifakara Experimental Huts (IEH) [8], and examined how these huts can be used to monitor behavioral and physiological responses of wild, free-flying disease-transmitting mosquitoes, focusing on African malaria vectors of the *Anopheles gambiae* and *Anopheles funestus* species complexes. Important characteristics of the Ifakara experimental huts include: 1) interception traps fitted onto eave spaces and windows, 2) use of eave baffles (panels that direct mosquito movement) to prevent loss of live mosquitoes through the eave spaces, 3) use of replaceable wall panels and ceilings that can be correctly disposed of after use [2] and prevent cross-contamination when the huts are used to test different insecticides in successive periods, 4) the kit format of the huts allowing portability and shipment of huts to multiple sites for standardized multi-site comparison 5) an suite of standard operating procedures (SOPs) to maximize data quality.

Comparative experimental hut evaluation of LLINs and IRS:
Comparative evaluations were conducted in experimental huts fitted with LLINs alone, IRS alone, or combinations of LLINs and IRS. To limit complications of having to rotate treated and untreated mud panels and ceilings between huts, the huts with IRS treatments were fixed for the entire duration of each spray round, and instead only the LLINs were rotated on a nightly basis. The study was conducted in two spray rounds, the first round being four months long during the dry season (May 2010 to August 2010) and the second being six months during the wet season (November 2010 to April 2011). In round 1 each combination was repeated 40 times and in round 2 each combination was repeated 60 times. Outcome measures were: 1) number of mosquitoes entering huts, 2) proportion and number killed after exposure to each treatment, 3) proportions prevented from blood-feeding, 4) time when mosquitoes exited the huts, and 5) proportions caught exiting. Two individuals slept in each of the huts[3], fixed to each hut for the duration of the study (so individuals and huts were included in analysis as a single source of bias), underneath the LLINs or an untreated bednet. Each individual was provided with a single size LLIN or untreated net, which was not deliberately holed to represent programmatic conditions where universal coverage of intact nets is achieved.

Experiments were conducted from 19.00 hours to 07.00 hours each night. Mosquitoes were collected from eave and window exit traps at 23.00hrs, 03.00hrs and 07.00hrs (ensuring that those mosquitoes attempting to exit the huts were not confined, thus potentially being exposed to the insecticides for a longer period than would occur in local houses with a similar open design) and indoor resting collections from the inside surfaces and floors of the huts at 18.30hrs and 07.00hrs.

All collected mosquitoes (dead and live) were kept in small netting cages (15cm × 15 cm × 15cm), and provided with 10% glucose solution for 24 hours inside a field insectary (29.1°C ± 3.0°C, 70.6% ± 17.9% R.H. during the day and 26.7°C ± 2.3°C, 75.7% ± 13.7% R.H. at night). Live mosquitoes were killed with ethyl acetate after which each group was sorted by taxon and counted. A sub-sample of *An. gambiae* s.l [9] and *An. funestus* s.l [10] mosquitoes were randomly selected for speciation by PCR.

Statistical analysis

Power calculation:
Baseline data [11] were used to calculate the number of replicates required to observe a 23% difference in mosquito hut entry relative to the control, chosen as the average effect size observed from LLINs [11] using a non-central two-sided t-distribution in STATA 11.0 (StataCorp) [12]. Deterrence was selected as the outcome to calculate power, given that it is the smallest effect generally observed in experimental hut trials, and mortality was considered as generally exceeding 50%, so as to avoid under-powering of the study. Power calculations

[2] Stockholm convention of persistent organic pollutants. http://www.pops.int/documents/convtext/convtext_en.pdf. Stockholm, United Nations Environmental Programme, 2001, pp. 34. Accessed 31/08/2012
[3] Ethical approval for this study was granted by the Institutional Review Board of the Ifakara Health Institute (IHRDC/IRB/No.A019), the Tanzania National Institute of Medical Research (NIMR/HQ/R.8aNo1.W710) and the London School of Hygiene and Tropical Medicine Ethical Review Board (Ethics Clearance No. 5552).

showed that a minimum of 60 replicates were required to see a significant difference in the mean number of mosquitoes in huts with 95% confidence and 80% power.

Analysis of number of mosquitoes entering huts:

Data were analyzed using R statistical software version 2.13.0, using lme4 [35]. The nightly total number of mosquitoes of each taxon live or dead was compared between huts having the various insecticidal treatments (IRS, LLINs or IRS/ LLINs combinations) and the controls (untreated nets in unsprayed huts). Data were fitted to generalized linear mixed effects models (GLMM), with Poisson errors, a log link and a random factor for each individual data point (i.e. a log normal Poisson model) to account for over-dispersion in the count data. Data were analyzed as a function of the three fixed factors, treatment (insecticidal combinations), time (number of months since the start of the experiment), and day order (a variable representing the fact that net rotations were conducted on consecutive nights between Mondays and Fridays, but not on Saturdays and Sundays). Random factors in the model included hut and day of mosquito collection. Satisfactory model fits were confirmed using a Wald function test, and the estimated mean number of mosquitoes entering huts, and their 95% confidence intervals, were calculated as exponentials from the coefficients. This way it was possible to determine mosquito density in huts with different insecticidal treatments relative to the controls, whilst accounting for data structure and design factors that might influence the results.

Mosquito mortality:

Data was analyzed using R statistical software version 2.13.0 with the statistical package lme4 [35]. Mortality associated with the different insecticidal applications was analyzed in two different ways: 1) percentage mortality by hut, a measure suitable for estimating household-level protection and 2) actual numbers of mosquitoes killed by the different treatments relative to the controls, a measure suitable for estimating community level protection. 1) Percentage mortality: data were fitted to GLMMs with binomial errors and a logit link and analyzed as a function of insecticidal combinations, month and day order, including hut and date as random factors. A Wald function test was used to assess the best model fit. Due to high mortalities in the controls, data from the second spray round was corrected using Abbots formula [36]; 2) actual number of mosquitoes killed by each treatment were analyzed using Poisson-lognormal GLMMs with the same fixed and random factors as above.

Timing of mosquito exit:

Analysis was performed using SPSS version 16 (SPSS inc.). To assess whether the insecticidal treatments affected the times when mosquitoes naturally exited the huts, the mosquito catches in the exit traps at the different periods of the night (6pm collections, 7pm - 11pm, 11pm - 3am and 3am - 7am), were computed as percentages of the total exit trap catches each night, in the different huts. Chi-square analysis was performed to determine if any of the observed percentage increases in early exit were significantly different from controls.

Mosquito escape:

To ensure that huts were retaining all those mosquitoes that entered, the correlation between mosquito density and proportional mortality was explored with linear regression on the log transformed *An. arabiensis* catches and proportional mortality using SPSS version 16 (SPSS inc.).

Assessment of bio-efficacy and residual activity of insecticides used for LLINs and IRS:

WHO bioassays were performed using cones and wire balls to assess residual activity of insecticides in LLINs, and those sprayed on mud walls and palm-thatched ceilings of experimental huts [13]. WHO-susceptibility tests [14] were also performed, using diagnostic concentrations of candidate insecticides, against wild mosquitoes collected in the study area. Lastly, molecular analysis was performed to detect knock-down resistance genes associated with resistance against DDT and pyrethroids.

Simulation of community level effects of LLIN/IRS combinations:

An existing deterministic mathematical model of mosquito life cycle processes was adapted [15] and used to estimate how malaria transmission might be affected, if LLINs are combined with IRS, and whether such combinations would be synergistic or redundant, relative to the use of either method alone. The model was modified to allow use of data derived directly from experimental hut evaluations where untreated bed nets are used as the experimental controls. A scenario was simulated to represent a closed community where residents own cattle, and where the main malaria vector is *An. arabiensis*, an increasingly dominant vector species in Africa, which remains a significant challenge to control even in areas with high LLINs and IRS coverage [16].

Considering situations with either LLINs or IRS as the pre-existing intervention, we then calculated relative improvements in transmission control whenever the complementary intervention is introduced.

Results

Review of existing evidence on LLINs and IRS

Based on our review of previously published reports [6], we determined that while the efficacy of IRS applications is mainly due to repellency and toxicity to mosquitoes, Insecticide Treated Nets (ITNs) (including LLINs) mainly inhibit feeding and kill mosquitoes. Full data are presented in Appendix 1.The review also revealed that the available evidence remained inconclusive on whether using both LLINs and IRS concurrently would confer significant additional benefits relative to using either method alone. Even though there had been no specific studies that expressly tested this hypothesis, previous IRS and ITN trials and a number of mathematical models gave mixed results showing improved benefits in some situations and redundancy in others. Nevertheless, there were still a number of reasons that theoretically justified combination of IRS and LLINs in households. Based on the available evidence at the time, we strongly recommended to maximize household level protection where residents already use pyrethroid treated LLIN, the IRS product to be sprayed in houses to supplement the nets must be of completely different mode of action and preferably a different class of insecticides rather than pyrethroids. The overall epidemiological outcome of such co-applications at community level would however depend on factors such as level of intervention coverage achieved, baseline epidemiological conditions, behavior of malaria vectors, nature of insecticides used for IRS and the actual type of nets being used. Figure 1. Shows the various possible outcomes when a mosquito encounters a house with both LLINs and IRS. Therefore, to maximize any possible additional benefits from IRS/ITN co-applications, we recommended that rigorous field evidence, supported by mathematical modeling where necessary, should be pursued to support the entire process of decision making (Figure 2), including the selection of which insecticides to be used for IRS and what type of LLINs to use [6].

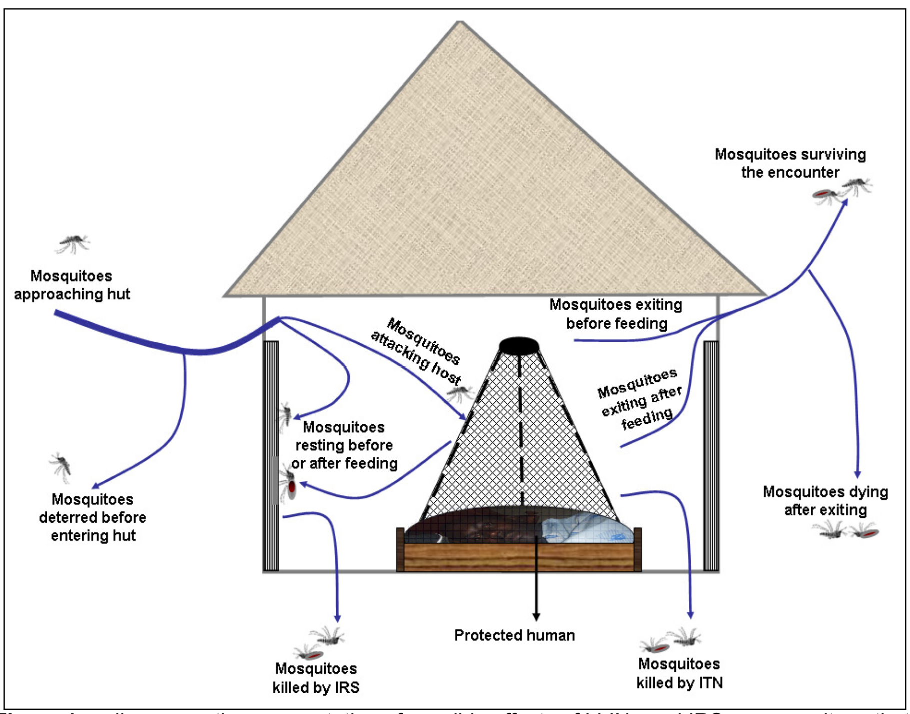

Figure 1: a diagrammatic representation of possible effects of LLINs and IRS on mosquitoes that enter or attempt to enter houses. Insecticides used on nets or for IRS effect mosquitoes at different levels along the path towards the individual human inside the sprayed hut. Mosquitoes can be deterred and diverted before they enter houses, killed by the IRS or LLINs, or they can be irritated so that they exit the huts earlier than normal. Exit may occur before or after the mosquitoes have fed, but both the fed and the unfed mosquitoes may die later after they have left the huts due to sub-lethal effects of the ITN or IRS insecticides. The net and the IRS may also inhibit mosquitoes' ability to successfully take blood meals from the hut dwellers.

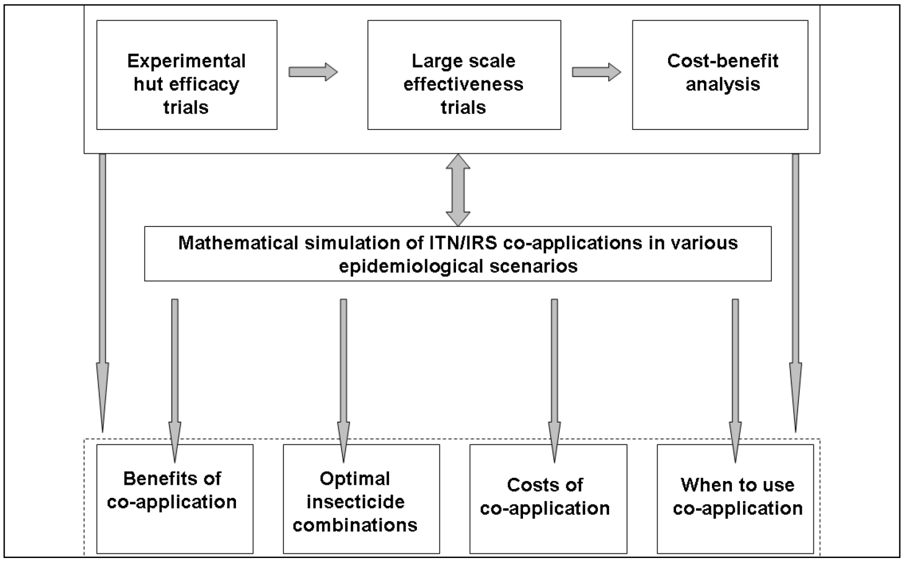

Figure 2: Evidence generation for decision making on combined use of LLINs and IRS. From direct measurements in experimental huts, efficacious combinations of LLINs and IRS are identified and subjected to community level effectiveness trials. This data can then be used for cost benefit analyses. Where necessary, mathematical models can utilize data from all the three studies (efficacy, effectiveness and cost-benefit analyses). Such simulations can: 1) help identify favorable single or combinations of insecticides for LLINs and IRS; 2) advise on design and implementation of new effectiveness trials and cost-benefit analyses; 3) enable extrapolation of efficacy and effectiveness of combinations in different epidemiological scenarios (including places with insecticide resistance). Results of these studies may then be examined to assess potential benefits of co-application, suitable insecticides for the combinations, and potential costs of the co applications in order to determine the most appropriate strategy

Design, construction and evaluation of experimental huts for testing LLINs and IRS

In preparation for evaluation of candidate LLINs and IRS, and in response to limitations of existing experimental hut designs, we designed an improved experimental hut design – now known as *The Ifakara Experimental Huts*- and described how these huts can be used to more realistically monitor behavioral and physiological responses of wild, free-flying disease-transmitting mosquitoes, including the African malaria vectors of the species complexes *An. gambiae* and *An. funestus*, to indoor vector control-technologies including LLINs and IRS (Figure 3).

Figure 3: Ifakara experimental huts. Panel A shows the main framework of the huts under construction at the workshop. Panel B shows technicians fitting the wall panels, (made of chicken wire on wooden frames), onto the inside walls of the huts. Panel C shows the inside surfaces of the huts after fitting the wall panels and also the palm woven (*mikeka*) ceiling on the underside of the steel roof, but before the inside walls are covered with mud, and Panel D shows a completed and functional Ifakara experimental hut, fitted with interception traps on windows and eave spaces and thatched over the steel roof with grass to match indoor temperatures of local dwellings. The overall shape and dimensions are those of typical local houses. The hut is suspended on water-filled metal bowls to prevent predator ants, which would otherwise prey on the trapped or dead mosquitoes in the huts.

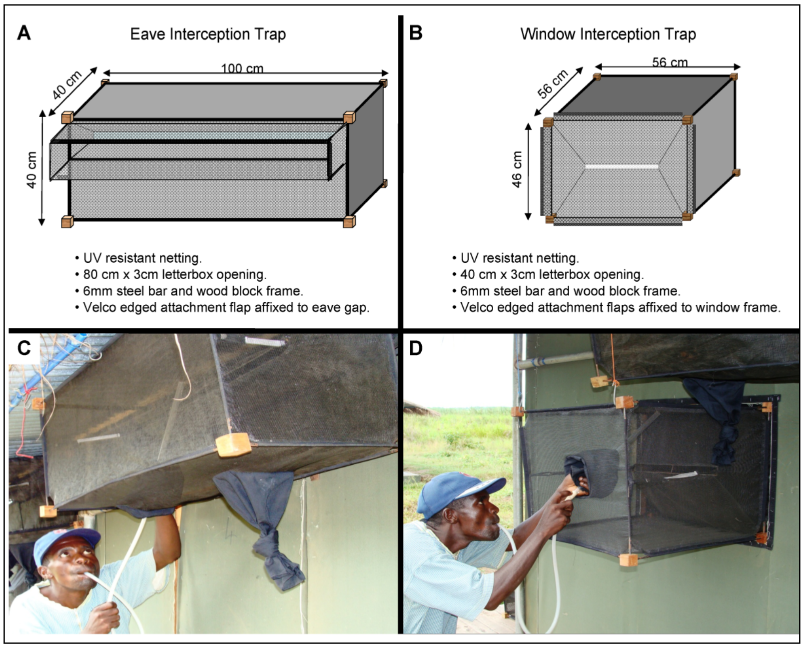

Figure 4: Diagrammatic illustration of eave trap and window trap. Panel A and B shows the dimensions and materials used to construct these traps, while panel C and D shows the eave and window traps fitted onto an Ifakara experimental hut during collection.

Baseline evaluations conducted on these experimental huts prior to introduction of any of our candidate LLINs or IRS showed that the huts (when fitted with the interception traps and baffles) and the improved suite of entomological procedures, were satisfactorily representative of local human dwellings; and were suitable for assessing all the important behavioral and physiological responses of malaria mosquitoes in and around houses having LLINs and/or IRS. These responses may include mosquito deterrence prior to entering the houses, irritancy of mosquitoes causing early un-programmed exit from treated houses and mortality of mosquitoes that are exposed to the indoor interventions.

Comparative experimental hut evaluation of LLINs and IRS or their combinations:

Bite prevention (feeding inhibition):

During both rounds, all the IRS treatments, LLINs and the controls (which consisted of intact untreated mosquito nets), provided greater than 99% protection from potentially infectious bites by the malaria vector, *An. arabiensis*, for the entire duration of the study. Most of the mosquitoes were caught inside the exit traps as opposed to inside the experimental huts, regardless of whether the huts were had LLINs, IRS or non-insecticidal nets. More than 95% of *An. arabiensis, Culex pipiens quinquefasciatus* and *Mansonia africana / uniformis* mosquitoes were caught inside the exit traps while exiting the huts.

Indoor mosquito density:

None of the *IRS treatments on their own* did reduced the number of mosquitoes entering the huts, though there was a non significant reduction in *An. arabiensis* catches in huts sprayed with DDT relative to unsprayed huts (Relative Rate (RR) and 95% CI = 0.650 (0.351 - 1.202), P = 0.170) in round 1. In round 2 here were marginal increases in *An. arabiensis* catches in huts having either pirimiphos methyl IRS, lambda cyhalothrin IRS. None of the *LLINs on their own* reduced the number of malaria vector catches in the huts, except for a non-significant decrease in huts fitted with PermaNet 2.0® nets (RR = 0.731 (0.481 - 1.109), P = 0.140) in round 1. Analysis of *combinations of IRS and LLINs* showed no significant reduction in mosquito catches in huts with any combination LLINs plus IRS relative to huts having any LLIN or IRS used alone. The complete data set by round is included in Table 1&2 of the appendix.

Toxicity:

All IRS treatments, all the LLINs and the majority of LLIN/IRS combinations significantly increased proportion of dead *An. arabiensis* mosquitoes, relative to the control huts. The most toxic IRS relative to the controls was PM (RR = 2.21 (1.82 – 2.68), P < 0.001), followed by ICON CS (RR = 1.55 (1.27 – 1.89), P < 0.001) and then DDT

(RR = 1.44 (1.18 – 1.77), P < 0.001). The most toxic LLIN relative to the controls was PermaNet 2.0® nets (RR = 1.65 (1.58 – 1.74), P < 0.001), followed by Icon Life® nets (RR = 1.55 (1.42 – 1.69), P < 0.001) and then Olyset® nets (RR = 1.33 (1.12 – 1.47), P < 0.001). The complete data set by round is included in the Table 3&4 of the appendix.

Combinations of IRS and LLINs relative to LLINs alone:

In most cases, there was no significant increase in *An. arabiensis* mortality in huts combining LLINs plus IRS, relative to huts having LLINs only, except in cases where the specific IRS treatment was PM. Addition of PM significantly increased proportional mortality of *An. arabiensis* when combined with Olyset® nets (RR = 1.38 (1.14 – 1.65), P = 0.001), PermaNet 2.0® nets (RR = 1.42 (1.18 – 1.71), P <0.001) and Icon Life® (RR = 1.24 (1.03 – 1.49), P = 0.023). Combinations of LLINs and DDT or lambda cyhalothrin resulted in marginal increases in *An. arabiensis* mortality relative to huts with LLINs alone although none of these combinations resulted in a statistically significant increase.

Combinations of IRS and LLINs relative to IRS alone:

There was a trend of significant increases in *An. arabiensis* mortality in huts having IRS plus LLINs, relative to huts having just the IRS alone, except for the combinations of 1) Olyset® with ICON CS, 2) DDT with Olyset® or 3) DDT with Icon Life® nets. In the huts that had been sprayed with PM, there was a significant increase in *An. arabiensis* mortality whenever Icon Life® nets (RR = 1.39 (1.18 – 1.63), P < 0.001), PermaNet 2.0® nets (RR = 1.26 (1.08 – 1.48), P = 0.004), or Olyset® nets (RR = 1.32 (1.13 – 1.55), P = 0.001) were added, relative to the huts where PM IRS was used alone. Similarly, in the huts that had been sprayed with ICON CS, there was a significant increase in *An. arabiensis* mortality in combination with Icon Life® nets (RR = 1.43 (1.19 – 1.73), P < 0.001) or PermaNet 2.0® nets (RR = 1.70 (1.35 – 2.13), P < 0.001), but not Olyset® nets (RR = 1.16 (0.92 – 1.45), P = 0.210), relative to the IRS alone.
In huts sprayed with DDT, none of the LLINs significantly improved proportional mortality of the *An. arabiensis* mosquitoes, except PermaNet 2.0® nets (RR = 1.18 (1.06 – 1.32), P = 0.003).

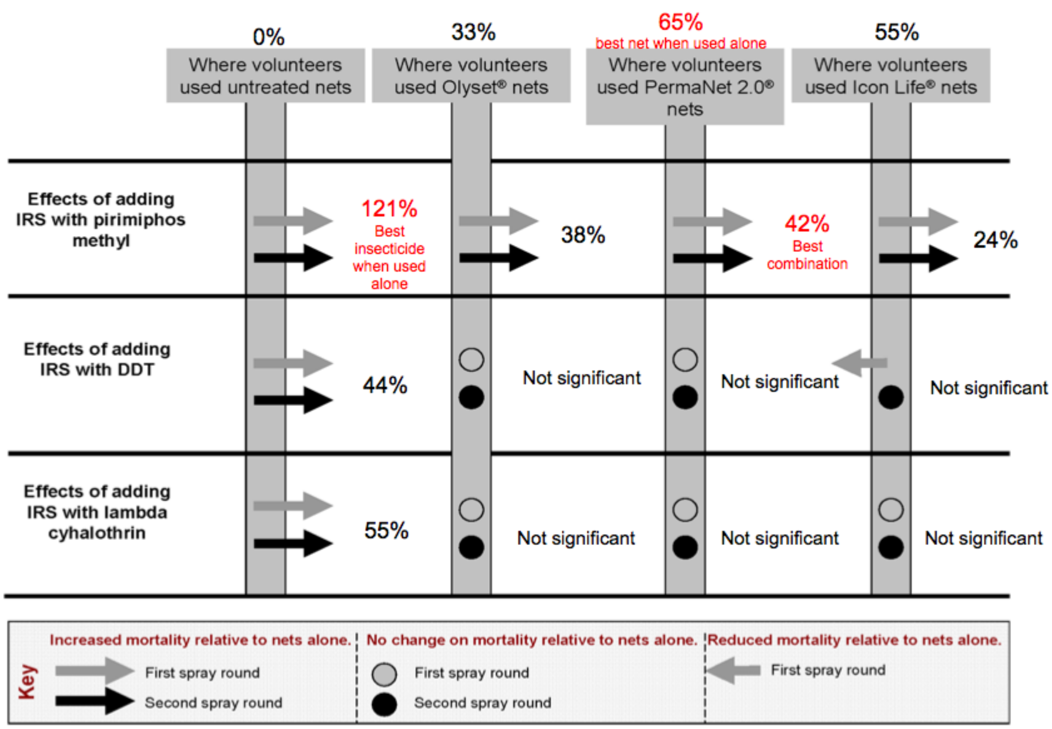

Figure 5: Summary of the observed changes on proportional mortality of the malaria vector, *Anopheles arabiensis,* when different IRS insecticides are introduced in situations where volunteers were already using different net types. Summaries are shown for both first and second spray rounds.

Residual efficacy bioassays of IRS:

All IRS formulations were highly effective during the first month after spraying and rapidly decayed losing most activity within 1-3 months. In month 1, all *An. arabiensis* exposed to palm ceilings sprayed with either PM or ICON CS died, and DDT killed 85% (despite full susceptibility most likely because it flaked away). On mud walls sprayed with the same chemicals, 100%, 90.0% and 97.5% mortality was observed, respectively, during the first month. Activity of the IRS declined significantly so that by the third month, PM on palm and mud killed 42.5% and 55.0% of exposed *An. arabiensis*, respectively. ICON CS killed only 46.3% on palm and 52.5% on mud walls. By month 6, PM had nearly entirely decayed, killing only 7.5% of *An. arabiensis* exposed to sprayed palm ceilings and 27.5% of those exposed to sprayed mud walls; ICON CS killed 30.0% on ceilings and 27.5% on walls. DDT had a longer residual action, killing 42.5% of *An. arabiensis* exposed to sprayed ceilings, and 36.3% of those exposed to sprayed walls after 6 months.

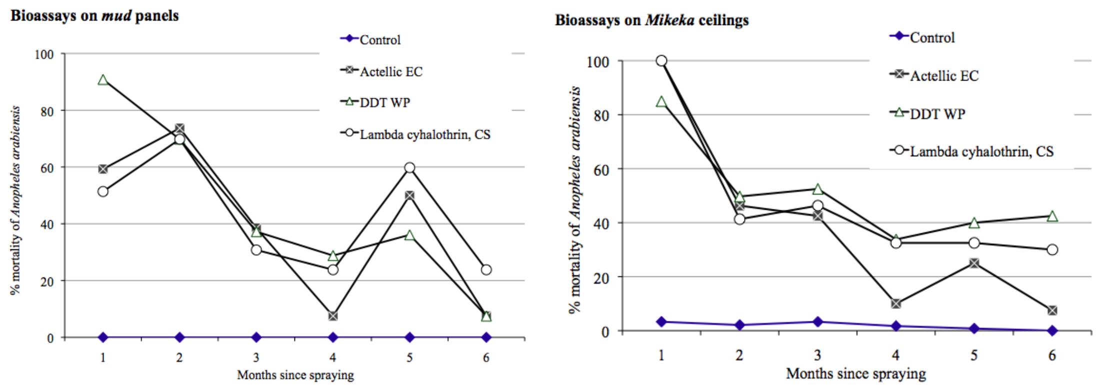

Figure 6: Results of monthly bioassays showing residual activity of IRS compounds sprayed on mud walls and *Mikeka* ceilings and of experimental huts

Residual efficacy bioassays of LLINs:

While all the LLINs generally performed better (i.e. killed more mosquitoes) on wire frame assays than on the cone assays, their activity rapidly deteriorated by the second month of use relative to new nets. Only PermaNet® nets retained efficacy of 80% by the sixth month of net use (killing 92.7% on wire ball tests and 84% on cone assays). All the LLINs however retained very high knock-down rates (> 90% in wire ball tests and >80% in cone tests) on the exposed mosquitoes, except Olyset® nets whose knock-down activity reduced to 72.7% on wire ball tests and 62% on cone tests by the sixth month.

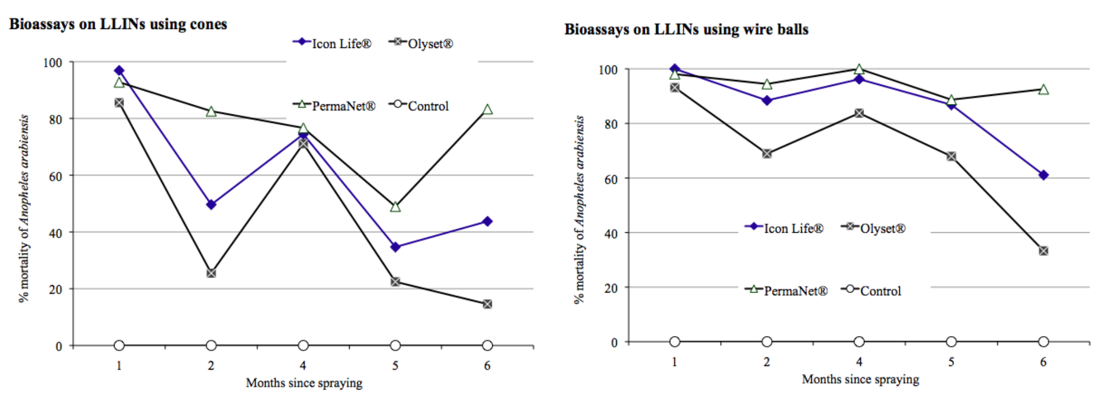

Figure 7: Monthly bioassays showing residual activity of various LLINs when tested using either the standard WHO cone assays or the wire ball method. NB. No assays were conducted on the third month due to lack of mosquitoes

Mosquito exit behavior:

During the first spray round (dry season), most of the mosquitoes caught exiting the control huts consisted of those caught between 7pm and 11pm. During the second spray round (wet season), more of the *An. arabiensis* exit from the control huts occurred at dawn. This data is consistent with other studies from Tanzania where *An. arabiensis* is more endophillic during the wet season [17]. In both spray rounds, the greatest shift towards early exit was observed in huts having pirimiphos methyl IRS combined with PermaNet 2.0® nets (P < 0.001). In both spray rounds, there were also apparent but marginal increases in early exit when the IRS and LLINs were used together, relative to whenever either the LLINs or the IRS were used alone (Figures 8 and 9).

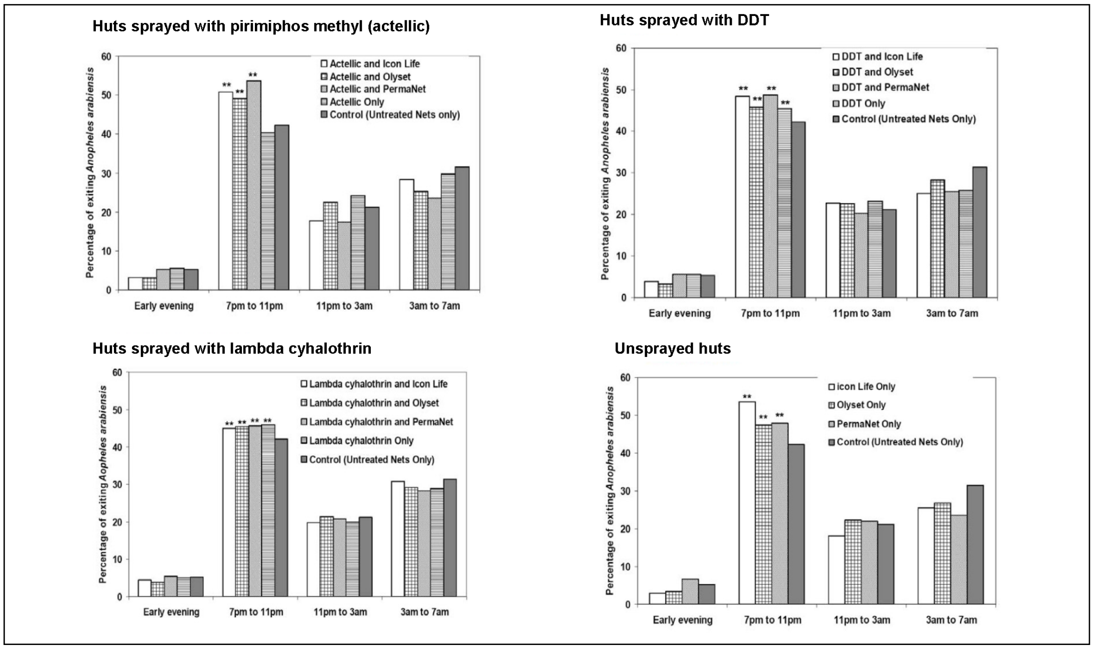

Figure 8: Effects of IRS/LLIN applications on the time when *Anopheles arabiensis* exited volunteer-occupied experimental huts during the **first spray round.** Bars marked with two stars (**) denote irritant applications that caused significantly more mosquitoes (P < 0.05) to exit earlier than in controls.

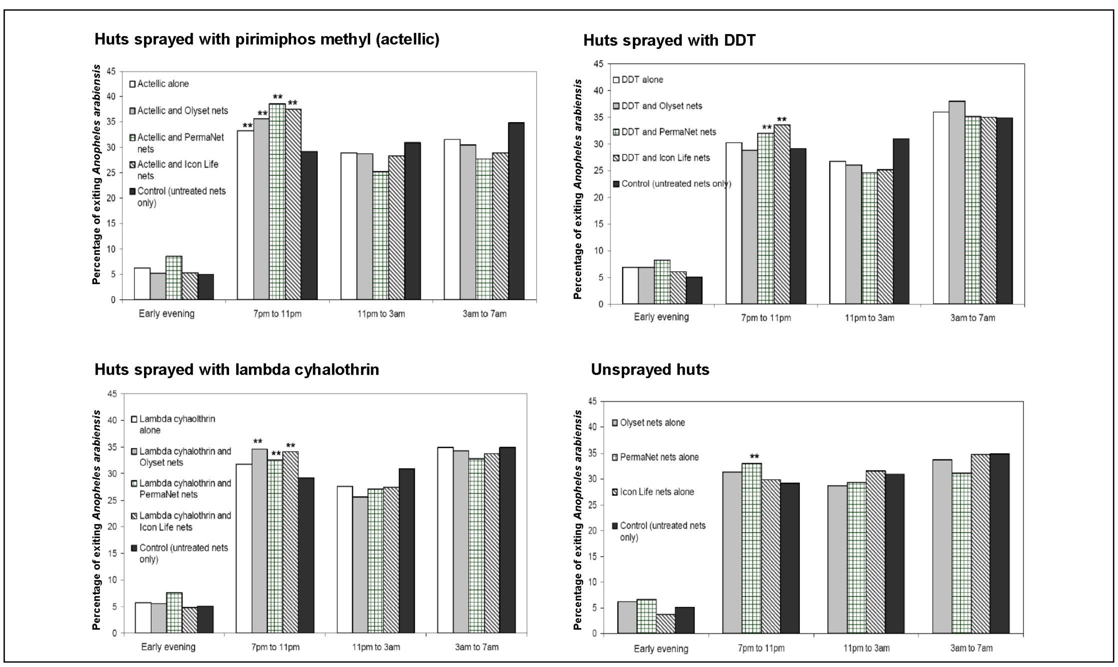

Figure 9: Effects of IRS/LLIN applications on the time when *Anopheles arabiensis* exited volunteer-occupied experimental huts during the **second spray round.** Bars marked with two stars (**) denote irritant applications that caused significantly more mosquitoes (P < 0.05) to exit earlier than in the controls

Insecticide resistance:

An. arabiensis in this study area were fully susceptible to DDT (Table 3) but showed weakening susceptibility [18] to lambda cyhalothrin, deltamethrin and permethrin was observed, necessitating vigilance against emerging pyrethroid resistance.

Of 122/141 successful amplifications of *An. arabiensis* females obtained from the colony that had been established using wild caught females from the study area, all were *kdr*-negative even though, these mosquitoes included those that had survived the standard bioassays on the hut walls and nets. Of the 383/522 successful amplifications 522 of mosquitoes obtained from our experimental huts during the LLIN/IRS combination study described earlier, all were *An. arabiensis* and all were *kdr*-negative. Of 15/43 successful amplifications from mosquitoes collected directly from local houses in the study area, using CDC light traps set near bed nets all were *An. arabiensis* and all were *kdr*-negative.

Table 3: Results of the insecticide susceptibility tests conducted on wild *Anopheles arabiensis* mosquitoes

		Knock Down (KD)								Mortality		
	Total No. exposed	KD 10mins	KD 15mins	KD 20mins	KD 30mins	KD 40mins	KD 50mins	KD 60mins	%KD 60mins	Total No. Dead	% Dead	Susceptibility Class
Control	123	0	0	0	0	0	1	1	0.8	1.0	0.8	-
DDT, 4%	124	0	0	7	44	94	114	118	95.2	124.0	100.0	***
Permethrin, 0.75%	125	0	8	20	71	95	122	124	99.2	119.0	95.2	**
Lambda cyhalothrin, 0.05%	123	0	0	2	23	60	81	92	74.8	111.0	90.2	**
Deltamethrin, 0.05%	96	0	5	16	37	60	74	79	85.9	92	95.8	**
Dieldrin, 0.4%	124	0	0	0	0	0	2	3	2.4	120.0	96.8	**

Susceptibility classification
*** The percentage mortality value indicates 100% susceptibility
** The percentage mortality value indicates insecticide tolerance and possibility of resistance that needs to be confirmed

Simulation of community level effects of LLIN/IRS combinations

We used an optimized version of a deterministic model based on mosquito life cycle processes, to estimate how malaria transmission might be affected, if LLINs are combined with IRS. For this purpose, we consider closed communities dominated by *An. arabiensis* as the main malaria vector. It also assumes perfect LLIN coverage where LLINs are used.

The two most important results from this simulation were that: 1) combining LLINs with IRS does not always result in improved community level malaria transmission control relative to the use of either method alone, and 2) whereas introduction of LLINs into a community with pre-existing IRS generally results in improved malaria transmission control, introduction of IRS into communities with pre-existing LLIN use, is in most cases redundant except where the IRS compound is highly toxic to malaria mosquitoes.

Figures 10 and 11 show the simulation results in situations where IRS is the pre-existing intervention and where nets are the pre-existing intervention respectively. For example, where there is no IRS but most people use intact untreated nets, replacing the untreated nets with two of the most common LLINs, Olyset® nets and PermaNet® nets can improve transmission control by 31% and 45% respectively, relative to the baseline. Similarly, where actellic IRS is already being combined with untreated nets, the two net types would provide an additional 14% and 35% transmission control respectively.

However, where IRS with DDT or lambda cyhalothrin is already in use with untreated intact nets, addition of these two LLIN types would be likely be redundant, except for an estimated 15% improvement when PermaNet® nets are combined with lambda cyhalothrin. Interestingly, these simulations show that in these same scenarios, replacing the untreated nets with Icon Life® net, would improve the impacts of IRS, providing 68%, 51%, 18% and 40% improvement in community wide transmission control when combined with no IRS, actellic, DDT or lambda cyhalothrin respectively (Figure 10).

On the contrary, situations where correct use of intact nets is already high there is little additional benefit to spraying IRS, except where the IRS chemical is very highly toxic to the mosquito populations, such as actellic. This strategy is also useful for resistance management since actellic is an organophosphate and therefore has a different mode of action to the pyrethroid insecticides used on LLINs.

For example, the simulations here show that introduction of DDT based IRS in such scenarios would either be redundant (when combined with untreated nets) or even worse, reduce the existing potential of transmission control if the pre-existing intervention were Olyset®, PermaNet® or Icon Life® nets. This may be the irritant mode of action of DDT reducing the amount of time that insects spend in contact with LLINs due to excitation. Similarly, addition of lambda cyhalothrin IRS would be redundant in places where most people already correctly use any of the three LLINs, but the same IRS would result in marginal improvement where the pre-existing net coverage was with untreated nets.

Actellic, the only one of the three IRS compounds that demonstrated additional benefit for *An. arabiensis* control based upon data from the experimental huts studies, is estimated to improve transmission control by 42%, 24%, 32% and 28% where the pre-existing intervention is untreated nets, Olyset®, PermaNet®, or Icon Life® nets, respectively (Figure 11).

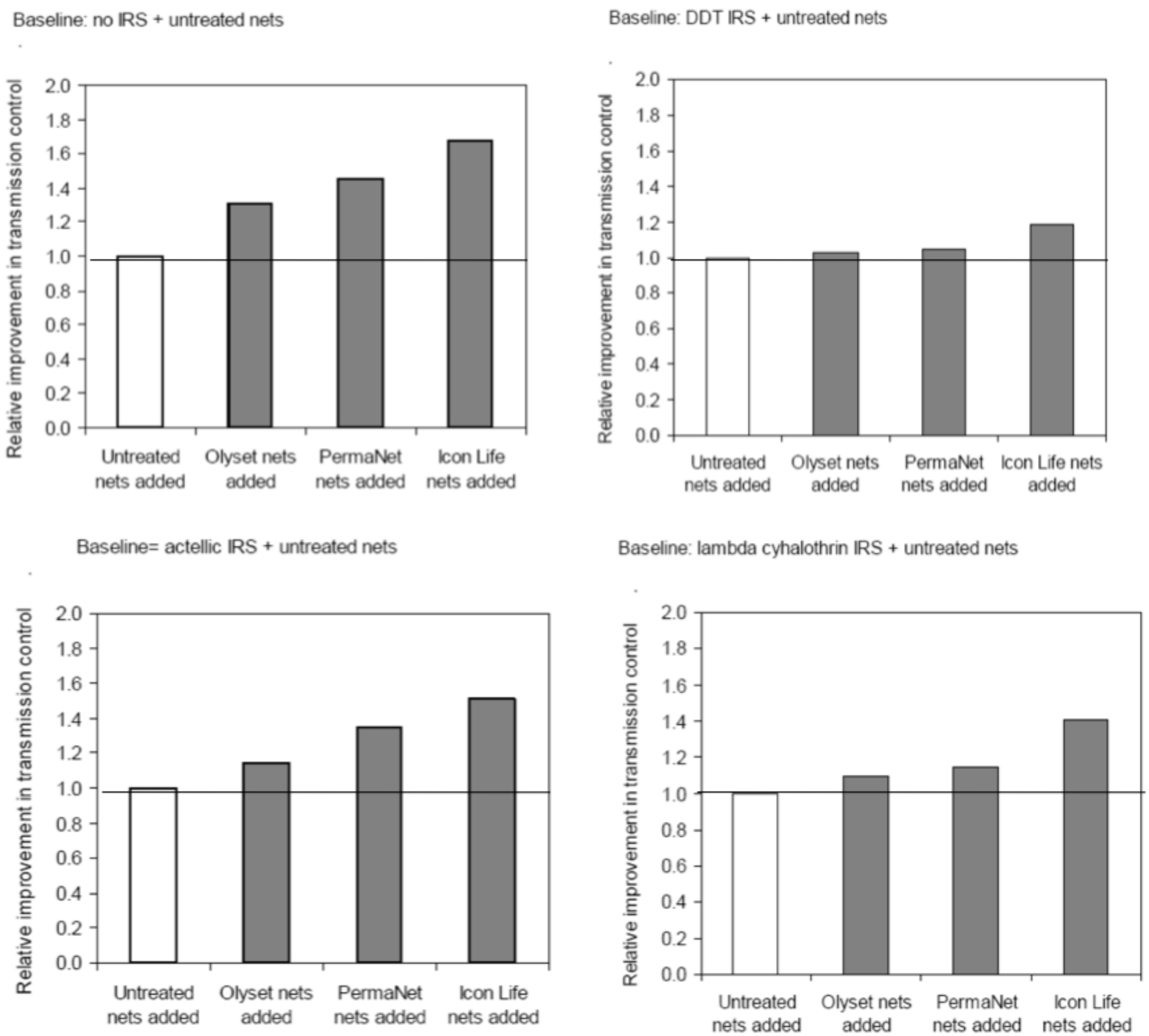

Figure 10: Relative change in malaria transmission control, whenever LLINs are introduced into communities with pre-existing high coverage of IRS and untreated nets. Values on the Y-axis can also be interpreted as the estimated 'fold' increase in transmission control relative to the respective baselines

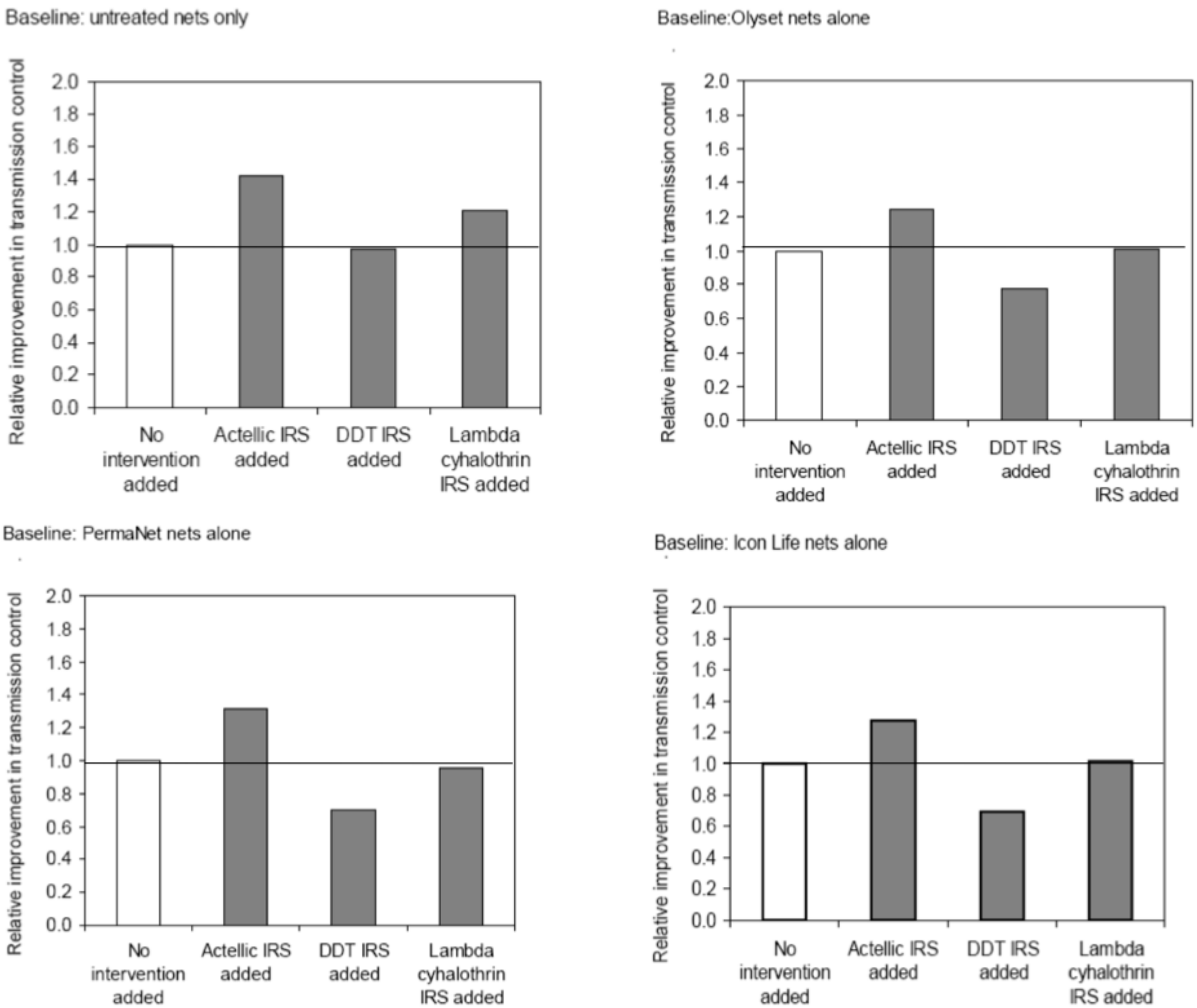

Figure 11: Relative change in malaria transmission control, whenever IRS is introduced into communities with pre-existing high coverage of nets. Values on the Y-axis can also be interpreted as the estimated 'fold' increase in transmission control relative to the respective baselines

General discussion of the results

Prior to this study, there was limited, inconclusive field evidence to support LLIN-IRS combinations relative to the use of either method alone [6, 19]. In the course of the research reported here, there have been at least three separate field studies that have addressed this question, one of which assessed effects of a single combination of LLINs with one type of insecticide, against resistant mosquitoes in Benin [20], and another which examined clinical outcomes of LLIN-IRS combinations in non-randomized prospective cohorts in Kenya [21]. The non-randomized prospective study, which had been embedded in a programmatic anti-malaria operation in western Kenya concluded that the protective efficacy of LLINs combined with IRS was 62% compared to LLINs alone [21]. However, a recent randomized controlled trial conducted across 58 villages in Benin has shown no significant reduction in malaria morbidity, infection and transmission in villages using a combination of LLINs with either IRS or durable wall lining as compared to villages using only LLINs [22]. Though this trial was conducted in an area with high pyrethroid resistance, the insecticide used for IRS was bendiocarb, which is known to remain effective in Benin despite the high pyrethroid resistance [23]. One would therefore expect this study to show some added advantages of the LLIN/IRS combinations relative to LLINs alone.

Our study has extended this limited evidence base, providing the first set of experimental huts data conducted for the entire duration of the insecticides (representing one spray round) that directly compares household level effects of multiple combinations of different IRS and LLIN types versus either the nets alone or the IRS alone. Mathematical simulations of community–level effects where the LLINs are combined with IRS and the primary vector is An. arabiensis, have augmented these household level results. The study clearly indicates that combining LLINs and IRS can enhance protective efficacy, and that there is need to carefully select the methods based on their modes of action, to achieve maximum benefits. Moreover, given the rapid decay of some of the insecticides from treated surfaces, the combinations are necessary to confer some temporal overlap of protection, for example LLINs continuing to provide protection where IRS has decayed. Most importantly, the study has shown the greatest communal impact would be obtained if the IRS being used together with the LLINs were highly toxic to malaria mosquitoes. This finding supports recent clinical evidence from Bioko demonstrating a clear increase in the odds of having malaria for each month that passes after IRS is conducted, and an additive effect of combining IRS with additional use of LLIN [24]. An additional reason why IRS may have an important role to play in malaria control even in areas where LLINs are available is compliance. In Tanzania, the proportion of households with at least one ITN increased from 62.6% before the Universal Coverage Campaign (UCC) to 90.8% afterwards and the mean number of LLINs owned per household almost doubled to 2.1. Even though the number of residents reporting that they slept under an ITN the previous night improved, only 55.7% of all residents had used their net the previous night after the UCC [25]. In addition, nets become worn and thus their protective efficacy declines [26]. Furthermore, mosquitoes that bite early in the evening, usually before people go to bed may not be sufficiently targeted by the nets. As a result, the proportion of malaria transmission that nets can actually prevent is always lower than 1, and this proportion is not expected to exceed 0.9 outside experimental conditions [27, 28]. Therefore, combination of interventions is warranted due to it's mass effect even for those in houses that are not sprayed, who are not using LLINs [26], but must be carefully planned to maximize efficiency and minimize the risk of insecticide resistance developing.

Implementation of IRS is a resource intensive exercise. It often requires extensive planning for transport and storage of the chemicals to be used and for management of the spray teams during the campaigns. In some communities, not all homes are accessible to the spray teams, meaning that the desired coverage may not always be achieved. IRS may therefore not be suitable for every setting and is often implemented only in selected locations and during selected times of the year, in which case it is not always expected to provide protection all year round [29]. Lastly, both IRS and LLINs target mainly those mosquitoes that enter or those that attempt to enter human dwellings. Therefore, other than the accumulated communal benefits [30, 31], which result from the fact that these interventions also kill mosquitoes that come into contact with them, the two interventions are not always directly effective against vectors that bite humans outdoors or those that rest outdoors so a thorough knowledge of local vector ecology is necessary before embarking on a spray program.

The data presented in this report show that indeed the additional benefits obtainable from IRS-LLIN combinations occur mainly due to the excess killing effect and the direct protection against bites. Mathematical simulations of control scenarios where the different interventions are used either alone or in together also suggest that the most efficacious combinations would be those that consist of the current pyrethroid-based LLINs, used alongside highly toxic IRS compounds such as actellic. Data showed that this compound is not deterrent and therefore kills maximal numbers of mosquitoes. The recommendation from this work is that LLINs were surprisingly effective when unholed. Therefore, by maximizing compliance and correct use of LLINs excellent health gains may be made. In addition it is beneficial to apply toxic IRS carefully timed to kill as many mosquitoes as possible i.e in malaria hotspots [32].

The work demonstrated excellent efficacy from LLINs. We suggest that maintenance of high coverage of intact LLINs be the primary goal of PMI because under programmatic circumstances, IRS cannot be expected to provide full protection. It is only sprayed periodically in selected areas, and usually not more than twice annually [29]. In other words, the practical limits of what can be expected from IRS under normal circumstances are much lower than the limits for LLINs. However, in epidemic situations, well-timed, regularly repeated and quality controlled IRS treatments with non-irritant, non-pyrethroid insecticides can kill significant proportions of vector populations and therefore dramatically drive down malaria transmission at malaria foci, at least on the short term. Moreover, where the public health systems are adequately organized and well funded enough to tackle the logistical challenges associated with repeated IRS campaigns, it will significantly impact upon the vector population and malaria burden as has been demonstrated in South Africa [33].

A key concern that has featured in this study is the poor performance of some of the most common vector control applications. For example, Olyset® nets which are currently the most common LLIN in Tanzania had extremely low toxicity and also low deterrence against the malaria vectors in both the two spray rounds. Moreover, standard bioassay tests performed on this net showed that its toxicity against malaria vectors was significantly reduced after six months. It was therefore clear that any protection from Olyset® nets was mainly due to the physical barrier that it provides against mosquito bites, rather than its insecticidal properties. The products that we tested were obtained from the regular supply chain in-country and therefore represent the products that are actually being used by the target population. Given that that approximately 30 million of these nets are being produced every year in Tanzania alone [34], and also the fact that this is the most widely distributed LLIN in the region, its poor performance should be considered a major challenge and quality assurance measures addressed promptly by the public health authorities.

Summary recommendations and implications of the research findings for malaria control policy in Africa

At the early stages of this work, it was determined that there are numerous theoretical justifications for the application of IRS combined with LLINs. This research then generated direct evidence to support or disprove combinations relevant to the most common situations where LLINs and IRS are combined. It should be noted that the view adopted here is purely based on the research evidence and is not in anyway aimed at promoting any of the LLIN or IRS products. Give potential public health and economic implications of LLIN/IRS combinations for malaria vector control in different places, these findings should be used with the full understanding of experimental and epidemiological circumstances under which our studies have been conducted. The key recommendations are summarized below:

1. Combinations of LLINs with IRS can be synergistic or redundant, depending on the types of insecticides used. Nonetheless, they would be most effective if any of the current LLINs are combined with highly toxic IRS treatments, one example being actellic.

2. Where people already have LLINs, addition of IRS would likely be less effective because: a) mosquitoes prevented from feeding may not rest indoors for long enough to pick up lethal IRS doses, and b) the high rates of decay of commonly used IRS insecticides from sprayed surfaces common in Africa, coupled with logistical challenges usually associated with re-spraying campaigns would make it impractical to maintain an all year round continuous coverage with effective IRS. Therefore, such an addition of IRS onto LLINs should be considered worthwhile only where adequate extra financial resources are guaranteed and where there are sufficient logistical mechanisms that would allow optimal IRS implementation with a highly toxic IRS. It is likely to be more cost effective to use resources to ensure LLIN coverage and compliance is high e.g. for behavior change such as hang up campaigns in remote or inaccessible regions.

3. Where IRS is the pre-existing intervention, addition of LLINs is beneficial, and should be encouraged, particularly to provide direct personal protection from mosquito bites, but also to provide continued protection when the activity of IRS has decayed. Where LLINs are considerably expensive or unavailable, then untreated nets should be considered as the basic minimum personal protection, so that IRS is never used as a stand-alone protection.

4. Where resources are limited, priority should be given to providing everybody with LLINs and ensuring that these nets are consistently and appropriately used, and replaced at sufficiently frequent time intervals, rather than trying to implement both LLINs and IRS in the same community at the same time.

5. The promotion of long lasting nets that provide a robust and long lasting physical barrier against mosquito bites even after their insecticidal activity has waned should be a funding priority. Having periods where nets are intact but no longer insecticidal may even provide an insecticide resistance management strategy.

6. Insecticides used in IRS and LLINs should be of different chemical classes, to generate maximum impact while at the same time minimizing the risk of proliferation of insecticide resistance. Given that all existing LLINs are currently pyrethroid based, and because of possibilities of cross-resistance between DDT and pyrethroids, IRS with either DDT or pyrethroids should be discouraged in Africa. Even in this mainly susceptible mosquito population in southern Tanzania, DDT and Lambda-cyhalothrin are less effective as IRS than the organophosphate pyrimiphos methyl.

Limitations and recommendations for future research

The experimental hut study evaluating LLINs and IRS was conducted in two spray rounds. Although the overall trends in the data were consistent between the two rounds, some variability in data obtained was observed. In the first round DDT elicited moderate levels of deterrence against the malaria vectors, this was not apparent during the second spray round. Also, the apparent increase in mosquito catches inside huts with pirimiphos-methyl alone or in combination with Icon Life® nets or PermaNet® nets, was more pronounced in the second round relative to the first round. It is unclear what the likely cause of these difference could be, given that the two spray round were not only conducted at different times but that the second spray round also incorporated a number of incremental improvements relative to the first round.

1. Round 1 was conducted in the dry season and round 2 in the wet season affecting mosquito densities behavior so that there was more exophily in round 1 and higher numbers in round 2.
2. In the second round, IRS huts had been randomly assigned, this had not been the case during the first round. It can therefore be argued that some of these differences could have been reduced or eliminated if the experiment had included more replicates and complete randomization in both spray rounds.
3. The mathematical simulations presented here, relied on a number parameter values obtained from a variety of sources, not necessarily representative of Africa-wide epidemiological scenario, and also a number of assumptions that may not necessarily proven in real life. These are common challenges in most mathematical models are must be considered when making inferences from results of any such simulations. Nevertheless, in the work presented here, significant attempts were made to ensure that all assumptions and parameter values incorporated in the simulations were carefully evaluated and that they reasonably matched the desired epidemiological characteristics. Moreover, the key intervention parameter values used in the model, which describes community-wide effects of LLIN/IRS combinations, were obtained from the single experimental hut study (spray round II data). It is therefore not a surprise that results of these simulations generally mirrored those of this experimental hut study.
4. In the field experiments, the human volunteers sleeping in the experimental huts were not rotated, but were instead fixed to their hut locations. This was done to minimize logistical challenges associated with rotating the 18 volunteers over 9 experimental huts during the course of the study, a situation which would significantly increase variability and reduce statistical power in the data set. The variations associated with the human volunteers and those associated with the actual position of the experimental huts were treated as a single source of variation.
5. A large number of treatments were evaluated (i.e. 3 IRS insecticides and one unsprayed house, plus 4 LLIN types and an untreated net). This practice enabled comparison of combinations of a variety of insecticides classes currently available for malaria vector control [35], with up to 16 different IRS-net combinations tested, but it also meant that the experimental design was weakened due to reduced replication. Even so round 1 n=40, round 2 n=60 for each combination, and both rounds were sufficiently powered to see an effect.

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Appendix

Abstracts of manuscripts completed as part of this research

Abstract 1: Published Malaria Journal 2011 **http://www.malariajournal.com/content/10/1/208/**

Combining indoor residual spraying and insecticide-treated nets for malaria control in Africa: a review of possible outcomes and an outline of suggestions for the future

Fredros O Okumu and Sarah J Moore

Insecticide-treated nets (LLINs) and indoor residual spraying (IRS) are currently the preferred methods of malaria vector control. In many cases, these methods are used together in the same households, especially to suppress transmission in holoendemic and hyperendemic scenarios. Though widespread, there has been limited evidence suggesting that such co-application confers greater protective benefits than either LLINs or IRS when used alone. Since both methods are insecticide-based and intradomicilliary, it is hypothesized that outcomes of their combination would depend on effects of the candidate active ingredients on mosquitoes that enter or those that attempt to enter houses. It is suggested here that enhanced household level protection can be achieved if the LLINs and IRS have divergent yet complementary properties, e.g. highly deterrent IRS compounds coupled with highly toxic LLINs. To ensure that the problem of insecticide resistance is avoided, the LLINs and IRS products should preferably be of different insecticide classes, e.g. pyrethroid-based nets combined with organophosphate or carbamate based IRS. The overall community benefits would however depend also on other factors such as proportion of people covered by the interventions and the behavior of vector species. This article concludes by emphasizing the need for basic and operational research, including mathematical modeling to evaluate IRS/ITN combinations in comparison to IRS alone or LLINs alone.

Abstract 2: Published PLoS ONE 2012
file://localhost/www.plosone.org:article:info/doi:10.1371:journal.pone.0030967

A modified experimental hut design for studying responses of disease-transmitting mosquitoes to indoor interventions: the Ifakara Experimental Huts

Fredros O. Okumu, Jason Moore, Edgar Mbeyela, Mark Sherlock, Robert Sangusangu Godfrey Ligamba, Tanya Russell, and Sarah J. Moore

Differences between individual human houses can confound results of studies aimed at evaluating indoor vector control interventions such as insecticide treated nets (LLINs) and indoor residual insecticide spraying (IRS). Specially designed and standardized experimental huts have historically provided a solution to this challenge, with an added advantage that they can be fitted with special interception traps to sample entering or exiting mosquitoes. However, many of these experimental hut designs have a number of limitations, for example: 1) inability to sample mosquitoes on all sides of huts, 2) increased likelihood of live mosquitoes flying out of the huts, leaving mainly dead ones, 3) difficulties of cleaning the huts when a new insecticide is to be tested, and 4) the generally small size of the experimental huts, which can misrepresent actual local house sizes or airflow dynamics in the local houses. Here, we describe a modified experimental hut design - The Ifakara Experimental Huts- and explain how these huts can be used to more realistically monitor behavioral and physiological responses of wild, free-flying disease-transmitting mosquitoes, including the African malaria vectors of the species complexes Anopheles gambiae and Anopheles funestus, to indoor vector control-technologies including LLINs and IRS. Important characteristics of the Ifakara experimental huts include: 1) interception traps fitted onto eave spaces and windows, 2) use of eave baffles (panels that direct mosquito movement) to control exit of live mosquitoes through the eave spaces, 3) use of replaceable wall panels and ceilings, which allow safe insecticide disposal and reuse of the huts to test different insecticides in successive periods, 4) the kit format of the huts allowing portability and 5) an improved suite of entomological procedures to maximize data quality.

Abstract 3: Submitted Malaria Journal

Comparative evaluation of combinations of long lasting insecticidal nets and indoor residual spraying, relative to the use of either method alone, for malaria vector control in an area dominated by Anopheles arabiensis

Fredros O. Okumu, Edgar Mbeyela, Godfrey Ligamba, Jason Moore, Alex Ntamatungiro, Deo Roman, Mike Kenward, Elizabeth Turner, Lena Lorenz and Sarah J. Moore

Background: Malaria vector control in sub-Saharan Africa currently relies mainly on long lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS). In several highly endemic regions, the methods are used together in the same households, despite limited empirical evidence to suggest protective advantages of such combinations. This study assessed whether there is any such additional protection, relative to using either method alone.
Methods: Comparative evaluations were conducted in experimental huts fitted with LLINs alone, IRS alone, or combinations of LLINs and IRS, in an area where Anopheles arabiensis is the predominant malaria vector species. Indicators of protection included: 1) number of mosquitoes entering huts, 2) proportion and number killed after exposure to each treatment, 3) proportions prevented from blood-feeding, 4) time when mosquitoes exited the huts, and 5) proportions caught exiting. Three LLIN types, Olyset®, PermaNet 2.0® and Icon Life® nets and three IRS treatments, pirimiphos-methyl (organophosphate), DDT (organochloride) and lambda cyhalothrin (synthetic pyrethroid), were assessed singly or in combinations, relative to each other and to non-insecticidal nets alone. The study was conducted in 2 rounds, I and II.
Findings: All LLINs and the untreated nets, used with or without any IRS, provided near-absolute protection from mosquito bites (>99% feeding inhibition). Addition of PermaNet 2.0® and Icon Life® nets into huts with IRS also increased the proportions of malaria mosquitoes killed. LLINs did not reduce mosquito entry into huts, except for a 30% reduction of An. arabiensis catches in huts with PermaNet 2.0® nets in first spray round. Of the IRS treatments, only pirimiphos-methyl significantly increased proportional mortality relative to LLINs alone. No IRS significantly reduced mosquito entry, except DDT during first spray round. More than 95% of mosquitoes were collected in exit traps rather than inside huts.
Conclusions: Adding IRS into houses where people already use LLINs (all of which are currently pyrethroid-based) does not enhance household-level protection, except where the IRS employs highly toxic and less irritant non-pyrethroids such as pirimiphos-methyl, combinations which would also control insecticide resistance by combining the different insecticide classes. In contrast, adding intact nets onto IRS enhances household-level protection by preventing mosquito bites (even if the nets are non-insecticidal) and by killing excess mosquitoes (in case of LLINs). Therefore, where resources are limited, priority should be to ensure that all people at risk have LLINs and that they use them consistently, rather than trying to implement both LLINs and IRS. However since this study involved only correct and consistent use of the nets, it is possible that IRS may remain beneficial in places where people do not correctly and consistently use their nets or where the nets are old and torn. Perhaps long lasting non-insecticidal nets should also be explored as a potential means of complementing IRS and reducing insecticide use in future vector control.

Abstract 4: Submitted Malaria Journal

Bio-efficacy and persistence of insecticides used for indoor residual spraying and long lasting insecticide nets: results from laboratory and field evaluations against the malaria vector, Anopheles arabiensis in southern-eastern Tanzania

Fredros Okumu, Edith Madumla, Edgar Mbeyela, Geoffrey Ligamba, Jason Moore, Beatrice Chipwaza and Sarah Moore
Background: We assessed the bio-efficacy and residual activity of insecticides used for indoor residual spraying (IRS) and long lasting insecticidal nets (LLINs), against laboratory-reared and wild populations of the malaria vector, Anopheles arabiensis in south-eastern Tanzania. This was a complementary study conducted alongside an experimental hut study aimed at assessing synergies and redundancies in household level protection, when IRS is combined with LLINs.

Methods: WHO bioassays were performed using cones and wire balls to assess residual activity of insecticides in LLINs, and those sprayed on mud walls and palm-thatched ceilings of experimental huts. WHO-susceptibility tests were also performed using diagnostic concentrations of candidate insecticides, against wild mosquitoes collected in the study area. Lastly, molecular analysis was performed to detect knock-down resistance genes associated with resistance against DDT and pyrethroids.
Results: Whereas all candidate IRS formulations (DDT wettable powder, lambda cyhalothrin capsule suspension and pirimiphos-methyl (actellic) emulsified concentrate), were highly effective during the first month after spraying (killing $\geq$ 85% of mosquitoes exposed in cone bioassays) these treatments rapidly decayed losing most activity within 1-3 months. The tested LLINs (Olyset®, PermaNet® and Icon Life®) also lost insecticidal efficacy, in some cases by > 50% in six months, although they were not washed in this period. Malaria vectors in this study area were fully susceptible to DDT and no knock-down resistance gene mutations were detected. However, weakening susceptibility to lambda cyhalothrin and permethrin was observed, necessitating vigilance against emerging pyrethroid resistance.
Conclusions: Existing pyrethroid-based LLINs remain the most efficacious intervention against malaria vectors in this area. Given the rapid decay of insecticidal activity on the mud surface, and possibility that mosquitoes might not rest long enough on treated surfaces to pick up lethal doses, IRS when used alone is minimally appropriate for vector control in this scenario. If these results are interpreted in the context of the more general objective, to determine if there are any added advantages of combining LLINs with IRS, there is clear justification for adding LLINs where IRS is the only existing intervention, especially to provide continued protection when the IRS decays. There is however, no evidence to support introduction of IRS into houses where LLINs are already being used. The potential for resistance emerging in the area should be carefully monitored.

Abstract 5: Completed, Ready for submission to journal

Simulated community-level effects of combining long lasting insecticidal nets with indoor residual spraying for malaria control in Africa

Fredros Okumu, Sarah J. Moore and Gerry, F. Killeen

Background: Even though it is common practice to combine indoor residual spraying (IRS) with long lasting insecticide nets (LLINs) in highly endemic communities, there is limited evidence to suggest that such strategies confer greater protection against malaria than either method when used alone. Experimental hut trials have already demonstrated improved personal and household level protection with certain LLIN/IRS combinations, but it remains unclear whether such findings can also translate to proportionately greater benefits at community level.
Methods: an existing deterministic mathematical model of mosquito life cycle processes is adapted and used to estimate how malaria transmission might be affected, if LLINs are combined with IRS, and whether such combinations would be synergistic or redundant, relative to the use of either method alone. The model was modified to allow use of data derived directly from experimental hut evaluations where untreated bed nets are used as the experimental controls. A scenario was simulated to represent a closed community where residents own cattle, and where the main malaria vector is Anopheles arabiensis, an increasingly dominant vector species in Africa, which remains a significant challenge to control even with high LLINs and IRS use rate. Considering situations with either LLINs or IRS as the pre-existing intervention, we then calculated a relative improvement in transmission control achievable when the complementary intervention is introduced.
Findings: Transmission control is improved when the common pyrethroid based LLINs are added onto IRS treatments such as actellic and lambda cyhalothrin, but not DDT, which is known to be less toxic but highly deterrent against mosquitoes. On the other hand, the outcome remains unchanged when lambda cyhalothrin IRS is added to communities already using LLINs. Nevertheless, addition of highly toxic IRS such as with actellic vastly improves transmission control relative to just the LLINs alone.
Conclusions: This in-silico assessment shows that whereas introduction of LLINs into communities with pre-existing IRS will generally result in improved control of malaria transmission, introduction of IRS into communities with pre-existing LLIN use will most likely be redundant unless the IRS is highly toxic to malaria mosquitoes.

Table 1: Median numbers (and inter-quartile ranges (IQR)), and the sum of mosquitoes of different taxa caught per night in experimental huts fitted with different IRS and LLIN treatments during the **first spray round.**

	Anopheles arabiensis			***Culex* species**			***Mansonia* species**		
IRS/LLIN combinations	**Median**	**IQR**	**Sum(n)^**	**Median**	**IQR**	**Sum(n) $**	**Median**	**IQR**	**Sum(n) $**
Untreated nets only**	66.5	20.3 - 103.8	4596 (60)	26.0	9.0 - 62.3	2388	9.5	4.0 - 21.0	802
Olyset alone	89.0	62.3 - 128.5	6047 (60)	26.5	10.0 - 65.8	2701	11.0	3.3 - 15.8	743
PermaNet alone	67.0	46.8 - 95.0	4420 (60)	28.0	10.3 - 56.3	2257	7.0	4.0 - 16.0	627
Icon Life	79.0	47.3 - 130.0	6492 (60)	23.0	11.3 - 66.8	2434	13.5	4.5 - 23.0	910
Actellic only	89.0	57.5 -162.8	4512 (40)	25.5	10.5 - 51.5	1437	13.0	6.3 - 27.3	669
Actellic and Olyset	119.5	71.3 -175.5	5466 (40)	27.0	9.8 - 71.3	1555	10.0	3.0 - 16.0	496
Actellic and PermaNet	87.5	60.3 - 139.3	4691 (40)	22.5	11.0 - 57.5	1438	13.0	7.3 - 23.0	656
Actellic and Icon Life	124.5	78.0 - 216.5	6022 (40)	33.5	14.5 - 66.5	1884	13.5	7.0 - 30.8	800
DDT only	45.0	32.3 -94.3	2605 (40)	21.5	10.3 - 48. 3	1380	10.0	3.3 - 15.8	414
DDT and Olyset	74.5	45.5 - 102.8	3162 (40)	26.0	7.3 - 54.5	1650	8.0	3.0 - 15.0	366
DDT and PermaNet	55.5	38.3 - 74.8	2728 (40)	24.5	10.3 - 44.8	1530	6.5	3.3 - 17.5	414
DDT and Icon Life	94.0	62.5 - 128.0	4017 (40)	22.5	10.0 - 48.5	1709	10.0	4.0 - 14.8	422
Lambda cyhalothrin alone	82.0	60.8 - 137.8	4212 (40)	34.0	9.5 - 67.3	1673	9.5	6.0 - 15.0	533
Lambda cyhalothrin and Olyset	99.0	61.0 - 186.8	5323 (40)	29.0	7.3 - 51.8	1355	6.5	2.0 - 12.5	361
Lambda cyhalothrin and PermaNet	85.5	41.5 - 141.0	3931 (40)	31.0	9.3 - 64.8	1596	7.0	3.0 - 17.0	494
Lambda cyhalothrin and Icon Life	106.0	59.3 - 174.5	5434 (40)	28.5	7.0 - 56.3	1477	11.5	5.0 - 23.0	598

^ The term 'n' refers to total number of replicates
$ The number of replicates (n) was the same as for *Anopheles arabiensis*
**Controls refer to unsprayed huts in which volunteers used untreated nets

Table 2: Median numbers (and inter-quartile ranges (IQR)), and the sum of mosquitoes of different taxa caught per night in experimental huts fitted with different IRS and LLIN treatments during the **second spray round.**

	Anopheles arabiensis			***Culex* species**			***Mansonia* species**		
IRS/LLIN combinations	**Median**	**IQR**	**Sum (n)^**	**Median**	**IQR**	**Sum$**	**Median**	**IQR**	**Sum$**
Untreated nets only**	64.0	36.5 - 95.0	7181 (90)	22.0	11.0 - 39.5	2461	5.0	3.0 - 8.0	537
Olyset alone	84.0	43.0 - 145.8	9789 (90)	23.0	10.0 - 39.3	2498	3.0	1.0 - 5.3	380
PermaNet alone	61.0	41.5 - 118.3	8240 (90)	23.0	10.0 - 41.5	2544	3.5	1.0 - 6.3	412
Icon Life	105.0	57.0 - 164.3	11279 (90)	22.5	13.8 - 43.3	2668	6.0	3.0 - 11.0	703
Actellic only	85.0	52.3 - 141.8	6751 (60)	33.5	14.5 - 65.8	3102	9.0	3.3 - 13.0	652
Actellic and Olyset	136.0	74.8 - 208.3	9988 (60)	33.5	16.5 - 74.0	3384	6.0	3.0 - 9.8	437
Actellic and PermaNet	94.5	59.0 - 191.3	7978 (60)	30.0	17.0 - 62.3	3032	7.0	3.3 - 11.8	518
Actellic and Icon Life	144.5	72.5 - 197.5	9621 (60)	37.5	16.3 - 59.5	3023	9.0	5.0 - 17.0	722
DDT only	67.0	38.3 - 107.8	4983 (60)	23.0	12.3 - 46.3	1828	4.0	2.0 - 8.0	365
DDT and Olyset	76.0	51.3 - 129.5	6053 (60)	25.5	10.3 - 40.8	1894	3.0	1.0 - 5.8	256
DDT and PermaNet	72.0	41.3 - 135.0	5528 (60)	27.0	10.3 - 40.5	1909	4.0	2.0 - 6.8	271
DDT and Icon Life	82.0	48.5 - 148.5	6176 (60)	29.0	15.0 - 43.8	1925	4.0	2.3 - 9.0	438
Lambda cyhalothrin alone	100.5	51.3 - 178.5	7535 (60)	20.5	10.3 - 38.0	1950	7.5	4.0 - 13.0	620
Lambda cyhalothrin and Olyset	115.5	65.5 - 207.0	8947 (60)	23.0	9.8 - 34.0	1916	5.0	2.0 - 9.8	438
Lambda cyhalothrin and PermaNet	100.5	58.3 - 173.8	7622 (60)	22.0	9.5 - 37.8	2018	6.0	3.0 - 12.0	548
Lambda cyhalothrin and Icon Life	120.0	71.8 - 243.5	9784 (60)	23.5	9.0 - 34.8	1981	8.0	5.0 - 15.0	706

^ The term ‘n’ refers to total number of replicates
$ The number of replicates (n) was the same as for *Anopheles arabiensis*
**Controls refer to unsprayed huts in which volunteers used untreated nets

Table 3: Median percentage mortality (and inter-quartile ranges (IQR)), and the sums of mosquitoes of different taxa killed per night in experimental huts fitted with different IRS and LLIN treatments during the **first spray round.**

	Mortality of *Anopheles arabiensis*		**Mortality of *Culex* species**		**Mortality of *Mansonia* species**	
IRS/LLIN combinations	**Median % (IQR)**	**Total number killed (n)^**	**Median% (IQR)**	**Total number killed$**	**Median% (IQR)**	**Total number killed$**
Untreated nets only**	7.1 (3.8 - 14.0)	403 (60)	1.0 (0.0 - 06.2)	77	16.5 (5.5 - 36.9)	170
Olyset only	11.8 (7.1 - 17.2)	709 (60)	3.9 (0.0 - 08.8)	121	33.3 (6.2 - 50.0)	285
PermaNet only	19.5 (13.6 - 26.5)	844 (60)	2.4 (0.0 - 09.0)	87	50.0 (39.6 - 70.1)	343
Icon Life only	19.0 (12.4 - 27.5)	1028 (60)	2.7 (0.0 - 11.1)	111	50.0 (29.6 - 62.8)	444
Actellic and untreated nets	16.6 (12.1 - 28.7)	836 (40)	9.8 (2.6 - 20.4)	136	42.9 (20.4 - 51.1)	300
Actellic and Olyset	16.4 (13.1 - 24.9)	980 (40)	7.4 (2.3 - 16.7)	102	41.2 (22.2 - 68.0)	255
Actellic and PermaNet	29.0 (18.8 - 36.2)	1196 (40)	6.9 (2.3 - 15.3)	98	71.8 (53.3 - 79.1)	433
Actellic and Icon Life	21.0 (13.3 - 32.2)	1338 (40)	3.3 (0.3 - 12.5)	108	56.5 (36.6 - 70.3)	433
DDT and untreated nets	14.0 (7.7 - 24.4)	369 (40)	1.4 (0.0 - 13.3)	52	50.0 (18.8 - 66.7)	192
DDT and Olyset	13.2 (8.8 - 17.2)	411 (40)	3.0 (0.0 - 11.0)	53	46.7 (21.1 - 62.4)	162
DDT and PermaNet	17.2 (12.0 - 25.7)	431 (40)	4.2 (0.0 - 12.9)	94	53.8 (36.7 - 66.7)	220
DDT and Icon Life	12.3 (9.3 - 18.6)	581 (40)	1.8 (0.0 - 08.8)	69	36.1 (20.2 - 50.0)	165
Lambda cyhalothrin and untreated nets	14.8 (10.6 - 22.2)	634 (40)	6.3 (0.3 - 09.9)	106	50.0 (25.0 - 66.9)	304
Lambda cyhalothrin and Olyset	14.9 (9.6 - 20.6)	755 (40)	6.8 (2.0 - 17.7)	98	66.7 (42.9 - 91.6)	232
Lambda cyhalothrin and PermaNet	20.6 (15.3 - 26.5)	802 (40)	6.3 (0.3 - 13.6)	110	64.3 (50.0 - 80.0)	307
Lambda cyhalothrin and Icon Life	21.6 (16.8 - 26.9)	1055 (40)	5.1 (1.4 - 18.9)	114	62.7 (46.6 - 77.6)	364

^ The term 'n' refers to total number of replicates
$ The number of replicates (n) was the same as for *Anopheles arabiensis*
**Controls refer to unsprayed huts in which volunteer used untreated nets

Table 4: Median percentage mortality (and inter-quartile ranges (IQR)), and the sums of mosquitoes of different taxa killed per night in experimental huts fitted with different IRS and LLIN treatments during the **second spray round.**

	Mortality of *Anopheles arabiensis*		**Mortality of *Culex***		**Mortality of *Mansonia* species**	
IRS/LLIN combinations	**Median % (IQR)**	**Total number killed (n)^**	**Median% (IQR)**	**Total number killed$**	**Median% (IQR)**	**Total number killed$**
Untreated nets only**	10.4 (04.2 - 18.1)	968 (90)	3.3 (0.0 - 10.0)	137	0.0 (0.0 - 27.1)	85
Olyset only	14.8 (09.3 - 23.9)	1742 (90)	2.9 (0.0 - 10.0)	128	0.0 (0.0 - 42.5)	86
PermaNet only	19.7 (11.2 - 30.1)	1644 (90)	3.8 (0.0 - 13.7)	177	26.1 (0.0 - 50.0)	147
Icon Life only	16.7 (07.2 - 26.4)	2121 (90)	2.3 (0.0 - 11.5)	187	20.0 (0.0 - 46.6)	198
Actellic and untreated nets	23.4 (12.9 - 36.7)	1599 (60)	5.7 (2.5 - 31.8)	272	21.1 (3.9 - 50.0)	119
Actellic and Olyset	20.3 (12.4 - 31.2)	2171 (60)	7.1 (3.6 - 21.0)	291	31.7 (12.7 - 56.2)	149
Actellic and PermaNet	25.0 (14.6 - 36.9)	2146 (60)	9.7 (4.1 - 28.6)	284	50.0 (29.4 - 97.7)	262
Actellic and Icon Life	21.8 (11.9 - 34.2)	2305 (60)	9.6 (3.8 - 33.6)	316	45.0 (28.6 - 79.5)	282
DDT and untreated nets	17.1 (08.0 - 28.3)	943 (60)	3.6 (0.0 - 14.0)	109	8.0 (0.0 - 38.3)	68
DDT and Olyset	19.2 (11.6 - 28.1)	1201 (60)	4.3 (0.0 - 11.1)	124	22.5 (0.0 - 50.0)	65
DDT and PermaNet	19.4 (12.6 - 34.1)	1171 (60)	4.8 (0.0 - 24.3)	150	33.3 (0.0 - 66.7)	97
DDT and Icon Life	14.7 (09.7 - 24.1)	1255 (60)	4.6 (0.0 - 10.6)	151	1.5 (0.0 - 30.6)	60
Lambda cyhalothrin and untreated nets	17.8 (10.4 - 28.6)	1431 (60)	9.7 (4.9 - 22.5)	197	21.1 (9.2 - 45.7)	138
Lambda cyhalothrin and Olyset	14.2 (09.0 - 27.7)	1578 (60)	5.5 (0.0 - 15.4)	157	25.0 (0.0 - 50.0)	136
Lambda cyhalothrin and PermaNet	19.0 (10.8 - 33.4)	1768 (60)	7.7 (2.6 - 23.6)	189	50.0 (8.5 - 80.0)	264
Lambda cyhalothrin and Icon Life	18.4 (09.3 - 26.2)	1893 (60)	8.0 (1.5 - 16.9)	155	33.3 (16.2 - 50.0)	210

^ The term 'n' refers to total number of replicates
$ The number of replicates (n) was the same as for *Anopheles arabiensis*
**Controls refer to unsprayed huts in which volunteer used untreated nets

Table S1: Effects of insecticides commonly used for indoor residual spraying (IRS) in Africa, on mosquitoes that enter or those that attempt to enter human occupied huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit [¢].

Insecticide	Country	Major vector	Dosage	Duration	Deterrence (%)	Feeding inhibition (%)	Toxicity (%)	Excess % exit	Reference[r]
DDT	Uganda	*An. funestus*	2g/ m^2 WP	7 months	80.2	-	71.0	-	[50]
			2g/ m^2 WP	7 months	88.9	-	83.6	-	
			2g/ m^2 WP	7 months	81.4	-	50.9	-	
			2g/ m^2 WP	7 months	95.2	-	79.8	-	
		An. gambiae	2g/ m^2 WP	7 months	74.4	-	-	-	[50]
			2g/ m^2 WP	7 months	89.0	-	-	-	
			2g/ m^2 WP	9 months	69.5	-	-	-	[85]
	Nigeria	*An. gambiae*	2g/ m^2 WP	6 months	68.1	-	52.0	50.3	[52] **^**
	South Africa	*An. arabiensis*	2g/ m^2 WP	3 months	52.4	67.1	55.9	26.3	[51] **^**
			2g/ m^2 WP	3 months	96.6	0.0	-	-	[51] **^***
			2g/ m^2 WP	5 months	-	36.0	32.0	-	[88] **^**
	Tanzania	*An. gambiae*	2g/ m^2 WP	5 months	56.4	35.1	17.0	46.2	[86] [+]
	Kenya	*An. gambiae*	2g/ m^2 WP	6 months	50.3	13.9	15.7	32.3	
		An. funestus	2g/ m^2 WP	6 months	36.3	22.1	41.2	8.75	
Lambda cyhalothrin	Benin	*An. gambiae s.l*	0.03g/m^2 CS	3 months	20.7	25.8	72.1	8.9	[30]
		An. gambiae	0.03g/m^2 CS	6 months	50.0	8.8	8.8	-	[71]
	South Africa	*An. arabiensis*	0.03g/m^2 CS	5 months	56.3	39.0	48.0	-	[88] **^**
	Tanzania	*An. gambiae & An. funestus*	0.03g/m^2 CS	7 months	71.7	59.0	-	-	[87]
Bendiocarb	Benin	*An. gambiae s.s*	0.02g/ m^2	2 Months	20.8	87.5	92.9	10.0	[53]

[¢] Table includes studies conducted in Africa, in areas where no resistance against DDT or pyrethroids had been reported. In studies where parameter values were not explicitly stated in the original publication, values were calculated from summary tables in those publications. ***Deterrence*** is calculated as the proportion of mosquitoes entering treated huts and number relative to the control hut. ***Feeding inhibition*** is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed and ***toxicity***, as the percentage of mosquitoes entering the treated hut that die. ***Excess exit*** is derived as the difference between percentage exit rates in sprayed and unsprayed huts, based on values presented in the original publications. The column for ***duration*** refers to the period after spraying, for which the data included in the analysis was collected.

^ Studies by Service *et al* 1964 [52] and Sharp *et al* 1990 [51] were conducted in local houses fitted with exit traps, unlike in all the other studies where specially designed experimental huts were used.

***** Only mosquitoes collected from the floors are included in this row

[+] The formula used by Smith and Webley [86] , to calculate deterrence is slightly different from that used in the other publications. That is, instead of using parallel catches in control huts as the reference, deterrence is determined by comparing number of mosquitoes entering treated huts with an expected number (N), which is calculated as N= (C x E)/C_1, where C is the number of mosquitoes entering control hut after spraying, E is the number entering treated hut after spraying and C_1 is the number entering control hut prior to the spraying of any hut. Also the results presented here are averages for all the months during which the experiments were conducted and may not exactly match the summary values in the original publication. For example, it should be noted that the deterrency value stated in the original publication is 60-70% which excludes the first month of the study.

[r] The study on the carbamate, Bendiocarb, was conducted in an area with high frequency of pyrethroid resistance, but with no resistance against the carbamates themselves [53], thus permitting its inclusion in this review, which otherwise considered only studies in areas where mosquitoes were susceptible to DDT and pyrethroids.

Table S2: Properties of conventionally treated nets (ordinary home-treated ITNs) commonly used in Africa, on mosquitoes that enter or those that attempt to enter human huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit [¢]. The nets are grouped as per the active ingredients (insecticides) used to treat them.

Insecticide	Country	Major Vector	Washing	Dosage	Holes	Deterrence (%)	Feeding inhibition (%)	Toxicity (%)	Excess % exit	Reference[r]
Alpha cypermethrin	The Gambia	*An. gambiae s.l*	Unwashed	100mg/m^2	Yes	0	92.0	94.0	-	[78]
			Washed	100mg/m^2	Yes	0	91.0	74.0	-	
	Tanzania	*An. arabiensis*	Unwashed	25mg/m^2	Yes	25.0	82.6	32.8	1.9	[72] [δ]
		An. gambiae & An funestus	Unwashed	10mg/m^2	Yes	45.8	81.5	59.5	-	[96]
			Washed	10mg/m^2	Yes	27.9	67.7	24.8	-	
			Unwashed	20mg/m^2	Yes	21.2	68.5	63.4	-	
			Washed	20mg/m^2	Yes	13.6	66.7	43.5	-	
			Unwashed	40mg/m^2	Yes	11.4	79.1	50.1	-	
			Washed	40mg/m^2	Yes	44.2	79.2	43.5	-	
		An. gambiae	Unwashed	20mg/m^2	Yes	21.1	67.9	72.0	3.5	[74]
		An funestus	Unwashed	20mg/m^2	Yes	32.7	69.3	70.6	8.4	
		An. gambiae	Washed	20mg/m^2	Yes	0	29.9	69.6	6.0	
		An funestus	Washed	20mg/m^2	Yes	7.7	9.9	58.4	4.8	
Permethrin	Tanzania	*An. arabiensis*	Unwashed	200mg/m^2	Yes	33.7	72.0	49.8	-	[75] [+]
			Unwashed	200mg/m^2	No	20.6	61.0	41.9	-	
			Unwashed	80mg/m^2	No	10.6	71.2	-	28.3	[76] [c]
		An. arabiensis	Unwashed	25mg/m^2	Yes	35.3	85.8	15.2	5.9	[72]
			Unwashed	200mg/m^2	No	57.1	75.0	89.0	27.0	[76]
			Unwashed	1000mg/m^2	No	66.6	63.0	70.0	56.0	
		An. gambiae & An funestus	Unwashed	200mg/m^2	Yes	38.7	97.8	46.3	-	[73]
			Unwashed	200mg/m^2	Yes	20.5	82.2	29.8	-	
	Kenya	*An. gambiae*	Unwashed	500mg/m^2	No	15.0	83.9	-	50.8	[97] [γ]
		An. arabiensis	Unwashed	500mg/m^2	No	0	66.7	-	13.9	
		An. funestus	Unwashed	500mg/m^2	No	35.7	85.9	-	49.6	
		An. gambiae s.s	Unwashed	500mg/m^2	No	94.6	-	-	-	[98] [γ]
		An. funestus.	Unwashed	500mg/m^2	No	96.7	-	-	-	
	The Gambia	*An. gambiae s.l.*	Unwashed	5mg/m^2	Yes	33.0	96.3	74.0	2.0	[77] [λ]
		An. gambiae s.l.	Unwashed	50mg/m^2	Yes	45.1	98.2	75.0	4.0	

		An. gambiae s.l.	Unwashed	500mg/m^2	Yes	69.9	98.7	79.0	10.0	
Lambda Cyhalothrin	The Gambia	*An. gambiae s.l.*	Unwashed	25mg/m^2	Yes	33.3	97.8	89.0	0	[77] ʎ
	Tanzania	*An. gambiae & An funestus*	Unwashed	10mg/m^2	Yes	33.6	63.3	71.4	-	[96]
			Washed	10mg/m^2	Yes	31.8	54.8	61.3	-	
			Unwashed	20mg/m^2	Yes	32.6	63.3	74.8	-	
			Washed	20mg/m^2	Yes	23.0	62.3	56.0	-	
		An. gambiae s.l.	Unwashed	18mg/m^2	Yes	26.4	96.1	98.5	10.7	[30]
Deltamethrin	The Gambia	*An. gambiae s.l*	Unwashed	25mg/m^2	Yes	11	93	88	-	[78]
			Washed	25mg/m^2	Yes	-	87	74	-	
			Unwashed	500mg/m^2	Yes	60	98	72	-	
			Washed	500mg/m^2	Yes	-	87	54	-	
			Unwashed	25mg/m^2	Yes	22	98	86	-	
			Washed	25mg/m^2	Yes	0	87	87	-	
	Tanzania	*An. arabiensis*	Unwashed	25mg/m^2	Yes	30.7	81.4	33.0	2.5	[72]
		An. gambiae	Washed	25mg/m^2	Yes	22.5	89.0	69.0	6	[69]
			Unwashed	25mg/m^2	No	0	90.3	83.9	-	[70]
			Washed	25mg/m^2	No	0	91.2	70.2	-	
		An. gambiae & An funestus	Washed	25mg/m^2	No	0	95.2	88.0	-	

¢ This table includes a section of studies conducted in Africa, in areas where no resistance against DDT or pyrethroids had been reported. In studies where parameter values were not explicitly stated in the original publication, these values have been calculated from summary tables given in those original publications. ***Deterrence*** is calculated as the difference between number of mosquitoes entering treated huts and number entering control huts and is presented as a percentage of the number entering the control hut. ***Feeding inhibition*** is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed. For purposes of uniformity, this formula was also applied to recalculate feeding inhibition for those studies where the authors had originally corrected the percentage feeding rates in treatment huts on the basis of feeding rates in control huts e.g. in Tungu *et al*., 2010 [69]. ***Toxicity*** on the other hand has been calculated as the percentage of mosquitoes entering the treated hut that die and ***excess exit*** is derived as the difference between percentage exit rates in sprayed and unsprayed huts, based on values presented in the original publications.

δ In the study by Mosha *et al* 2008 [72], the percentage mortality observed among mosquitoes collected in control huts was greater than 20%, therefore the toxicity values represented here are statistically corrected percentages.

+ In studies by Lines *et al* 1985 and Lines *et al* 1987, the vector species are reported as *An. gambiae s.l.* though the original publications also had statements indicating that these mosquito populations were almost entirely *An. arabiensis* [75, 76].

c Results represented in this raw from the study by Lines *et al* [76] were obtained from tests of nets made of cotton rather than polyester as used in the rest of the studies

ʎ Deterrency and feeding rates in the Lindsay *et al.,* 1991 paper were recalculated, by subjecting the log numbers presented in the original publication to a microsoft excel function (z = IMEXP) that returns the actual number of mosquitoes (z) as an exponential of complex numbers originally in x + y_i or x + y_j format.

ɣ In the studies by Mathenge *et al.,* 2001[97] and Bogh *et al*., 1998[98], the data used was based on pyrethrum spray catches done inside local huts and also from catches of exiting mosquitoes trapped using Colombian curtains [57] installed around village huts that were allocated (or not allocated) nets.

Table S3: Properties of different long lasting insecticidal nets (LLINs) commonly used in Africa, on mosquitoes that enter or those that attempt to enter human occupied huts. The effects are classified as deterrence, feeding inhibition, toxicity, and excess exit [¢].

Type [¥]	***Insecticide***	***Country***	***Major Vector***	***Washing***	***Holes***	**Deterrence (%)**	**Feeding inhibition (%)**	**Toxicity (%)**	**Excess % exit**	**Reference** [r]
PermaNet 2.0™	Deltamethrin	Tanzania	*An. gambiae*	Unwashed	Yes	20.6	90.0	95.0	0	[69]
				Washed	Yes	18.9	91.0	85.0	2	
			An. gambiae	Unwashed	No	0	93.0	97.7	-	[70]
				Washed	No	0	96.4	96.6	-	
			An. gambiae &	Unwashed	No	0	93.4	85.5	-	[70]
			An funestus	Washed	No	0	98.2	93.0	-	
PermaNet 3.0™	Deltamethrin	Tanzania	*An. gambiae*	Unwashed	Yes	41.2	97.0	95.0	0	[69]
				Washed	Yes	22.8	90.0	94.0	0	
Interceptor™	Alpha cypermethrin	Benin	*An. gambiae s.l*	Unwashed	No	22.5	90.0	95.0	22.5	[71] [ß]
				Washed	No	22.5	90.0	95.0	22.5	
		Tanzania	*An. gambiae*	Unwashed	No	0	88.0	93.0	15.0	
				Washed	No	0	82.0	73.0	15.0	
			An. funestus	Unwashed	No	0	-	76.0	-	
				Washed	No	0	86.0	60.0	-	
			An. gambiae s.l.	Unwashed	No	-	93.0	88.0	-	
				Washed	No	-	79.0	84.0	-	
			An. funestus	Unwashed	No	-	67.0	-	-	
				Washed	No	-	61.0	96.0	-	
Olyset™	Permethrin	Tanzania	*An. arabiensis*	Unwashed	Yes	0	96.3	11.8	25.6	[72] [δ]
			An. gambiae &	Unwashed	No	5.4	87.2	56.0	-	[73]
			An funestus	Unwashed	No	0	90.3	55.0	-	
				Washed	No	0	97.2	70.0	-	
				Unwashed	No	0	80.4	49.0	-	
			An. gambiae	Unwashed	Yes	0	40.9	62.7	7.2	[74]
			An funestus	Unwashed	Yes	28.9	49.9	73.9	1.4	
			An. gambiae & An funestus	Washed	No	0	81.1	57.5	-	[73] [µ]
			An. gambiae	Washed	Yes	0	0	40.0	5.9	[74] [µ]
			An funestus	Washed	Yes	30.8	0	58.9	4.2	

[¢] This table includes a section of studies conducted in Africa, in areas where no resistance against DDT or pyrethroids had been reported. In studies where parameter values were not explicitly stated in the original publication, these values have been calculated from summary tables given in those original publications. ***Deterrence*** is calculated as the difference between number of mosquitoes entering treated huts and number entering control huts and is presented as a percentage of the number entering the control hut. ***Feeding inhibition*** is calculated as the percentage of all mosquitoes entering the treated huts that do not manage to feed. For purposes of uniformity, this formula was also applied to recalculate feeding inhibition for those studies where the authors had originally corrected the percentage feeding rates in treatment huts on the basis of feeding rates in control huts e.g. in Tungu *et al*., 2010 [69]. ***Toxicity*** on the other hand has been calculated as the percentage of mosquitoes entering the treated hut that die and ***excess exit*** is derived as the difference between percentage exit rates in sprayed and unsprayed huts, based on values presented in the original publications.

[¥] **PermaNet 2.0**™ is a 100% polyester LLIN coated with 55-62mg of synthetic deltamethrin per square metre. **PermaNet 3.0**™ on the other hand is a mosaic-style LLIN specifically designed for the control of insecticide resistant mosquito populations. Its side panels, which unlike PermaNet 2.0™ have strengthened borders, are made of deltamethrin-coated-polyester (with approximately 118 mg/m^2 of deltamethrin), while the top panel is made of monofilament polyethylene fabric into which a higher dose of deltamethrin (approx. 180 mg/m^2) and approximately 1100mg/m^2 of a synergist, piperonyl butoxide (PBO) are incorporated. This synergist inhibits mixed function oxidases, which are known to be associated with pyrethroid resistance. PermaNet 3.0™ is also manufactured by Vestergaard Frandsen, Denmark. **Interceptor™** is a long lasting insecticidal net made of polyester coated with alpha cypermethrin (200mg/m^2). It is manufactured by BASF, Germany. Finally, **Olyset**™ is made of a polyethylene netting (150 deniers), that is impregnated during manufacture with synthetic permethrin at a concentration of 2% (equivalent to 1000mg of active ingredient per square metre). It is manufactured by A to Z company, Tanzania.

[ß] The results for Interceptor™ nets evaluation in Benin are reported in the WHO report in very general terms as follows: high mortality (above 95%), high blood feeding inhibition (above 90%), 15-30% deterrence and 10-35% increase in exophilly [71]. Values reported in this table are therefore estimated as minimum mortality (95%) minimum feeding inhibition (90%), mean deterrence (22.5%) and mean excess exit (22.5%).

[δ] In the study by Mosha *et al* 2008 [72], the percentage mortality observed among mosquitoes collected in control huts was greater than 20%, therefore the toxicity values represented here are statistically corrected percentages.

[µ] The data represented in these specific rows were collected from studies where the Olyset™ nets tested had already been in use for 4 years [73] or 7 years [74].

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