Cochrane Library

Cochrane Database of Systematic Reviews

Piperonyl butoxide (PBO) combined with pyrethroids in long-lasting insecticidal nets (LLINs) to prevent malaria in Africa (Protocol)

Gleave K, Lissenden N, Richardson M, Ranson H

Gleave K, Lissenden N, Richardson M, Ranson H.

Piperonyl butoxide (PBO) combined with pyrethroids in long-lasting insecticidal nets (LLINs) to prevent malaria in Africa.

Cochrane Database of Systematic Reviews 2017, Issue 8. Art. No.: CD012776.

DOI: 10.1002/14651858.CD012776.

www.cochranelibrary.com

TABLE OF CONTENTS

HEADER 1
ABSTRACT 1
BACKGROUND 1
OBJECTIVES 3
METHODS 3
ACKNOWLEDGEMENTS 6
REFERENCES 6
ADDITIONAL TABLES 8
APPENDICES 13
CONTRIBUTIONS OF AUTHORS 17
DECLARATIONS OF INTEREST 17
SOURCES OF SUPPORT 17

[Intervention Protocol]

Piperonyl butoxide (PBO) combined with pyrethroids in long-lasting insecticidal nets (LLINs) to prevent malaria in Africa

Katherine Gleave[1a], Natalie Lissenden[1b], Marty Richardson[2], Hilary Ranson[1]

[1]Vector Biology, Liverpool School of Tropical Medicine, Liverpool, UK. [2]Cochrane Infectious Diseases Group, Liverpool School of Tropical Medicine, Liverpool, UK

[a]These authors contributed equally to this work.. [b]These authors contributed equally to this work.

Contact address: Katherine Gleave, Vector Biology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK. Katherine.Gleave@lstmed.ac.uk.

Editorial group: Cochrane Infectious Diseases Group.
Publication status and date: New, published in Issue 8, 2017.

Citation: Gleave K, Lissenden N, Richardson M, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in long-lasting insecticidal nets (LLINs) to prevent malaria in Africa. *Cochrane Database of Systematic Reviews* 2017, Issue 8. Art. No.: CD012776. DOI: 10.1002/14651858.CD012776.

ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

1. Evaluate whether adding PBO to pyrethroid LLINs increases the epidemiological and entomological effectiveness of the nets.

2. Compare the effects of PBO-LLINs currently in commercial development or on the market with their non-PBO equivalent in relation to:

a. malaria infection (prevalence or incidence);

b. entomological outcomes.

BACKGROUND

Description of the condition

Substantial progress has been made in malaria control in the 21st century. Some studies estimate that the clinical incidence of *Plasmodium falciparum* malaria has dropped by 40% since 2000 equating to the prevention of 663 million cases (Bhatt 2015; WHO-GMP 2015). The main focus of malaria control relies on the use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and treatment with artemisinin-based combination therapy (ACT). Of these methods, 68% of cases averted have been attributed to the use of LLINs (Bhatt 2015). This method of malaria prevention is particularly effective in Africa where the major malaria vectors, *Anopheles gambiae* and *Anopheles funestus*, are largely endophagic (feed indoors) and endophilic (rest indoors after blood-feeding).

Currently only one insecticide class, the pyrethroids, is commonly used to treat LLINs; pyrethroids have the required dual properties of low mammalian toxicity and rapid insecticidal activity (Zaim 2000), and their repellent or contact irritant effects may

enhance the personal protection of LLINs. Unfortunately, resistance to pyrethroids is now widespread in African malaria vectors (Ranson 2016). This may be the result of mutations in target-site proteins (target-site resistance) (Ranson 2011; Ridl 2008), which results in reduced sensitivity or increased activity of detoxification enzymes (metabolic resistance) (Mitchell 2012; Stevenson 2011), or other as yet poorly-described resistance mechanisms, or both. The evolution of insecticide resistance and its continuing spread threatens the operational success of malaria vector control interventions. The current impact of this resistance on malaria transmission is largely unquantified and will vary depending on the level of resistance, malaria endemicity, and proportion of the human population using LLINs (Churcher 2016). However, it is generally accepted that resistance will eventually erode the efficacy of pyrethroid-only LLINs and that innovation in the LLIN market is essential to maintain the efficacy of this preventative measure (MPAC 2016).

Description of the intervention

One way of controlling insecticide-resistant mosquito populations is through the use of insecticide synergists. Synergists are generally non-toxic and act by enhancing the potency of insecticides. Piperonyl butoxide (PBO) is a synergist that inhibits specific metabolic enzymes within mosquitoes, and has been incorporated into pyrethroid-treated LLINs to form PBO-combination nets (thereafter referred to as PBO-LLINs). Insecticide-synergist combination nets represent a new product class with the capacity to impact against insecticide resistant populations. However, there is no consensus of their effectiveness in reducing malaria cases in comparison to "standard" LLINs (WHO-GMP 2015).

Currently there are five PBO-LLINs that are either in development or on the market: Olyset® Plus, PermaNet® 3.0, Veeralin® LN, and DawaPlus 3.0 and 4.0. Olyset Plus, which is manufactured by Sumitomo Chemical Company Ltd, is a polyethylene net treated with permethrin (20 g/kg ± 25%) and PBO (10 g/kg ± 25%) across the whole net (Sumitomo 2013). PermaNet 3.0, which is manufactured by Vestergaard Frandsen, is a mixed polyester (sides) polyethylene (roof) net treated with deltamethrin and PBO; PBO is only found on the roof of the net (25 g/kg ± 25%) and the concentration of deltamethrin varies depending on location (roof: 4.0 g/kg ± 25%) and yarn type (sides: 75 denier with 70 cm lower border 2.8 g/kg ± 25%, 100 denier without border 2.1 g/kg ± 25%) (Vestergaard 2015). Veeralin LN, manufactured by Vector Control Innovations Private Ltd, is a polyethylene net treated with alpha-cypermethrin (6.0 g/kg) and PBO (2.2 g/kg) across the whole net (WHOPES 2016). DawaPlus 3.0 and 4.0 are manufactured by Tana Netting, UAE and contain PBO on the roof only (3.0) or on all sides (4.0); Deltamethrin suspension concentrate (SC) is coated on knitted multifilament polyester fibres, at the target dose of 1.33 g/kg in 75-denier yarn and 1 g/kg in 100- denier yarn, corresponding to 40 mg of deltamethrin per LN m², using a polymer as a binder.

How the intervention might work

PBO inhibits metabolic enzyme families, in particular the cytochrome P450 enzymes that detoxify or sequester pyrethroids. Increased production of P450s is thought to be the most potent mechanism of pyrethroid resistance in malaria vectors, and pre-exposure to PBO has been shown to restore susceptibility to pyrethroids in laboratory bioassays on multiple pyrethroid-resistant vector populations (Churcher 2016).

Widespread use of conventional LLINs provides both personal and community protection from malaria (Bhatt 2015; Lengeler 2004). However, in areas where the mosquito populations are resistant to pyrethroids, experimental hut trials (as described in the 'Types of studies' section) have shown that mosquito mortality rates and protection from blood-feeding are substantially reduced (Abílio 2015; Awolola 2014; Bobanga 2013; N'Guessan 2007; Riveron 2015; Yewhalaw 2012). The addition of PBO to pyrethroids in LLINs can restore the killing effects of LLINs in areas where this has been eroded by insecticide resistance. LLINs that contain PBO have been evaluated in multiple experimental hut trials across Africa (Adeogun 2012; Corbel 2010; Koudou 2011; N'Guessan 2010; Pennetier 2013; Tungu 2010). In most settings, the PBO-LLINs resulted in higher rates of mosquito mortality and greater blood-feeding inhibition than conventional LLINs, although the magnitude of this effect was variable.

Why it is important to do this review

All LLINs approved by the World Health Organization Pesticide Evaluation Scheme (WHOPES) contain pyrethroids as the sole active ingredient. Five LLINs that contain PBO have interim approval but these are not in widespread use (WHOPES 2016). As PBO-LLINs are more expensive than conventional LLINs, it is important to determine if they are superior to conventional LLINs and, if so, under what circumstances, to enable cost effectiveness studies to be performed to inform procurement decisions.

An Expert Review Group (ERG) commissioned by the World Health Organization (WHO) previously recommended conditions for the use of pyrethroid LLINs treated with PBO (WHO-GMP 2015), and advised that although "PBO LLINs appear to have an increased efficacy in certain settings…the evidence is still limited to justify a complete switch from pyrethroid-only LLINs to PBO LLINs across all settings". A meta-analysis of PBO-LLIN performance in experimental hut studies was used to parameterize a malaria transmission model to predict the public health benefit of PBO-LLINs (Churcher 2016). Although it provided a tool to predict the malaria transmission setting in which PBO-LLINs would have greatest impact, the reliance on models was not widely

accepted by the ERG, which emphasized the need for pilot exploratory implementation with robust evaluation. More recently, the Vector Control Advisory Group has issued guidance on the evidence requirements for new vector control tools and recommended at least two trials with epidemiological endpoints for 'first in class' products (WHO 2017).

In an attempt to assess the evidence of the effectiveness of PBO-LLINs against African malaria vectors in areas of insecticide resistance, we will conduct a systematic review of all relevant studies and we will examine both epidemiological and entomological endpoints. We appreciate that the evaluation of PBO will depend on studies where the background insecticide and dose is the same in both intervention and control groups. We are aware that some studies will have evaluated PBO-LLINs against pyrethroid-only LLINs with different background insecticides and doses, which confound the effects. We will include and describe these studies in this Cochrane Review, and appraise the findings in the light of the comparisons and potential confounding using GRADE.

One of the problems with the field is that PBO-LLINs are commercial products. The PBO-LLINs currently undergoing clinical trials have had additional alterations made to them, such as changing the concentration or rate at which the pyrethroid is released. However, these are the products for which policy decisions are needed, based on evidence related to their relative effectiveness. There are no clinical trials underway comparing nets that are identical except for the addition of PBO. Thus in this Cochrane Review we seek direct evidence for PBO alone impacting on the effectiveness of nets, and also will examine the evidence concerning the effectiveness of commercial products. These comparisons will carefully detail other potential confounding factors to be sure that generic recommendations that might be made take this into account.

OBJECTIVES

1. Evaluate whether adding PBO to pyrethroid LLINs increases the epidemiological and entomological effectiveness of the nets.

2. Compare the effects of PBO-LLINs currently in commercial development or on the market with their non-PBO equivalent in relation to:

a. malaria infection (prevalence or incidence);

b. entomological outcomes.

METHODS

Criteria for considering studies for this review

Types of studies

For objective 1 we will include a) laboratory bioassay (for example, cone bioassays, tunnel tests) studies; b) experimental hut trials, and c) village trials (WHOPES Phase I, II and III studies).

For objective 2 (a and b) we will include experimental hut studies and village trials (WHOPES Phase II studies and Phase III studies).

See Table 1 for WHOPES definitions in detail.

Types of participants

Mosquitoes

Anopheles gambiae complex or *Anopheles funestus* group. Included studies must test a minimum of 50 mosquitoes per study arm. We will include all mosquito populations and we will examine the insecticide resistance level (measured using phenotypic resistance) during data analysis.

Humans

Adults and children living in malaria-endemic areas where pyrethroid resistances is suspected in the local malaria vector population.

Types of interventions

Intervention

Combination LLIN treated with both PBO and a pyrethroid insecticide. LLINs must have received a minimum of interim-WHO approval (Table 2), and LLINs must be treated with a WHO-recommended dose of pyrethroid (Table 3).

Control

Conventional LLINs that contain pyrethroid only.

For objective 1 nets of the same fabric must be treated with the same insecticide, dose and release rate as the intervention net to allow direct evaluation of the addition on PBO.

For objective 2 (a and b), nets can be treated with the same insecticide at different doses than the intervention net to allow critical appraisal of all PBO-LLINs currently in development or on the market, and not singular products.

Types of outcome measures

Studies must include at least one of the following outcomes to be eligible for inclusion: mosquito mortality, blood-feeding, sporozoite rate, parasite presence, clinical malaria confirmation, not passed through net, deterrence, exophily, mosquito density, or parity rate.

Primary outcomes

Epidemiological

- Parasite presence: presence of malaria parasites through microscopy of blood or Rapid Diagnostic Tests (RDTs).
- Clinical malaria confirmation: clinical diagnosis based on the participant's symptoms and on physical findings at examination.

Entomological

- Mosquito mortality: immediate death or delayed death (up to 24 hours), or both, measured as a proportion of total mosquito number. A mosquito is classified as dead if it is immobile, cannot stand or fly, or shows no sign of life.
- Mosquito knock-down: mosquito 'mortality' recorded one hour post-insecticide exposure, termed 'knock-down' as some mosquitoes may recover during the 24-hour recovery period before mosquito mortality is recorded at 24 hours post-exposure.
- Blood-feeding success: number of mosquitoes that have blood-fed (alive or dead).
- Sporozoite rate: percentage of mosquitoes with sporozoites in salivary glands.

Secondary outcomes

Entomological

- Not passed through net: the number of mosquitoes that do not pass through a holed PBO-LLIN to reach a human bait relative to a standard LLIN.
- Deterrence: the number of mosquitoes that enter a hut that is using a PBO-LLIN relative to the number of mosquitoes found in a control hut that is using a standard LLIN.
- Exophily: the proportion of mosquitoes found in exit/veranda traps of a hut that is using a PBO-LLIN, relative to the control hut that is using a standard LLIN.
- Mosquito density: measured using all standard methods, such as window exit traps, indoor resting collections, floor sheet collections, pyrethrum spray catch, and light traps.
- Parity rate: percentage of parous mosquitoes which are detected by mosquito ovary dissections.

Search methods for identification of studies

We will attempt to identify all relevant trials regardless of language or publication status (published, unpublished, in press, and in progress). We have presented the MEDLINE search strategy in Appendix 1.

Electronic searches

Vittoria Lutje, the CIDG Information Specialist, will search the following databases using the search terms and strategy described in Appendix 1: the Cochrane Infectious Diseases Group (CIDG) Specialized Register; the Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE (Pubmed); Embase (OVID); Web of Science Citation Index Expanded; and BIOSIS Previews. We will also search the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/en/) and ClinicalTrials.gov (https://clinicaltrials.gov/ct2/home) for trials in progress, and will use "piperonyl butoxide", bednet*, ITN, and LLIN as search terms.

Searching other resources

We will contact the following organizations for unpublished data: the President's Malaria Initiative (PMI); the Innovative Vector Control Consortium (IVCC); Vestergaard Frandsen; Sumitomo Chemical Company Ltd; Vector Control Innovations Private Ltd; Endura SpA; and the WHO Pesticide Evaluation Scheme (WHOPES). We will check the reference lists of studies identified by the above methods.

Data collection and analysis

For the primary analysis we will stratify data by study type only. During subgroup analysis we will analyse the data by both study design and mosquito insecticide resistance level (Table 4; Table 5), or study design and product type if applicable.

Selection of studies

Two review authors (KG and NL) will independently screen titles and abstracts of all retrieved references based on the inclusion criteria (Table 6). We will resolve any inconsistencies between the review authors' selections by discussion. If we are unable to reach an agreement, we will consult the third review author (HR). We will retrieve the full-text study reports for all potentially relevant citations. Two review authors will independently screen the full-text articles and identify studies for inclusion, and identify and record reasons for exclusion of ineligible studies in a 'Characteristics of excluded studies' table. We will resolve any disagreements through discussion or, if required, we will consult the third review author (HR). We will identify and exclude duplicates and collate multiple reports of the same study so that each study, rather than each report, is the unit of interest in the review. We will record the selection process in sufficient detail to complete a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Moher 2009).

Data extraction and management

After selection, we will summarize all included studies according to the tables in Appendix 2. Two review authors (KG and NL) will independently extract data from the included studies using the pre-designed data extraction form (Appendix 3). If data are missing from an included study, we will contact the study authors for further information. We will enter data into Review Manager (RevMan) (RevMan 2014).

Assessment of risk of bias in included studies

Two review authors (KG and NL) will independently assess the risk of bias of each included study using a set of predetermined criteria specific to each study type adapted from Strode 2014 (Appendix 4). We will assign a classification of either low, high, or unclear risk of bias for each component. For all included studies we will assess whether any study authors had submitted any conflicts of interest that may have biased the study methodology or results.

Laboratory bioassays

For laboratory bioassays we will assess four criteria: comparability of mosquitoes between LLIN/PBO-LLIN arms (for example, all female, same age, and non-blood-fed), observers blinded, incomplete outcome data, and raw data reported for both net treatments.

Experimental hut trials

For experimental hut trials we will assess 10 criteria: comparability of mosquitoes between LLIN/PBO-LLIN arms (for example, species composition), collectors blinded, sleepers blinded, allocation of treatments, rotation of treatments, sleeper bias accounted for, standardized hut design, cleaning of huts between treatments, incomplete outcome data, and raw data reported.

Village trials

We will assess six criteria for village trials: comparability of mosquitoes between LLIN/PBO-LLIN households (for example, species composition), collectors blinded, household blinded, allocation of treatments, incomplete outcome data, and raw data reported.

Measures of treatment effect

For dichotomous data we will present the risk ratio (RR), for continuous data we will present the mean difference, and for count data we will present the rate ratios. We will present all results with 95% confidence intervals (CIs).

Unit of analysis issues

For human studies randomized by hut or village, or both (for example, cluster-randomized trials). We will use the adjusted measure of effect reported in the paper if available. If not, we will adjust the effect estimate using an intracluster correlation coefficient (ICC), and average cluster size (Higgins 2011, Section 16.3.4). If the included study does not report the ICC value, we will either use an ICC value reported by a similar study or we will estimate the ICC value. We will investigate the impact of estimating the ICC or using an ICC value from another study in sensitivity analyses.

Dealing with missing data

In the case of missing data, we will contact the study authors to retrieve this information. Should this information be unavailable, where applicable to the study design, we will conduct a complete case-analysis, and we will investigate the impact of missing data via imputation using a best-worst case scenario analysis.

Where information on mosquito insecticide resistance was not collected at the time of the study, the study authors will determine a suitable proxy. The proxy resistance data must be from the same area, conducted within three years of the study, and using the same insecticide, dose, and mosquito species. More than 50 mosquitoes per insecticide should have been tested using an appropriate control. Where no resistance data is available, we will class the resistance status as unclassified. If the proxy-resistance places the study in a borderline (within 1%) position for the subgroup analysis by resistance level, we will consider it in both the level it is in and the level it borderlines with.

Assessment of heterogeneity

We will present the results of the included studies in forest plots, which we will inspect visually to assess heterogeneity (that is, non-overlapping CIs generally signify statistical heterogeneity). We will also use the Chi^2 test with a P value of less than 0.1 to indicate statistical heterogeneity. We will quantify heterogeneity using the I^2 statistic and we will interpret a value greater than 75% to indicate considerable heterogeneity.

Assessment of reporting biases

To analyse the possibility of publication bias, we will use funnel plots provided there are at least 10 included studies.

Data synthesis

Where appropriate, we will pool the results of the included studies using meta-analysis. We will analyse the effect of adding PBO to pyrethroid LLINs, and then compare the effects of PBO-LLINs currently in development with their non-PBO equivalent. We will stratify results by type of study and we will examine mosquito resistance status or net type (i.e. product, e.g. Olyset Plus) through

subgroup analyses. For human outcomes we will assess whether use of PBO-LLINs resulted in reduced malaria infection (for example, prevalence, incidence) compared to conventional pyrethroid-only LLINs. One review author (MR) will analyse the data using Review Manager 5 (RevMan 5) (RevMan 2014), and we will use the random-effects model (if we detect heterogeneity; the I^2 statistic value is more than 50%) or the fixed-effect model (for no heterogeneity; the I^2 statistic value is less than 50%). We will not pool studies in a meta-analysis if it is not clinically meaningful to do so due to clinical or methodological heterogeneity. If the study authors stated the adjusted estimates for cluster-RCTs or if we can calculate these, we will combine these with RCTs; otherwise we will exclude them.

We will assess the certainty of the evidence using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. We will construct 'Summary of findings' tables using the GRADEpro Guideline Development Tool (GDT) software (GRADEpro GDT 2014).

Subgroup analysis and investigation of heterogeneity

We will stratify the results by study type.

We will determine whether mosquito populations are susceptible or resistant to pyrethroid insecticides based on WHO definitions (WHOPES 2013; Table 4). Where available, we will use 24-hour mosquito mortality. However, if this is unavailable, we will use knock-down 60 minutes after the end of the assay. We will conduct a subgroup analysis to examine susceptible versus resistant mosquitoes using WHO definitions (Table 4). If we observe that heterogeneity is present, we will perform a second subgroup analysis to examine the stratification of resistance; we will stratify resistant populations into low, moderate, or high prevalence resistance (Table 5).

Sensitivity analysis

If applicable, we will perform sensitivity analyses to determine the effect of exclusion of trials that we consider to be at high risk of bias. Also, we will perform a sensitivity analysis for missing data during imputation with best-worst case scenarios.

ACKNOWLEDGEMENTS

We thank Vittoria Lutje, Cochrane Infectious Diseases Group (CIDG) Information Specialist, for her assistance in conducting the literature search and Paul Garner, CIDG Co-ordinating Editor, for his guidance during the protocol preparation process.

This work was supported through a grant from the World Health Organization (WHO) Global Malaria Programme.

Marty Richardson is supported by the Effective Health Care Research Consortium. This Consortium and the CIDG editorial base are funded by UK aid from the UK Government for the benefit of developing countries (Grant: 5242). The views expressed in this review do not necessarily reflect UK government policy.

REFERENCES

Additional references

Abílio 2015
Abílio AP, Marrune P, de Deus N, Mbofana F, Muianga P, Kampango A. Bio-efficacy of new long-lasting insecticide-treated bed nets against Anopheles funestus and Anopheles gambiae from central and northern Mozambique. *Malaria Journal* 2015;**14**:352. [DOI: 10.1186/s12936-015-0885-y]

Adeogun 2012
Adeogun AO, Olojede JB, Oduola AO, Awolola TS. Efficacy of a combination long lasting insecticidal net (PermaNet® 3.0) against pyrethroid resistant Anopheles gambiae s.s. and Culex quinquefasciatus: an experimental hut trial in Nigeria. *Nigerian Journal of Clinical & Biomedical Research* 2012;**6**:37–50.

Awolola 2014
Awolola ST, Adeogun AO, Olojede JB, Oduola AO, Oyewole IO, Amajoh CN. Impact of PermaNet 3.0 on entomological indices in an area of pyrethroid resistant Anopheles gambiae in south-western Nigeria. *Parasites & Vectors* 2014;**7**:236. [DOI: 10.1186/1756-3305-7-236]

Bhatt 2015
Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature* 2015;**526**(7572):207–11.

Bobanga 2013
Bobanga T, Ayieko W, Zanga M, Umesumbu S, Landela A, Fataki O, et al. Field efficacy and acceptability of PermaNet® 3.0 and OlysetNet® in Kinshasa, Democratic Republic of the Congo. *Journal of Vector Borne Diseases* 2013;**50**(3):206–14.

Churcher 2016
Churcher TS, Lissenden N, Griffin JT, Worrall E, Ranson H. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. *eLife* 2016;**5**:e16090. [DOI: http://dx.doi.org/10.7554/eLife.16090]

Corbel 2010
Corbel V, Chabi J, Dabiré RK, Etang J, Nwane P, Pigeon O, et al. Field efficacy of a new mosaic long-lasting mosquito net (PermaNet 3.0) against pyrethroid-resistant malaria

vectors: a multi centre study in Western and Central Africa. *Malaria Journal* 2010;**9**:113. [DOI: 10.1186/1475-2875-9-113]

GRADEpro GDT 2014 [Computer program]
GRADE Working Group, McMaster University. GRADEpro GDT. Version accessed 5 May 2017. Hamilton (ON): GRADE Working Group, McMaster University, 2014.

Higgins 2011
Higgins JPT, Deeks JJ, Altman DG, Cochrane Statistical Methods Group. Chapter 16: Special topics in statistics. In: Higgins JP, Green S, editor(s). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Koudou 2011
Koudou BG, Koffi AA, Malone D, Hemingway J. Efficacy of PermaNet® 2.0 and PermaNet® 3.0 against insecticide-resistant Anopheles gambiae in experimental huts in Côte d'Ivoire. *Malaria Journal* 2011;**10**:172. [DOI: 10.1186/1475-2875-10-172]

Lengeler 2004
Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. *Cochrane Database of Systematic Reviews* 2004, Issue 2. [DOI: 10.1002/14651858.CD000363.pub2]

Mitchell 2012
Mitchell SN, Stevenson BJ, Müller P, Wilding CS, Egyir-Yawson A, Field SG, et al. Identification and validation of a gene causing cross-resistance between insecticide classes in Anopheles gambiae from Ghana. *Proceedings of the Nationall Academy of Sciences of the United States of America* 2012;**109**(16):6147–52. [DOI: 10.1073/pnas.1203452109]

MPAC 2016
Malaria Policy Advisory Committee. WHO Malaria Policy Advisory Committee (MPAC) meeting report (September 2016). www.who.int/malaria/publications/atoz/mpac-september2016-report.pdf?ua=1 (accessed 1 May 2017).

N'Guessan 2007
N'Guessan R, Corbel V, Akogbéto M, Rowland M. Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area, Benin. *Emerging Infectious Diseases* 2007;**13**(2):199–206. [DOI: 10.3201/eid1302.060631]

N'Guessan 2010
N'Guessan R, Asidi A, Boko P, Odjo A, Akogbeto M, Pigeon O, et al. An experimental hut evaluation of PermaNet (®) 3.0, a deltamethrin-piperonyl butoxide combination net, against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes in southern Benin. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2010;**104**(12):758–65. [DOI: 10.1016/j.trstmh.2010.08.008]

Pennetier 2013
Pennetier C, Bouraima A, Chandre F, Piameu M, Etang J, Rossignol M, et al. Efficacy of Olyset® Plus, a new long-lasting insecticidal net incorporating permethrin and piperonyl-butoxide against multi-resistant malaria vectors [corrected]. *PLoS ONE* 2013;**8**(10):e75134. [DOI: 10.1371/journal.pone.0075134]

Ranson 2011
Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control?. *Trends in Parasitology* 2011;**27**(2):91–8. [DOI: 10.1016/j.pt.2010.08.004]

Ranson 2016
Ranson H, Lissenden N. Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends in Parasitology* 2016;**32**(3):187–96. [DOI: 10.1016/j.pt.2015.11.010]

RevMan 2014 [Computer program]
Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager 5 (RevMan 5). Version 5.3. Copenhagen: Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Ridl 2008
Ridl FC, Bass C, Torrez M, Govender D, Ramdeen V, Yellot L, et al. A pre-intervention study of malaria vector abundance in Rio Muni, Equatorial Guinea: their role in malaria transmission and the incidence of insecticide resistance alleles. *Malaria Journal* 2008;**7**:194. [DOI: 10.1186/1475-2875-7-194]

Riveron 2015
Riveron JM, Chiumia M, Menze BD, Barnes KG, Irving H, Ibrahim SS, et al. Rise of multiple insecticide resistance in Anopheles funestus in Malawi: a major concern for malaria vector control. *Malaria Journal* 2015;**14**:344. [DOI: 10.1186/s12936-015-0877-y]

Stevenson 2011
Stevenson BJ, Bibby J, Pignatelli P, Muangnoicharoen S, O'Neill PM, Lian LY, et al. Cytochrome P450 6M2 from the malaria vector Anopheles gambiae metabolizes pyrethroids: sequential metabolism of deltamethrin revealed. *Insect Biochemistry and Molecular Biology* 2011;**41**(7):492–502. [DOI: 10.1016/j.ibmb.2011.02.003]

Strode 2014
Strode C, Donegan S, Garner P, Enayati AA, Hemingway J. The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: systematic review and meta-analysis. *PLoS Medicine* 2014;**11**(3):e1001619. [DOI: 10.1371/journal.pmed.1001619]

Sumitomo 2013
Sumitomo Chemical Co. Ltd. Olyset Plus Technical Brochure 2013. http://sumivector.com/sites/default/files/

site-content/pdf/Olyset_Plus_Technical_Brochure_Jan_2013_ENG.pdf (accessed 1 May 2017).

Tungu 2010
Tungu P, Magesa S, Maxwell C, Malima R, Masue D, Sudi W, et al. Evaluation of PermaNet 3.0 a deltamethrin-PBO combination net against Anopheles gambiae and pyrethroid resistant Culex quinquefasciatus mosquitoes: an experimental hut trial in Tanzania. *Malaria Journal* 2010;**9**: 21. [DOI: 10.1186/1475-2875-9-21]

Vestergaard 2015
Vestergaard. Technical basis for deployment of PermaNet® 3.0 in areas with pyrethroid-resistant malaria vectors. PermaNet® 3.0 Technical Brochure. January 2015. www.vestergaard.com/images/pdf/PN3_Tech_Eng_2015.pdf (accessed 1 May 2017).

WHO 2017
World Health Organization/Department of Control of Neglected Tropical Diseases. Design of epidemiological trials for vector control products, Report of a WHO Expert Advisory Group; Château de Penthes, Geneva, 24-25 April 2017. http://www.who.int/neglected_diseases/vector_ecology/resources/WHO_HTM_NTD_VEM_2017.04/en/ (accessed 11 July 2017).

WHO-GMP 2015
World Health Organization Global Malaria Programme. Conditions for use of long-lasting insecticidal nets treated with a pyrethroid and piperonyl butoxide. December 2015. www.who.int/malaria/publications/atoz/use-of-pbo-treated-llins.pdf?ua=1 2015.

WHOPES 2013
World Health Organization Pesticide Evaluation Scheme. Guidelines for laboratory and field testing of long-lasting insecticidal nets. http://apps.who.int/iris/bitstream/10665/80270/1/9789241505277_eng.pdf. World Health Organization.

WHOPES 2016
WHO/Department of control of neglected tropical diseases. Report of the nineteenth WHOPES working group meeting: WHO/HQ, Geneva, 8-11 February 2016. Review of Veeralin LN, VectoMax GR, Bactivec SC. http://apps.who.int/iris/bitstream/10665/205588/1/9789241510400_eng.pdf (accessed 1 May 2017).

Yewhalaw 2012
Yewhalaw D, Asale A, Tushune K, Getachew Y, Duchateau L, Speybroeck N. Bio-efficacy of selected long-lasting insecticidal nets against pyrethroid resistant Anopheles arabiensis from South-Western Ethiopia. *Parasites & Vectors* 2012;**5**:159. [DOI: 10.1186/1756-3305-5-159]

Zaim 2000
Zaim M, Aitio A, Nakashima N. Safety of pyrethroid-treated mosquito nets. *Medical and Veterinary Entomology* 2000;**14**(1):1–5.

* *Indicates the major publication for the study*

ADDITIONAL TABLES

Table 1. WHOPES classification

WHOPES Phase	Definition
WHOPES Phase I. Laboratory bioassays	Cone bioassays: these are studies that are conducted in the laboratory setting and use standard WHO protocols (WHOPES 2013, Section 2.2.1), where mosquitoes are exposed to a suitable LLIN (treated intervention or untreated control) for three minutes using a standard plastic WHO cone. Following net exposure, mosquitoes are transferred to a holding container and maintained on a sugar solution diet while entomological outcomes (mosquitoes knocked down one hour post-exposure, and mosquito mortality 24 hours post-exposure) are measured Tunnel tests: these are studies conducted in the laboratory setting that use standard WHO protocols (WHOPES 2013, Section 2.2.2). Mosquitoes are released into a glass tunnel covered at each end with untreated netting. The intervention or control LLIN net sample is placed one-third down the length of the tunnel and the net contains nine holes that enable mosquitoes to pass through. A suitable bait is immobilized in the shorter section of the tunnel where it is available for mosquito biting. Mosquitoes are released into the opposite end of the tunnel and must make contact with the net and locate holes before being able to feed on the bait. After 12 to 15 hours, mosquitoes are removed from both sections of the tunnel and entomological outcomes (the number of mosquitoes in each section, mortality, and blood-feeding inhibition at the end of the assay and 24 hours post-exposure) are recorded

Table 1. WHOPES classification (*Continued*)

	Wire-ball bioassays: these are studies conducted in the laboratory setting where mosquitoes are introduced into a wire-ball frame that has been covered with either the intervention or control LLIN. Mosquitoes are exposed for three minutes, after which they are transferred to a holding container and entomological outcomes (mosquitoes knocked down one hour post-exposure, and mosquito mortality 24 hours post-exposure) are measured
WHOPES Phase II. Experimental hut trials	WHOPES Phase II experimental hut trials are field trial studies conducted in Africa where wild mosquito populations or local colonized populations are evaluated. Volunteers or livestock sleep in experimental huts under a purposefully holed LLIN, with one person or animal per hut. Huts are designed to resemble local housing based on a West or East African design (WHOPES 2013; Section 3.3.1-2). However they have identical design features, such as eave gaps or entry slits to allow mosquitoes to enter, and exit traps to capture exiting mosquitoes. LLINs and volunteers are randomly allocated to huts and rotated in a Latin square to avoid bias, with huts cleaned between rotations to avoid contamination. Several nets, including an untreated control net, can be tested at the same time. Dead and alive mosquitoes are collected each morning from inside the net, inside the hut, and inside the exit traps. They are then scored as either blood-fed or non-blood-fed, and either alive or dead, and live mosquitoes are maintained for a further 24 hours to assess delayed mosquito mortality
WHOPES Phase III. Village trials	WHOPES Phase III village trials are village trials conducted in Africa where wild mosquito populations are evaluated. Villages chosen to be included in the study are similar in terms of size, housing structure, location, and the data available on the insecticide resistance status of the local malaria vectors. Households are assigned either conventional LLINs or PBO-LLINs. Randomization can be at the household or village level. Adult mosquitoes are collected from the study houses and mosquito density is measured. An indication of malaria transmission is measured in the study sites either by recording infections in mosquitoes, malaria prevalence, or malaria incidence

Table 2. WHO-recommended LLINs

Product name	Product type	Status of WHO recommendation
DawaPlus 2.0	Deltamethrin coated on polyester	Interim
DawaPlus 3.0	Combination of deltamethrin coated onto polyester (side panels), and deltamethrin and PBO incorporated into polyester (roof)	Interim
DawaPlus 4.0	Deltamethrin and PBO incorporated into polyester	Interim
Duranet	Alpha-cypermethrin incorporated into polyethylene	Full
Interceptor	Alpha-cypermethrin coated on polyester	Full
LifeNet	Deltamethrin incorporated into polypropylene	Interim

Table 2. WHO-recommended LLINs (*Continued*)

MAGNet	Alpha-cypermethrin incorporated into polyethylene	Full
MiraNet	Alpha-cypermethrin incorporated into polyethylene	Interim
Olyset Net	Permethrin incorporated into polyethylene	Full
Olyset Plus	Permethrin and PBO incorporated into polyethylene	Interim
Panda Net 2.0	Deltamethrin incorporated into polyethylene	Interim
PermaNet 2.0	Deltamethrin coated on polyester	Full
PermaNet 3.0	Combination of deltamethrin coated on polyester with strengthened border (side panels), and deltamethrin and PBO incorporated into polyethylene (roof)	Interim
Royal Sentry	Alpha-cypermethrin incorporated into polyethylene	Full
SafeNet	Alpha-cypermethrin coated on polyester	Full
Veeralin	Alpha-cypermethrin and PBO incorporated into polyethylene	Interim
Yahe	Deltamethrin coated on polyester	Interim
Yorkool	Deltamethrin coated on polyester	Full

Abbreviations: LLIN: long-lasting insecticidal net; WHO: World Health Organization.

Table 3. WHO-recommended insecticide products for treatment of mosquito nets for malaria vector control

Insecticide	**Formulation**[1]	**Dosage**[2]
Alpha-cypermethrin	SC 10%	20 to 40
Cyfluthrin	EW 5%	50
Deltamethrin	SC 1% WT 25% WT 25% + binder[3]	15 to 25
Etofenprox	EW 10%	200
Lambda-cyhalothrin	CS 2.5%	10 to 15

Table 3. WHO-recommended insecticide products for treatment of mosquito nets for malaria vector control (*Continued*)

Permethrin	EC 10%	200 to 500

[1]Abbreviations: EC: emulsifiable concentrate; EW: emulsion, oil in water; CS: capsule suspension; SC: suspension concentrate; WT: water dispersible tablet.
[2]Active ingredient/netting (mg/m²).
[3]K-O TAB 1-2-3.

Table 4. Definition of resistance level

Outcome	Confirmed resistance	Suspected resistance	Susceptible	Unclassified
WHO mosquito mortality	< 90%	90% to 97 %	98% to 100%	Unknown
CDC knock-down	< 90%	80% to 97%	98% to 100%	Unknown

Abbreviations: CDC: Centers for Disease Control and Prevention; WHO: World Health Organization.
Definition of resistance level based on mosquito mortality (%) after exposure to insecticide in a WHO diagnostic dose assay (WHO mosquito mortality), or a CDC bottle bioassay (CDC knock-down) using the methodology, diagnostic doses, and diagnostic times recommended by each test respectively.

Table 5. Stratification of resistance level

Outcome	Low	Moderate	High	Unclassified
Mosquito mortality[1]	61% to 90%	31% to 60%	< 30%	Unknown

[1]24-hour post-exposure mortality (%).

Table 6. Study inclusion screening form

Criteria	Assessment			Comments
	Yes	No	Unclear	
Mosquito population				
Did the study test *Anopheles gambiae* complex or *Anopheles funestus* group mosquitoes?		-		State mosquito species
Were a minimum of 50 mosquitoes tested per study arm?		-		

Table 6. Study inclusion screening form (*Continued*)

Intervention				
Did the study include an long-lasting insecticidal net (LLIN) or insecticide treated net (ITN)?		-		State net LLIN or ITN
Was the intervention net either of the following? • A piperonyl butoxide (PBO) LLIN which received a minimum of interim World Health Organization (WHO) approval. • An ITN impregnated with WHO-recommended dose of pyrethroid + PBO.		-		State net type
Was the control net either of the following? • A pyrethroid LLIN of the same fabric impregnated with the same insecticide and dose as intervention net (objective 1). • A pyrethroid LLIN impregnated with the same insecticide at any dose (objective 2 (a and b)).		-		State which objective study meets
Study design				
Was the study one of the following? • Laboratory bioassay (cone, tunnel, ball) • Experimental hut study • Village trial		-		State study type

Table 6. Study inclusion screening form (*Continued*)

For laboratory bioassay. Did the study use standard-WHO protocol?		-		
For experimental hut study and village trial. Was the study conducted in Africa?		-		State country
Outcome				
Did the study include at least one of the following outcome measures? • Mortality. • Blood feeding. • Sporozoite rate. • Not passed through net. • Deterrence. • Exophily. • Mosquito density. • Parity rate.		-		
Decision				
Is the study eligible for inclusion?	-	-		State reason(s) for exclusion
	Discuss with authors			

APPENDICES

Appendix 1. MEDLINE search strategy

1) "Piperonyl Butoxide"[Mesh]
2) piperonyl butoxide or PBO [Title/Abstract]
3) 1 or 2
4) Net* OR bednet* OR hammock* OR curtain* OR ITN* OR LLIN* or "Insecticide-Treated Bednet*" or "Insecticide-Treated net*" [Title/Abstract]
5) Olyset* or PermaNet* or Veeralin [Title/Abstract]
6) "Insecticide-Treated Bednets" [Mesh]
7) 4 or 5 or 6
8) 3 and 7

This is the preliminary search strategy for MEDLINE (PubMed). It will be adapted for other electronic databases. We will report all search strategies in full in the final version of the Cochrane Review.

Appendix 2. Study characteristics tables

Table 2.1 Study characteristics of the included cone bioassays

Study ID	Study name	Mosquito species (strain/ origin)	Resistance level	Resistance status	Mosquito age	Blood-fed status	Intervention	Washing	Measured outcome; mosquito mortality

Table 2.2 Study characteristics of the included ball bioassays

Study ID	Study name	Mosquito species (strain/ origin)	Resistance level	Resistance status	Mosquito age	Blood-fed status	Intervention	Washing	Measured outcome; mosquito mortality

Table 2.3 Study characteristics of the included tunnel tests

Study ID	Study name	Mosquito species (strain/ origin)	Resistance level	Resistance status	Mosquito age	Blood-fed status	Intervention	Washing	Measured outcome			
									KD	M	BF	NPTN

Abbreviations: KD: knock-down; M: mortality; BF; blood-feeding; NPTN: not passed though net.

Table 2.4 Study characteristics of the included experimental hut trials

Study ID	Study name	Study location	Mosquito species (strain/ origin)	Resistance level	Resistance status	Study start/ end date	Intervention	Net washed	Net holed	Measured outcome			
										M	BF	D	E

Abbreviations: M: mortality; BF; blood-feeding; D: deterrence; E: exophily.

Table 2.5 Study characteristics of the included village trials

Study ID	Study name	Study location	Mosqu species (strain ori-gin)	Re-sis-tance level	Re-sis-tance sta-tus	Study start/ end date	In-ter-ven-tion	Net washed	Net holed	Measured outcome						
										M	BF	SR	MD	PR	PP	CMC

Abbreviations: M: mortality; BF: blood-feeding; SR: sporozoite rate; MD: mosquito density; PR: parity rate; PP: parasite presence; CMC: clinical malaria confirmation.

Appendix 3. Data extraction tables

Table 3.1 Data extracted from laboratory bioassays.

Study ID	Study name	Net type	Net washed	Mosquit species	Resis-tance level	Resis-tance status	Total mosquit	Dead	Mosquit mor-tality (%)	BF	BF (%)	NPTN	NPTN (%)

Abbreviations: BF: blood-feeding; NPTN: not passed through net.

Table 3.2 Data extracted from experimental hut trials

Study ID	Study name	Net type	Net washe	Net holed	Mosqu specie	Re-sis-tance level	Re-sis-tance sta-tus	To-tal mosqu	Dead	Mosqu mor-tal-ity (%)	BF	BF (%)	BFI	Num-ber of mosqu de-terred	De-ter-rence (%)	Exit trap	Ex-ophily (%)	To-tal num-ber of peo-ple (N)

Abbreviations: BF: blood-feeding; BFI: blood-feeding inhibition; N: number of people.

Table 3.3 Data extracted from village trials

Study ID	Study name	Net type	Net washe	Net holed	Mosq specie	Resistance level	Resistance status	Total mosq	Dead	Mosq mortality (%)	BF	BF (%)	BFI	Sporozoite (%)	Mosq density (%)	Parity (%)	Total number of people (N)	PP (%)	CMC (%)

Abbreviations: BF: blood-feeding; PP: parasite prevalence; CMC: clinical malaria confirmation; N: number of people.

Appendix 4. 'Risk of bias' tables

Table 4.1 'Risk of bias' assessment for laboratory bioassays

Study ID	Study name	Comparability of mosquitoes in LLIN and LLIN + PBO groups	Observers blinded	Incomplete outcome data	Raw data reported for LLIN and LLIN + PBO groups	Authors' conflicting interest

Abbreviations: LLIN: long-lasting insecticidal nets; PBO: piperonyl butoxide.
Table 4.2 'Risk of bias' assessment for experimental hut trials

Study ID	Study name	Comparability of mosquitc in LLIN and LLIN+PI groups	Collectors blinded	Sleepers blinded	Sleeper bias	Treatment allocation	Treatment rotation	Standardized hut design	Hut cleaning between treatments	Incomplete outcome data	Raw data reported for both treatment groups	Authors' conflicting interest

Table 4.3 'Risk of bias' assessment for village trials

Study ID	Study name	Comparability of mosquitoes in LLIN and LLIN + PBO households	Collectors blinded	Household blinded	Allocation of treatments	Incomplete outcome data	Raw data reported for both groups	Authors' conflicting interest

Abbreviations: LLIN: long-lasting insecticidal nets; PBO: piperonyl butoxide.

CONTRIBUTIONS OF AUTHORS

KG, NL, and HR conceived and designed the protocol.

MR provided statistical input.

KG, NL, HR, and MR read and approved the final protocol draft.

DECLARATIONS OF INTEREST

KG has no known conflicts of interest.

NL has acted as rapporteur since 2015 for the Innovative Vector Control Consortium (IVCC) at their External Scientific Advisory Committee (ESAC) meetings.

MR has no known conflicts of interest.

HR has served on a WHO committee to consider the evidence for PBO nets in malaria control. Preparation of the background work presented at this WHO meeting was funded by the Global Fund for AIDS, TB and Malaria. Although HR interacts regularly with bednet manufacturers through her own research and her role on the IVCC's advisory panels, neither HR nor her research group have received direct funding from these companies.

SOURCES OF SUPPORT

Internal sources

- Liverpool School of Tropical Medicine, UK.

External sources

- Department for International Development, UK.

Grant: 5242

- World Health Organization (WHO), Switzerland.

WHO Global Malaria Programme Agreement for Performance of Work (APW) Grant 2017 (number 709319)