Conditions for use of long-lasting insecticidal nets treated with a pyrethroid and piperonyl butoxide

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WHO Evidence Review Group Meeting report
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SUMMARY

Long-lasting insecticidal nets (LLINs) treated with a pyrethroid insecticide and the synergist piperonyl butoxide (PBO) have become available. Two of these nets have a WHO Pesticide Evaluation Scheme (WHOPES) interim recommendation as LLINs. Moreover, the technology of combining a pyrethroid with PBO on LLINs for use in areas of substantive pyrethroid resistance was considered a new paradigm by the WHO Vector Control Advisory Group.

PBO is not an insecticide. It acts by inhibiting certain metabolic enzymes (e.g., mixed-function oxidases [MFOs]) within the mosquito that detoxify or sequester insecticides before they can have a toxic effect on the mosquito. In the presence of PBO, the pyrethroid on the LLINs can still have a toxic effect on mosquitoes even if they harbour resistance mutations that affect these metabolic enzymes. However, due to the wide variation in insecticide resistance levels and mechanisms in malaria vector species, the expected increase in the impact of LLINs treated with a pyrethroid and PBO (referred to as PBO LLINs below) over that of pyrethroid-only LLINs on entomological and epidemiological outcomes is not yet well defined.

A meeting of experts, the Evidence Review Group (ERG), was therefore convened by the WHO Global Malaria Programme (GMP) in September 2015 to review the evidence base on PBO LLINs and to make recommendations on the conditions that would govern their use. This information will be used to prepare WHO guidance for Member States.

Conclusions

The meeting reached consensus on the current knowledge on PBO LLINs for malaria control (see Box 1), and concluded that:

1. While PBO LLINs appear to have an increased efficacy in certain settings, at this point, the evidence is still limited to justify a complete switch from pyrethroid-only LLINs to PBO LLINs across all settings.

2. PBO LLINs with a WHOPES interim or full recommendation can be considered to be at least an equivalent option to other LLINs in all settings, and probably superior in some settings. However, there is neither evidence to assume their higher efficacy nor greater utility in a resistance management strategy in all settings.

3. PBO LLINs should be used only where universal coverage with effective vector control (LLINs and/or indoor residual spraying – IRS) of populations at risk of malaria will not be reduced, as PBO LLINs may be more expensive than pyrethroid-only LLINs.

4. Due to the potential for an antagonistic effect between PBO and organophosphates, PBO LLINs should not be used in areas programmed for IRS with pirimiphos-methyl CS. Further data to confirm or disprove any antagonistic effect should be collected promptly.

5. In order to build the evidence base that would support accelerated deployment of PBO LLINs, pilot “exploratory” implementation is necessary. However, it should only be undertaken in areas where prevalence of malaria...
in children aged 2–10 years is > 20% and mosquito mortality in bioassay with pyrethroids is < 80%. Pilot implementation should not be undertaken unless it is accompanied by robust evaluation.

6. In order to guide potential deployment of PBO LLINs, countries considering pilot exploratory implementation should be supported to:

(i) collect data on the presence, level, intensity and mechanisms of resistance to all insecticide classes at representative sentinel sites;

(ii) design an evaluation with appropriate indicators based on detailed guidance.

7. To manage insecticide resistance, in addition to the rotational use of insecticides with different modes of action in IRS, WHO urgently calls for the development and evaluation of non-pyrethroid LLINs and other innovative vector control tools for use across all settings.

**Recommendations and rationale**

The following are recommendations that should be subject to periodic review as additional results become available from ongoing community cluster randomized effectiveness trials, WHOPES phase–III trials, economic analyses and from pilot “exploratory” implementation of PBO LLINs with concurrent robust evaluations. The first review should be conducted within 12 months of publication of this report.

1. While PBO LLINs appear to have an increased efficacy in certain settings, at this point, the evidence is still limited to justify a complete switch from pyrethroid-only LLINs to PBO LLINs across all settings.

While entomological end-points provide some indication that PBO LLINs may be more effective than pyrethroid-only LLINs in some settings, the limited evidence at this point does not justify a complete switch from pyrethroid-only LLINs to PBO LLINs across all settings. Box 1 summarizes the quality of the current evidence.

2. PBO LLINs with a WHOPES interim or full recommendation can be considered to be at least an equivalent option to other LLINs in all settings, and probably superior in some settings. However, there is neither evidence to assume their higher efficacy nor greater utility in a resistance management strategy in all settings.

There is no evidence or reason to suspect that PBO LLINs are inferior in efficacy to pyrethroid-only LLINs.

3. PBO LLINs should be used only where universal coverage with effective vector control (LLINs and / or IRS) of all persons at risk of malaria will not be reduced.

The evidence for greater efficacy of PBO LLINs is not yet adequate to justify any reduction in overall LLIN coverage by allocating available funds to purchase fewer, more expensive nets.

4. Due to the potential for an antagonistic effect between PBO and organophosphates, PBO LLINs should not be used in areas programmed for IRS with pirimiphos–methyl CS.

Consideration should also be given to a potential antagonistic effect between PBO and IRS with the organophosphate pirimiphos–methyl, but this possibility will require further research. Until experimental hut data are available to confirm or disprove any
antagonistic effect, control programmes are advised not to use PBO LLINs in areas earmarked for IRS with pirimiphos-methyl CS.

5. In order to build the evidence base further and support accelerated deployment, initial pilot “exploratory” implementation of PBO LLINs should be supported in specific areas, linked to robust evaluation.

Areas in which pilot exploratory implementation could be considered are those for which current evidence suggests that PBO LLINs may be more effective than pyrethroid-only LLINs, i.e. where the prevalence of malaria in 2–10-year olds is > 20% and mosquito mortality in bioassays with pyrethroids is < 80%. Fig. 8 shows mosquito mortality in bioassays with pyrethroids and PBO plus pyrethroids that would indicate an area that might be appropriate for pilot “exploratory” implementation.

Pilot “exploratory” implementation should be done as follows:

1. Implementation should be undertaken only if accompanied by robust evaluation and/or operational research. In many cases, this will require greater collaboration between research institutes and national malaria control programmes. Details of recommended evaluation approaches are given in the main body of this report.

2. Ongoing WHOPES phase-III trials of PBO LLINs, provide a unique opportunity to gather additional data that may prove to be useful to build the evidence base further. This would include requesting samples of PBO LLINs and positive controls (pyrethroid-only LLINs) for testing in cone bioassays with well-characterized laboratory strains with metabolic resistance as well as against known susceptible strains. If feasible, wild female mosquitoes collected indoors in rooms and possibly from specially fitted window traps in on-going WHOPES phase-III trials could be used, coupled with molecular characterization and assessment of their knockdown and delayed mortality.

3. Furthermore, data from evaluations of the differential impact of PBO LLINs compared to pyrethroid-only LLINs as the physical integrity of the net deteriorates should be made available – preferably from the ongoing cluster randomized trial run for at least 2 years, and ideally 3 years.

6. In order to guide potential deployment of PBO LLINs, countries considering pilot exploratory implementation should be supported to:

   (i) collect data on the presence, level, intensity and mechanisms of resistance to all insecticide classes at representative sentinel sites; and

   (ii) design an evaluation with appropriate indicators, based on detailed guidance.

It is essential that countries collect data on insecticide resistance in malaria vectors in different eco-epidemiological settings to allow broad vector control decision-making.

7. There is a need for next generation of non-pyrethroid LLINs to manage insecticide resistance.

The threat of insecticide resistance is real, and has huge potential to undermine progress in malaria control. Management of insecticide resistance currently relies on the rotational use in IRS of insecticides with different modes of action. The ERG therefore calls for the urgent development and evaluation of non-pyrethroid LLINs and other innovative vector control tools, for use in all settings.
BOX 1
QUALITY OF CURRENT EVIDENCE

1. Laboratory data on the comparative efficacy of a pyrethroid-only LLIN versus a PBO LLIN against pyrethroid-resistant Anopheles spp. are available from 28 studies, providing 137 data points. These bioassay data show that PBO LLINs can kill most resistant mosquito strains, except those with very high resistance and with mechanisms unaffected by PBO.

2. Semi-field data from nine experimental hut trials support this finding, although few were conducted in areas of documented high insecticide resistance, and data were available only for An. gambiae s.l.

3. While there appears to be some correlation between the data from the bioassays and the limited experimental hut data, at this point, the ERG does not recommend reliance on predictions (made by modelling) of the entomological or epidemiological impact of PBO LLINs.

4. Data are available from six village trials with entomological, but not epidemiological, outcomes. Two of the trials are not yet completed. The results correlated to some extent with the modelling predictions, although there are too few trials to confirm the accuracy at village level of the predictions from resistance bioassay data.

5. No data are available from high-quality cluster randomized trials of the epidemiological impact of PBO LLINs. One trial is under way, and results from the first year will be available in early 2016. It is important that this trial continues for at least 2 and preferably 3 years in order to answer the outstanding questions in point 6.

6. The following outstanding questions were identified as important:

   i. Do the bioassay data correlate with the results of experimental trials in all scenarios of vector susceptibility and transmission levels, and how is the correlation affected by the number of data points available?

   More data points in areas of high resistance would be particularly useful. Initial results from one trial in the United Republic of Tanzania give cause for concern, as they suggest that the greater efficacy of pyrethroids in bioassays might not be correlated with better performance of PBO LLINs in experimental huts. The mosquito numbers were low, and further trials should be conducted during the mosquito season.

   ii. What is the relative performance of PBO LLINs and pyrethroid-only LLINs as each type of net ages?

   This question arises for two reasons. The first is concern about how long PBO will be released during field use, given that the level of PBO in one product with WHOPES interim recommendation remained stable after 10 laboratory washes, indicating that no PBO was being released after 10 washes and therefore was not bioavailable on the net surface. The second reason is the prediction that a PBO LLIN may be more effective than a pyrethroid-only LLIN only once the LLINs are old and damaged, when the physical barrier is less effective. This is a key point for predicting the potential additional benefit of PBO LLINs. Experimental hut data on LLINs of different ages would help answer this question. However, it is only in cluster randomized trials that a definitive answer will be possible.

   iii. Do PBO LLINs have a greater impact on the epidemiology of malaria than pyrethroid-only LLINs, and how much additional impact do they have?

   The ERG experts agreed that the evidence for a correlation between bioassay results, experimental hut results and epidemiological impact is not yet strong enough to justify epidemiological predictions from models based on experimental data. This question can therefore be answered only by more evidence for a strong correlation between bioassay, experimental hut and epidemiological outcomes, and/or direct epidemiological data from the ongoing cluster randomized trial.

   iv. Does the presence of PBO on LLINs reduce the efficacy of IRS with pirimiphos-methyl CS?

   The biological reason for this concern is based on bioassay data with non-malaria vector mosquitoes and other insects. Nevertheless, it is not certain that these bioassay data would translate into a meaningful effect in the field.
1 BACKGROUND

The rapid scale-up in coverage with long-lasting insecticidal nets (LLINs) has significantly contributed to the marked reductions in the numbers of malaria cases and deaths seen in the past 15 years. All LLINs currently recommended by WHO are treated with either a pyrethroid insecticide alone or with a pyrethroid plus the synergist piperonyl butoxide (PBO). However, pyrethroid resistance is now widespread in malaria vectors in many countries where this disease is endemic and has increased rapidly in intensity in some areas. Such increases in the distribution and intensity of pyrethroid resistance may jeopardize the significant gains made in malaria control.

LLINs treated with a single or multiple non-pyrethroid insecticides or a combination of pyrethroid and non-pyrethroid insecticides are urgently needed. These are being developed or evaluated and will be of utility in a broader resistance management strategy.

Currently available are LLINs treated with a pyrethroid insecticide and PBO. PBO is not an insecticide. It acts by inhibiting some metabolic enzymes in the mosquito that detoxify or sequester insecticides so that they have no toxic effect on the mosquito. These enhanced metabolic enzymes (i.e. certain MFOs) represent some of the strongest mechanisms of resistance. In the presence of PBO, the pyrethroid insecticide on an LLIN is still toxic to mosquitoes that harbour these resistance mechanisms. Because of the wide variation in insecticide resistance status and mechanisms in malaria vector species, the expected impact of PBO LLINs on entomological and epidemiological indices is not yet well defined.

1.1 The problem of insecticide resistance

Insecticide resistance in malaria vectors has spread geographically and increased in intensity in recent years, particularly to pyrethroid insecticides. Fig. 1 shows the currently known occurrence of pyrethroid resistance globally (WHO, 2015a).

Initial results of an on-going five-country trial of the impact of pyrethroid resistance on the effectiveness of pyrethroid LLINs (and IRS) were presented to the ERG (presentation 3, Annex 2). While insecticide resistance is an increasingly important problem for malaria control, the initial results of this trial do not indicate that pyrethroid resistance has significantly diminished the epidemiological impact of LLINs. While the final results are expected in 2016, it should be noted that in this trial, only the insecticide resistance frequency and not the intensity were analysed.

The ERG noted that there is no definitive example of failure of LLINs to provide adequate protection in the presence of pyrethroid resistance. The urgency to deploy a tool which may be potentially expensive and yet lacking definitive evidence of a substantially better impact than a pyrethroid-only LLIN in all settings was questioned. The situation would be different if the tool acted at a different target site from pyrethroids, thus representing a true alternative for use in a resistance management strategy. It is emphasized, however, that effective alternative insecticide resistance management tools are an urgent priority in order to mitigate and/or manage the potential failure of LLINs.
1.2 PBO LLINs and other potential new LLIN types

As PBO inhibits the oxidase enzymes that detoxify or sequester pyrethroids before they have a toxic effect on the mosquito, LLINs with both PBO and a pyrethroid might be more effective than LLINs with pyrethroids alone, particularly in areas where the mosquito population has developed this type of resistance.

Two PBO LLINs have already received WHOPEES interim recommendation: PermaNet® 3.0 and Olyset® Plus. With the WHOPEES recommendation, these products can be procured as standard LLINs. Whether the PBO component effectively improves malaria control as compared with pyrethroid-only LLINs in areas of moderate or high insecticide resistance has not yet been determined.

Other LLIN types are under development that, unlike PBO LLINs, will have one or more active ingredients with different modes of action. It is hoped that when these LLINs become available they will be used as part of a resistance management strategy. Development and evaluation of such LLINs should be a priority, in line with the recommendation of the Malaria Policy Advisory Group to WHO in their report in March 2015 (WHO, 2015b).

1.3 The cost of PBO LLINs

Estimates provided by manufacturers and procurers indicate that PBO LLINs are currently sold at a higher price than pyrethroid-only LLINs (see also section 3.3). There is currently a major funding gap in malaria control, with only about half the estimated global funding needs currently being met through commitments (Table 1). Hence paying a higher price with a fixed LLIN commodity budget would mean lower LLIN coverage rates. Procurement negotiations should work towards substantially reducing the price margin of PBO LLINs to avoid coverage losses while enabling deployment.
of PBO LLINs in areas where they are likely to have improved effectiveness. Cost-effectiveness modelling analysis could help to determine thresholds and conditions where any improved effectiveness of PBO LLINs may outweigh the additional cost.

TABLE 1.
Financial and LLIN commodity needs and gaps for malaria control in Africa, 2015–2017. B = billion; M = Million

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
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<tbody>
<tr>
<td>Overall Africa needs and gaps in USD</td>
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<tr>
<td>Need</td>
<td>3.42 B</td>
<td>3.24 B</td>
<td>3.46 B</td>
</tr>
<tr>
<td>Financed</td>
<td>2.38 B</td>
<td>1.69 B</td>
<td>1.12 B</td>
</tr>
<tr>
<td>Gap</td>
<td>1.03 B</td>
<td>1.55 B</td>
<td>2.33 B</td>
</tr>
<tr>
<td>LLIN commodity gap</td>
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<td>Need</td>
<td>255.6 M</td>
<td>185.4 M</td>
<td>216.9 M</td>
</tr>
<tr>
<td>Financed</td>
<td>206.0 M</td>
<td>112.3 M</td>
<td>54.5 M</td>
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<tr>
<td>Gap</td>
<td>49.6 M</td>
<td>74.1 M</td>
<td>162.4 M</td>
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</tbody>
</table>

Source: RBM harmonization working group

1.4 Preparation of the Evidence Review Group meeting

The mandate of the WHO Vector Control Advisory Group, created in 2013, is to review and assess the public health value and “proof of principle” (epidemiological impact) of new tools, paradigms, approaches and technologies and to make recommendations on their use for vector control in the context of integrated vector management of one or several diseases.

The Group has reviewed 16 submissions, including several prototypes with specific claims, and recognized eight new paradigms for vector control. One submission was for a PBO LLIN (PermaNet® 3.0) which was developed with the aim of having improved efficacy in areas with substantive pyrethroid resistance. In November 2014, the Group supported the modest claim of the manufacturers that this net had increased bio-efficacy compared with pyrethroid-only LLINs in areas where malaria vectors have P450-based metabolic resistance mechanisms that reduce the efficacy of pyrethroid-only LLINs. It recommended that the WHOPES phase-III evaluation proceed towards full recommendation (WHO, 2014).

In March 2015, the Group’s recommendations were presented to the WHO Malaria Policy Advisory Committee, after input from the Vector Control Technical Expert Group. The Advisory Committee considered that the evidence presented was not strong enough for unconditional recommendation for deployment of PBO LLINs, and asked the GMP to consolidate all the available data and to commission new studies if necessary, in order to identify areas and conditions in which PBO LLINs could be deployed (WHO, 2015c). The GMP consolidated the evidence and constituted the independent Evidence Review Group (ERG) which met in September 2015.
2 OBJECTIVES

The specific objectives of the ERG were to:

- review the available field and laboratory data on the effectiveness of nets treated with a pyrethroid and the synergist PBO;
- identify the field conditions under which PBO LLINs could be deployed; and
- make recommendations on the operational requirements for deployment of PBO LLINs.

3 EVIDENCE REVIEWED

Brief summaries of the evidence presented are given here, comprising the methods used and key findings reported. The findings do not necessarily represent the views of the ERG. The key conclusions made by the ERG are provided in a box at the end of each summary.

3.1 Identification of geographical areas in which LLINs treated with a pyrethroid plus PBO might be more effective than pyrethroid-only LLINs

Methods

The evidence presented included insecticide susceptibility bioassays using standard procedures (i.e., WHO tube tests, CDC bottle bioassays), and net bioassays (WHO cone tests), experimental hut trials and village studies with PBO LLINs (Annex 4). The data points were used to:

- compare mosquito mortality in bioassays with pyrethroid-only LLINs versus in bioassays with PBO LLINs;
- compare mosquito mortality in experimental huts with pyrethroid-only LLINs versus those with PBO LLINs;
- analyse the relationship between bioassay results and experimental hut results to determine whether bioassay data alone are sufficient to predict the results of experimental hut trials; and
- predict the differential impacts of PBO LLINs and pyrethroid-only LLINs in contexts with different malaria prevalence, LLIN coverage and level of insecticide resistance.

Findings of the review

- Appropriate evidence is available from 28 bioassays, providing 137 data points (see Annex 4, Table 4.1). The assays included WHO tube tests and CDC bottle bioassays, in which the mosquitoes were exposed to PBO before exposure to pyrethroid, and WHO cone tests in which exposure to PBO and a pyrethroid was simultaneous.
  - The results (Fig. 2) show that, in certain settings, PBO can increase the efficacy of pyrethroids against pyrethroid-resistant mosquito strains.
• In *An. funestus*, susceptibility to pyrethroids appeared to be almost completely restored by PBO, regardless of the level of resistance in the population. This is to be expected, given that MFO resistance is the only mechanism so far observed in *An. funestus*.

• In *An. gambiae* s.l., PBO appears to increase the pyrethroid susceptibility of strains with moderate resistance. The greatest differential impact is seen for mosquito strains that have a mortality rate of 40–80% in bioassays without PBO. PBO LLINs do not appear to restore the susceptibility of mosquitoes with very high resistance.

• No data for other mosquito species were presented.

• While the resistance mechanism (see Annex 3) probably plays a role in the effect of exposure to PBO on mosquito mortality, the data suggest that confirmation of the resistance mechanism is not necessary for predicting the differential impact of PBO on bioassay results. Evidence of the resistance mechanism was not considered for the 137 data points; rather, the data were used to show that the overall level of mortality in a bioassay can be used to predict whether PBO LLINs have a useful differential impact relative to pyrethroid-only LLINs.

• Data from experimental hut studies comparing PBO LLINs with pyrethroid-only LLINs are available from nine studies in seven countries at 10 sites (Annex 4, Table A4.2).

  • These data are available only for *An. gambiae* s.l. There have been only limited attempts to identify members of the *An. gambiae* group of species that are known to show behavioural heterogeneity in hut trials.

  • The results of the studies support the suggestion of the bioassay results that PBO increases the efficacy of LLINs in areas of moderate insecticide resistance.

  • There are too few studies, particularly in areas with very high pyrethroid resistance.

  • There appears to be a correlation between the results of bioassays and of experimental hut trials (Fig. 3), although the correlation is not strong.

• Data were available from six village trials with entomological end-points that were of varying quality and conducted with different methods, preventing direct comparisons. Four of the six trials found higher efficacy of PBO LLINs compared to pyrethroid-only LLINs. Three of these were in areas with moderate resistance, and the fourth was in an area of very high resistance for which the model predicted little additional benefit of PBO LLINs. In fact, little benefit seen in this trial was a result of less blood-feeding in the presence of PBO LLINs at one trial site, rather than of an increase in mosquito mortality linked to PBO LLINs. Of the two village studies that did not show a benefit of PBO LLINs, one is still under way and is in an area of high resistance; initial reports suggest that pyrethroid susceptibility is not restored by PBO, as observed in bioassays. The second study, in Malawi, had inconclusive initial results and is also still under way. Data from these studies are expected to be available in early 2016.

• A model was developed by extrapolation from bioassay data and was used to demonstrate correlation between bioassays and experimental hut trials data. Assumptions were made about the impact of mosquito behaviour in experimental hut trials on malaria transmission dynamics. The model supported a potential impact of PBO LLINs on entomological inoculation rates and clinical incidence (parasitologically confirmed cases per 1000 population). When parameters including levels of insecticide resistance, LLIN coverage
and malaria prevalence were varied, the model predicted that additional benefits in the absolute number of cases averted would be greatest in areas of moderate insecticide resistance and high malaria prevalence (Fig. 4). The incremental effectiveness of PBO LLINs over pyrethroid-only LLINs was predicted to be higher as LLIN coverage rates increased.

FIG. 2
Bioassay results from 28 studies (114 points) showing An. gambiae s.l. mosquito mortality in bioassays without and with PBO. Green, WHO tube; red, WHO cone; blue, CDC bottle. Solid line shows the best fit relationship.

FIG. 3.
Correlations between mosquito mortality in WHO tube bioassays and experimental hut trials. Solid line shows best fit relationship. Point colour indicate mosquito species, be it An. gambiae s.l. (purple) or An. funestus (orange).
FIG. 4. Three-dimensional relations between endemicity (x axis), mosquito mortality in bioassays (y axis) and the reduction in predicted clinical incidence (coloured contours) with the introduction of PBO LLINs at a coverage rate of 80%. (A), (B) and (C) show the absolute differences in incidence (per 1000 people per year), while (D), (E) and (F) show the percentage reduction. The different panels show different mosquito species.

An. funestus

An. arabiensis

An. gambiae s.s.

Reduction in clinical incidence with PBO LLIN (cases per 1000 per year)

Percentage reduction in clinical incidence with PBO LLIN
Limitations reported in the studies

- Durability studies should be conducted to determine the life-span (physical integrity and bio-availability) and changing efficacy of PBO LLINs and of pyrethroid-only LLINs.

- Studies with epidemiological end-points would be useful, although it is likely that most cases of malaria will be in people who do not use LLINs; therefore, in areas or trials with high LLIN coverage rates, large sample sizes will be required to measure accurately the differential impact of pyrethroid-only LLINs versus PBO LLINs.

Further supporting evidence could be obtained from well-designed, sufficiently powered studies.

BOX 2
KEY CONCLUSIONS OF THE ERG

- A large number of bioassays confirm that PBO can increase the efficacy of pyrethroids in all but highly resistant mosquito populations.

- Some data from experimental hut studies also suggest that PBO can increase the efficacy of pyrethroids, although there are few studies. Very little data from experimental hut studies are available for An. funestus.

- Bioassay data are fairly consistent with those for experimental huts, suggesting that bioassay results might be used to model the effectiveness of PBO LLINs for personal protection. There are, however, currently too few experimental hut studies for sufficient confidence in this correlation. Initial experimental hut results from ongoing studies in the United Republic of Tanzania (section 3.2) do not fit this pattern as the mosquito numbers in these studies were low. These studies should be repeated and reviewed in order to strengthen the evidence for a correlation.

- Modelling data suggest that PBO LLINs provide an additional benefit, except in settings where resistance levels are very high, perhaps because of the presence of additional resistance mechanisms in these situations. It appears most likely that PBO LLINs will confer significant additional protection in areas of moderate-to-high resistance, where oxidase-based mechanisms are important (i.e. where PBO can significantly increase susceptibility in bioassays) and where the malaria prevalence is high. As discussed above, the evidence for this possibility is not yet definitive. While modelling studies can predict areas in which PBO LLINs are most likely to provide additional protection, this approach has not yet been validated.

3.2 Defining strategies for controlling malaria transmission in areas of pyrethroid resistance with PBO LLINs and IRS with pirimiphos-methyl CS

Methods and timeframes

A community cluster randomized trial of the impact of pyrethroid-only and PBO LLINs, with and without IRS using the organophosphate pirimiphos-methyl CS is currently under way in Muleba, United Republic of Tanzania. The four arms are:

i) Olyset® Net
ii) Olyset® Plus
iii) Olyset® Net + IRS with pirimiphos-methyl CS
iv) Olyset® Plus + IRS with pirimiphos-methyl CS.
The trial will have the following outcomes:

- *P. falciparum* prevalence in children;
- the entomological inoculation rate;
- type and frequency of resistance mechanisms (potential for resistance management); and
- cost-effectiveness.

The trial has reached the end of its first year, and the cross-sectional survey that will provide data on epidemiological indicators was completed in August–September 2015. The results are likely to be available in late 2015 or early 2016.

At the same time as the cluster randomized trial, an experimental hut study was conducted in Muheza, United Republic of Tanzania, and also in an area with similar insecticide resistance levels and vectors to those of Muleba. At both sites, pre-exposure to PBO resulted in an increase in the efficacy of pyrethroids.

**Findings reported**

The experimental hut trials showed that although PBO enhanced the efficacy of pyrethroids in bioassays, there was no increase in mosquito mortality with PBO LLINs compared to pyrethroid-only LLINs. The trials were conducted at the end of the rainy season when the mosquito numbers were very low. Experimental hut trials will be repeated in 2016 to determine the robustness of these initial results.

It was postulated that free-flying host-seeking mosquitoes in the experimental huts were not receiving a sufficient dose of PBO to affect their mortality. WHO cone irritability tests were conducted to compare the irritancy of pyrethroid-only LLINs versus PBO LLINs. The results (Fig. 5) suggest that the presence of PBO does result in shorter contact with the LLIN than when only a pyrethroid is present.

**FIGURE 5**

Results of irritability tests. Contact time (time to first take off) in seconds.
3.3 Economic comparison of PBO LLINs with pyrethroid-only LLINs

Methods
This economic evaluation included:

- a cost data search to determine the price of PBO LLINs and pyrethroid-only LLINs on the basis of actual sales and manufacturers’ quotes;
- an analysis to compare the cost-effectiveness of PBO LLINs and pyrethroid-only LLINs, based on the estimates of effectiveness derived by modelling (section 3.1 and Annex 1), and calculation of the incremental cost-effectiveness of PBO LLINs as compared with other vector control interventions; and
- quantification of the potential impact on LLIN coverage of a shift to more expensive PBO LLINs at varied relative PBO and pyrethroid-only LLIN prices.

Findings reported
In the analysis of the cost of PBO LLINs, 11 paired data points were found to support direct comparison of WHO-recommended PBO LLINs and pyrethroid-only LLINs. In all cases, the PBO LLINs were more expensive. This is not surprising, given the high cost of PBO; anecdotal reports suggest that cost is one reason that PBO has not been used for resistance management in agriculture. The price of PBO LLINs was 27–104% higher than that of pyrethroid-only LLINs. Higher price margins were found in the quotations (as opposed to actual transactions). To reduce this margin, while not guaranteed, procurement negotiations may be considered.

The average cost-effectiveness of PBO LLINs was predicted to be better than that of pyrethroid-only LLINs, although not in settings of low endemicity or high insecticide resistance. Fig. 6 illustrates the incremental cost per case averted by use of PBO LLINs in different scenarios. These findings are based on the efficacy data derived by modelling (section 3.1), which predicted a lower additional impact on malaria...
incidence in areas of low endemicity and high insecticide resistance. However, the impact of reduced coverage due to procurement of higher priced PBO LLINs under a fixed budget envelope was not analysed.

Analysis of the impact of procurement of higher-priced PBO LLINs on overall LLIN coverage levels suggested that, if PBO LLINs were 50% more expensive than pyrethroid-only LLINs, about 11.5% LLIN coverage would be lost, if the budget were constant. This result is linked to the fact that delivery cost constitutes an important proportion of the total cost of achieving LLIN coverage; therefore, a 50% increase in the commodity cost does not necessarily lead to a 50% increase in the total cost of LLIN distribution. However, the Global Technical Strategy estimated that the procurement costs for a net was higher than the delivery cost, so with a 50% higher cost for a PBO LLIN there would be an approximate increase of 29% in the cost of a delivered net (WHO 2015b).

FIG. 6
Incremental cost-effectiveness of PBO LLINs with different possible “mark-ups” (price increases as compared with pyrethroid-only LLINs). L, low; M, medium; H, high
3.4 Cost-effectiveness of PBO LLINs and pyrethroid-only LLINs in villages in Nigeria

Methods
In this cost-effectiveness study, a 25-month trial in two villages in Nigeria was the source of efficacy data for the model. The efficacy of the PBO LLINs was estimated as the difference between the average prevalence of confirmed cases of malaria seen in the village that received PBO LLINs versus the village that received pyrethroid-only LLINs. The difference between the two averages was taken to indicate the number of cases potentially averted by PBO LLINs, and the relative reduction was used in cost-effectiveness modelling. The numbers of months during which the PBO LLIN village had fewer cases than the pyrethroid-only LLIN village were compared statistically. Incremental cost-effectiveness, as assessed by the impact on cost per disability-adjusted life-year (DALY) averted, was estimated for contexts with different malaria incidence and assuming levels of 5% to 30% greater effectiveness of PBO LLINs over pyrethroid-only LLINs. The costs for PBO LLIN and pyrethroid-only LLIN were provided by the manufacturer.

Findings of the review

- The incidence of malaria in the PBO LLIN village was determined to be lower than that in the pyrethroid-only LLIN village on the basis of the average number of cases per month and from the statistically significant result of the McNemar sign test used to compare the number of months in which each village had fewer cases.

- PBO LLINs were considered to be cost-effective as a public health tool in all settings. The incremental cost-effectiveness of PBO LLINs over pyrethroid-only LLINs depended on the projected incremental efficacy of PBO LLINs and on the incidence of malaria. It was projected that a PBO LLIN would have to avert an additional 4.1 cases per 1000 population per year in order to be considered highly cost-effective.

- Fig. 7 shows the approach taken to plotting incremental cost-effectiveness for a range of increased levels of effectiveness of PBO LLINs, in settings with different malaria incidences.

Box 4

**KEY CONCLUSIONS OF THE ERG**

- This economic evaluation has important components, particularly the impact of the price of PBO LLINs as compared with pyrethroid-only LLINs on LLIN coverage. Inclusion of the public health impact in a cost-effectiveness analysis of deployment of PBO LLINs would be useful.

- The ERG concluded that the evidence for the efficacy of PBO LLINs is not yet sufficiently robust. Thus, economic analyses cannot yet be used to make recommendations and decisions. This work should, however, be extended to a number of scenarios: to predict the cost and consequences of deploying PBO LLINs in different contexts, and, assuming different degrees of increased efficacy (as yet unproven), to determine the prices that would be justified in different settings. This would allow rapid decision-making once more definitive evidence on the comparative efficacy of PBO LLINs versus pyrethroid-only LLINs in different settings is available.

- Specific prices on PBO LLINs are not relevant to the discussion at this point, given that this is anticipated to change based on demand and/or changes in procurement practices. It is likely that absolute prices will change and therefore the price relative to that of pyrethroid-only LLINs will also change.
BOX 5
KEY CONCLUSIONS OF THE ERG

- The study of efficacy was limited to two villages. The incidence of malaria per month varied considerably, as would be expected in small communities of about 600 people each, and the incidence patterns in the two villages before the intervention were not sufficiently similar to permit a confident conclusion that any difference between the two after the intervention was due solely to the use of PBO LLINs and not to confounding factors or natural variation. A more useful design would comprise more units for comparison randomly assigned to intervention or control.

- The incremental benefits of PBO LLINs over their full 3-year expected life-span have not yet been demonstrated in any trial. This information will be essential for predicting the incremental cost-effectiveness of this tool in comparison with pyrethroid-only LLINs. It is possible that, as LLINs age and their physical condition deteriorates, the reduced physical barrier will result in greater incremental effectiveness of PBO LLINs relative to pyrethroid-only LLINs 2 or 3 years after deployment. However, WHOPES phase-I trials with PBO LLINs suggested that the PBO component does not last as long as the pyrethroid component on all PBO LLINs. Thus, the bioavailability of PBO on PermaNet® 3.0 did not last beyond 10 laboratory washes, although PBO in Olyset® Plus appeared to withstand 20–25 washes. Thus, it is not yet understood whether PBO LLINs are actually more effective than pyrethroid-only LLINs in their third year of life or whether the bioavailability of PBO in fact declines.

- The cost-effectiveness analysis took into account reduced costs for treating malaria, which included not only the cost of drugs but also diagnostics and service delivery. There is concern that this approach overestimates the benefit of a vector control tool such as this, as most programmatic costs will not decrease proportionate to the reduction in the number of malaria cases.

- The analysis included cost-effectiveness at different assumed levels of incremental effectiveness of PBO LLINs. This approach would be useful for future studies in various settings, once stronger evidence becomes available for PBO LLIN effectiveness.

- The conclusions on cost-effectiveness are highly dependent on commodity and delivery costs, with the former being subject to variation as explained in section 3.4.
3.5 VecNet model simulations of the effects of PermaNet® 2.0 and of PermaNet® 3.0 against pyrethroid-resistant *An. gambiae* s.l. transmission of *P. falciparum* malaria

Methods

Two simulation models – the Epidemiologic Model of Disease (v1.8) and OpenMalaria (v32) – were used to analyse malaria transmission in highly specified and in more broadly defined geographical areas (administrative units). The model versions used simulate the impact on transmission of *P. falciparum* only.

Analyses of the efficacy of pyrethroid-only and PBO LLINs were based on the results of experimental hut studies in areas of resistant and susceptible vectors. Data on the area with resistance were from Burkina Faso and those for the susceptible context were from Kisumu, Kenya. The climate, geography and population characteristics in western Kenya were used for the model simulation.

The model included the impact of pyrethroid-only and PBO LLINs on the entomological inoculation rate and the incidence of severe cases, clinical cases and deaths among children under 5 years. The models were run for contexts that differed by: the starting entomological inoculation rate, the proportion of mosquitoes biting indoors, proportion of resistant or susceptible local vectors and rates of LLIN use.

DALYs were calculated as a proxy for projected health improvements. The predicted numbers of cases and DALYs averted were used for a cost-benefit analysis of use of PBO LLINs.

Findings of the review

- Against susceptible mosquitoes, PBO LLINs were predicted to have no benefit over pyrethroid-only LLINs.

- Against resistant populations, PBO LLINs were predicted to have a greater impact than pyrethroid-only LLINs for all outcome measures. The predicted benefit was highest in areas of high transmission (where the starting entomological inoculation rate was 30 or 300) and where over 75% of bites were indoors.

- The results of the OpenMalaria simulation indicate that use of PermaNet® 3.0 against resistant *An. gambiae* s.l. averted more cases, deaths and DALYs due to *P. falciparum* than PermaNet® 2.0 and is highly cost-effective.

BOX 6

**KEY CONCLUSIONS OF THE ERG**

- The predictions of the model for incremental benefits of PBO LLINs are based on the assumption that experimental hut data can predict epidemiological outcomes. The ERG is not convinced that there is as yet sufficient evidence to support this assumption.

- The data on efficacy used in this model were obtained from only two study sites, and hence the representativeness of conclusions considering the wide range of vector resistance profiles in malaria-endemic areas was questioned. Incorporating a wider range of efficacy data into the simulations may be more informative, particularly if data from additional studies supports a clear correlation between experimental hut and epidemiological data.
4 RECOMMENDATIONS AND THEIR RATIONALES

4.1 General considerations

Current sources of evidence

The effectiveness of new malaria control interventions can be estimated in a number of ways. In order of increasing complexity, these are: (i) bioassays in which adult mosquitoes of laboratory strains or local populations are exposed to proposed chemicals or insecticide-treated materials; (ii) exposure of free-flying, host-seeking mosquitoes in experimental huts to insecticide-treated materials; (iii) trials in villages to determine the impact on entomological outcomes of the proposed tool; and (iv) cluster randomized trials to determine the impact on both entomological and epidemiological outcomes of the proposed tool, such as LLINs distributed to individual households or communities.

Evidence from cluster randomized trials is the global standard for establishing the public health value of any new intervention.

Evidence for the efficacy of PBO LLINs is currently limited to 28 bioassay studies, 9 studies conducted in experimental huts and 6 trials in villages, all of which measured only entomological indicators (Annex 4). Data from the first year of the only currently running community cluster randomized trial will be available in early 2016.

The PBO LLINs currently being evaluated by WHOPES are:

- Olyset® Plus: phase-III trial with data collection to end in October 2017 (three sites);
- PermaNet® 3.0: phase-III trial with data collection to end in August 2018 (three sites); and
- Veeralin® LN: phase-II trial with data collection to end in December 2015, and phase-III trials likely to start in late 2016.

A lesson learnt from the review of evidence is that decisions on operationalization of new vector control tools must be based on comprehensive, diverse evidence. It will take time to gather this evidence, thus delaying the introduction of potentially beneficial tools for widespread use. In the future, development of potential new vector control tools should include parallel and sufficiently powered trials in a range of settings as soon as technically possible (e.g. these include randomized by household or community, pilot “exploratory” implementation after completion of phase-II evaluation).

The importance of innovation within the LLIN market

The global malaria control community depends on the development of new products by manufacturers. This continues to be important as the context of malaria transmission and control shifts, with a need to respond to threats such as insecticide resistance. There is therefore a pressing need for LLINs with non-pyrethroids and/or combinations of active ingredients, for more physically durable LLINs, and for tools that use innovative approaches. The ERG is aware that innovation should be supported and rewarded. At the same time, it is important to ensure that fostering innovation and introducing new products does not preclude the requirement for a strong evidence base to inform deployment.
The available evidence provides some indication that PBO LLINs may confer an additional benefit in certain settings, but the available evidence is limited and insufficient for a broad recommendation on their deployment. Nevertheless, there is no indication that PBO LLINs would be inferior in efficacy to pyrethroid-only LLINs. Therefore, if PBO LLINs cost the same as pyrethroid-only LLINs and were of the same field durability, it could be argued that a complete switch to PBO LLINs should be recommended on the basis of anticipated improved efficacy and impact. However, the ERG does not support this argument.

It is hoped that the initial recommendation – to begin pilot exploratory implementation of PBO LLINs in order to build the evidence base across different settings – achieves a balance between the desire to support and encourage innovation and the need for more robust evidence.

Summary of current evidence

- Appropriate evidence is available from 28 bioassay studies, providing 137 data points. These data clearly show that PBO can increase the susceptibility to pyrethroids of resistant mosquito strains to some extent when tested in bioassays, except for those with very high resistance.

- Data from nine experimental hut trials support this finding, although there are few studies, particularly in settings with high insecticide resistance, and data are limited to An. gambiae s.l. only.

- There appears to be some correlation between the results of bioassays and of experimental hut trials, but as the number of experimental hut studies is limited, at this point the ERG is not sufficiently confident to rely on projections of the impact of PBO LLINs based on this correlation in order to extrapolate from bioassay results.

- The six village trials included only entomological outcomes and not epidemiological outcomes. Two are still under way. Some correlation was found with the predictions from modelling, although there are too few trials to convince the ERG that extrapolations from bioassay data to experimental hut trials to village-level impact would be robust.

- No high-quality cluster randomized trials have been completed that clearly demonstrate the epidemiological impact of PBO LLINs. One trial is under way, and the results of the first year of implementation will become available in the coming months. It is important that this trial continue for at least 2 and preferably 3 years to help answer the questions below.

Knowledge gaps

Some important gaps in knowledge about PBO LLINs remain. The main questions identified by the ERG are listed and discussed below.

Do the different bioassay data correlate with the results of experimental hut trials in all scenarios (and strata) of vector susceptibility and transmission intensity, and how is the correlation affected by the number of data points available?

In addition to performing correlation analysis, stratifying the correlation by susceptibility level and transmission level is important as this could reveal trends that may not be apparent if the data is not stratified. For this reason, more data points in areas of high resistance would be particularly useful. However, initial results from the ongoing trial in the United Republic of Tanzania are cause for concern, as they suggest that the mitigation of resistance by PBO seen in bioassays may not translate into better
performance of PBO LLINs in experimental huts. The mosquito numbers were low, and further trials should be conducted during the mosquito season.

What is the relative performance of PBO LLINs and pyrethroid-only LLINs as each type of net ages?

This question arises because:

- There is concern about the residual efficacy of PBO LLINs under field conditions. In at least one product (PermaNet® 3.0), the PBO component was no longer bioavailable in LLINs after they were washed 10 times (WHO, 2008), while the pyrethroid remained bioavailable for at least 20 laboratory washes.
- The predicted greater efficacy of a PBO LLIN than a pyrethroid-only LLIN may be most apparent when the LLINs are old and damaged.

Answers to this question would help predict the additional benefit that PBO LLINs may confer relative to pyrethroid-only LLINs. Experimental hut data on LLINs of different ages would help, but the question may be addressed through continuation of the ongoing cluster randomized trial as well as in WHOPES Phase III trials.

Do PBO LLINs have a greater impact on the epidemiology of malaria than pyrethroid-only LLINs, and how much additional impact do they have?

The ERG agreed that the evidence for a correlation between bioassay results, experimental hut results and epidemiological impact is not yet strong enough to justify epidemiological predictions from models based on bioassay and experimental hut data.

This question can therefore be answered only by: (i) increasing the evidence for a strong correlation between bioassay, experimental hut and epidemiological outcomes, and/or (ii) obtaining direct epidemiological data from cluster randomized trials.

Does the presence of PBO on LLINs reduce the effectiveness of IRS with pirimiphos-methyl CS?

While no data relevant to this point were presented or considered during the ERG meeting, the issue was raised briefly. Further expert opinion was sought after the meeting, and the ERG experts had the opportunity to review and discuss the potential relevance of the issue after the meeting.

Some insecticides (“pro-insecticides”) require activation by specific mosquito enzymes (P450 isozymes) in order to be toxic, and this is the case for the organophosphate pirimiphos-methyl CS. PBO acts by inhibiting P450 enzymes that are responsible for metabolic resistance to pyrethroids, and PBO can therefore reduce the toxicity of pro-insecticides by blocking their metabolic activation (Rider & LeBlanc, 2005). Some bioassay data suggest an antagonistic interaction in some non-malaria vector mosquitoes and other insects (Ankley et al., 1991; El-Merhibi et al., 2004; Raghavendra et al., 2011), but most of the work was conducted for the agricultural industry, and no data were found for mosquito vectors. It should be noted, however, that PBO can also prevent the metabolic enzymes from detoxifying pirimiphos-methyl, therefore resulting in no net antagonistic result.

The biology of mosquitoes and indications from non-malaria vector bioassays suggest that a host-seeking mosquito exposed to PBO on an LLIN that does not die would be less likely to be killed by pirimiphos-methyl CS on the walls of the house or
a neighbouring house. While this is pure speculation, until experimental hut data are available, control programmes should not use PBO LLINs in areas programmed for IRS with pirimiphos-methyl CS.

In the trial in the United Republic of Tanzania (described in section 3.2), PBO LLINs are compared with pyrethroid-only LLINs; the effect of each type of net in conjunction with IRS with pirimiphos-methyl CS is also compared. The first round of experimental hut trials showed no indication of antagonism between PBO and pirimiphos-methyl CS; however, the evaluation was conducted at the end of the mosquito season when there were low mosquito numbers. More experimental hut studies are required to determine whether this issue should be taken into account in an operational setting.

4.2 Proposed recommendations

The following recommendations were proposed by the ERG. These recommendations should be reviewed periodically as additional results become available from cluster randomized effectiveness trials, entomology trials, WHOPES phase-III trials, economic analyses and pilot exploratory implementation.

By early 2016, data for the first year of the cluster randomized trial in the United Republic of Tanzania will be available. Entomological results from trials in Malawi and Mali may also be available in 2016. It is recommended that the evidence be reviewed again, within 12 months of publication of this report.

Recommendation 1: Only pilot “exploratory” implementation of PBO LLINs is supported.

The evidence that PBO LLINs may perform better than pyrethroid-only LLINs in specific settings is so far based largely on experimental hut and laboratory studies on African vectors. There is very limited evidence of effectiveness, or that the effectiveness persists in field settings. The limited evidence does not yet justify a complete switch from pyrethroid-only LLINs to PBO LLINs in all settings at this point, especially if their price is higher. However, the evidence is sufficient to justify limited, pilot “exploratory” implementation of PBO LLINs in Africa or in other regions outside of Africa, if accompanied by robust evaluation of the impact. It should be noted that the evidence for the effectiveness of PBO LLINs available so far relates only to potential increased mortality in An. gambiae s.l.

In order to build the evidence base further, the ERG recommends that pilot exploratory implementation be focused in areas in which PBO LLINs are likely to be effective on the basis of current information from modelling predictions, although the limitations of modelling should be taken into account. The areas in which PBO LLINs are likely to be effective on the basis of available data are where:

- the prevalence of malaria among children aged 2–10 years is > 20%; and
- the mortality rate of local African vectors in WHO tube or CDC bottle bioassays with a pyrethroid is < 80% and has been shown to be increased by pre-exposure to PBO; specifically, the results of the bioassays should fall within the grey area on Fig. 8.

Recommendation 2: PBO LLINs with a WHOPES interim or full recommendation can be considered at least an equivalent option with other LLINs in all settings and probably superior in other settings. However, there is no evidence to assume higher efficacy nor greater utility in a resistance management strategy in all settings.
There is no evidence or reason to suspect that PBO LLINs are inferior in efficacy to pyrethroid-only LLINs. PBO LLINs that have a WHOPES interim or full recommendation can be considered for use in any setting as an LLIN.

**FIG. 8.**

Mortality rates in bioassays with pyrethroids with and without PBO exposure, which should determine whether an area is appropriate for exploratory implementation of PBO LLINs.

Recommendation 3: PBO LLINs should be used only where universal coverage with effective vector control (LLINs and/or IRS) of all people at risk of malaria will not be reduced.

PBO LLINs are currently more expensive than pyrethroid-only LLINs. The evidence for the effectiveness of PBO LLINs is not sufficiently strong to risk a decrease in overall LLIN coverage (WHO 2015d).

Recommendation 4: Further evidence of the impact of PBO LLINs should be generated and reviewed.

It is important that the evidence base on PBO LLINs be built. Evidence could be generated from independent trials (including that currently under way in the United Republic of Tanzania) or from robust evaluations conducted during pilot exploratory implementation.

It is recommended that the following approaches be used to ensure that an adequate evidence base is built.

(a) Pilot exploratory implementation of PBO LLINs should be accompanied by robust evaluation and/or operational research. In many cases, this will require greater collaboration between research institutes and national malaria control programmes. Evaluations should be designed to ensure the following:

i. comparison of PBO LLINs with pyrethroid-only LLINs;

ii. randomization at community level or higher (to ensure that a community effect can be measured);
iii. clustering and intervention randomization take into account and can demonstrate equivalence between areas with PBO LLINs and pyrethroid-only LLINs, with careful determination of the minimum number of clusters required for adequate statistical power; and

iv. outcomes include entomological and epidemiological indicators, i.e.,:
   - epidemiological indicators: incidence of confirmed malaria cases and/or prevalence of infection in 2–10-year olds and/or slide positivity rate; and
   - entomological outcome indicators: entomological inoculation rate and/or sporozoite rate.

It is recommended that research institutes be engaged to provide support in the design and evaluation phases.

(b) WHOPES phase-III trials should be leveraged to answer questions that go beyond their standard requirements and procedures. This would include requesting samples of PBO LLINs and positive control LLINs for testing in cone bioassays with well-characterized Anopheles spp. laboratory strains that have metabolic resistance, as well as with susceptible Anopheles spp. strains. If feasible, testing in WHOPES phase-III trial sites should also include mosquito samples collected indoors (for example, using CDC light traps or knockdown catches alternating with window exit traps) for molecular characterization and assessment of knockdown and delayed mortality. Selection of such sites must ensure that there are high enough densities of the vectors to yield robust results.

The PBO LLINs currently being evaluated in WHOPES are:

- Olyset® Plus: phase-III trial with data collection to end in October 2017 (three sites);
- PermaNet® 3.0: phase-III trial with data collection to end in August 2018 (three sites); and
- Veeralin® LN: phase-II trial with data collection to end in December 2015 and phase-III trials likely to start in late 2016.

(c) In addition to evidence generated by evaluations and operational research accompanying pilot exploratory implementation of PBO LLINs, evidence from high-quality community cluster randomized trials would be desirable. Such trials should run for at least 2 years, and ideally 3 years, in order to demonstrate the effectiveness of PBO LLINs over time, as the physical integrity of the nets deteriorates. It is strongly recommended that the current cluster randomized trial in the United Republic of Tanzania be supported for a further 2–3 years to ensure that evidence can be collected on the impact over the lifespan of the LLINs.

Recommendation 5: To guide and support countries in pilot exploratory implementation, the following activities are recommended:

(i) collect data on the presence, level, intensity and mechanisms of pyrethroid resistance at representative sentinel sites; and

(ii) design an evaluation of PBO LLINs that includes appropriate indicators based on detailed guidance.

Further information on insecticide resistance throughout malaria-endemic regions should be obtained to inform decisions on deployment of insecticide-based control tools.
Countries should ensure that data on insecticide resistance in malaria vectors is collected from eco-epidemiologically representative sites stratified by transmission and resistance levels, as well as by history of use of pesticides in both agriculture/veterinary and public health. The data should be effectively consolidated and managed at national level. This will be crucial for local decisions on vector control tools and to inform policy. Specifically, to support the deployment of PBO LLINs, countries should collect data on the presence, level, intensity and mechanisms of pyrethroid resistance.

5 ENDNOTES

1. Universal coverage is defined as the provision of an effective vector control intervention to all people at risk of malaria in a defined area. An area is determined by availability of reliable disaggregated disease surveillance data and is not necessarily based on administrative boundaries.

2. WHOPES has received letters of interest for submission of two new PBO LLINs, and phase-I studies are likely to begin in early 2016.

3. Comments from Jeffrey Scott (Professor of Entomology at the School of Agriculture at Cornell University, USA), Michael Macdonald (independent consultant, Baltimore, USA) and Mark Rowland (London School of Hygiene and Tropical Medicine) were particularly useful.

4. J. Scott, personal communication

5. WHOPES has received letters of interest for submission of two new PBO LLINs, and phase-I studies are likely to begin in early 2016.

6 REFERENCES


WHO (2015d) Information note on the risks associated with the scale back of vector control in areas where transmission has been reduced, Geneva (http://who.int/malaria/publications/atoz/scale-back-vector-control.pdf).

7 ABBREVIATIONS

DALY  disability-adjusted life year
ERG  Evidence Review Group
GMP  Global Malaria Programme
IRS  indoor residual spraying
LLIN  long-lasting insecticidal net
MFOs  mixed-function oxidases
PBO  piperonyl butoxide
WHOPES  WHO Pesticide Evaluation Scheme
8 ANNEXES

Annex 1 Working papers

Churc her T, Lissenden N, Worrall E, Ranson H. Identification of geographic areas where long-lasting insecticidal nets (LLINs) treated with a pyrethroid + PBO combination may be more effective than conventional LLINs impregnated with pyrethroid only.


Shepard DS and STA w olo. Cost-effectiveness of pyrethroid plus PBO bed nets compared to pyrethroid-only nets in villages in Nigeria and Ghana.

Worrall E, Lissenden N, Churcher T, Ranson H. Economic evaluation of pyrethroid plus PBO LLINs in comparison to pyrethroid LLINs.

Annex 2 Presentations

1. Objectives of the meeting and policy-setting process leading to the constitution of the Evidence Review Group (Abraham Mnzava and Raman Velayudhan)

2. Issues to consider in deciding where and when PBO nets are preferable (Jo Lines)

3. Impact of insecticide resistance on the effectiveness of LLINs – current knowledge and gaps (Immo Kleinschmidt)

4. Where might PBO LLINs be most effective at reducing malaria? (Hilary Ranson)

5. WHO Expert Review Group: PBO LLINs (Tom Churcher)

6. Economic evaluation of pyrethroid plus PBO LLINs in comparison to pyrethroid LLINs (Eva Worrall)

7. Defining strategies for controlling malaria transmission in areas of pyrethroid resistance using PBO-LLIN and OP-IRS (Mark Rowland)

8. Cluster randomized trial to evaluate the impact of pyrethroid plus pyriproxyfen nets on malaria infections – lessons from Burkina Faso: AvecNet Trial Update (Steve Lindsay)

9. Cost-effectiveness of pyrethroid plus PBO nets and pyrethroid nets in villages in Nigeria (Donald Shepherd)

10. VecNet model simulations of the impact of PermaNet 2.0 versus PermaNet 3.0 against pyrethroid resistant Anopheles gambiae transmission of P. falciparum malaria (Robert Farlow)
Annex 3  The rationale for using PBO LLINs in areas of pyrethroid resistance

Mixed-function oxidases (MFOs) are present in all insects and detoxify or sequester pyrethroids (even in susceptible insects). Some genes that confer insecticide resistance in *An. gambiae* s.l. and *An. funestus* increase the expression of MFOs so that pyrethroids are broken down more quickly in insect tissues, and a larger dose of insecticide is needed to kill the insect. This is one of the types of pyrethroid resistance mechanisms, and is referred to as “oxidase-based resistance”.

PBO inhibits MFOs and thus reduces an insect’s pyrethroid tolerance. PBO thus reduces the tolerance of both susceptible and resistant insects but to a greater degree in the latter if the mechanism of resistance involves MFOs. This fact can be used as a simple way to demonstrate the presence of an oxidase-based resistance mechanism: assays of discriminating doses of pyrethroid can be conducted against the mosquito population of interest to determine the resistance level. These are then repeated on mosquitoes that have been pre-exposed to PBO. If PBO pre-exposure is seen to have increased the susceptibility of the vector, then oxidase-based resistance is likely to be involved.

From these basic facts, we can infer that:

- If the dose of pyrethroid is regarded as fixed, addition of PBO can always be expected to increase the mortality rate in exposed insects.

- The effect of PBO depends on the insect’s MFO activity, and it is expected to be greater in insects with resistance genes that increase MFO expression. Hence, the addition of PBO is expected to have a greater differential impact on oxidase-based resistance, which will be more important in driving overall resistance levels.

- Even in areas with susceptible mosquitoes, PBO LLINs may have a differential impact.

- Note that this impact would not be demonstrable in susceptible populations in a discriminating dose bioassay, in which the mortality rate would already be 100%. Susceptible populations in experimental huts would also have a ceiling effect of 100% mortality, which would complicate demonstration of a differential impact of PBO LLINs. Trials in villages, with entomological or epidemiological outcomes, may, however, demonstrate a differential impact of PBO LLINs even in susceptible areas.
### Annex 4  Studies from which data were used for modelling (section 3.1)

#### TABLE A4.1  Bioassay studies

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<td>27</td>
<td>Mzilahowa et al. (2015). PMI.</td>
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<td>28</td>
<td>PMI (2014). PMI Africa IRS (AIRS) Project.</td>
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<td>30</td>
<td>Adeogun et al. (2012). Malar Chemother Control Elimination 1.</td>
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<td>32</td>
<td>Adeogun et al. (2012). Nig J Clin Biomed Res 6(1).</td>
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**TABLE A4.2**

*Experimental hut studies (see section 3.1)*

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<td>1</td>
<td>Corbel et al. (2010). Malar J. 9:113</td>
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<td>Ketoh (2014). Unpublished</td>
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<td>Field evaluation of PermaNet® 3.0 in controlling pyrethroid-resistant Anopheles gambiae in the Chirana Area – Western Region, Ghana</td>
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<td>5</td>
<td>Efficacy of the PermaNet 3.0 and the Olyset Plus against pyrethroid resistant A. funestus and A. arabiensis</td>
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<td>6</td>
<td>Progress Report for the study of the impact of new combination long-lasting insecticidal net products on entomological measures of malaria transmission in southern Mali</td>
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</table>
Annex 5

List of participants

ERG members
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Annex 6 Declarations of interests

All participants completed their declaration of interest forms for assessment by the WHO Secretariat. The following reported conflict of interests:

**ERG Members**

**John Gimnig**

Received in 2013 non-monetary support from Sumitomo Chemical (6000 LLINs) for research work on durability in Malawi. This interest was assessed as non-personal, non-specific but financially significant.

**Temporary advisers**

**Mark Rowland**

Receives significant research grants from Sumitomo Chemical and BASF for field and laboratory testing of next generation vector control tools (combination nets and LL IRS formulations) in Tanzania and Benin. The grant holder is LSHTM, and Mark Rowland receives no remuneration or salary replacement. This interest was assessed as non-personal, non-specific but financially significant.

**Steve Lindsay**

Receives non-monetary support from Sumitomo Chemical in the form of DUO nets for a clinical trial and durability study in Burkina Faso. This interest was assessed as non-personal, non-specific but financially significant.

**Robert Farlow**

Through R. Farlow Consulting LLC, Robert Farlow receives grant support from net manufacturers for a VecNet modelling study. This interest was assessed as personal,