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Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa (Review)

Gleave K, Lissenden N, Chaplin M, Choi L, Ranson H

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[Intervention Review]

Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa

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ABSTRACT

Background

Pyrethroid long-lasting insecticidal nets (LLINs) have been important in the large reductions in malaria cases in Africa, but insecticide resistance in *Anopheles* mosquitoes threatens their impact. Insecticide synergists may help control insecticide-resistant populations. Piperonyl butoxide (PBO) is such a synergist; it has been incorporated into pyrethroid-LLINs to form pyrethroid-PBO nets, which are currently produced by five LLIN manufacturers and, following a recommendation from the World Health Organization (WHO) in 2017, are being included in distribution campaigns. This review examines epidemiological and entomological evidence on the addition of PBO to pyrethroid nets on their efficacy.

Objectives

To compare effects of pyrethroid-PBO nets currently in commercial development or on the market with effects of their non-PBO equivalent in relation to:

1. malaria parasite infection (prevalence or incidence); and
2. entomological outcomes.

Search methods

We searched the Cochrane Infectious Diseases Group (CIDG) Specialized Register, CENTRAL, MEDLINE, Embase, Web of Science, CAB Abstracts, and two clinical trial registers (ClinicalTrials.gov and WHO International Clinical Trials Registry Platform) up to 25 September 2020. We contacted organizations for unpublished data. We checked the reference lists of trials identified by these methods.

Selection criteria

We included experimental hut trials, village trials, and randomized controlled trials (RCTs) with mosquitoes from the *Anopheles gambiae* complex or the *Anopheles funestus* group.

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Data collection and analysis

Two review authors assessed each trial for eligibility, extracted data, and determined the risk of bias for included trials. We resolved disagreements through discussion with a third review author. We analysed data using Review Manager 5 and assessed the certainty of evidence using the GRADE approach.

Main results

Sixteen trials met the inclusion criteria: 10 experimental hut trials, four village trials, and two cluster-RCTs (cRCTs). Three trials are awaiting classification, and four trials are ongoing.

Two cRCTs examined the effects of pyrethroid-PBO nets on parasite prevalence in people living in areas with highly pyrethroid-resistant mosquitoes (< 30% mosquito mortality in discriminating dose assays). At 21 to 25 months post intervention, parasite prevalence was lower in the intervention arm (odds ratio (OR) 0.79, 95% confidence interval (CI) 0.67 to 0.95; 2 trials, 2 comparisons; moderate-certainty evidence).

In highly pyrethroid-resistant areas, unwashed pyrethroid-PBO nets led to higher mosquito mortality compared to unwashed standard-LLINs (risk ratio (RR) 1.84, 95% CI 1.60 to 2.11; 14,620 mosquitoes, 5 trials, 9 comparisons; high-certainty evidence) and lower blood feeding success (RR 0.60, 95% CI 0.50 to 0.71; 14,000 mosquitoes, 4 trials, 8 comparisons; high-certainty evidence). However, in comparisons of washed pyrethroid-PBO nets to washed LLINs, we do not know if PBO nets had a greater effect on mosquito mortality (RR 1.20, 95% CI 0.88 to 1.63; 10,268 mosquitoes, 4 trials, 5 comparisons; very low-certainty evidence), although the washed pyrethroid-PBO nets did decrease blood-feeding success compared to standard-LLINs (RR 0.81, 95% CI 0.72 to 0.92; 9674 mosquitoes, 3 trials, 4 comparisons; high-certainty evidence).

In areas where pyrethroid resistance is moderate (31% to 60% mosquito mortality), mosquito mortality was higher with unwashed pyrethroid-PBO nets compared to unwashed standard-LLINs (RR 1.68, 95% CI 1.33 to 2.11; 1007 mosquitoes, 2 trials, 3 comparisons; moderate-certainty evidence), but there was little to no difference in effects on blood-feeding success (RR 0.90, 95% CI 0.72 to 1.11; 1006 mosquitoes, 2 trials, 3 comparisons; moderate-certainty evidence). For washed pyrethroid-PBO nets compared to washed standard-LLINs, we found little to no evidence for higher mosquito mortality or reduced blood feeding (mortality: RR 1.07, 95% CI 0.74 to 1.54; 329 mosquitoes, 1 trial, 1 comparison, low-certainty evidence; blood feeding success: RR 0.91, 95% CI 0.74 to 1.13; 329 mosquitoes, 1 trial, 1 comparison; low-certainty evidence).

In areas where pyrethroid resistance is low (61% to 90% mosquito mortality), studies reported little to no difference in the effects of unwashed pyrethroid-PBO nets compared to unwashed standard-LLINs on mosquito mortality (RR 1.25, 95% CI 0.99 to 1.57; 1580 mosquitoes, 2 trials, 3 comparisons; moderate-certainty evidence), and we do not know if there was any effect on blood-feeding success (RR 0.75, 95% CI 0.27 to 2.11; 1580 mosquitoes, 2 trials, 3 comparisons; very low-certainty evidence). For washed pyrethroid-PBO nets compared to washed standard-LLINs, we do not know if there was any difference in mosquito mortality (RR 1.39, 95% CI 0.95 to 2.04; 1774 mosquitoes, 2 trials, 3 comparisons; very low-certainty evidence) or on blood feeding (RR 1.07, 95% CI 0.49 to 2.33; 1774 mosquitoes, 2 trials, 3 comparisons; low-certainty evidence).

In areas where mosquito populations are susceptible to insecticides (> 90% mosquito mortality), there may be little to no difference in the effects of unwashed pyrethroid-PBO nets compared to unwashed standard-LLINs on mosquito mortality (RR 1.20, 95% CI 0.64 to 2.26; 2791 mosquitoes, 2 trials, 2 comparisons; low-certainty evidence). This is similar for washed nets (RR 1.07, 95% CI 0.92 to 1.25; 2644 mosquitoes, 2 trials, 2 comparisons; low-certainty evidence). We do not know if unwashed pyrethroid-PBO nets had any effect on the blood-feeding success of susceptible mosquitoes (RR 0.52, 95% CI 0.12 to 2.22; 2791 mosquitoes, 2 trials, 2 comparisons; very low-certainty evidence). The same applies to washed nets (RR 1.25, 95% CI 0.82 to 1.91; 2644 mosquitoes, 2 trials, 2 comparisons; low-certainty evidence).

In village trials comparing pyrethroid-PBO nets to LLINs, there was no difference in sporozoite rate (4 trials, 5 comparisons) nor in mosquito parity (3 trials, 4 comparisons).

Authors' conclusions

In areas of high insecticide resistance, pyrethroid-PBO nets have greater entomological and epidemiological efficacy compared to standard LLINs, with sustained reduction in parasite prevalence, higher mosquito mortality and reduction in mosquito blood feeding rates 21 to 25 months post intervention. Questions remain about the durability of PBO on nets, as the impact of pyrethroid-PBO nets on mosquito mortality was not sustained over 20 washes in experimental hut trials, and epidemiological data on pyrethroid-PBO nets for the full intended three-year life span of the nets is not available. Little evidence is available to support greater entomological efficacy of pyrethroid-PBO nets in areas where mosquitoes show lower levels of resistance to pyrethroids.

PLAIN LANGUAGE SUMMARY

Pyrethroid-PBO nets to prevent malaria

Background

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Bed nets treated with pyrethroid insecticides are an effective way to reduce malaria transmission and have been deployed across Africa. However, mosquitoes that spread malaria are now developing resistance to this type of insecticide. One way to overcome this resistance is to add another chemical - piperonyl butoxide (PBO) - to the net. PBO is not an insecticide, but it blocks the substance (an enzyme) inside the mosquito that stops pyrethroids from working.

What is the aim of this review?

The aim of this Cochrane Review was to find out if pyrethroid-PBO nets provide additional protection against malaria when compared to standard pyrethroid-only nets.

Key messages

Pyrethroid-PBO nets were more effective than standard pyrethroid-only nets in killing mosquitoes and preventing blood feeding in areas where mosquito populations are very resistant to pyrethroid insecticides (high-certainty evidence). Pyrethroid-PBO nets reduced the number of malaria infections in areas of high pyrethroid resistance (moderate-certainty evidence), although further studies are needed to measure clinical outcomes for the full lifetime of the net.

What was studied in the review?

We included 16 trials conducted between 2010 and 2020 that compared standard pyrethroid nets to pyrethroid-PBO nets. These consisted of 10 experimental hut trials that measured the impact of pyrethroid-PBO nets on a wild population of mosquitoes, four village trials, and two cRCTs. The two cRCTs measured the impact of pyrethroid-PBO nets on malaria infection in humans; all other studies recorded their impact on mosquito populations. We analysed hut and village studies to determine whether pyrethroid-PBO nets were better for killing mosquitoes and preventing them from blood feeding. For both cRCT trials, we examined whether pyrethroid-PBO nets reduced the number of malaria infections. As the benefit of adding PBO to nets is likely to depend on the level of pyrethroid resistance in the mosquito population, we performed separate analyses for studies conducted in areas of high, medium, and low levels of pyrethroid resistance.

What are the main results of the review?

When mosquitoes show high levels of resistance to pyrethroids, pyrethroid-PBO nets perform better than standard pyrethroid-only nets for killing mosquitoes and preventing them from blood feeding. As expected, this effect is not seen in areas where mosquitoes show low or no resistance to pyrethroid-only insecticides. Two trials looked at the impact of using pyrethroid-PBO nets on the number of people infected with the malaria parasite. These trials, involving 10,603 participants in total and conducted in an area where mosquitoes are very resistant to pyrethroids, found that fewer people were infected with malaria when the population used pyrethroid-PBO nets than when standard pyrethroid-only nets were used.

How up-to-date is this review?

We searched for all studies and trials that had been published up to 25 September 2020.

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SUMMARY OF FINDINGS

Summary of findings 1. Summary of findings table 1

Pyrethroid-piperonyl butoxide (PBO) nets compared to long-lasting insecticidal nets (LLINs) for malaria control when insecticide resistance is high

Patient or population: adults and childen living in malaria-endemic areas, *Anopheles gambiae* complex or *Anopheles funestus* group
Setting: areas of high insecticide resistance
Intervention: pyrethroid-PBO nets
Comparison: LLIN

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Number of participants, (trials)	Certainty of the evidence (GRADE)	Comments
	Risk with LLIN	Risk with pyrethroid-PBO nets				
Parasite prevalence (4- to 6-month follow-up)	254 per 1000[a]	201 per 1000 (174 to 233)[a]	OR 0.74 (0.62 to 0.89)	11,582 people (2 trials, 2 comparisons, 61 PBO clusters, 64 non-PBO clusters)	⊕⊕⊕⊕ HIGH	Pyrethroid-PBO nets at 4- to 6-month follow-up reduce parasite prevalence in areas of high insecticide resistance
Parasite prevalence (9- to 12-month follow-up)	224 per 1000[a]	172 per 1000 (150 to 199)[a]	OR 0.72 (0.61 to 0.86)	11,370 people (2 trials, 2 comparisons, 61 PBO clusters, 64 non-PBO clusters)	⊕⊕⊕⊖ MODERATE[b] *due to inconsistency*	Pyrethroid-PBO nets at 9- to 12-month follow-up reduce parasite prevalence in areas of high insecticide resistance
Parasite prevalence (16- to 18-month follow-up)	248 per 1000[a]	225 per 1000 (196 to 255)[a]	OR 0.88 (0.74 to 1.04)	11,822 people (2 trials, 2 comparisons, 61 PBO clusters, 64 non-PBO clusters)	⊕⊕⊕⊖ MODERATE[b] *due to inconsistency*	Pyrethroid-PBO nets at 16- to 18-month follow-up reduce parasite prevalence in areas of high insecticide resistance
Parasite prevalence (21- to 25-month follow-up)	350 per 1000[a]	298 per 1000 (265 to 338)[a]	OR 0.79 (0.67 to 0.95)	10,603 people (2 trials, 2 comparisons, 54 PBO clusters, 60 non-PBO clusters)	⊕⊕⊕⊖ MODERATE[b] *due to inconsistency*	Pyrethroid-PBO nets at 21- to 25-month follow-up reduce parasite prevalence in areas of high insecticide resistance

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Mosquito mortality (unwashed nets)	238 per 1000[a]	438 per 1000 (381 to 503)[a]	RR 1.84 (1.60 to 2.11)	14,620 mosquitoes (5 trials, 9 comparisons)	⊕⊕⊕⊕ HIGH[c]	Mosquito mortality is higher with unwashed pyrethroid-PBO nets compared to standard unwashed LLINs in areas of high insecticide resistance
Mosquito mortality (washed nets)	201 per 1000[a]	242 per 1000 (177 to 328)[a]	RR 1.20 (0.88 to 1.63)	10,268 mosquitoes (4 trials, 5 comparisons)	⊕⊖⊖⊖ VERY LOW[d,e] *due to imprecision and inconsistency*	We do not know whether pyrethroid-PBO nets have an effect on mosquito mortality in areas of high insecticide resistance when the nets have been washed
Blood-feeding success (unwashed nets)	438 per 1000[a]	263 per 1000 (241 to 311)[a]	RR 0.60 (0.50 to 0.71)	14,000 mosquitoes (4 trials, 8 comparisons)	⊕⊕⊕⊕ HIGH[c]	Mosquito blood-feeding success is decreased with unwashed pyrethroid-PBO nets compared to standard unwashed LLINs in areas of high insecticide resistance
Blood-feeding success (washed nets)	494 per 1000[a]	400 per 1000 (356 to 454)[a]	RR 0.81 (0.72 to 0.92)	9674 mosquitoes (3 trials, 4 comparisons)	⊕⊕⊕⊕ HIGH[c]	Mosquito blood-feeding success is decreased with washed pyrethroid-PBO nets compared to standard washed LLINs in areas of high insecticide resistance

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).
CI: confidence interval; LLINs: long-lasting insecticidal nets; OR: odds ratio; PBO: pyrethroid-piperonyl butoxide; RR: risk ratio.

GRADE Working Group grades of evidence.
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Original numbers were used in this table; however in pooled analysis, events and total numbers were generated from cluster-adjusted results, which use the effective sample size. Note that cluster adjustments do not change the point estimate of the effect size - just the standard error.
[b]Downgraded by one for inconsistency.
[c]Not downgraded for imprecision: both best- and worst-case scenarios in this situation are important effects.
[d]Downgraded by one for imprecision due to wide CIs.
[e]Downgraded by two for inconsistency due to unexplained qualitative heterogeneity.

Summary of findings 2. Summary of findings table 2

Pyrethroid-piperonyl butoxide (PBO) nets compared to long-lasting insecticidal nets (LLINs) for malaria control when insecticide resistance is moderate

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Patient or population: *Anopheles gambiae* complex or *Anopheles funestus* group
Setting: areas of moderate insecticide resistance
Intervention: pyrethroid-PBO nets
Comparison: LLIN

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Number of mosquitoes (experimental hut trials)	Certainty of the evidence (GRADE)	Comments
	Risk with LLIN	Risk with pyrethroid-PBO nets				
Mosquito mortality (unwashed nets)	180 per 1000[a]	303 per 1000 (259 to 411)[a]	RR 1.68 (1.33 to 2.11)	1007 (2 trials, 3 comparisons)	⊕⊕⊕⊖ MODERATE[b] *due to imprecision*	Mosquito mortality is probably higher with unwashed pyrethroid-PBO nets compared to standard unwashed LLINs in areas of moderate insecticide resistance
Mosquito mortality (washed nets)	287 per 1000[a]	307 per 1000 (213 to 443)[a]	RR 1.07 (0.74 to 1.54)	329 (1 trial, 1 comparison)	⊕⊕⊖⊖ LOW[b,c,d] *due to imprecision and indirectness*	There may be little to no difference in the effect of washed pyrethroid-PBO nets on mosquito mortality compared to standard washed LLINs (washed) in areas of moderate insecticide resistance
Blood-feeding success (unwashed nets)	258 per 1000[a]	232 per 1000 (197 to 304)[a]	RR 0.90 (0.72 to 1.11)	1006 (2 trials, 3 comparisons)	⊕⊕⊕⊖ MODERATE[b] *due to imprecision*	There is probably little to no difference in the effect of pyrethroid-PBO nets (unwashed) on mosquito blood-feeding success compared to standard LLINs in areas of moderate insecticide resistance
Blood-feeding success (washed nets)	586 per 1000[a]	533 per 1000 (434 to 662)[a]	RR 0.91 (0.74 to 1.13)	329 (1 trial, 1 comparison)	⊕⊕⊖⊖ LOW[b,c,d] *due to imprecision and indirectness*	There may be little to no difference in the effect of washed pyrethroid-PBO nets on mosquito blood-feeding success compared to standard washed LLINs in areas of moderate insecticide resistance

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; LLIN: long-lasting insecticidal net; PBO: pyrethroid-piperonyl butoxide; RR: risk ratio.

GRADE Working Group grades of evidence.
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.

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Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Original numbers are used in this table; however for the pooled analysis, we generated events and total numbers from cluster-adjusted results, which used the effective sample size. Note that cluster adjustments do not change the point estimate of the effect size, just the standard error.
[b]Downgraded by one for imprecision due to wide CIs.
[c]Not downgraded for inconsistency, as only one trial measured this outcome in this setting.
[d]Downgraded by one for indirectness: the outcome is highly context-specific, and only one trial is included.

Summary of findings 3. Summary of findings table 3

Pyrethroid-piperonyl butoxide (PBO) nets compared to long-lasting insecticidal nets (LLINs) for malaria control when insecticide resistance is low

Patient or population: *Anopheles gambiae* complex or *Anopheles funestus* group
Setting: areas of low insecticide resistance
Intervention: pyrethroid-PBO nets
Comparison: LLINs

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Number of mosquitoes (experimental hut trials)	Certainty of the evidence (GRADE)	Comments
	Risk with LLINs	Risk with pyrethroid-PBO nets				
Mosquito mortality (unwashed nets)	527 per 1000[a]	659 per 1000 (613 to 972)[a]	RR 1.25 (0.99 to 1.57)	1580 (2 trials, 3 comparisons)	⊕⊕⊕⊖ MODERATE[b] *due to imprecision*	There is probably little to no difference in the effect of unwashed pyrethroid-PBO nets on mosquito mortality compared to standard unwashed LLINs in areas of low insecticide resistance
Mosquito mortality (washed nets)	394 per 1000[a]	547 per 1000 (437 to 938)[a]	RR 1.39 (0.95 to 2.04)	1774 (2 trials, 3 comparisons)	⊕⊖⊖⊖ VERY LOW[c,d] *due to imprecision and inconsistency*	We do not know if pyrethroid-PBO nets have an effect on mosquito mortality in areas of low insecticide resistance when the nets have been washed
Blood-feeding success (unwashed nets)	201 per 1000[a]	151 per 1000 (58 to 456)[a]	RR 0.75 (0.27 to 2.11)	1580 (2 trials, 3 comparisons)	⊕⊖⊖⊖ VERY LOW[c,d] *due to imprecision and inconsistency*	We do not know if unwashed pyrethroid-PBO nets have an effect on mosquito blood-feeding success in areas of low insecticide resistance
Blood-feeding success (washed nets)	161 per 1000[a]	172 per 1000 (122 to 578)[a]	RR 1.07 (0.49 to 2.33)	1774 (2 trials, 3 comparisons)	⊕⊕⊖⊖ LOW[d]	Mosquito blood-feeding success may decrease with washed pyrethroid-PBO nets compared to standard washed LLINs in areas of low insecticide resistance

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						due to inconsistency

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; LLIN: long-lasting insecticidal net; PBO: pyrethroid-piperonyl butoxide; RR: risk ratio.

GRADE Working Group grades of evidence.
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Original numbers are used in this table; however for the pooled analysis, events and total numbers were generated from cluster-adjusted results, which use the effective sample size. Note that cluster adjustments do not change the point estimate of the effect size, just the standard error.
[b]Downgraded by one for imprecision due to wide CIs.
[c]Downgraded by one for inconsistency due to unexplained heterogeneity.
[d]Downgraded by two for imprecision due to extremely wide CIs.

Summary of findings 4. Summary of findings table 4

Pyrethroid-piperonyl butoxide (PBO) nets compared to long-lasting insecticidal nets (LLINs) for malaria control when mosquitoes are susceptible

Patient or population: *Anopheles gambiae* complex or *Anopheles funestus* group
Setting: areas of insecticide-susceptible mosquitoes

Intervention: pyrethroid-PBO nets
Comparison: LLINs

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Number of mosquitoes (experimental hut trials)	Certainty of the evidence (GRADE)	Comments
	Risk with LLINs	Risk with pyrethroid-PBO nets				
Mosquito mortality (unwashed nets)	392 per 1000[a]	471 per 1000 (251 to 887)[a]	RR 1.20 (0.64 to 2.26)	2791 (2 trials, 2 comparisons)	⊕⊕⊖⊖ LOW[b] *due to imprecision*	There may be little to no difference in the effect of unwashed pyrethroid-PBO nets on mosquito mortality compared to standard unwashed LLINs in areas of no insecticide resistance

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Mosquito mortality (washed nets)	457 per 1000[a]	489 per 1000 (420 to 571)[a]	RR 1.07 (0.92 to 1.25)	2644 (2 trials, 2 comparisons)	⊕⊕⊖⊖ LOW[b] *due to imprecision*	There may be little to no difference in the effect of washed pyrethroid-PBO nets on mosquito mortality compared to standard washed LLINs in areas of no insecticide resistance
Blood-feeding success (unwashed nets)	57 per 1000[a]	29 per 1000 (6 to 132)[a]	RR 0.52 (0.12 to 2.22)	2791 (2 trials, 2 comparisons)	⊕⊖⊖⊖ VERY LOW[b,c] *due to imprecision and inconsistency*	We do not know if unwashed pyrethroid-PBO nets have an effect on mosquito blood-feeding success in areas of no insecticide resistance
Blood-feeding success (washed nets)	64 per 1000[a]	82 per 1000 (52 to 131)[a]	RR 1.25 (0.82 to 1.91)	2644 (2 trials, 2 comparisons)	⊕⊖⊖⊖ VERY LOW[b,c] *due to imprecision and inconsistency*	We do not know if washed pyrethroid-PBO nets have an effect on mosquito blood-feeding success in areas of no insecticide resistance

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).
CI: confidence interval; LLINs: long-lasting insecticidal nets; PBO: pyrethroid-piperonyl butoxide; RR: risk ratio.

GRADE Working Group grades of evidence.
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Original numbers are used in this table; however for the pooled analysis, events and total numbers were generated from cluster-adjusted results, which use the effective sample size. Note that cluster adjustments do not change the point estimate of the effect size, just the standard error.
[b]Downgraded by two for imprecision due to extremely wide CIs.
[c]Downgraded by one for inconsistency due to unexplained heterogeneity.

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BACKGROUND

Description of the condition

Substantial progress has been made in reducing the burden of malaria in the 21st century. It is estimated that the clinical incidence of *Plasmodium falciparum* malaria in Africa dropped by 40% between 2000 and 2015, equating to prevention of 663 million cases (Bhatt 2015; WHO-GMP 2015). However progress has stalled in recent years (WHO 2019a). Targeting the mosquito vector has proved to be the most effective method of malaria prevention in Africa, with over two-thirds of malaria cases averted in the first 15 years of this century attributed to scale-up in the use of long-lasting insecticidal nets (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 all LLINs contain pyrethroids; 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 result in reduced sensitivity to the insecticide or increased activity of detoxification enzymes (metabolic resistance) (Mitchell 2012; Stevenson 2011), or other as yet poorly described resistance mechanisms, or a combination of all or some of these factors. The evolution of insecticide resistance and its continuing spread threaten the operational success of malaria vector control interventions. The current impact of this resistance on malaria transmission is largely unquantified and varies depending on level of resistance, malaria endemicity, and proportion of the human population using LLINs (Churcher 2016). A multi-country trial found no evidence that pyrethroid resistance reduced the personal protection provided by the use of LLINs (Kleinschmidt 2018). 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 (hereafter referred to as pyrethroid-PBO nets). Insecticide-synergist combination nets represent a new product class with the capacity to affect insecticide-resistant populations. In 2017, the World Health Organization (WHO) gave pyrethroid-PBO nets an interim endorsement as a new vector control class and recommended that countries consider deploying these nets in areas where pyrethroid resistance has been confirmed among main malaria vectors (WHO-GMP 2017a).

Currently six pyrethroid-PBO nets are in production: Olyset® Plus; PermaNet® 3.0; Veeralin® LN; Tsara Plus (previously DawaPlus 3.0); Tsara Boost (previously DawaPlus 4.0); and DuraNet Plus. 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 found only 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 (thickness) yarn with 70-cm lower border 2.8 g/kg ± 25%, 100-denier yarn 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). Tsara Plus and Tsara Boost are manufactured by NRS Moon Netting FZE. Tsara Plus is treated with deltamethrin (3 g/kg) and PBO (11 g/kg) on the roof, and with deltamethrin only (2.5 g/kg) on its sides. Tsara Boost is treated with deltamethrin (120 mg/m^2) and PBO (440 mg/m^2) on all panels. DuraNet Plus, manufactured by Shobikaa Impex Private Limited, is a polyethylene net treated with alpha-cypermethrin (6.0 g/kg) and PBO (2.2 g/kg) across the whole net.

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). In areas where 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 when conventional LLINs are used (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; Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010). In most settings, pyrethroid-PBO nets resulted in higher rates of mosquito mortality and greater blood-feeding inhibition than conventional LLINs, although the magnitude of this effect was variable. Village trials have measured the impact on sporozoite infection rates in mosquitoes with mixed results (Awolola 2014; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013). Recently, two separate cluster-randomized trials (cRCTs) in Tanzania and Uganda demonstrated that use of pyrethroid-PBO nets can reduce parasite prevalence in children (Protopopoff 2018; Staedke 2020).

Why it is important to do this review

All LLINs approved by the WHO Prequalification Team (formerly the WHO Pesticide Evaluation Scheme (WHOPES)) contain pyrethroids. Six bed nets that contain PBO have received WHO pre-qualification and have been recognized as a new product class by WHO (WHO-GMP 2017a). As pyrethroid-PBO nets are generally more expensive than conventional LLINs, it is important to determine if they are

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superior to conventional LLINs, and under what circumstances, to enable cost-effectiveness trials to be performed to inform procurement decisions.

An Expert Review Group (ERG) commissioned by the WHO has recommended pyrethroid-PBO nets be considered for use in areas where the major malaria vectors are resistant to pyrethroids (WHO-GMP 2017a). This guidance has been adopted by some net providers, for example, the President's Malaria Initiative (PMI) (PMI 2018). The WHO recommendation was largely based on a single randomized controlled trial (RCT) of one pyrethroid-PBO net type conducted in Tanzania (Protopopoff 2018), but it was also supported by a meta-analysis of performance of pyrethroid-PBO nets in experimental hut trials, which was used to parameterize a malaria transmission model to predict the public health benefit of pyrethroid-PBO nets (Churcher 2016). The WHO recommendation is that countries should consider deployment of this new product class in areas with intermediate levels of pyrethroid resistance, but it calls for further evidence, including data from a second clinical trial (WHO 2019b). Results of a second RCT evaluating the epidemiological impact of pyrethroid-PBO nets in Uganda were published in 2020, and this review has been updated to include these data (Staedke 2020).

In an attempt to assess evidence of effectiveness of pyrethroid-PBO nets against African malaria vectors in areas with differing levels of insecticide resistance, we have conducted a systematic review of all relevant trials and examined both epidemiological and entomological endpoints. We appreciate that evaluation of PBO will depend on trials in which the background insecticide and dose are the same in both intervention and control groups; we are aware that most trials have evaluated pyrethroid-PBO nets against pyrethroid-only LLINs with different background insecticides and doses, which confounds the effects.

OBJECTIVES

To compare effects of pyrethroid-PBO nets currently in commercial development or on the market with effects of their non-PBO equivalent in relation to:

1. malaria parasite infection (prevalence or incidence); and
2. entomological outcomes

METHODS

Criteria for considering studies for this review

Types of studies

We included:

1. randomized trials that measured epidemiological outcomes, entomological outcomes, or both; and
2. experimental hut trials.

See Table 1 for detailed WHOPES definitions.

Types of participants

Mosquitoes

Anopheles gambiae complex or *Anopheles funestus* group. Included trials had to test a minimum of 50 mosquitoes per trial arm. We examined the insecticide resistance level (measured by phenotypic resistance) during data analysis.

Humans

Adults and children living in malaria-endemic areas.

Types of interventions

Intervention

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

Control

Conventional LLINs that contain pyrethroid only. Nets could be treated with the same insecticide at different doses from the intervention net to allow critical appraisal of all pyrethroid-PBO nets currently in development or on the market. For both intervention and control arms, nets could be unholed, holed, unwashed, or washed, provided the trials adhered to WHO guidelines (WHO 2013).

Types of outcome measures

Trials had to include at least one of the following primary outcomes to be eligible for inclusion.

Primary outcomes

Epidemiological

1. Parasite prevalence: presence of malaria parasites detected through microscopy of blood or rapid diagnostic tests (RDTs)
2. Incidence of clinical malaria: clinical diagnosis based on participants' symptoms and on physical findings at examination

Entomological

1. 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
2. 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
3. Blood-feeding success: number of mosquitoes that have blood-fed (alive or dead)
4. Sporozoite rate: percentage of mosquitoes with sporozoites in the salivary glands

Secondary outcomes

Entomological

1. Deterrence: the number of mosquitoes that enter a hut that is using a pyrethroid-PBO net relative to the number of mosquitoes found in a control hut that is using a standard LLIN (experimental hut trials only)
2. Exophily: the proportion of mosquitoes found in exit/veranda traps of a hut that is using a pyrethroid-PBO net relative to the control hut that is using a standard LLIN (experimental hut trials only)

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3. Mosquito density: measured by all standard methods, such as window exit traps, indoor resting collections, floor sheet collections, pyrethrum spray catch, and light traps (village trials)
4. Parity rate: percentage of parous mosquitoes detected by mosquito ovary dissections (village trials)

Search methods for identification of studies

We identified all relevant trials regardless of language or publication status (published, unpublished, in press, and in progress). We have presented the search strategies in Appendix 1.

Electronic searches

Vittoria Lutje, the Cochrane Infectious Diseases Group (CIDG) Information Specialist, searched the following databases on 25 September 2020 using the search terms and strategy described in Appendix 1: the CIDG Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL; 2018, Issue 8), included in the Cochrane Library; MEDLINE (PubMed); Embase (OVID); Web of Science Core Collection; and CAB Abstracts. She also searched for trials in progress at the WHO International Clinical Trials Registry Platform (WHO ICTRP; www.who.int/ictrp/en/) and ClinicalTrials.gov (clinicaltrials.gov/ct2/home).

Searching other resources

We contacted the following organizations for unpublished data: the PMI; the Innovative Vector Control Consortium (IVCC); Vestergaard Frandsen; Sumitomo Chemical Company Ltd.; Vector Control Innovations Private Ltd.; Endura SpA; and WHOPES. We checked the reference lists of trials identified by the above methods.

Data collection and analysis

All analyses were stratified by trial design and mosquito insecticide resistance level when possible. We performed analyses for the primary outcomes stratified by follow-up time (4 to 6 months, 9 to 12 months, 16 to 18 months, and 21 to 25 months).

We determined whether mosquito populations are susceptible or resistant to pyrethroid insecticides based on WHO definitions (WHO 2016; Table 4). We used 24-hour mosquito mortality to determine resistance status; however if this had been unavailable, we intended to use knock-down 60 minutes after the end of the assay. We stratified resistant populations into low-, moderate-, and high-prevalence resistance groups (Table 5), by dividing resistant mosquitoes (i.e. those with < 90% mortality) into three equal groups, with the lower third being most resistant and the upper third most susceptible.

Selection of studies

Two review authors (KG and NL or LC) independently screened titles and abstracts of all retrieved references based on the inclusion criteria (Table 6). We resolved any inconsistencies between review authors' selections by discussion. If we were unable to reach an agreement, we consulted a third review author (HR). We retrieved full-text trial reports for all potentially relevant citations. Two review authors independently screened the full-text articles and identified trials for inclusion, and identified and recorded reasons for exclusion of ineligible trials in a Characteristics of excluded studies table. We resolved any disagreements through discussion or, if required, we consulted a third review author (HR). We identified and excluded duplicates and collated multiple reports of the same trial, so that each trial, rather than each report, was the unit of interest in the review. We recorded the selection process in sufficient detail to complete a PRISMA flow diagram (Moher 2009).

Data extraction and management

After selection, we summarized all included trials according to the tables in Appendix 2. Two review authors (KG and NL or LC) independently extracted data from included trials using the pre-designed data extraction form (Appendix 3). If data were missing from an included trial, we contacted the trial authors to ask for further information. We entered data into Review Manager 5 (RevMan 5) (Review Manager 2014).

Assessment of risk of bias in included studies

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

Randomized trials and village trials

We assessed 12 criteria for village and RCTs: recruitment bias, comparability of mosquitoes between LLIN/pyrethroid-PBO net households (e.g. species composition), collectors blinded, household blinded, treatment allocation, allocation concealment, incomplete outcome data, raw data reported, clusters lost to follow-up, selective reporting, adjustment for data clustering, and trial authors' conflicting interests.

Experimental hut trials

For experimental hut trials, we assessed 11 criteria: comparability of mosquitoes between LLIN/pyrethroid-PBO net arms (e.g. species composition), collectors blinded, sleepers blinded, sleeper bias accounted for, treatment allocation, treatment rotation, standardized hut design, hut cleaning between treatments, incomplete outcome data, raw data reported, and trial authors' conflicting interests.

Measures of treatment effect

For dichotomous data, we preferentially presented the risk ratio (RR). For the outcome of parasite prevalence from cRCTs, we used the odds ratio (OR) as the measure of effect, as one study presented adjusted ORs that could not be converted to adjusted RRs using the standard formula presented in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). We found no continuous or count data; however if we had, we would have used mean differences (MDs) and rate ratios, respectively. We have presented all results with 95% confidence intervals (CIs).

Unit of analysis issues

For trials randomized by hut or village, we used the adjusted measure of effect reported in the paper if available. For the outcome of parasite prevalence from cRCTs, we converted adjusted RRs presented in one study - Staedke 2020 - to adjusted ORs using the standard formula presented in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011), so that this study could be pooled with Protopopoff 2018.

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When adjusted measures of effect were not reported, we used an intracluster correlation coefficient (ICC) and average cluster size to adjust the data ourselves (Higgins 2011 Section 16.3.4). If the included trial did not report the ICC value, we estimated the ICC value and performed sensitivity analyses to investigate the impact of estimating the ICC. When ICCs have been used to adjust results for clustering, forest plots for both hut and village trials show the effective number of events and the number of mosquitoes after adjustments for clustering.

To adjust results of experimental hut trials for clustering, we treated each 'hut and night' combination as the unit of randomization, as each hut was tested with each type of net over a series of nights. Sleepers inside the huts were rotated each night, so by using "hut/night" as the unit of randomization, sleeper effects were also accounted for. We calculated effective sample sizes by estimating an ICC and a corresponding design effect. We divided both the number of mosquitoes and the number experiencing the event by this design effect.

Dealing with missing data

In the case of missing data, we contacted trial authors to request this information. If we had identified trials in which participants were lost to follow-up, we would have investigated the impact of missing data via imputation using a best/worst-case scenario analysis.

When information on mosquito insecticide resistance was not collected at the time of the trial, review authors determined a suitable proxy. Proxy resistance data had to be taken from the same area and conducted within three years of the trial, and the same insecticide, dose, and mosquito species had to be used. More than 50 mosquitoes per insecticide should have been tested against an appropriate control. When no resistance data were available, we determined that resistance status was unclassified.

Assessment of heterogeneity

We presented the results of included trials in forest plots, which we inspected visually, to assess heterogeneity (i.e. non-overlapping CIs generally signify statistical heterogeneity). We used the Chi^2 test with a P value less than 0.1 to indicate statistical heterogeneity. We quantified heterogeneity by using the I^2 statistic (Higgins 2003), and we interpreted a value greater than 75% to indicate considerable heterogeneity (Deeks 2017).

Assessment of reporting biases

To analyse the possibility of publication bias, we intended to use funnel plots if 10 trials with epidemiological endpoints were included in any of the meta-analysis. However, no analyses included 10 or more trials, so this plan was not applicable.

Data synthesis

When appropriate, we pooled the results of included trials using meta-analysis. We stratified results by type of trial, mosquito resistance status, and net type (i.e. by product, e.g. Olyset Plus).

Four review authors (KG, NL, LC, and MC) analysed the data using RevMan 5 (Review Manager 2014), using the random-effects model (if we detected heterogeneity; or if the I^2 statistic value was greater than 75%) or the fixed-effect model (for no heterogeneity; or if the I^2 statistic value was less than 75%). The exception to this is that for the primary outcome of parasite prevalence from cluster trials, we pooled results using the fixed-effect model, although heterogeneity between study results was substantial. For additional information, see 'Effects of Interventions: Epidemiological results'. We would have refrained from pooling trials in meta-analysis if it was not clinically meaningful to do so, due to clinical or methodological heterogeneity.

Subgroup analysis and investigation of heterogeneity

We performed subgroup analyses according to whether nets were washed or unwashed.

Sensitivity analysis

We intended to perform sensitivity analyses to determine the effect of exclusion of trials that we considered to be at high risk of bias; however this approach was not applicable, as no trials were deemed at high risk. We would have performed a sensitivity analysis for missing data during imputation with best/worst-case scenarios, but again this was not applicable.

We performed sensitivity analyses to investigate the impact of estimating an ICC to adjust trial results for clustering. We performed analyses using ICCs of 0.01, 0.05, and 0.1. Because results were robust to these adjustments, we used the most conservative ICC (0.1), and we adjusted all results from unadjusted cluster trials using this ICC. We have not presented analyses using the smaller ICCs (0.01 and 0.05).

Summary of findings and assessment of the certainty of the evidence

We assessed the certainty of evidence using the GRADE approach (Schünemann 2013). We constructed 'Summary of findings' tables using GRADEpro Guideline Development Tool (GDT) software (GRADEpro GDT 2015).

RESULTS

Description of studies

Results of the search

We identified 389 records through our searches. We removed duplicates, leaving 347 records, and we screened all articles for possible inclusion. After abstract and title screening, we excluded 322 ineligible trials. We assessed 25 full-text articles for eligibility and excluded nine articles for the following reasons: three trials did not share full data sets, two were laboratory studies, and four are ongoing. Sixteen trials met the inclusion criteria (Figure 1).

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Figure 1. Study flow diagram.

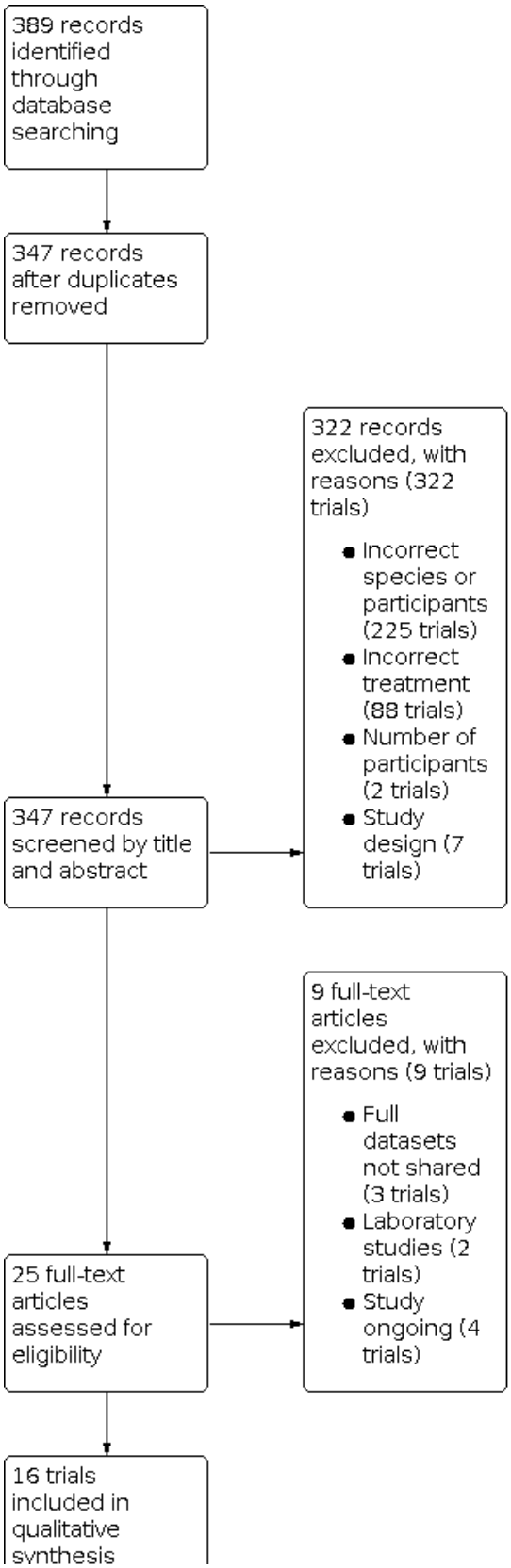

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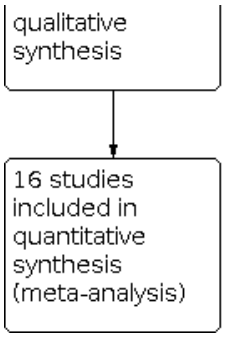
Figure 1. (Continued)



Included studies

Sixteen trials met the inclusion criteria; we have described them in the Characteristics of included studies tables. Ten trials were experimental hut trials (Bayili 2017 (Burkina Faso); Corbel 2010 (Burkina Faso, Benin, Cameroon); Koudou 2011 (Côte d'Ivoire); Menze 2020 (Cameroon); Moore 2016 (Tanzania); N'Guessan 2010 (Benin); Oumbouke 2019 (Côte d'Ivoire); Pennetier 2013 (Benin); Toé 2018 (Burkina Faso); Tungu 2010 (Tanzania)). Four trials were village trials (Awolola 2014 (Nigeria); Cisse 2017 (Mali); Mzilahowa 2014 (Malawi); Stiles-Ocran 2013 (Ghana)). Two were cRCTs (Protopopoff 2018 (Tanzania); Staedke 2020 (Uganda)). All trials were conducted in Africa.

Interventions

Six trials compared Permanet 2.0 to Permanet 3.0 (Awolola 2014; Corbel 2010; Koudou 2011; N'Guessan 2010; Stiles-Ocran 2013; Tungu 2010); two trials compared Olyset Net to Olyset Plus (Pennetier 2013; Protopopoff 2018); two trials compared MAGNet LN to Veeralin LN (Moore 2016; Oumbouke 2019); five trials compared both Olyset Net to Olyset Plus and Permanet 2.0 to Permanet 3.0 (Cisse 2017; Menze 2020; Mzilahowa 2014; Staedke 2020; Toé 2018); and one trial compared DawaPlus 2.0 to DawaPlus 3.0 and DawaPlus 4.0 (Bayili 2017).

Excluded studies

We assessed 25 full-text articles for eligibility and excluded nine articles for the following reasons: three trials are awaiting classification because we were unable to obtain the full data sets after we contacted trial authors (see Characteristics of studies awaiting classification table); four trials are ongoing (see Characteristics of ongoing studies section); and two trials included only laboratory data (Darriet 2011; Darriet 2013).

Risk of bias in included studies

We have provided a 'Risk of bias' assessment summary in Figure 2. The criteria we used to assess risk of bias are provided in Appendix 5 (experimental hut trials) and in Appendix 6 (village trials).

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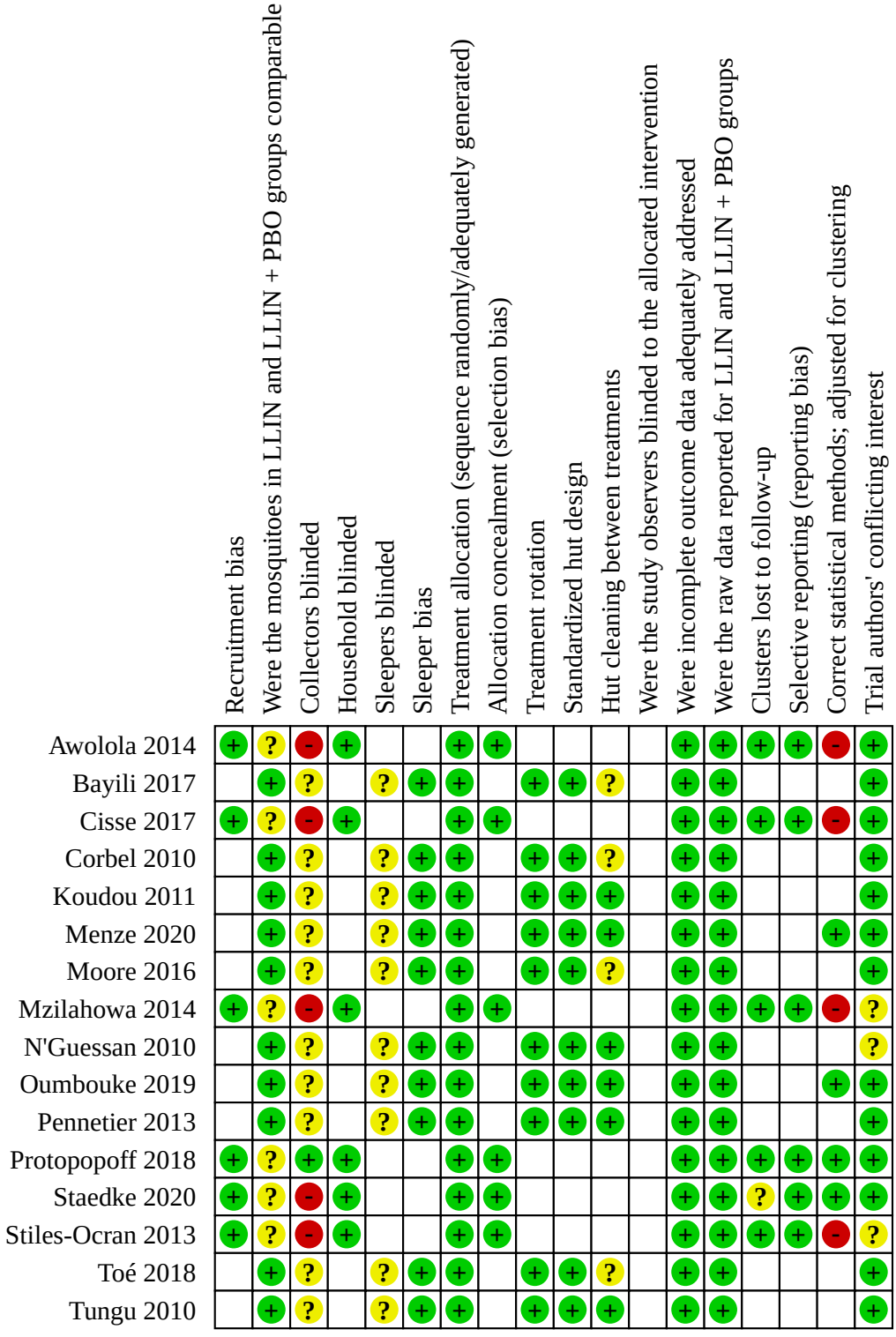
Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



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Allocation

Recruitment bias

We assessed all four village trials as having low risk of recruitment bias, as recruitment bias is related to human participants and so is not applicable to this review (Awolola 2014; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013). We assessed the two cRCTs as having low risk, as no participants were recruited after clusters had been randomized (Protopopoff 2018; Staedke 2020).

Mosquito group comparability

We judged all 10 experimental hut trials to be at low risk (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010), as the huts were situated in the same trial area and therefore were accessible to the same mosquito populations. We judged all four village trials and both cRCTs to be at unclear risk, as for six trials, species composition and resistance status varied slightly between treatment arms (Awolola 2014; Cisse 2017; Menze 2020; Oumbouke 2019; Protopopoff 2018; Stiles-Ocran 2013); for one trial, species and resistance data were not separated by village (Mzilahowa 2014); and for one trial, the size of the area covered made it difficult to classify resistance status in all areas (Staedke 2020).

Blinding

We assessed the 10 hut trials to be at unclear risk, as they did not specify whether observers, collectors and sleepers (hut trials) were blinded (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010). This is not standard protocol for these trial designs and is thought unlikely to affect the results. We judged four village trials to be at high risk of bias, as it was not stated whether collectors were blinded, and this may have affected searching efforts during collection (Awolola 2014; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013). We judged one cRCT as having high risk, as it was stated that LLIN allocation was not masked to collectors (Staedke 2020), and the other as having low risk because collectors were masked to treatment (Protopopoff 2018). For household blinding, we judged all four village trials and both cRCTs to be at low risk of bias. Four village trials and one cRCT did not state whether households were blind to the intervention; however this was unlikely to influence the results (Awolola 2014; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013; Staedke 2020). We judged one cRCT as having low risk, as inhabitants and field collectors were blinded to intervention arms (Protopopoff 2018).

Sleeper bias

We assessed the 10 hut trials to be at low risk for sleeper bias, as sleepers were rotated between huts according to a Latin square design (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010).

Treatment allocation, rotation, and concealment

We assessed the 10 hut trials to be at low risk for treatment allocation and rotation, as treatments were rotated between huts according to a Latin square design (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010). We assessed all four village trials and both cRCTs to be at low risk for treatment allocation (Awolola 2014; Cisse 2017; Mzilahowa 2014; Protopopoff 2018; Staedke 2020; Stiles-Ocran 2013), as villages were randomly assigned to treatment arms. We assessed all four village trials and both cRCTs as having low risk of bias for allocation concealment (Awolola 2014; Cisse 2017; Mzilahowa 2014; Protopopoff 2018; Staedke 2020; Stiles-Ocran 2013).

Hut design

We assessed all 10 hut trials to be at low risk of bias, as huts were built to standard West or East African specifications (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010), or they used modified but standardized designs (Moore 2016).

Cleaning

We assessed four hut trials to be at unclear risk, as they did not state whether huts were cleaned between treatment arms (Bayili 2017; Corbel 2010; Moore 2016; Toé 2018). We assessed six to be at low risk, as cleaning was conducted between treatment rotations (Koudou 2011; Menze 2020; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Tungu 2010).

Incomplete outcome data

We assessed all hut trials - Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010, village trials - Awolola 2014; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013, and cRCTs - Protopopoff 2018; Staedke 2020 - to be at low risk for both incomplete outcome data and raw data reporting, as there were no incomplete outcome data, or missing data were later provided by trial authors. In cases when raw data were not reported, we were able to calculate them from the percentages and sample sizes given. When these data were not available, we did not include the trials.

Clustering bias

Staedke 2020 lost 14 clusters to follow-up at the latest time point and was therefore assessed as having unclear risk of bias. In the other village and cRCT trials, no clusters were lost to follow-up, and these trials were assessed as having low risk (Awolola 2014; Cisse 2017; Mzilahowa 2014; Protopopoff 2018; Staedke 2020; Stiles-Ocran 2013). We assessed four village trials as having high risk of bias for statistical methods used, as they did not adjust for clustering (Awolola 2014; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013). We assessed the two cRCTs as having low risk of bias, as they took clustering into account and adjusted for it in their statistical methods (Protopopoff 2018; Staedke 2020).

Selective reporting

We assessed all village trials and cRCTs as having low risk of bias regarding selective reporting, as they appear to have reported all measured outcomes (Awolola 2014; Cisse 2017; Mzilahowa 2014; Protopopoff 2018; Staedke 2020; Stiles-Ocran 2013).

Other potential sources of bias

Conflicting interests

We judged nine hut trials - Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; Oumbouke 2019; Pennetier 2013; Toé

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2018; Tungu 2010, two village trials - Awolola 2014; Cisse 2017, and both cRCTs - Protopopoff 2018; Staedke 2020 - as having low risk, as trial authors reported no conflicting interests. We assessed one hut trial to be at unclear risk (N'Guessan 2010), as trial authors stated that they had received funding from LLIN manufacturers when conducting the trials, and the same funders provided comments on the manuscript. We assessed one village trial as having unclear risk, as trial authors did not state whether there were conflicting interests (Mzilahowa 2014), and another trial as having unclear risk, as the trial was conducted to form part of the manufacturer's product dossier (Stiles-Ocran 2013).

Effects of interventions

See: **Summary of findings 1** Summary of findings table 1; **Summary of findings 2** Summary of findings table 2; **Summary of findings 3** Summary of findings table 3; **Summary of findings 4** Summary of findings table 4

We compared the effects of pyrethroid-PBO nets currently in commercial development or on the market with their non-PBO equivalent in relation to malaria infection and entomological outcomes. This review is based on results from 16 trials.

Epidemiological results

Two trials examined the effects of pyrethroid-PBO nets (Olyset Plus and PermaNet 3.0) on parasite prevalence (Protopopoff 2018; Staedke 2020). Pooling the latest endpoint after the intervention from both trials revealed that parasite prevalence was decreased in the intervention arm (Olyset Plus and PermaNet 3.0) (OR 0.79, 95% CI 0.67 to 0.95; 2 trials, 2 comparisons; Analysis 1.1).

There was little variation of effect from the earliest time point (4 to 6 months after: OR 0.74, 95% CI 0.62 to 0.89) to the latest time point (21 to 25 months after: OR 0.79, 95% CI 0.67 to 0.95) (Analysis 1.2).

We used a fixed-effect model to pool data from the two studies. Although heterogeneity between study results was considerable, both studies demonstrated clear beneficial effects with PBO nets. Performing random-effects meta-analysis accounted for differences between study results to the extent that identified benefits disappeared in the pooled analysis, indicating failure of the random-effects model.

Entomological results

Experimental hut trials

Ten experimental hut trials (phase 2 trials) examined the effects of pyrethroid-PBO nets on mosquito mortality, blood feeding, exophily, and deterrence (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010). We subgrouped the data by net washing into unwashed and washed groups. All washed nets were washed 20 times according to WHO specifications (WHO 2013). We pooled the results initially and then stratified them by insecticide resistance level and by net type. Two trials did not wash their nets and so did not report any data for the washed subgroup (Menze 2020 Toé 2018). One trial did not introduce holes into the nets and so did not report blood-feeding success data (Koudou 2011).

Pooled analysis

Pooled analysis of all experimental hut trials using both unwashed nets - Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010 - and washed nets - Bayili 2017; Corbel 2010; Koudou 2011; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Tungu 2010 - revealed that pyrethroid-PBO nets significantly increased mosquito mortality by 43% (risk ratio (RR) 1.43, 95% confidence interval (CI) 1.26 to 1.62) and reduced blood-feeding success by 25% (RR 0.75, 95% CI 0.66 to 0.85). The magnitude of the effect was reduced by net washing. Unwashed pyrethroid-PBO nets increased mosquito mortality by 63% compared to unwashed LLINs (RR 1.63, 95% CI 1.29 to 2.05; 10 trials, 18 comparisons; Analysis 2.1); when nets were washed, this effect was decreased to 19% (RR 1.19, 95% 1.04 to 1.38; 8 trials, 12 comparisons; Analysis 2.1). Unwashed pyrethroid-PBO nets reduced mosquito blood-feeding success by 32% (RR 0.68, 95% CI 0.57 to 0.80; 9 trials, 17 comparisons; Analysis 2.2; Bayili 2017; Corbel 2010; Moore 2016; N'Guessan 2010; Pennetier 2013; Toé 2018; Tungu 2010); however this effect was lost when nets were washed (7 trials, 11 comparisons; Analysis 2.2; Bayili 2017; Corbel 2010; Moore 2016; N'Guessan 2010; Pennetier 2013; Tungu 2010). There was no effect on mosquito exophily in either unwashed (10 trials, 17 comparisons; Analysis 2.3) or washed groups (8 trials, 12 comparisons; Analysis 2.3). Mosquito deterrence data were presented relative to an untreated control and hence are not included as a forest plot. There was considerable variation in deterrence rates but no clear relationship with resistance level, net type, or washing status (Table 7).

Heterogeneity in this pooled analysis was considerable, particularly for estimates of mortality. We therefore performed a pre-specified, stratified analysis, dividing the results into trials conducted in areas of low, moderate, or high resistance in the *Anopheles* population.

Stratified analysis: mosquito resistance status

We used WHO and Centers for Disease Control and Prevention (CDC) definitions of mosquito mortality from WHO tube assays or CDC bottle tests to classify mosquito resistance (Table 4). Both tests define mosquitoes as resistant when mortality is less than 90%. We further stratified resistance based on the following mortality levels: < 30%, high resistance; 31% to 60%, moderate resistance; and 61% to 90%, low resistance (Table 5). When resistance data were not collected at the time of the trial, we identified a suitable proxy based on previously described criteria (see Dealing with missing data section); when we could not identify a suitable proxy, we deemed the trial as 'unclassified' and did not include it in the resistance stratification.

Five trials were conducted in four areas where mosquito populations exhibited high resistance to pyrethroids (Bayili 2017; Corbel 2010; Koudou 2011; Pennetier 2013; Toé 2018). Under these conditions, unwashed pyrethroid-PBO nets increased mosquito mortality by 84% in comparison to unwashed LLINs (RR 1.84, 95% CI 1.60 to 2.11; 5 trials, 9 comparisons; Analysis 2.4); however this effect was lost when nets were washed (4 trials, 5 comparisons; Analysis 2.4; Bayili 2017; Corbel 2010; Koudou 2011; Pennetier 2013). Blood-feeding success was reduced by 40% in unwashed pyrethroid-PBO net groups compared to unwashed LLIN groups (RR 0.60, 95% CI 0.50 to 0.71; 4 trials, 8 comparisons; Analysis 2.5; Bayili 2017; Corbel 2010; Pennetier 2013; Toé 2018), and was reduced by 19% when nets were washed (RR 0.81, 95% CI 0.72 to

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0.92; 3 trials, 4 comparisons; Analysis 2.5; Bayili 2017; Corbel 2010; Pennetier 2013).

Two trials at three different sites were conducted in areas with moderate insecticide resistance (Menze 2020; N'Guessan 2010). With unwashed nets, mosquito mortality was increased by 68% in comparison to mosquito mortality with unwashed LLINs (RR 1.68, 95% CI 1.33 to 2.11; 2 trials, 3 comparisons; Analysis 2.6); however there was minimal effect on blood-feeding success. No effect on mosquito mortality (1 trial, 1 comparison; Analysis 2.6) or on blood-feeding success (1 trial, 1 comparison; Analysis 2.7) was observed with washed treatments.

Two trials at three different sites were conducted in areas with low insecticide resistance (Corbel 2010; Oumbouke 2019). A small effect on mosquito mortality was observed with unwashed nets (RR 1.25, 95% CI 0.99 to 1.57; 2 trials, 3 comparisons; Analysis 2.8) and was also seen with washed nets (RR 1.39, 95% CI 0.95 to 2.04; 2 trials, 3 comparisons; Analysis 2.8). No effect on blood-feeding success was noted (2 trials, 3 comparisons; Analysis 2.9).

At susceptible sites (Moore 2016; Tungu 2010), no effect on mosquito mortality (2 trials, 2 comparisons; Analysis 2.10) nor on blood-feeding success (2 trials, 2 comparisons; Analysis 2.11) was observed.

Stratified analysis: net type

After stratifying by resistance status, we performed a secondary analysis stratified according to net type. Due to the limited number of trials, we performed this analysis only for trials using PermaNet 3.0 or Olyset Plus. Although additional trials utilising Veeralin LN, DawaPlus 3.0, and DawaPlus 4.0 have been conducted, not all data were made available to us for the purposes of this Cochrane Review. Futhermore, the analysis was restricted to trials conducted in areas of high resistance, as this analysis indicated an impact of only pyrethroid-PBO nets in these settings. Three trials compared PermaNet 2.0 (LLIN) to PermaNet 3.0 (pyrethroid-PBO nets), and two compared Olyset Nets (LLIN) to Olyset Plus (pyrethroid-PBO nets).

In the PermaNet group, in high-resistance settings, unwashed PermaNet 3.0 increased mosquito mortality by 81% compared to PermaNet 2.0 (RR 1.81, 95% CI 1.56 to 2.10; 3 trials, 4 comparisons; Analysis 2.12; Corbel 2010; Koudou 2011; Toé 2018). After washing, there was no significant increase in mortality in the PermaNet 3.0 arm (2 trials, 2 comparisons; Analysis 2.12; Corbel 2010; Koudou 2011). Blood-feeding success was reduced by 47% when unwashed PermaNet 3.0 was used (RR 0.53, 95% CI 0.40 to 0.69; 2 trials, 3 comparisons; Analysis 2.13; Corbel 2010; Toé 2018); only one trial was available for washed nets (Corbel 2010), and in this trial, PermaNet 3.0 also reduced blood-feeding success (RR 0.76, 95% 0.61 to 0.93; 1 trial, 1 comparison; Analysis 2.13).

In high-resistance settings, Olyset Plus increased mosquito mortality by 72% when nets were unwashed (RR 1.72, 95% CI 1.48 to 1.99; 2 trials, 3 comparisons; Analysis 2.14; Pennetier 2013; Toé 2018). Only one trial compared washed Olyset Plus with washed Olyset (Pennetier 2013); in this trial, enhanced mortality (81%) was still observed in the Olyset Plus arm after washing (RR 1.81, 95% CI 1.25 to 2.61; 1 trial, 1 comparison; Analysis 2.14). There was no impact on blood-feeding success when unwashed Olyset Plus was compared with Olyset (2 trials, 3 comparisons; Analysis 2.15); the single trial that looked at washed Olyset Plus showed decreased blood feeding compared to Olyset (RR 0.50, 95% 0.27 to 0.93; 1 trial, 1 comparison; Analysis 2.15).

Village trials

In the village trials, there was no decrease in sporozoite rate in trial arms receiving pyrethroid-PBO nets (RR 0.82, 95% CI 0.24 to 2.75; 4 trials, 5 comparisons; Analysis 1.3; Awolola 2014; Cisse 2017; Protopopoff 2018; Stiles-Ocran 2013). Mosquito parity was not reduced in pyrethroid-PBO villages (3 trials, 4 comparisons; Analysis 1.4; Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013). It was not possible to stratify these data by resistance status due to the variability in resistance levels between villages within the same trial. Mosquito density was measured by a variety of methods and was summarized in different ways (e.g. mean number caught per house, mean number caught per village). When baseline data were collected, we calculated a percentage reduction. Higher reductions in mosquito densities were observed in pyrethroid-PBO net villages compared to LLIN villages (Table 8).

DISCUSSION

See Summary of findings 1, Summary of findings 2, Summary of findings 3, and Summary of findings 4.

Summary of main results

Two cluster-randomized controlled trials (cRCTs) were performed on pyrethroid-piperonyl butoxide (PBO) nets. The first trial, which compared parasite prevalence in children using Olyset Plus nets with that in children using Olyset nets, in a region of Tanzania where mosquito vectors are highly resistant to pyrethroids, found that pyrethroid-PBO nets reduced parasite prevalence by 60% at the final time point (21 months) (Protopopoff 2018). The second cRCT compared parasite prevalence in children using Olyset Plus or Permanet 3.0 nets with that in children using Olyset or Permanet 2.0 nets across East and West Uganda, where mosquito vectors are also highly resistant to pyrethroids, and found that pyrethroid-PBO nets reduced parasite prevalence by 17% at the latest time point (25 months) (Staedke 2020).

All other trials included in this review measured entomological endpoints. Four village trials measured sporozoite rates in mosquitoes collected from houses using pyrethroid-PBO nets and standard pyrethroid long-lasting insecticidal nets (LLINs), but the results were highly heterogeneous and no evidence suggests that pyrethroid-PBO nets reduced the mosquito infection rate derived from this pooled analysis (Awolola 2014; Cisse 2017; Protopopoff 2018; Stiles-Ocran 2013). Similarly, the proportion of parous mosquitoes (i.e. mosquitoes that have survived past one gonotrophic cycle; used as an indirect measure of longevity) was not significantly affected by the presence of pyrethroid-PBO nets (Cisse 2017; Mzilahowa 2014; Stiles-Ocran 2013).

When we pooled the results from 10 experimental hut trials (Bayili 2017; Corbel 2010; Koudou 2011; Menze 2020; Moore 2016; N'Guessan 2010; Oumbouke 2019; Pennetier 2013; Toé 2018; Tungu 2010), data showed improved performance of pyrethroid-PBO LLINs over standard LLINs in both increasing mosquito mortality and reducing blood feeding, but these results were highly heterogeneous. Stratifying experimental hut data by resistance levels in this population reduced heterogeneity. In areas where mosquitoes are highly resistant to pyrethroids, pyrethroid-PBO nets will reduce mosquito blood-feeding rates (i.e. users will be

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better protected against mosquito bites by using pyrethroid-PBO nets). This impact on blood feeding is reduced when nets have been through the standard 20 washes recommended by the World Health Organization (WHO) to assess chemical durability, but it remains significant (high-certainty evidence). When resistance is high and new unwashed nets are used, mosquito mortality is substantially higher when the nets contain PBO compared to pyrethroid only (high-certainty evidence). However this effect on mosquito mortality, which is important for the community-level protection afforded by LLIN usage (Hawley 2003; Maxwell 2002), is not sustained when nets have been washed multiple times. In this Cochrane Review, we classified mosquitoes as highly resistant if less than 30% were killed in a standard bioassay. When mortality rates exceeded 30%, we found little evidence to suggest that pyrethroid-PBO nets provided greater personal protection or resulted in greater mosquito mortality than standard pyrethroid-only nets. This result is not unexpected, given that in areas where resistance is uncommon or absent, exposure to pyrethroids alone would be expected to negatively affect the mosquito; it is only in areas where the efficacy of pyrethroids has been eroded by the development of high levels of resistance that the addition of a synergist might be needed.

We found no evidence for any difference in the performance of pyrethroid-PBO nets from different manufacturers against highly pyrethroid-resistant mosquitoes. We stratified results by net type only for trials that were conducted in areas of high resistance. We have not reported comparisons for DawaPlus-PBO and Veeralin-PBO nets in this sub-analysis, as there was only a single data point for these net types. We did not stratify data from the cRCTs by net type, as one trial used only one net type (Protopopoff 2018), and the second was not powered to detect differences between nets from different manufacturers and assigned an uneven number of clusters to each net type (Staedke 2020). Unwashed PermaNet 3.0 and Olyset Plus resulted in similar increases in mosquito mortality compared to pyrethroid-only LLINs from the same manufacturer, although this effect on mortality was not always sustained after washing (Corbel 2010; Koudou 2011; Pennetier 2013; Toé 2018). A significant improvement in personal protection for unwashed pyrethroid-PBO nets was observed only for PermaNet 3.0 (Corbel 2010; Toé 2018), but after washing, pyrethroid-PBO nets from both manufacturers provided greater personal protection than the equivalent pyrethroid-only nets (Corbel 2010; Pennetier 2013). Results from comparisons between pyrethroid-PBO nets from different manufacturers should be taken with great caution, given the very limited number of data points available, particularly for washed nets. Further trials, in which nets from different manufacturers are directly compared in the same trial, are needed to address the issue of equivalence between different pyrethroid-PBO nets.

Certainty of the evidence

We appraised the certainty of evidence using the GRADE approach (Summary of findings 1 Summary of findings 2 Summary of findings 3 Summary of findings 4). The two cRCTs provided moderate-certainty evidence that pyrethroid-PBO nets reduced parasite prevalence for the duration of the trial (high-certainty evidence after four to six months) (Protopopoff 2018; Staedke 2020).

This result was obtained from two independent studies, conducted in different locations and settings; therefore the evidence adheres to the WHO recommendation that at least two cRCTs must be completed to demonstrate public health value (WHO-GMP 2017b).

The certainty of evidence from trials using entomological endpoints varied. Data from village trials were difficult to assess, as there was considerable heterogeneity in the level of pyrethroid resistance and presumably also in the resistance mechanisms, both within and between trials. Analysis of data from experimental hut trials yielded high-certainty evidence for superior performance of pyrethroid-PBO nets in areas of high resistance, but evidence from trials conducted in other settings was of low or very low certainty.

Overall completeness and applicability of evidence

All trials included in this review compared pyrethroid-PBO nets with the nearest equivalent pyrethroid-only LLINs. Further changes to net specifications were often included when manufacturers incorporated the synergist. For example, the pyrethroid-PBO net manufactured by Vestergaard (PermaNet 3.0) contains higher levels of deltamethrin and yarn of a different denier (thickness) compared to the pyrethroid-only equivalent, PermaNet 2.0; the pyrethroid in Olyset Plus (Sumitomo Chemical Co. Ltd.) is released from the yarn at a different rate than that in the Olyset nets. These additional variations in chemical or physical composition, or both, of the nets make it difficult to directly assess the added value of the addition of PBO. Furthermore, the concentration of PBO and its site of application differ markedly between nets received from different manufacturers. Two of the currently available pyrethroid-PBO nets (PermaNet 3.0 and Tsara Plus 3.0) contain PBO only on the roof of the netting, exploiting the behavioural patterns of host-seeking mosquitoes to attempt to reach the net user by approaching from above (Parker 2015), whilst the remaining pyrethroid-PBO nets contain the synergist on all sides of the net. The amount of PBO contained within the net differs by a factor of 25-fold. It is not known how net manufacturers selected the doses of PBO applied to the netting.

With currently available data, it is not possible to draw any conclusions on which strategy for producing pyrethroid-PBO nets will prove the most effective under field conditions. The optimum PBO:pyrethroid ratio will likely differ depending on the level of resistance in the mosquito and underpinning resistance mechanisms. Data from experimental hut trials suggest that the PBO component of pyrethroid-PBO nets is lost after repeated washing, as enhanced mortality caused by the synergist nets is not maintained after 20 washes. As yet, no trials on the durability of pyrethroid-PBO nets under operational conditions have been published, although monitoring is under way. It is encouraging to note that both RCTs of pyrethroid-PBO nets found that the superior protective efficacy of Olyset Plus compared to standard Olyset nets was maintained at 21 months of use; the trial in Tanzania is being extended to establish whether this effect lasts the full duration of an LLIN's intended 36-month life span. No plans are under way to continue monitoring in the Uganda trial past the 25-month collections (Staedke 2020).

Most available data evaluated the performance of pyrethroid-PBO LLINs against *Anopheles gambiae* s.l., with very limited data available for the second major species complex in Africa, *An funestus*, and none for other minor vector species. As different mosquito species may differ in their behaviour and in the strength and underpinning mechanisms of pyrethroid resistance, this represents an important data gap that may have implications for

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practice in areas where *An gambiae* complex is not the predominant malaria vector.

Potential biases in the review process

As the addition of PBO to pyrethroid LLINs is expected to enhance their performance only in areas where mosquitoes are resistant to pyrethroid insecticides, it was important to stratify the results by resistance status. To do this, we used the WHO definition of resistance as mosquito populations with less than 90% mortality in a discriminating dose assay (WHO 2016), and then we split the resistant populations into three groups, depending on the percentage of mortality observed. Discriminating dose assays provide an estimate of the prevalence of resistance in a population but do not indicate the strength of this resistance nor give any indication of the mechanism(s) underpinning the resistance. As PBO works primarily by inhibiting the metabolism of pyrethroids by cytochrome P450s, this synergist is likely to have had greatest impact in populations where resistance was primarily conferred by elevated P450 activity and further stratification according to resistance mechanisms might have proved informative. However, in reality, characterization of resistance in mosquitoes is still primarily performed by bioassays alone and the relevant contributions of different resistance mechanisms to the phenotype remain unknown. An exception to this is seen in *An funestus*, where pyrethroid resistance is almost entirely due to elevated P450 activity (Churcher 2016). Unfortunately, only one data set from experimental hut trials conducted where *An funestus* was the primary vector was made available to us at the time of this review.

Other examples of missing data that may have influenced study results include the absence of data on resistance status in some settings. Three experimental hut trials did not measure resistance at the time of the trial (Moore 2016; N'Guessan 2010; Pennetier 2013). For two of these trials, we used proxies for resistance; however, no proxy data were available for *An funestus* in Moore 2016, and hence we did not include this population in the stratified analysis. Three trials did not share their data with the review authors; these included trials on nets from two of the more recent manufacturers to produce pyrethroid-PBO nets (N'Guessan 2016; Tungu 2017), which precluded stratified analysis for these net types. For clinical trials, both species composition and resistance level may vary between clusters and/or over the duration of the trial (e.g. the Uganda trial - Staedke 2020 - involved 104 clusters across the country as part of the national LLIN campaign). The population was classified as highly pyrethroid resistant based on data provided by the study authors (WHO tube bioassay conducted in Banangaizi East: deltamethrin 0.05%, 20.7% mosquito mortality, n = 163), but the resistance phenotype of the vector population is likely to vary considerably between clusters.

One key finding of this trial was the decline in performance of pyrethroid-PBO nets after washing. However, as discussed above, it is not clear how the standardized washing protocol employed in experimental hut trials of LLINs reflects the actual chemical retention of active ingredients under operational use. It is encouraging to note that the impact of pyrethroid-PBO nets in reducing parasite prevalence was sustained over two years, hence the policy implications of the loss in bio efficacy after washing remain to be determined.

Agreements and disagreements with other studies or reviews

This is an update of the first Cochrane Review of pyrethroid-PBO nets (Gleave 2018). An earlier meta-analysis of experimental hut data indicated that pyrethroid-PBO nets would have the greatest impact against mosquito populations with intermediate levels of resistance (Churcher 2016). Using transmission models to convert entomological outputs into estimates of public health benefit, the authors noted that the impact of pyrethroid-PBO nets would vary depending on mosquito species, resistance levels, parasite prevalence, and LLIN usage. The importance of taking these key parameters into account when predicting the public health impact of a switch to pyrethroid-PBO nets has been somewhat lost in policy documents and operational guidelines, which seek to provide a simple decision rule to aid net selection. Hence, in the WHO report from the 2017 Evidence Review Group on 'Conditions for deployment of mosquito nets treated with pyrethroid and piperonyl butoxide', it is recommended that "National malaria control programmes and their partners should consider deployment of pyrethroid-PBO nets in areas where pyrethroid resistance has been confirmed in the main malaria vectors" (WHO 2017). In technical guidelines from one of the major net distributors, the PMI, the conditions for deployment of PBO nets include "moderate levels of pyrethroid resistance (defined as 35% to 80% mortality), evidence that PBO restores pyrethroid susceptibility, and moderate to high malaria prevalence" (PMI 2018). The PMI definition of moderate resistance overlaps with our definitions of moderate and low resistance. However in our review, the best evidence for superior efficacy of pyrethroid-PBO nets is derived from areas with high resistance (< 30% mortality), and very little evidence suggests improved performance in areas with moderate or low levels of resistance. The differences between these trials may have arisen from incorporation of a large data set of laboratory bioassays comparing mosquito mortality with or without pre-exposure to PBO in the modelling study. These laboratory bioassays rely on use of a single discriminating dose and identified multiple trials where highly resistant populations were not impacted by PBO. In the current review, the mosquito populations included were limited to sites in which experimental hut trials had been conducted, and this may not have fully captured the full diversity of resistance mechanisms in *Anopheles* mosquitoes. This again highlights the importance of further trials on the influence of resistance mechanisms on the impact of pyrethroid-PBO LLINs.

AUTHORS' CONCLUSIONS

Implications for practice

The findings of this review support the recent WHO policy recommendation that pyrethroid-piperonyl butoxide (PBO) nets should be considered for deployment in areas where pyrethroid resistance has been confirmed in the main malaria vectors (WHO-GMP 2017a). It is encouraging to note that both randomized controlled trials (RCTs) of pyrethroid-PBO nets found that the superior protective efficacy of Olyset Plus compared to that of standard Olyset nets was maintained at 21/25 months of use; the Tanzania trial has been extended further to establish whether this effect lasts the full duration of an LLIN's intended 36-month life span, but results are not yet publicly available. The WHO has declared Olyset Plus as first-in-class for pyrethroid-PBO nets; as a result, pyrethroid-PBO nets from other manufacturers will not

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be required to generate epidemiological evidence showing their efficacy.

When evaluating these trials, it is important to remember that the PBO is an additive to the nets that is intended to increase their efficacy against pyrethroid-resistant mosquito populations. No evidence suggests that pyrethroid-PBO nets are less effective than standard LLINs for inducing mosquito mortality in any setting. For personal protection, blood-feeding rates are similarly decreased under all resistance scenarios when unwashed PBO nets are used, although this has not been shown for washed nets in low-resistance or susceptible areas (low-certainty evidence). Hence if pyrethroid-PBO nets perform as well as, or better than, standard LLINs, the decision on whether to switch to nets incorporating the synergist is largely a question of economics. With fixed budgets, there is a risk that the target of universal coverage of LLINs may be more difficult to reach if more expensive pyrethroid-PBO nets are deployed. Indeed, the WHO clearly states that countries should consider deploying pyrethroid-PBO nets only in situations where coverage with standard vector-control interventions is not reduced (WHO-GMP 2017c). Trials of the cost-effectiveness of pyrethroid-PBO nets have not yet been possible due to uncertainties over the price differential between pyrethroid-PBO nets and LLINs.

Implications for research

Experimental hut trials simultaneously comparing different pyrethroid-PBO nets in areas where mosquitoes have high levels of pyrethroid resistance are needed to demonstrate equivalency and to inform procurement decisions, particularly given the very different approaches used to incorporate PBO into LLINs employed by different manufacturers. The issue of durability of bioactive levels of the synergist on the nets also needs further study; current WHO protocols for measuring LLIN durability will need to be adjusted to utilize pyrethroid-resistant colonies of mosquitoes, so that the impact of PBO, and not just of the insecticide, can be measured over the net's intended life span. The issue of the value of entomological endpoints in estimating the public health value of new types of nets remains contentious (Killeen 2018; WHO-GMP 2017c). Performing experimental hut trials alongside future randomized controlled trials of nets containing synergists, or other novel active ingredients, would help resolve this issue.

In relation to reporting trial results, study authors need to record the level of resistance in the local mosquito population at the time of the trial and should include this when reporting the results. Data on resistance mechanisms would also be of value toward a improved understanding of how this influences the performance of pyrethroid-PBO nets.

Limitations of this review

One of the problems in this research field is that pyrethroid-PBO nets are commercial products. The pyrethroid-PBO nets currently undergoing RCTs 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 that are based on evidence related to their relative effectiveness. Thus, in this Cochrane Review, we examined the evidence concerning the effectiveness of commercial products. During these comparisons, we considered other potential confounding factors.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [author-defined order]

Staedke 2020

Study characteristics	
Methods	Cluster-randomized controlled village trial
Participants	Households with at least 1 adult resident and 1 child aged 2 to 10 years, *Anopheles* species
Interventions	Control: LLIN, PermaNet 2.0 Intervention: LLIN, PermaNet 3.0 Control: LLIN, Olyset Net Intervention: LLIN, Olyset Plus
Outcomes	Primary outcomes; parasite prevalence (proportion of thick blood smears that are positive for asexual parasites) in children ages 2 to 10 years, assessed before net distribution and 3 times after nets are distributed Secondary outcomes: prevalence of anaemia; mean haemoglobin in children ages 2 to 10 years; vector density; measures of LLIN ownership; coverage, use, and integrity
Mosquito resistance status	Resistance - high
Net treatment	Nets unholed and unwashed
Location(s)	Uganda - East and West, 104 sub-districts
Notes	
Risk of bias	

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Staedke 2020 *(Continued)*

Bias	Authors' judgement	Support for judgement
Recruitment bias	Low risk	No participants were recruited after clusters had been randomized
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Unclear risk	Resistance monitoring was not conducted at all study sites due to the size of the RCT
Collectors blinded	High risk	LLIN allocation was not masked; therefore risk of detection bias was high for entomological outcomes
Household blinded	Low risk	LLIN allocation was not masked, but this is unlikely to affect the primary outcome (parasite prevalence)
Treatment allocation (sequence randomly/adequately generated)	Low risk	Randomization was used to allocate clusters to study groups
Allocation concealment (selection bias)	Low risk	Randomization was carried out to allocate treatments to clusters
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete; intention-to-treat analysis was conducted
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	No outcome data were missing
Clusters lost to follow-up	Unclear risk	14 clusters were lost to follow-up in the final time point (25 months) due to the COVID-19 pandemic
Selective reporting (reporting bias)	Low risk	All intended outcomes stated in the pre-published protocol were reported in the final publication
Correct statistical methods; adjusted for clustering	Low risk	Clustering was not taken into account and adjusted for during statistical analysis. Trial authors did however provide us with an ICC, so we could adjust for clustering
Trial authors' conflicting interest	Low risk	Trial authors declared no conflicting interests

Awolola 2014

Study characteristics	
Methods	Village trial
Participants	Ilara - *An gambiae* (100% S-form) Irolu - 95% *An gambiae* (100% S-form), 4.5% *An arabiensis* Ijesa - 98.1% *An gambiae* (80% S-form, 19% M-form), 1.6% *An arabiensis*
Interventions	Control: LLIN, PermaNet 2.0 Intervention: LLIN, PermaNet 3.0

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Awolola 2014 *(Continued)*

Outcomes	Mosquito mortality, blood feeding, sporozoite rate, mosquito density, parity rate
Mosquito resistance status	Ilara - resistant - low (deltamethrin, 72.5% mortality, N = 120) Irolu - resistant - low (deltamethrin, 62.5% mortality, N = 120) Ijesa - resistant - low (deltamethrin, 66.7% mortality, N = 120)
Net treatment	Nets unholed and unwashed
Location(s)	Ilara, Nigeria - untreated net Irolu, Nigeria - PermaNet 2.0 Ijesa, Nigera - PermaNet 3.0
Notes	Trial conducted: March 2012 to March 2013

Risk of bias

Bias	Authors' judgement	Support for judgement
Recruitment bias	Low risk	Recruiment bias is related to human participants and so is not applicable to this study
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Unclear risk	Mosquito species composition varied slightly pre-trial and post-trial between treatment villages. However, resistance level was the same
Collectors blinded	High risk	Not stated whether collectors where blinded; therefore judged as high risk, as this is likely to impact searching efforts
Household blinded	Low risk	Unclear whether households were blinded – not stated in the publication. We judged this as low risk, as it is unlikely to affect the outcome
Treatment allocation (sequence randomly/adequately generated)	Low risk	Villages were randomly assigned to treatment arms
Allocation concealment (selection bias)	Low risk	Allocation concealment procedures were not adhered to; however this is unlikely to affect the results
Were incomplete outcome data adequately addressed	Low risk	There were no incomplete data
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Clusters lost to follow-up	Low risk	No clusters were lost to follow-up
Selective reporting (reporting bias)	Low risk	It appears that all measured outcomes were reported
Correct statistical methods; adjusted for clustering	High risk	Study did not take clustering into account for statistical methods

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Awolola 2014 *(Continued)*

Trial authors' conflicting interest	Low risk	Trial authors declared no conflicting interests; however the study was funded by Vestergaard (net manufacturers). Views and findings in the publication are stated to be those of the trial authors

Bayili 2017

Study characteristics	
Methods	Experimental hut trial
Participants	*An coluzzii*
Interventions	Control: LLIN, DawaPlus 2.0 Intervention: LLIN, DawaPlus 3.0, DawaPlus 4.0
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito resistance status	Resistant - high (6% mortality, N = 98)
Net treatment	Nets holed, nets unwashed and washed (x 20)
Location(s)	Vallée du Kou, Burkina Faso
Notes	Trial conducted: August 2016 to October 2016

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	The hut trial was conducted in the same area; therefore characteristics are similar
Collectors blinded	Unclear risk	Paper does not state whether collectors were blinded
Sleepers blinded	Unclear risk	Paper does not state whether sleepers were blinded
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were not randomly allocated to huts; however the trial completed a full rotation through the huts
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design + 2 weeks
Standardized hut design	Low risk	Huts were built previously according to standard West African design
Hut cleaning between treatments	Unclear risk	Trial authors do not state whether huts were cleaned between treatments
Were incomplete outcome data adequately addressed	Low risk	No data were incomplete

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Bayili 2017 *(Continued)*

Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Trial authors' conflicting interest	Low risk	Trial authors declare no conflicting interest in the WHOPES report

Cisse 2017

Study characteristics		
Methods	Village trial	
Participants	*An gambiae* s.s.	
Interventions	Control: LLIN, Olyset Net, PermaNet 2.0 Intervention: LLIN, Olyset Plus, PermaNet 3.0	
Outcomes	Sporozoite rate, mosquito density, parity rate	
Mosquito resistance status	Olyset Net villages - resistance - high (1% mortality, N = 305) Olyset Plus villages - resistance - high (2% mortality, N = 411) PermaNet 2.0 villages - resistance - high (29% mortality, N = 410) PermaNet 3.0 villages - resistance - moderate (38% mortality, N = 408)	
Net treatment	Nets unholed and unwashed	
Location(s)	Sikasso region, Mali PermaNet 2.0 villages - Beko East, Dalabani, Berila, Dierila PermaNet 3.0 villages - Beko West, Farabacoura East, Kola Djokada, Tieblembougou Olyset Net villages - Karako, Geleba 2, Toula East, Toula West Olyset Plus villages - Dialake, Farabacoura West, Deneklin, Faradjele	
Notes	Trial conducted: January 2014 to January 2015	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Recruitment bias	Low risk	Recruiment bias is related to human participants and so is not applicable to this study
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Unclear risk	Mosquito species composition is constant between villages; however resistance level varies slightly
Collectors blinded	High risk	Not stated whether collectors where blinded; therefore judged as high risk, as this is likely to affect searching efforts
Household blinded	Low risk	Unclear whether households were blinded – not stated in the publication. We judged this as low risk, as this is unlikely to affect the outcome

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Cisse 2017 *(Continued)*

Treatment allocation (sequence randomly/adequately generated)	Low risk	Villages were randomly assigned to treatment arms
Allocation concealment (selection bias)	Low risk	Allocation concealment procedures were not adhered to; however this is unlikely to affect study results
Were incomplete outcome data adequately addressed	Low risk	No data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Clusters lost to follow-up	Low risk	No clusters were lost to follow-up
Selective reporting (reporting bias)	Low risk	It appears that all measured outcomes were reported
Correct statistical methods; adjusted for clustering	High risk	Study did not take clustering into account for statistical methods
Trial authors' conflicting interest	Low risk	Trial authors have no competing interests

Corbel 2010

Study characteristics	
Methods	Experimental hut trial
Participants	Vallée du Kou, Burkina Faso - 100% *An gambiae*: M-form (15%), S-form (85%) Malanville, Benin - 95% *An gambiae*: M-form (100%), 5% *An arabiensis* Pitoa, Cameroon - 5% *An gambiae*: S-form (100%), 95% *An arabiensis*
Interventions	Control: LLIN, PermaNet 2.0 Intervention: LLIN, PermaNet 3.0
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito resistance status	Vallée du Kou, Burkina Faso - resistant – high (deltamethrin, 23% mortality, N = 100) Malanville, Benin - resistant – low (deltamethrin, 85% mortality, N = 100) Pitoa, Cameroon - resistant – low (deltamethrin, 70% mortality, N = 100)
Net treatment	Nets holed, nets unwashed and washed (x 20)
Location(s)	Vallée du Kou, Burkina Faso Malanville, Benin

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Corbel 2010 *(Continued)*

	Pitoa, Cameroon
Notes	Trial conducted: Vallée du Kou, Burkina Faso - September 2007 to November 2007 Malanville, Benin - July 2008 to September 2008 Pitoa, Cameroon - July 2008 to September 2008

Risk of bias

Bias	Authors' judgement	Support for judgement
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area: mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether sleeper was blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were randomly allocated to huts
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Unclear risk	Unclear whether huts were cleaned between treatments – not stated in the publication
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Trial authors' conflicting interest	Low risk	Trial authors have no competing interests

Koudou 2011

Study characteristics

Methods	Experimental hut trial
Participants	*An gambiae* s.s.
Interventions	Control: LLIN, PermaNet 2.0

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Koudou 2011 *(Continued)*

	Intervention: LLIN, PermaNet 3.0
Outcomes	Mosquito mortality, deterrence, exophily
Mosquito resistance status	Resistant - high (deltamethrin, 10.6% mortality, N = 80 min)
Net treatment	Nets not holed, nets unwashed and washed (x 20)
Location(s)	Yaokoffikro, Côte d'Ivoire
Notes	Trial conducted: April 2009 to July 2009

Risk of bias

Bias	Authors' judgement	Support for judgement
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether sleeper was blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were not randomly allocated to the huts However, results from trials performed before this trial show no significant differences in attractiveness of the different huts
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Low risk	All huts were cleaned between treatments
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Trial authors' conflicting interest	Low risk	Trial authors declared they had no conflicting interests

Moore 2016

Study characteristics

Methods	Experimental hut trial

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Moore 2016 *(Continued)*

Participants	*An arabiensis* (100%), *An funestus* group (95% s.s.)
Interventions	Control: LLIN, MAGNet LN Intervention: LLIN, Veeralin LN
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito resistance status	*An arabiensis* - susceptible (alpha-cypermethrin, 100% mortality, N = 97) *An funestus* - unclassified
Net treatment	Nets holed, nets unwashed and washed (x 20)
Location(s)	Ifakara, Tanzania
Notes	Although additional data provided, they show resistance to deltamethrin and permethrin in *An gambiae s.l.*

Risk of bias

Bias	Authors' judgement	Support for judgement
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	The hut trial was conducted in the same area; therefore characteristics are similar
Collectors blinded	Unclear risk	Paper does not state whether collectors were blinded
Sleepers blinded	Unclear risk	Paper does not state whether sleepers were blinded
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were not randomly allocated to huts; however the trial completed a full rotation through the huts
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Study used the standard design of the Ifakara experimental huts
Hut cleaning between treatments	Unclear risk	The paper does not state whether huts were cleared between treatments
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	No outcome data were missing
Trial authors' conflicting interest	Low risk	Trial authors declared they received prescribed standard fees from Vestergaard Frandsen for evaluating its pesticide products; however this is standard practice

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Mzilahowa 2014

Study characteristics	
Methods	Village trial
Participants	*An gambiae* s. l., *An funestus* group
Interventions	Control: LLIN, Olyset Net, PermaNet 2.0 Intervention: LLIN, Olyset Plus, PermaNet 3.0
Outcomes	Mosquito density, parity rate
Mosquito resistance status	*An funestus* (Balaka district) Permethrin - resistant - moderate (55.5% mortality, N = unknown) Deltamethrin - resistant - high (14.9% mortality, N = unknown) *An gambiae* (Balaka district) Permethrin - resistant - low (84.4% mortality, N = unknown) (Machinga district) Deltamethrin - resistant - moderate (54.5% mortality, N = unknown)
Net treatment	Nets unholed and unwashed
Location(s)	Balaka district, Malawi (12 villages)
Notes	Trial conducted: December 2012 to June 2014

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Recruitment bias	Low risk	Recruiment bias is related to human participants and so is not applicable to this study
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Unclear risk	Mosquito species composition and resistance status are not recorded per village. Village names are not provided in the study; instead villages are grouped by treatment type
Collectors blinded	High risk	Not stated whether collectors were blinded; therefore judged as high risk, as this is likely to affect searching effort
Household blinded	Low risk	Unclear whether households were blinded – not stated in the publication. We judged this as low risk, as this is unlikely to affect the outcome
Treatment allocation (sequence randomly/adequately generated)	Low risk	Villages were randomly assigned to treatment arms
Allocation concealment (selection bias)	Low risk	Allocation concealment procedures were not adhered to; however this is unlikely to affect the results

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Mzilahowa 2014 *(Continued)*

Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Clusters lost to follow-up	Low risk	No clusters were lost to follow-up
Selective reporting (reporting bias)	Low risk	It appears that all measured outcomes were reported
Correct statistical methods; adjusted for clustering	High risk	Study did not take clustering into account when statistical methods were performed
Trial authors' conflicting interest	Unclear risk	No information on trial authors' possible conflicting interests is provided

N'Guessan 2010

Study characteristics		
Methods	Experimental hut trial	
Participants	*An gambiae*	
Interventions	Control: LLIN, PermaNet 2.0 Intervention: LLIN, PermaNet 3.0	
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily	
Mosquito resistance status	Proxy data. Adjara, Benin: resistant - moderate (deltamethrin, 50% mortality, N = 56) (Aïzoun 2013)	
Net treatment	Nets holed, nets unwashed and washed (x 20)	
Location(s)	Akron, Benin	
Notes	Trial conducted: October 2008 to January 2009	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts were situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether sleeper was blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design

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N'Guessan 2010 *(Continued)*

Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were randomly allocated to huts
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Low risk	All huts were cleaned between treatments
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Trial authors' conflicting interest	Unclear risk	The trial was sponsored by Vestergaard (net manufacturers), which also commented on the manuscript

Pennetier 2013

Study characteristics	
Methods	Experimental hut trial
Participants	95% *An gambiae*: M-form (100%), 5% *An arabiensis* (Corbel 2010)
Interventions	Control: LLIN, Olyset Net Intervention: LLIN, Olyset Plus
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito resistance status	Proxy data. Resistant - high (permethrin, 22% mortality, N = 100) (Djègbè 2011)
Net treatment	Nets holed, nets unwashed and washed (x 20)
Location(s)	Malanville, Benin
Notes	Trial conducted: September 2011 to December 2011

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether sleeper was blinded – not stated in the publication

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Pennetier 2013 *(Continued)*

Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were not randomized to huts but instead were rotated fully between all huts according to a Latin square design
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Low risk	All huts were cleaned between treatments
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Trial authors' conflicting interest	Low risk	Funders of the trial stated that they had no part in data collection, data analysis, or manuscript preparation

Protopopoff 2018

Study characteristics	
Methods	Cluster-randomized controlled village trial
Participants	3966 children analysed (21 months after intervention) aged 6 months to 14 years (excluding the severely ill), *Anopheles* species (pooled). Total core cluster population ranged from 14,845 to 16,358
Interventions	Control: LLIN, Olyset Net Intervention: LLIN, Olyset Plus
Outcomes	Malaria parasite prevalence, sporozoite rate, mosquito density
Mosquito resistance status	Resistance - high (17.8% mortality, N = 107)
Net treatment	Nets unholed and unwashed
Location(s)	Muleba District, Tanzania
Notes	Trial conducted: March 2014 to December 2016

Risk of bias

Bias	Authors' judgement	Support for judgement
Recruitment bias	Low risk	No participants were recruited after clusters had been randomized

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Protopopoff 2018 *(Continued)*

Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Unclear risk	Resistance level was available only for the whole district - not at the village level
Collectors blinded	Low risk	Field workers were masked to net treatment
Household blinded	Low risk	Inhabitants were masked to net treatment
Treatment allocation (sequence randomly/adequately generated)	Low risk	Restricted randomization was used to allocate clusters to study groups
Allocation concealment (selection bias)	Low risk	Restricted randomization was used to allocate treatments to clusters
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	No outcome data were missing
Clusters lost to follow-up	Low risk	No clusters were lost to follow-up
Selective reporting (reporting bias)	Low risk	It appears that all measured outcomes were reported
Correct statistical methods; adjusted for clustering	Low risk	Clustering was taken into account and was adjusted for during statistical analysis
Trial authors' conflicting interest	Low risk	Trial authors declared no conflicting interests

Stiles-Ocran 2013

Study characteristics	
Methods	Village trial
Participants	*An gambiae*
Interventions	Control: LLIN, PermaNet 2.0 Intervention: LLIN, PermaNet 3.0
Outcomes	Sporozoite rate, mosquito density, parity rate
Mosquito resistance status	Futa - resistant - moderate (33.3% mortality, N = 96) Abrabra- resistant - moderate (43.7% mortality, N = 126) Kunkumso - resistant - high (28.4% mortality, N = 109) Anyinabrim - resistant - moderate (53.2% mortality, N = 109) Wenchi - resistant - low (61.9% mortality, N =126)

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Stiles-Ocran 2013 *(Continued)*

Net treatment	Nets unholed and unwashed
Location(s)	Futa, Ghana - no net control Abrabra, Ghana - PermaNet 2.0 Kunkumso, Ghana - PermaNet 2.0 Anyinabrim, Ghana - PermaNet 3.0 Wench, Ghana - PermaNet 3.0
Notes	Trial conducted: November 2010 to August 2011

Risk of bias

Bias	Authors' judgement	Support for judgement
Recruitment bias	Low risk	Recruiment bias is related to human participants and so is not applicable to this study
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Unclear risk	Mosquito species composition varied slightly. Resistance level varies between villages. However, pre-trial and post-trial data are provided
Collectors blinded	High risk	Not stated whether collectors were blinded; therefore judged as high risk, as this is likely to affect searching efforts
Household blinded	Low risk	Unclear whether households were blinded – not stated in the publication. We judged this as low risk, as this is unlikely to impact the outcome
Treatment allocation (sequence randomly/adequately generated)	Low risk	Villages were randomly assigned to treatment arms
Allocation concealment (selection bias)	Low risk	Allocation concealment procedures were not adhered to; however this is unlikely to affect the results
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Clusters lost to follow-up	Low risk	No clusters were lost to follow-up
Selective reporting (reporting bias)	Low risk	It appears that all measured outcomes were reported
Correct statistical methods; adjusted for clustering	High risk	Study did not take clustering into account for statistical methods
Trial authors' conflicting interest	Unclear risk	Study data were collected for use in Vestergaard PermaNet 3.0 product dossier

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Toé 2018

Study characteristics	
Methods	Experimental hut trial
Participants	*An coluzzii*
Interventions	Control: LLIN, PermaNet 2.0, Olyset Net Intervention: LLIN, PermaNet 3.0, Olyset Plus
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito resistance status	Vallée du Kou 5 - resistant – high (deltamethrin, 2.5% mortality, N = 163; permethrin, 5% mortality, N = 153) Tengrela - resistant – high (deltamethrin, 34% mortality, N = 85; permethrin, 14% mortality, N = 101)
Net treatment	Nets holed, nets unwashed
Location(s)	Vallée du Kou 5, Burkina Faso Tengrela, Burkina Faso
Notes	Trial conducted: September 2014 to October 2014

Risk of bias

Bias	Authors' judgement	Support for judgement
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether sleeper was blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were not randomized to huts but instead were rotated fully between all huts according to a Latin square design
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Unclear risk	Unclear whether huts were cleaned between treatments – not stated in the publication
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported

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Toé 2018 *(Continued)*

Trial authors' conflicting interest	Low risk	Trial authors had no competing interests

Tungu 2010

Study characteristics		
Methods	Experimental hut trial	
Participants	*An gambiae*	
Interventions	Control: LLIN, PermaNet 2.0 Intervention: LLIN, PermaNet 3.0	
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily	
Mosquito resistance status	Susceptible (deltamethrin, 100% mortality, N = not stated)	
Net treatment	Nets holed, nets unwashed and washed (x 20)	
Location(s)	Zeneti, Muheza, Tanzania	
Notes	Trial conducted: July 2008 to October 2008	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether sleeper was blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were randomly allocated to huts
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Low risk	All huts were cleaned between treatments
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete

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Tungu 2010 *(Continued)*

Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	All necessary data were reported
Trial authors' conflicting interest	Low risk	Trial authors had no competing interests

Menze 2020

Study characteristics	
Methods	Experimental hut trial
Participants	*An funestus*
Interventions	Control: LLIN, PermaNet 2.0, Olyset Net Intervention: LLIN, PermaNet 3.0, Olyset Plus
Outcomes	Mosquito mortality, blood feeding, exophily
Mosquito resistance status	Moderate
Net treatment	Nets unwashed and holed
Location(s)	Mibellon, Cameroon
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers were rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were not randomized to huts but instead were rotated fully between all huts according to a Latin square design
Treatment rotation	Low risk	Treatments were rotated between huts according to a Latin square design
Standardized hut design	Low risk	Huts were built according to a standard West African design
Hut cleaning between treatments	Low risk	All huts were cleaned between treatments

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Menze 2020 *(Continued)*

Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	No outcome data were missing
Correct statistical methods; adjusted for clustering	Low risk	Clustering was not taken into account and adjusted for during statistical analysis. We adjusted for clustering by using an ICC value of 0.1
Trial authors' conflicting interest	Low risk	Trial authors state that they have no competing interests

Oumbouke 2019

Study characteristics	
Methods	Experimental hut trial
Participants	*An gambiae*
Interventions	Control: LLIN, MAGNet LN Intervention: LLIN, Veeralin LN
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito resistance status	Low resistance
Net treatment	Nets holed, nets unwashed and washed (x 20)
Location(s)	M'be Côte d'Ivoire
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Were the mosquitoes in LLIN and LLIN + PBO groups comparable	Low risk	Huts situated in the same area – mosquito characteristics will be the same
Collectors blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleepers blinded	Unclear risk	Unclear whether collectors were blinded – not stated in the publication
Sleeper bias	Low risk	Sleepers rotated between huts according to a Latin square design
Treatment allocation (sequence randomly/adequately generated)	Low risk	Treatments were randomly allocated to huts

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Oumbouke 2019 *(Continued)*

Treatment rotation	Low risk	Treatment were rotated between huts according to a Latin Square design
Standardized hut design	Low risk	Huts were built previously according to standard West African hut design
Hut cleaning between treatments	Low risk	Huts were thoroughly cleaned and aired for a day at the end of each rotation
Were incomplete outcome data adequately addressed	Low risk	No outcome data were incomplete
Were the raw data reported for LLIN and LLIN + PBO groups	Low risk	No outcome data were missing
Correct statistical methods; adjusted for clustering	Low risk	Clustering was not taken into account and adjusted for during statistical analysis. We adjusted for clustering using an ICC value of 0.1
Trial authors' conflicting interest	Low risk	Trial authors state that they have no conflicting interests

An arabiensis: *Anopheles arabiensis*; *An coluzzii*: *Anopheles coluzzii*; *An funestus: Anopheles funestus*; *An gambiae*: *Anopheles gambiae*; ITN: insecticide-treated net; LLIN: long-lasting insecticidal net; PBO: piperonyl butoxide.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Darriet 2011	Study included laboratory data only
Darriet 2013	Study included laboratory data only

Characteristics of studies awaiting classification *[ordered by study ID]*

Koudou 2012

Methods	Village trial
Participants	Bouaké - 100% *An gambiae*: (70% S-form, 30% M-form) Tiassalé - 100% *An gambiae*: (70% S-form, 30% M-form)
Interventions	Control: LLIN, PermaNet 2.0 Extra Intervention: LLIN, PermaNet 3.0
Outcomes	Blood feeding, mosquito density
Mosquito Resistance Status	Bouaké - resistant - moderate (43.9% mortality, N = 114) Tiassalé - resistant - moderate (7.5% mortality, N = 106)
Net Treatment	Nets unholed and unwashed
Location(s)	Bouaké, Côte d'Ivoire

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Koudou 2012 *(Continued)*

	Tiassalé, Côte d'Ivoire
Notes	Trial conducted: November 2009 to January 2012

Shono 2017

Methods	Not available
Participants	*An funestus: Anopheles funestus*; *An gambiae*: *Anopheles gambiae*
Interventions	
Outcomes	Not available
Mosquito Resistance Status	Not available
Net Treatment	Control: LLIN, Olyset Net Intervention: LLIN, Olyset Plus
Location(s)	Not available
Notes	

Tungu 2017

Methods	Experimental hut trial
Participants	*An funestus*
Interventions	Control: LLIN, DawaPlus 2.0 Intervention: LLIN, DawaPlus 3.0, DawaPlus 4.0
Outcomes	Mosquito mortality, blood feeding, deterrence, exophily
Mosquito Resistance Status	
Net Treatment	Nets holed, nets unwashed and washed (x 20)
Location(s)	Muheza, Tanzania
Notes	

Characteristics of ongoing studies *[ordered by study ID]*

ISRCTN99611164

Study name	Comparative evaluation of standard insecticide-treated bed nets and co-treated bed nets on malaria prevalence in Sud Ubangi, Democratic Republic of Congo: a cluster-randomised trial

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ISRCTN99611164 *(Continued)*

Methods	Cluster-randomized trial
Participants	Women (> 15 years) attending first ANC appointment at a clinic that is taking part in the study, who consent to be enrolled in the study 20 visitors per month at each of 7 antenatal clinics (held monthly) in each of 17 study clusters, which gives a total of approximately 2400 participants per month, 28,500 per year, and 86,000 in total
Interventions	Control: bed net treated with pyrethroid only Intervention: bed net treated with both pyrethroid and PBO
Outcomes	1. Determination of parasite prevalence in women visiting monthly antenatal clinics 2. Entomological collections for surveillance of insecticide resistance and mosquito abundance and parasite infection 3. Assessment of bed net durability (physical and chemical analysis) and bio-efficacy (against mosquitoes) over time
Starting date	November 2019 (recruitment start date 01/06/2020)
Contact information	Dr David Weetman
Notes	

NCT03289663

Study name	Effectiveness study of new-generation bed nets in the context of conventional insecticide resistance in the Democratic Republic of the Congo (Net-PBO)
Methods	Cluster-randomized trial
Participants	1680 participants; 0 to 10-year-old subjects in 30 villages
Interventions	Control: bed net treated with pyrethroid only Intervention: bed net treated with both pyrethroid and PBO (IRS and LSM included in trial)
Outcomes	Incidence rate of laboratory-confirmed clinical cases of malaria (time frame: participants will be actively followed up for 12 months, and any suspected case of clinical malaria will immediately lead to microscopy and RDT for confirmation). Microscopy to confirm the diagnosis of malaria sporozoite rate (time frame: *Anopheles* mosquitoes will be captured every 3 months during 1 year), sporozoite detection by ELISA to determine infectivity of *Anopheles*
Starting date	2 October 2017
Contact information	
Notes	

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NCT04182126

Study name	HS#2017-3512. Adaptive interventions for optimizing malaria control: a cluster-randomized SMART trial
Methods	Cluster-randomized trial
Participants	122,872 participants (6 months and older, all sexes)
Interventions	Other: regular long-lasting insecticidal nets (Olyset) Other: LLIN plus piperonyl butoxide-treated LLIN (Olyset Plus)
Outcomes	Annual clinical malaria incidence rate Malaria parasite prevalence Malaria vector density Malaria transmission intensity
Starting date	01/12/2019
Contact information	Dr Guiyun Yan
Notes	

UMIN000019971

Study name	A preliminary study on designing a cluster randomized control trial of two mosquito nets to prevent malaria parasite infection
Methods	Cluster-randomized trial
Participants	1360 target participants Children targeted for malaria transmission survey are aged between 7 and 131 months Children between 60 and 131 months old are schoolchildren; 170 children are randomly selected from each cluster for survey
Interventions	Control: bed net treated with pyrethroid only Intervention: bed net treated with both pyrethroid and PBO
Outcomes	*Plasmodium falciparum* parasite infection after distribution of bed nets with PBO: PCR-based infection Slide-based infection Haemoglobin amount
Starting date	
Contact information	Dr Noboru Minakawa
Notes	

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ELISA: enzyme-linked immunosorbent assay; PBO: piperonyl butoxide.

DATA AND ANALYSES

Comparison 1. Commercial pyrethroid-PBO nets versus commercial LLINs: village trials

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1.1 Parasite prevalence (pyrethroid-PBO nets vs non-PBO LLINs, latest end points in RCT)	2		Odds Ratio (IV, Fixed, 95% CI)	0.79 [0.67, 0.95]
1.2 Parasite prevalence (pyrethroid-PBO nets vs non-PBO LLINs, shown at 4 different time points)	2		Odds Ratio (IV, Fixed, 95% CI)	Subtotals only
1.2.1 4 to 6 months	2		Odds Ratio (IV, Fixed, 95% CI)	0.74 [0.62, 0.89]
1.2.2 9 to 12 months	2		Odds Ratio (IV, Fixed, 95% CI)	0.72 [0.61, 0.86]
1.2.3 16 to 18 months	2		Odds Ratio (IV, Fixed, 95% CI)	0.88 [0.74, 1.04]
1.2.4 21 to 25 months	2		Odds Ratio (IV, Fixed, 95% CI)	0.79 [0.67, 0.95]
1.3 Mosquito sporozoite-positive (adjusted ICC 0.1)	4	424	Risk Ratio (M-H, Random, 95% CI)	0.82 [0.24, 2.75]
1.4 Mosquito parous (adjusted ICC 0.1)	3	220	Risk Ratio (M-H, Random, 95% CI)	0.97 [0.82, 1.13]

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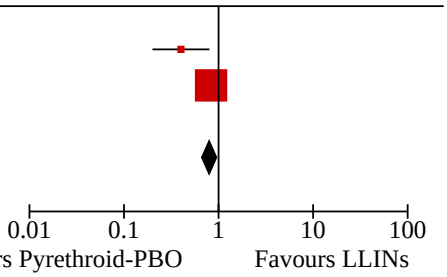
Analysis 1.1. Comparison 1: Commercial pyrethroid-PBO nets versus commercial LLINs: village trials, Outcome 1: Parasite prevalence (pyrethroid-PBO nets vs non-PBO LLINs, latest end points in RCT)

Study or Subgroup	log[OR]	SE	Weight	Odds Ratio IV, Fixed, 95% CI
Protopopoff 2018 (1)	-0.9163	0.3537	6.4%	0.40 [0.20 , 0.80]
Staedke 2020 (2)	-0.1827	0.0923	93.6%	0.83 [0.70 , 1.00]
Total (95% CI)			**100.0%**	**0.79 [0.67 , 0.95]**

Heterogeneity: Chi² = 4.03, df = 1 (P = 0.04); I² = 75%
Test for overall effect: Z = 2.57 (P = 0.01)
Test for subgroup differences: Not applicable



Footnotes

(1) 21 months after intervention
(2) 25 months after intervention

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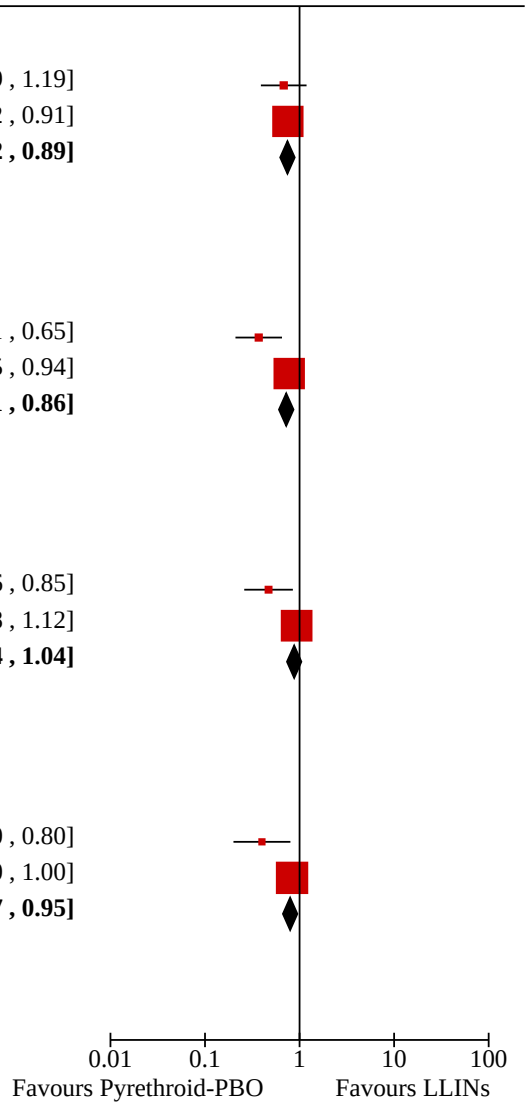
Analysis 1.2. Comparison 1: Commercial pyrethroid-PBO nets versus commercial LLINs: village trials, Outcome 2: Parasite prevalence (pyrethroid-PBO nets vs non-PBO LLINs, shown at 4 different time points)

Study or Subgroup	log[OR]	SE	Weight	Odds Ratio IV, Fixed, 95% CI
1.2.1 4 to 6 months				
Protopopoff 2018 (1)	-0.3857	0.2837	10.1%	0.68 [0.39 , 1.19]
Staedke 2020 (2)	-0.2845	0.095	89.9%	0.75 [0.62 , 0.91]
Subtotal (95% CI)			**100.0%**	**0.74 [0.62 , 0.89]**
Heterogeneity: Chi² = 0.11, df = 1 (P = 0.74); I² = 0%				
Test for overall effect: Z = 3.27 (P = 0.001)				
1.2.2 9 to 12 months				
Protopopoff 2018 (3)	-0.9943	0.289	9.8%	0.37 [0.21 , 0.65]
Staedke 2020 (4)	-0.2522	0.095	90.2%	0.78 [0.65 , 0.94]
Subtotal (95% CI)			**100.0%**	**0.72 [0.61 , 0.86]**
Heterogeneity: Chi² = 5.95, df = 1 (P = 0.01); I² = 83%				
Test for overall effect: Z = 3.60 (P = 0.0003)				
1.2.3 16 to 18 months				
Protopopoff 2018 (5)	-0.755	0.3021	8.7%	0.47 [0.26 , 0.85]
Staedke 2020 (6)	-0.0713	0.0934	91.3%	0.93 [0.78 , 1.12]
Subtotal (95% CI)			**100.0%**	**0.88 [0.74 , 1.04]**
Heterogeneity: Chi² = 4.68, df = 1 (P = 0.03); I² = 79%				
Test for overall effect: Z = 1.47 (P = 0.14)				
1.2.4 21 to 25 months				
Protopopoff 2018 (7)	-0.9163	0.3537	6.4%	0.40 [0.20 , 0.80]
Staedke 2020 (8)	-0.1827	0.0923	93.6%	0.83 [0.70 , 1.00]
Subtotal (95% CI)			**100.0%**	**0.79 [0.67 , 0.95]**
Heterogeneity: Chi² = 4.03, df = 1 (P = 0.04); I² = 75%				
Test for overall effect: Z = 2.57 (P = 0.01)				



Footnotes

(1) 4 months after intervention
(2) 6 months after intervention
(3) 9 months after intervention
(4) 12 months after intervention
(5) 16 months after intervention
(6) 18 months after intervention
(7) 21 months after intervention
(8) 25 months after intervention

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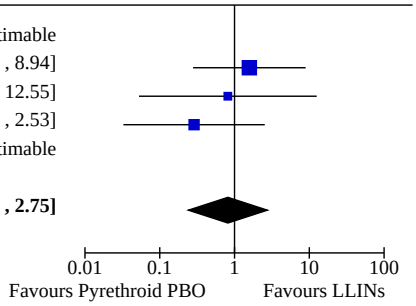
Analysis 1.3. Comparison 1: Commercial pyrethroid-PBO nets versus commercial LLINs: village trials, Outcome 3: Mosquito sporozoite-positive (adjusted ICC 0.1)

Study or Subgroup	Pyrethroid-PBO nets Events	Total	LLINs Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Awolola 2014 (1)	0	4	0	16		Not estimable
Cisse 2017 (2)	3	38	2	40	49.1%	1.58 [0.28 , 8.94]
Cisse 2017 (3)	1	43	1	35	19.7%	0.81 [0.05 , 12.55]
Protopopoff 2018 (2)	1	106	4	122	31.2%	0.29 [0.03 , 2.53]
Stiles-Ocran 2013 (3)	0	9	0	11		Not estimable
Total (95% CI)		**200**		**224**	**100.0%**	**0.82 [0.24 , 2.75]**
Total events:	5		7			

Heterogeneity: Tau² = 0.00; Chi² = 1.46, df = 2 (P = 0.48); I² = 0%
Test for overall effect: Z = 0.33 (P = 0.74)
Test for subgroup differences: Not applicable

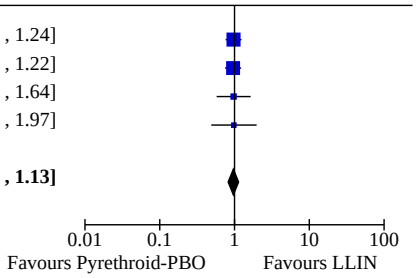


Footnotes
(1) Permanet 3.0, Low resistance
(2) Olyset Plus, High resistance
(3) Permanet 3.0, Moderate resistance

Analysis 1.4. Comparison 1: Commercial pyrethroid-PBO nets versus commercial LLINs: village trials, Outcome 4: Mosquito parous (adjusted ICC 0.1)

Study or Subgroup	Pyrethroid-PBO nets Events	Total	LLINs Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Cisse 2017 (1)	28	37	32	41	43.0%	0.97 [0.76 , 1.24]
Cisse 2017 (2)	30	40	29	37	42.3%	0.96 [0.75 , 1.22]
Mzilahowa 2014 (3)	10	18	16	28	9.4%	0.97 [0.58 , 1.64]
Stiles-Ocran 2013 (2)	5	8	7	11	5.3%	0.98 [0.49 , 1.97]
Total (95% CI)		**103**		**117**	**100.0%**	**0.97 [0.82 , 1.13]**
Total events:	73		84			

Heterogeneity: Tau² = 0.00; Chi² = 0.01, df = 3 (P = 1.00); I² = 0%
Test for overall effect: Z = 0.43 (P = 0.66)
Test for subgroup differences: Not applicable



Footnotes
(1) Olyset Plus, High resistance
(2) Permanet 3.0, Moderate resistance
(3) Permanet 3.0, *Anopheles funestus*

Comparison 2. Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Mosquito mortality (pooled) hut/night (adjusted ICC 0.1)	10	15614	Risk Ratio (M-H, Random, 95% CI)	1.43 [1.26, 1.62]
2.1.1 Unwashed	10	8647	Risk Ratio (M-H, Random, 95% CI)	1.63 [1.29, 2.05]

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Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1.2 Washed	8	6967	Risk Ratio (M-H, Random, 95% CI)	1.19 [1.04, 1.38]
2.2 Mosquito blood-feeding success (pooled) hut/night (adjusted ICC 0.1)	9	12351	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.66, 0.85]
2.2.1 Unwashed	9	7261	Risk Ratio (M-H, Random, 95% CI)	0.68 [0.57, 0.80]
2.2.2 Washed	7	5090	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.74, 1.02]
2.3 Mosquito exophily (pooled) hut/night (adjusted ICC 0.1)	10	13214	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.94, 1.06]
2.3.1 Unwashed	10	7699	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.91, 1.10]
2.3.2 Washed	8	5515	Risk Ratio (M-H, Random, 95% CI)	1.00 [0.93, 1.07]
2.4 Mosquito mortality (high resistance) hut/night (adjusted ICC 0.1)	5	7997	Risk Ratio (M-H, Random, 95% CI)	1.58 [1.34, 1.86]
2.4.1 Unwashed	5	4896	Risk Ratio (M-H, Random, 95% CI)	1.84 [1.60, 2.11]
2.4.2 Washed	4	3101	Risk Ratio (M-H, Random, 95% CI)	1.20 [0.88, 1.63]
2.5 Mosquito blood-feeding success (high resistance) hut/night (adjusted ICC 0.1)	4	7134	Risk Ratio (M-H, Random, 95% CI)	0.66 [0.57, 0.76]
2.5.1 Unwashed	4	4458	Risk Ratio (M-H, Random, 95% CI)	0.60 [0.50, 0.71]
2.5.2 Washed	3	2676	Risk Ratio (M-H, Random, 95% CI)	0.81 [0.72, 0.92]
2.6 Mosquito mortality (moderate resistance) hut/night (adjusted ICC 0.1)	2	1027	Risk Ratio (M-H, Fixed, 95% CI)	1.47 [1.21, 1.78]
2.6.1 Unwashed	2	751	Risk Ratio (M-H, Fixed, 95% CI)	1.68 [1.33, 2.11]
2.6.2 Washed	1	276	Risk Ratio (M-H, Fixed, 95% CI)	1.07 [0.74, 1.54]
2.7 Mosquito blood-feeding success (moderate resistance) hut/night (adjusted ICC 0.1)	2	1034	Risk Ratio (M-H, Random, 95% CI)	0.91 [0.78, 1.05]

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Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.7.1 Unwashed	2	752	Risk Ratio (M-H, Random, 95% CI)	0.90 [0.72, 1.11]
2.7.2 Washed	1	282	Risk Ratio (M-H, Random, 95% CI)	0.91 [0.74, 1.13]
2.8 Mosquito mortality (low resistance) hut/night (adjusted ICC 0.1)	2	1970	Risk Ratio (M-H, Random, 95% CI)	1.30 [1.09, 1.56]
2.8.1 Unwashed	2	948	Risk Ratio (M-H, Random, 95% CI)	1.25 [0.99, 1.57]
2.8.2 Washed	2	1022	Risk Ratio (M-H, Random, 95% CI)	1.39 [0.95, 2.04]
2.9 Mosquito blood-feeding success (low resistance) hut/night (adjusted ICC 0.1)	2	1970	Risk Ratio (M-H, Random, 95% CI)	0.94 [0.56, 1.57]
2.9.1 Unwashed	2	948	Risk Ratio (M-H, Random, 95% CI)	0.75 [0.27, 2.11]
2.9.2 Washed	2	1022	Risk Ratio (M-H, Random, 95% CI)	1.07 [0.49, 2.33]
2.10 Mosquito mortality (susceptible) hut/night (adjusted ICC 0.1)	2	1916	Risk Ratio (M-H, Random, 95% CI)	1.05 [0.96, 1.15]
2.10.1 Unwashed	2	948	Risk Ratio (M-H, Random, 95% CI)	1.20 [0.64, 2.26]
2.10.2 Washed	2	968	Risk Ratio (M-H, Random, 95% CI)	1.07 [0.92, 1.25]
2.11 Mosquito blood-feeding success (susceptible) hut/night (adjusted ICC 0.1)	2	1916	Risk Ratio (M-H, Random, 95% CI)	0.87 [0.40, 1.89]
2.11.1 Unwashed	2	948	Risk Ratio (M-H, Random, 95% CI)	0.52 [0.12, 2.22]
2.11.2 Washed	2	968	Risk Ratio (M-H, Random, 95% CI)	1.25 [0.82, 1.91]
2.12 Mosquito mortality (high resistance/Permanet) hut/night (adjusted ICC 0.1)	3	2806	Risk Ratio (M-H, Random, 95% CI)	1.59 [1.26, 2.01]
2.12.1 Not Washed	3	1877	Risk Ratio (M-H, Random, 95% CI)	1.81 [1.56, 2.10]
2.12.2 Washed	2	929	Risk Ratio (M-H, Random, 95% CI)	1.18 [0.61, 2.28]

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Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.13 Mosquito blood-feeding success (high resistance/Permanet) hut/night (adjusted ICC 0.1)	2	1943	Risk Ratio (M-H, Random, 95% CI)	0.58 [0.45, 0.76]
2.13.1 Unwashed	2	1439	Risk Ratio (M-H, Random, 95% CI)	0.53 [0.40, 0.69]
2.13.2 Washed	1	504	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.61, 0.93]
2.14 Mosquito mortality (high resistance/Olyset) hut/night (adjusted ICC 0.1)	2	1410	Risk Ratio (M-H, Random, 95% CI)	1.73 [1.51, 1.97]
2.14.1 Unwashed	2	1257	Risk Ratio (M-H, Random, 95% CI)	1.72 [1.48, 1.99]
2.14.2 Washed	1	153	Risk Ratio (M-H, Random, 95% CI)	1.81 [1.25, 2.61]
2.15 Mosquito blood-feeding success (high resistance/Olyset) hut/night (adjusted ICC 0.1)	2	1470	Risk Ratio (M-H, Random, 95% CI)	0.63 [0.40, 0.98]
2.15.1 Unwashed	2	1257	Risk Ratio (M-H, Random, 95% CI)	0.67 [0.38, 1.18]
2.15.2 Washed	1	213	Risk Ratio (M-H, Random, 95% CI)	0.50 [0.27, 0.93]

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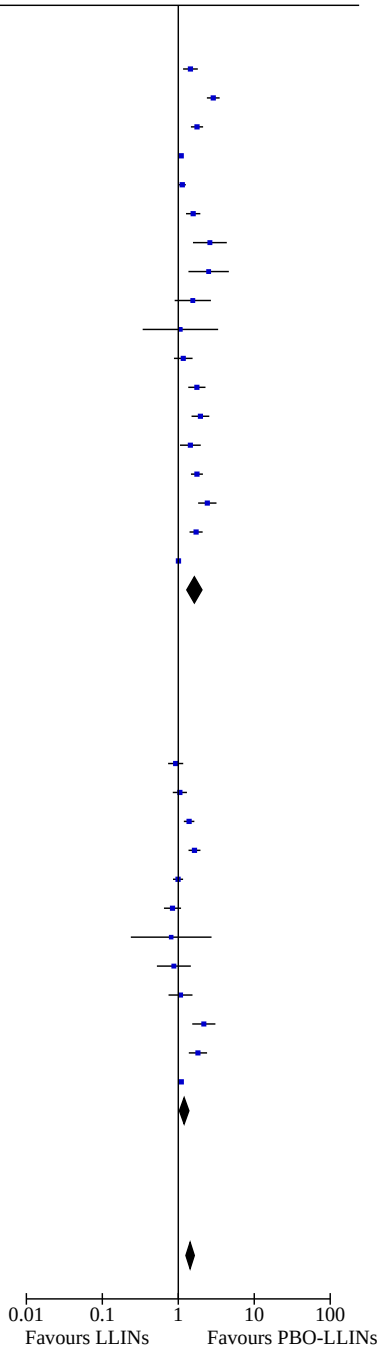
Analysis 2.1. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 1: Mosquito mortality (pooled) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.1.1 Unwashed						
Bayili 2017 (1)	144	664	113	753	3.7%	1.45 [1.16 , 1.81]
Bayili 2017 (2)	197	457	125	837	3.8%	2.89 [2.38 , 3.50]
Corbel 2010 (3)	181	232	73	165	3.8%	1.76 [1.47 , 2.12]
Corbel 2010 (4)	170	176	177	200	4.1%	1.09 [1.03 , 1.16]
Corbel 2010 (5)	110	117	70	84	4.1%	1.13 [1.01 , 1.25]
Koudou 2011 (6)	117	214	78	224	3.7%	1.57 [1.26 , 1.95]
Menze 2020 (7)	32	106	19	164	2.5%	2.61 [1.56 , 4.35]
Menze 2020 (8)	34	140	12	124	2.1%	2.51 [1.36 , 4.63]
Moore 2016 (9)	23	161	22	239	2.3%	1.55 [0.90 , 2.69]
Moore 2016 (10)	5	68	6	87	0.9%	1.07 [0.34 , 3.35]
N'Guessan 2010 (11)	59	115	45	102	3.5%	1.16 [0.88 , 1.54]
Oumbouke 2019 (12)	79	156	62	215	3.5%	1.76 [1.35 , 2.28]
Pennetier 2013 (13)	53	66	39	95	3.5%	1.96 [1.49 , 2.56]
Toé 2018 (14)	62	221	57	293	3.3%	1.44 [1.05 , 1.97]
Toé 2018 (15)	125	199	116	325	3.8%	1.76 [1.47 , 2.11]
Toé 2018 (16)	116	249	52	269	3.5%	2.41 [1.83 , 3.18]
Toé 2018 (17)	146	272	97	310	3.8%	1.72 [1.41 , 2.09]
Tungu 2010 (18)	223	233	300	315	4.2%	1.00 [0.97 , 1.04]
Subtotal (95% CI)		**3846**		**4801**	**60.2%**	**1.63 [1.29 , 2.05]**
Total events:	1876		1463			
Heterogeneity: Tau² = 0.22; Chi² = 750.61, df = 17 (P < 0.00001); I² = 98%						
Test for overall effect: Z = 4.17 (P < 0.0001)						
2.1.2 Washed						
Bayili 2017 (2)	105	683	149	895	3.7%	0.92 [0.73 , 1.16]
Bayili 2017 (1)	136	780	141	848	3.7%	1.05 [0.85 , 1.30]
Corbel 2010 (5)	82	105	112	199	3.9%	1.39 [1.18 , 1.63]
Corbel 2010 (3)	183	371	122	404	3.8%	1.63 [1.36 , 1.96]
Corbel 2010 (4)	101	144	94	133	3.9%	0.99 [0.85 , 1.16]
Koudou 2011 (6)	72	224	77	201	3.6%	0.84 [0.65 , 1.09]
Moore 2016 (10)	4	67	6	81	0.8%	0.81 [0.24 , 2.74]
Moore 2016 (19)	23	186	28	198	2.5%	0.87 [0.52 , 1.46]
N'Guessan 2010 (11)	40	130	42	146	3.1%	1.07 [0.74 , 1.54]
Oumbouke 2019 (12)	60	158	38	217	3.2%	2.17 [1.53 , 3.08]
Pennetier 2013 (13)	64	96	43	117	3.5%	1.81 [1.38 , 2.39]
Tungu 2010 (18)	271	285	260	299	4.1%	1.09 [1.04 , 1.15]
Subtotal (95% CI)		**3229**		**3738**	**39.8%**	**1.19 [1.04 , 1.38]**
Total events:	1141		1112			
Heterogeneity: Tau² = 0.04; Chi² = 63.76, df = 11 (P < 0.00001); I² = 83%						
Test for overall effect: Z = 2.46 (P = 0.01)						
Total (95% CI)		**7075**		**8539**	**100.0%**	**1.43 [1.26 , 1.62]**
Total events:	3017		2575			
Heterogeneity: Tau² = 0.10; Chi² = 615.89, df = 29 (P < 0.00001); I² = 95%						
Test for overall effect: Z = 5.56 (P < 0.00001)						
Test for subgroup differences: Chi² = 5.08, df = 1 (P = 0.02), I² = 80.3%						



Footnotes

(1) Valle du Kou, DawaPlus 3.0, High resistance
(2) Valle du Kou, DawaPlus 4.0, High resistance
(3) Vallée du Kou, Permanet 3.0, High resistance
(4) Malanville, Permanet 3.0, Low resistance
(5) Pitoa, Permanet 3.0, Low resistance

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Analysis 2.1. (Continued)

(4) Malanville, Permanet 3.0, Low resistance
(5) Pitoa, Permanet 3.0, Low resistance
(6) Yaokoffikro, Permanet 3.0, High resistance
(7) Mibellon, PermaNet 3.0, moderate resistance, *An funestus*
(8) Mibellon, Olyset Plus, Moderate resistance, *An funestus*
(9) Ifakara, Veeralin, Susceptible, *An arabiensis*
(10) Ifakara, Veeralin, Unclassified, *An funestus*
(11) Akron, Permanet 3.0, Moderate resistance
(12) Cote d'Ivoire, VEERALIN, Low resistance
(13) Malanaville, Olyset Plus, High resistance
(14) Tengrela, Olyset Plus, High resistance
(15) Vallee du Kou 5, Permanet 3.0, High resistance
(16) Tengrela, Permanet 3.0, High resistance
(17) Vallee du Kou 5, Olyset Plus, High resistance
(18) Zeneti, Permanet 3.0, Susceptible
(19) Ifakara, Veeralin, Susceptible, *An arabiensis*

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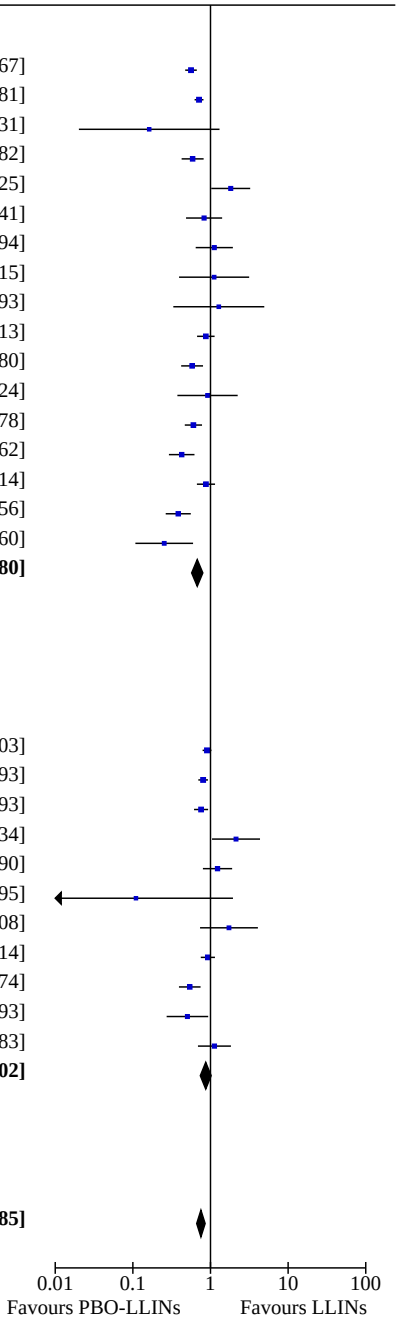
Analysis 2.2. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 2: Mosquito blood-feeding success (pooled) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.2.1 Unwashed						
Bayili 2017 (1)	102	302	333	553	5.9%	0.56 [0.47 , 0.67]
Bayili 2017 (2)	182	425	290	482	6.2%	0.71 [0.62 , 0.81]
Corbel 2010 (3)	1	176	7	200	0.3%	0.16 [0.02 , 1.31]
Corbel 2010 (4)	48	232	58	165	4.5%	0.59 [0.42 , 0.82]
Corbel 2010 (5)	33	117	13	84	2.7%	1.82 [1.02 , 3.25]
Menze 2020 (6)	17	106	32	165	3.0%	0.83 [0.48 , 1.41]
Menze 2020 (7)	24	140	19	124	2.9%	1.12 [0.64 , 1.94]
Moore 2016 (8)	6	161	8	239	1.2%	1.11 [0.39 , 3.15]
Moore 2016 (9)	4	68	4	87	0.8%	1.28 [0.33 , 4.93]
N'Guessan 2010 (10)	55	115	56	102	5.1%	0.87 [0.67 , 1.13]
Oumbouke 2019 (11)	37	156	88	215	4.6%	0.58 [0.42 , 0.80]
Pennetier 2013 (10)	7	66	11	95	1.5%	0.92 [0.37 , 2.24]
Toé 2018 (12)	63	249	113	269	5.2%	0.60 [0.47 , 0.78]
Toé 2018 (13)	31	272	83	310	4.1%	0.43 [0.29 , 0.62]
Toé 2018 (14)	62	221	94	293	5.1%	0.87 [0.67 , 1.14]
Toé 2018 (15)	28	199	119	325	4.2%	0.38 [0.26 , 0.56]
Tungu 2010 (16)	6	233	32	315	1.6%	0.25 [0.11 , 0.60]
Subtotal (95% CI)		**3238**		**4023**	**58.7%**	**0.68 [0.57 , 0.80]**
Total events:	706		1360			
Heterogeneity: Tau² = 0.06; Chi² = 53.70, df = 16 (P < 0.00001); I² = 70%						
Test for overall effect: Z = 4.66 (P < 0.00001)						
2.2.2 Washed						
Bayili 2017 (2)	215	474	259	515	6.2%	0.90 [0.79 , 1.03]
Bayili 2017 (1)	170	420	277	550	6.1%	0.80 [0.70 , 0.93]
Corbel 2010 (4)	88	241	127	263	5.6%	0.76 [0.61 , 0.93]
Corbel 2010 (3)	23	178	10	165	2.1%	2.13 [1.05 , 4.34]
Corbel 2010 (5)	26	105	40	199	3.7%	1.23 [0.80 , 1.90]
Moore 2016 (9)	0	67	5	81	0.2%	0.11 [0.01 , 1.95]
Moore 2016 (8)	13	186	8	198	1.6%	1.73 [0.73 , 4.08]
N'Guessan 2010 (10)	70	130	85	146	5.6%	0.92 [0.75 , 1.14]
Oumbouke 2019 (11)	37	158	94	217	4.6%	0.54 [0.39 , 0.74]
Pennetier 2013 (17)	12	96	29	117	2.5%	0.50 [0.27 , 0.93]
Tungu 2010 (16)	30	285	28	299	3.3%	1.12 [0.69 , 1.83]
Subtotal (95% CI)		**2340**		**2750**	**41.3%**	**0.87 [0.74 , 1.02]**
Total events:	684		962			
Heterogeneity: Tau² = 0.03; Chi² = 28.71, df = 10 (P = 0.001); I² = 65%						
Test for overall effect: Z = 1.73 (P = 0.08)						
Total (95% CI)		**5578**		**6773**	**100.0%**	**0.75 [0.66 , 0.85]**
Total events:	1390		2322			
Heterogeneity: Tau² = 0.06; Chi² = 103.45, df = 27 (P < 0.00001); I² = 74%						
Test for overall effect: Z = 4.48 (P < 0.00001)						
Test for subgroup differences: Chi² = 4.60, df = 1 (P = 0.03), I² = 78.3%						



Footnotes

(1) Vallee du Kou, DawaPlus 4.0, High resistance
(2) Vallee du Kou, DawaPlus 3.0, High resistance
(3) Malanville, Permanet 3.0, Low resistance
(4) Vallée du Kou, Permanet 3.0, High resistance
(5) Pitoa, Permanet 3.0, Low resistance
(6) Mibellon, PermaNet 3.0, moderate resistance. *An funestus*

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Analysis 2.2. (Continued)

(5) Pitoa, Permanet 3.0, Low resistance
(6) Mibellon, PermaNet 3.0, moderate resistance, *An funestus*
(7) Mibellon, Olyset Plus, Moderate resistance, *An funestus*
(8) Ifakara, Veeralin, Susceptible, *An arabiensis*
(9) Ifakara, Veeralin, Unclassified, *An funestus*
(10) Akron, Permanet 3.0, Moderate resistance
(11) Cote d'Ivoire, VEERALIN, Low resistance
(12) Tengrela, Permanet 3.0, High resistance
(13) Vallee du Kou 5, Olyset Plus, High resistance
(14) Tengrela, Olyset Plus, High resistance
(15) Vallee du Kou 5, Permanet 3.0, High resistance
(16) Zeneti, Permanet 3.0, Susceptible
(17) Malanville, Olyset Plus, High resistance

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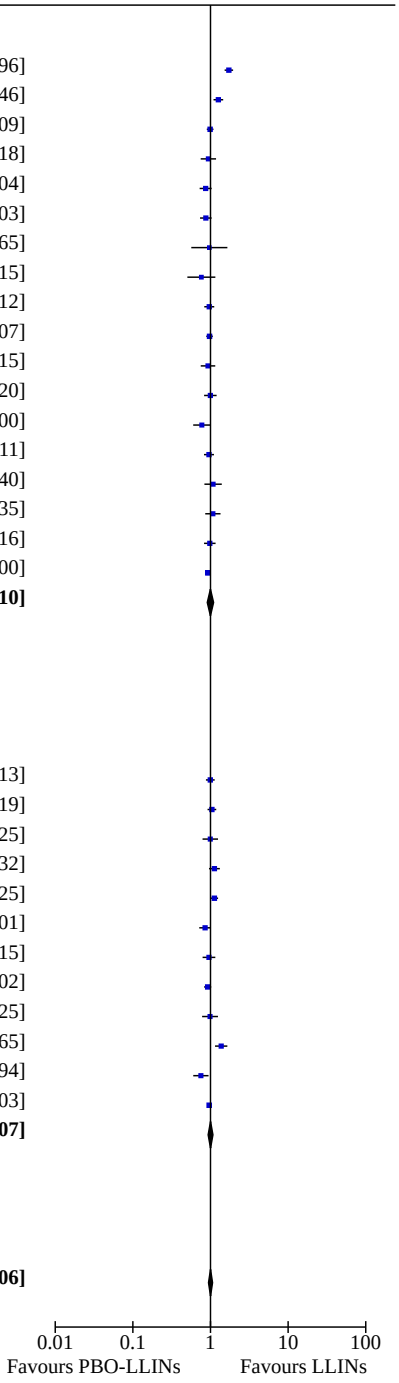
Analysis 2.3. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 3: Mosquito exophily (pooled) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLIN Events	PBO-LLIN Total	LLIN Events	LLIN Total	Weight	Risk Ratio M-H, Random, 95% CI
2.3.1 Unwashed						
Bayili 2017 (1)	210	302	223	553	3.9%	1.72 [1.52 , 1.96]
Bayili 2017 (2)	216	425	194	482	3.7%	1.26 [1.09 , 1.46]
Corbel 2010 (3)	185	232	133	165	4.2%	0.99 [0.90 , 1.09]
Corbel 2010 (4)	68	117	52	84	2.8%	0.94 [0.75 , 1.18]
Corbel 2010 (5)	93	176	122	200	3.3%	0.87 [0.72 , 1.04]
Koudou 2011 (6)	109	214	131	224	3.4%	0.87 [0.73 , 1.03]
Menze 2020 (7)	18	106	29	165	1.0%	0.97 [0.57 , 1.65]
Menze 2020 (8)	31	140	36	124	1.4%	0.76 [0.50 , 1.15]
Moore 2016 (9)	55	68	73	87	3.7%	0.96 [0.83 , 1.12]
Moore 2016 (10)	129	161	197	239	4.3%	0.97 [0.88 , 1.07]
N'Guessan 2010 (11)	67	115	64	102	2.9%	0.93 [0.75 , 1.15]
Oumbouke 2019	86	156	119	215	3.3%	1.00 [0.83 , 1.20]
Pennetier 2013 (12)	36	66	67	95	2.5%	0.77 [0.60 , 1.00]
Toé 2018 (13)	146	272	174	310	3.7%	0.96 [0.82 , 1.11]
Toé 2018 (14)	80	249	80	269	2.5%	1.08 [0.84 , 1.40]
Toé 2018 (15)	85	221	105	293	2.8%	1.07 [0.86 , 1.35]
Toé 2018 (16)	102	199	170	325	3.4%	0.98 [0.83 , 1.16]
Tungu 2010 (17)	184	233	269	315	4.4%	0.92 [0.85 , 1.00]
Subtotal (95% CI)		**3452**		**4247**	**57.5%**	**1.00 [0.91 , 1.10]**
Total events:	1900		2238			
Heterogeneity: Tau² = 0.03; Chi² = 100.21, df = 17 (P < 0.00001); I² = 83%						
Test for overall effect: Z = 0.04 (P = 0.97)						
2.3.2 Washed						
Bayili 2017 (2)	227	474	248	515	3.9%	0.99 [0.87 , 1.13]
Bayili 2017 (1)	211	420	265	550	3.9%	1.04 [0.92 , 1.19]
Corbel 2010 (4)	54	105	103	199	2.8%	0.99 [0.79 , 1.25]
Corbel 2010 (5)	120	178	99	165	3.5%	1.12 [0.96 , 1.32]
Corbel 2010 (3)	183	241	178	263	4.1%	1.12 [1.01 , 1.25]
Koudou 2011 (6)	113	224	119	201	3.4%	0.85 [0.72 , 1.01]
Moore 2016 (9)	49	67	62	81	3.2%	0.96 [0.79 , 1.15]
Moore 2016 (10)	140	186	162	198	4.2%	0.92 [0.83 , 1.02]
N'Guessan 2010 (11)	65	130	74	146	2.7%	0.99 [0.78 , 1.25]
Oumbouke 2019	102	158	102	217	3.3%	1.37 [1.14 , 1.65]
Pennetier 2013 (12)	50	96	81	117	2.8%	0.75 [0.60 , 0.94]
Tungu 2010 (17)	242	285	264	299	4.6%	0.96 [0.90 , 1.03]
Subtotal (95% CI)		**2564**		**2951**	**42.5%**	**1.00 [0.93 , 1.07]**
Total events:	1556		1757			
Heterogeneity: Tau² = 0.01; Chi² = 32.00, df = 11 (P = 0.0008); I² = 66%						
Test for overall effect: Z = 0.04 (P = 0.97)						
Total (95% CI)		**6016**		**7198**	**100.0%**	**1.00 [0.94 , 1.06]**
Total events:	3456		3995			
Heterogeneity: Tau² = 0.02; Chi² = 132.61, df = 29 (P < 0.00001); I² = 78%						
Test for overall effect: Z = 0.03 (P = 0.97)						
Test for subgroup differences: Chi² = 0.00, df = 1 (P = 0.96), I² = 0%						



Footnotes

(1) Vallee du Kou. DawaPlus 4.0, High resistance
(2) Vallee du Kou. DawaPlus 3.0, High resistance
(3) Vallée du Kou, Permanet 3.0, HIgh resistance
(4) Pitoa, Permanet 3.0, Low resistance

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Analysis 2.3. (Continued)

(3) Vallée du Kou, Permanet 3.0, HIgh resistance
(4) Pitoa, Permanet 3.0, Low resistance
(5) Malanville, Permanet 3.0, Low resistance
(6) Yaokoffikro, Permanet 3.0, High resistance
(7) Mibellon, PermaNet 3.0, moderate resistance, *An funestus*
(8) Mibellon, Olyset Plus, Moderate resistance, *An funestus*
(9) Ifakara, Veeralin, Unclassified, *An funestus*
(10) Ifakara, Veeralin, Susceptible, *An arabiensis*
(11) Akron, Permanet 3.0, Moderate resistance
(12) Malanville, Olyset Plus, High resistance
(13) Vallee du Kou 5 ,Olyset Plus, High resistance
(14) Tengrela, Permanet 3.0, High resistance
(15) Tengrela, Olyset Plus, High resistance
(16) Vallee du Kou 5, Permanet 3.0, High resistance
(17) Zeneti, Permanet 3.0, Susceptible

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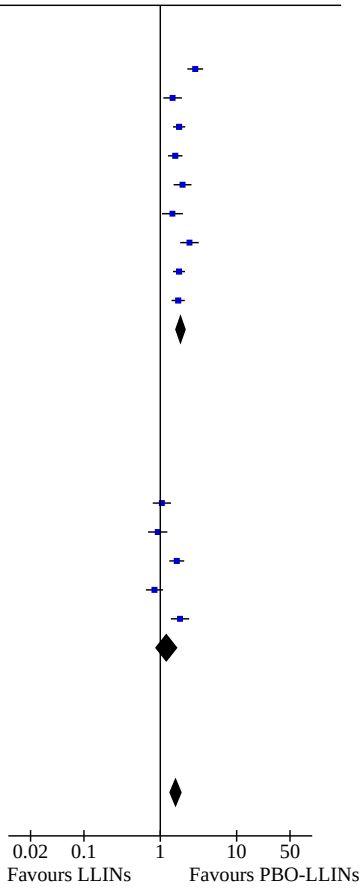
Analysis 2.4. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 4: Mosquito mortality (high resistance) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.4.1 Unwashed						
Bayili 2017 (1)	130	302	83	553	7.3%	2.87 [2.26 , 3.64]
Bayili 2017 (2)	92	425	72	482	6.9%	1.45 [1.10 , 1.92]
Corbel 2010 (3)	181	232	73	165	7.7%	1.76 [1.47 , 2.12]
Koudou 2011 (4)	117	214	78	224	7.4%	1.57 [1.26 , 1.95]
Pennetier 2013 (5)	53	66	39	95	7.0%	1.96 [1.49 , 2.56]
Toé 2018 (6)	62	221	57	293	6.5%	1.44 [1.05 , 1.97]
Toé 2018 (7)	116	249	52	269	6.9%	2.41 [1.83 , 3.18]
Toé 2018 (8)	125	199	116	325	7.8%	1.76 [1.47 , 2.11]
Toé 2018 (9)	146	272	97	310	7.6%	1.72 [1.41 , 2.09]
Subtotal (95% CI)		**2180**		**2716**	**65.0%**	**1.84 [1.60 , 2.11]**
Total events:	1022		667			
Heterogeneity: Tau² = 0.03; Chi² = 25.39, df = 8 (P = 0.001); I² = 68%						
Test for overall effect: Z = 8.65 (P < 0.00001)						
2.4.2 Washed						
Bayili 2017 (2)	83	474	86	515	6.9%	1.05 [0.80 , 1.38]
Bayili 2017 (1)	65	420	92	550	6.7%	0.93 [0.69 , 1.24]
Corbel 2010 (3)	119	241	79	263	7.4%	1.64 [1.31 , 2.06]
Koudou 2011 (3)	72	224	77	201	7.1%	0.84 [0.65 , 1.09]
Pennetier 2013 (3)	64	96	43	117	6.9%	1.81 [1.38 , 2.39]
Subtotal (95% CI)		**1455**		**1646**	**35.0%**	**1.20 [0.88 , 1.63]**
Total events:	403		377			
Heterogeneity: Tau² = 0.11; Chi² = 27.69, df = 4 (P < 0.0001); I² = 86%						
Test for overall effect: Z = 1.14 (P = 0.25)						
Total (95% CI)		**3635**		**4362**	**100.0%**	**1.58 [1.34 , 1.86]**
Total events:	1425		1044			
Heterogeneity: Tau² = 0.08; Chi² = 84.86, df = 13 (P < 0.00001); I² = 85%						
Test for overall effect: Z = 5.46 (P < 0.00001)						
Test for subgroup differences: Chi² = 6.11, df = 1 (P = 0.01), I² = 83.6%						



Footnotes

(1) Valle du Kou, DawaPlus 4.0, High resistance
(2) Valle du Kou, DawaPlus 3.0, High resistance
(3) Vallée du Kou, Permanet 3.0, High resistance
(4) Yaokoffikro, Permanet 3.0, High resistance
(5) Malanaville, Olyset Plus, High resistance
(6) Tengrela, Olyset Plus, High resistance
(7) Tengrela, Permanet 3.0, High resistance
(8) Vallee du Kou 5, Permanet 3.0, High resistance
(9) Vallee du Kou 5, Olyset Plus, High resistance

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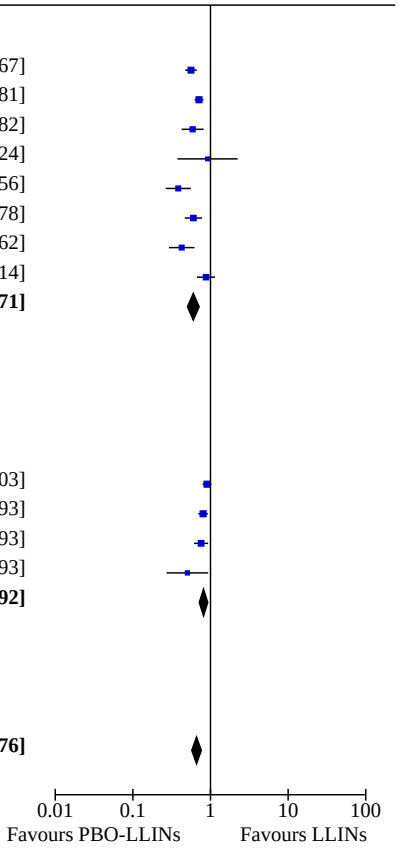
Analysis 2.5. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 5: Mosquito blood-feeding success (high resistance) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.5.1 Unwashed						
Bayili 2017 (1)	102	302	333	553	10.8%	0.56 [0.47 , 0.67]
Bayili 2017 (2)	182	425	290	482	11.5%	0.71 [0.62 , 0.81]
Corbel 2010 (3)	48	232	58	165	7.6%	0.59 [0.42 , 0.82]
Pennetier 2013 (4)	7	66	11	95	2.1%	0.92 [0.37 , 2.24]
Toé 2018 (5)	28	199	119	325	6.8%	0.38 [0.26 , 0.56]
Toé 2018 (6)	63	249	113	269	9.0%	0.60 [0.47 , 0.78]
Toé 2018 (7)	31	272	83	310	6.7%	0.43 [0.29 , 0.62]
Toé 2018 (8)	62	221	94	293	8.8%	0.87 [0.67 , 1.14]
Subtotal (95% CI)		**1966**		**2492**	**63.3%**	**0.60 [0.50 , 0.71]**
Total events:	523		1101			
Heterogeneity: Tau² = 0.04; Chi² = 23.11, df = 7 (P = 0.002); I² = 70%						
Test for overall effect: Z = 5.76 (P < 0.00001)						
2.5.2 Washed						
Bayili 2017 (2)	215	474	259	515	11.5%	0.90 [0.79 , 1.03]
Bayili 2017 (1)	170	420	277	550	11.3%	0.80 [0.70 , 0.93]
Corbel 2010 (3)	88	241	127	263	10.0%	0.76 [0.61 , 0.93]
Pennetier 2013 (4)	12	96	29	117	3.7%	0.50 [0.27 , 0.93]
Subtotal (95% CI)		**1231**		**1445**	**36.7%**	**0.81 [0.72 , 0.92]**
Total events:	485		692			
Heterogeneity: Tau² = 0.01; Chi² = 5.07, df = 3 (P = 0.17); I² = 41%						
Test for overall effect: Z = 3.24 (P = 0.001)						
Total (95% CI)		**3197**		**3937**	**100.0%**	**0.66 [0.57 , 0.76]**
Total events:	1008		1793			
Heterogeneity: Tau² = 0.04; Chi² = 48.33, df = 11 (P < 0.00001); I² = 77%						
Test for overall effect: Z = 5.72 (P < 0.00001)						
Test for subgroup differences: Chi² = 7.70, df = 1 (P = 0.006), I² = 87.0%						



Footnotes

(1) Vallee du Kou, DawaPlus 4.0, High resistance
(2) Vallee du Kou, DawaPlus 3.0, High resistance
(3) Vallée du Kou, Permanet 3.0, High resistance
(4) Malanville, Olyset Plus, High resistance
(5) Vallee du Kou 5, Permanet 3.0, High resistance
(6) Tengrela, Permanet 3.0, High resistance
(7) Vallee du Kou 5, Olyset Plus, High resistance
(8) Tengrela, Olyset Plus, High resistance

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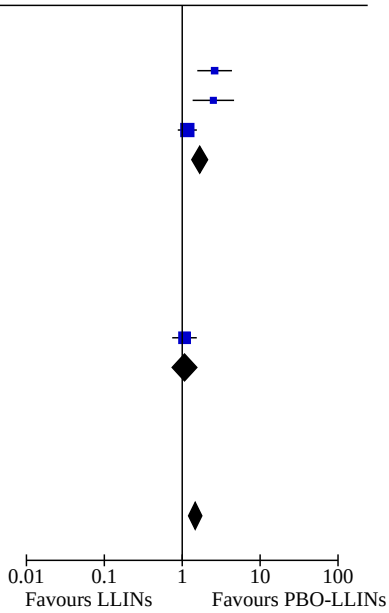
Analysis 2.6. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 6: Mosquito mortality (moderate resistance) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Fixed, 95% CI
2.6.1 Unwashed						
Menze 2020 (1)	32	106	19	164	13.0%	2.61 [1.56 , 4.35]
Menze 2020 (2)	34	140	12	124	11.1%	2.51 [1.36 , 4.63]
N'Guessan 2010 (3)	59	115	45	102	41.5%	1.16 [0.88 , 1.54]
Subtotal (95% CI)		**361**		**390**	**65.6%**	**1.68 [1.33 , 2.11]**
Total events:	125		76			
Heterogeneity: Chi² = 10.98, df = 2 (P = 0.004); I² = 82%						
Test for overall effect: Z = 4.35 (P < 0.0001)						
2.6.2 Washed						
N'Guessan 2010 (3)	40	130	42	146	34.4%	1.07 [0.74 , 1.54]
Subtotal (95% CI)		**130**		**146**	**34.4%**	**1.07 [0.74 , 1.54]**
Total events:	40		42			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.36 (P = 0.72)						
Total (95% CI)		**491**		**536**	**100.0%**	**1.47 [1.21 , 1.78]**
Total events:	165		118			
Heterogeneity: Chi² = 13.31, df = 3 (P = 0.004); I² = 77%						
Test for overall effect: Z = 3.86 (P = 0.0001)						
Test for subgroup differences: Chi² = 4.17, df = 1 (P = 0.04), I² = 76.0%						



Footnotes

(1) Mibellon, PermaNet 3.0, moderate resistance, *An funestus*

(2) Mibellon, Olyset Plus, Moderate resistance, *An funestus*

(3) Akron, Permanet 3.0, Moderate resistance

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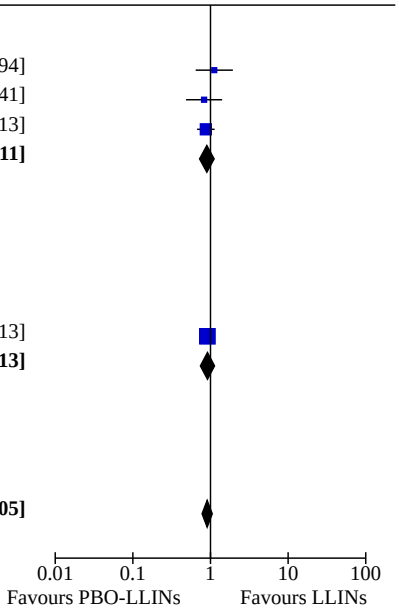
Analysis 2.7. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 7: Mosquito blood-feeding success (moderate resistance) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.7.1 Unwashed						
Menze 2020 (1)	24	140	19	124	7.4%	1.12 [0.64 , 1.94]
Menze 2020 (2)	17	106	32	165	7.8%	0.83 [0.48 , 1.41]
N'Guessan 2010 (3)	55	115	56	102	33.3%	0.87 [0.67 , 1.13]
Subtotal (95% CI)		**361**		**391**	**48.5%**	**0.90 [0.72 , 1.11]**
Total events:	96		107			
Heterogeneity: Tau² = 0.00; Chi² = 0.77, df = 2 (P = 0.68); I² = 0%						
Test for overall effect: Z = 0.99 (P = 0.32)						
2.7.2 Washed						
N'Guessan 2010 (3)	71	133	87	149	51.5%	0.91 [0.74 , 1.13]
Subtotal (95% CI)		**133**		**149**	**51.5%**	**0.91 [0.74 , 1.13]**
Total events:	71		87			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.84 (P = 0.40)						
Total (95% CI)		**494**		**540**	**100.0%**	**0.91 [0.78 , 1.05]**
Total events:	167		194			
Heterogeneity: Tau² = 0.00; Chi² = 0.77, df = 3 (P = 0.86); I² = 0%						
Test for overall effect: Z = 1.29 (P = 0.20)						
Test for subgroup differences: Chi² = 0.01, df = 1 (P = 0.90), I² = 0%						



Footnotes

(1) Mibellon, Olyset Plus, Moderate resistance, *An funestus*

(2) Mibellon, PermaNet 3.0, moderate resistance, *An funestus*

(3) Akron, Permanet 3.0, Moderate resistance

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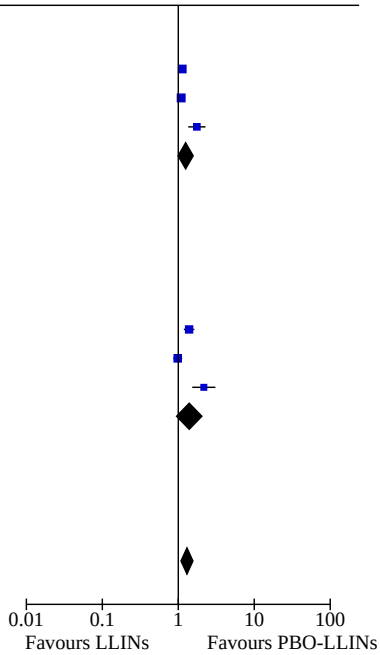
Analysis 2.8. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 8: Mosquito mortality (low resistance) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.8.1 Unwashed						
Corbel 2010 (1)	110	117	70	84	18.9%	1.13 [1.01 , 1.25]
Corbel 2010 (2)	170	176	177	200	19.8%	1.09 [1.03 , 1.16]
Oumbouke 2019	79	156	62	215	14.2%	1.76 [1.35 , 2.28]
Subtotal (95% CI)		**449**		**499**	**53.0%**	**1.25 [0.99 , 1.57]**
Total events:	359		309			
Heterogeneity: Tau² = 0.03; Chi² = 23.55, df = 2 (P < 0.00001); I² = 92%						
Test for overall effect: Z = 1.91 (P = 0.06)						
2.8.2 Washed						
Corbel 2010 (1)	82	105	112	199	17.5%	1.39 [1.18 , 1.63]
Corbel 2010 (2)	124	178	117	165	18.1%	0.98 [0.86 , 1.13]
Oumbouke 2019	60	158	38	217	11.4%	2.17 [1.53 , 3.08]
Subtotal (95% CI)		**441**		**581**	**47.0%**	**1.39 [0.95 , 2.04]**
Total events:	266		267			
Heterogeneity: Tau² = 0.10; Chi² = 23.97, df = 2 (P < 0.00001); I² = 92%						
Test for overall effect: Z = 1.70 (P = 0.09)						
Total (95% CI)		**890**		**1080**	**100.0%**	**1.30 [1.09 , 1.56]**
Total events:	625		576			
Heterogeneity: Tau² = 0.04; Chi² = 53.37, df = 5 (P < 0.00001); I² = 91%						
Test for overall effect: Z = 2.86 (P = 0.004)						
Test for subgroup differences: Chi² = 0.23, df = 1 (P = 0.63), I² = 0%						



Footnotes

(1) Pitoa, Permanet 3.0, Low resistance

(2) Malanville, Permanet 3.0, Low resistance

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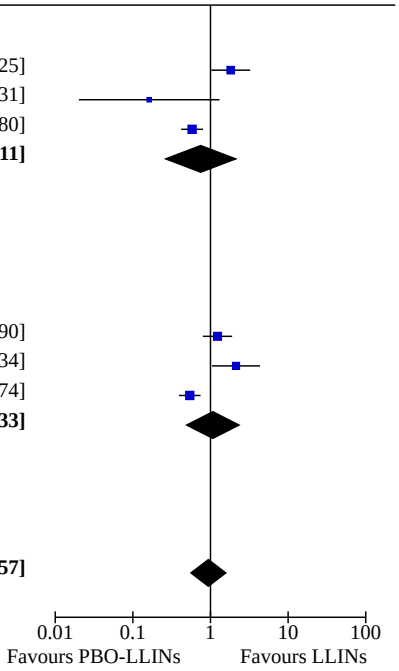
Analysis 2.9. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 9: Mosquito blood-feeding success (low resistance) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.9.1 Unwashed						
Corbel 2010 (1)	33	117	13	84	17.7%	1.82 [1.02 , 3.25]
Corbel 2010 (2)	1	176	7	200	4.8%	0.16 [0.02 , 1.31]
Oumbouke 2019	37	156	88	215	20.9%	0.58 [0.42 , 0.80]
Subtotal (95% CI)		**449**		**499**	**43.5%**	**0.75 [0.27 , 2.11]**
Total events:	71		108			
Heterogeneity: Tau² = 0.61; Chi² = 13.59, df = 2 (P = 0.001); I² = 85%						
Test for overall effect: Z = 0.55 (P = 0.58)						
2.9.2 Washed						
Corbel 2010 (1)	26	105	40	199	19.6%	1.23 [0.80 , 1.90]
Corbel 2010 (2)	23	178	10	165	15.9%	2.13 [1.05 , 4.34]
Oumbouke 2019	37	158	94	217	21.0%	0.54 [0.39 , 0.74]
Subtotal (95% CI)		**441**		**581**	**56.5%**	**1.07 [0.49 , 2.33]**
Total events:	86		144			
Heterogeneity: Tau² = 0.40; Chi² = 16.87, df = 2 (P = 0.0002); I² = 88%						
Test for overall effect: Z = 0.18 (P = 0.86)						
Total (95% CI)		**890**		**1080**	**100.0%**	**0.94 [0.56 , 1.57]**
Total events:	157		252			
Heterogeneity: Tau² = 0.30; Chi² = 30.72, df = 5 (P < 0.0001); I² = 84%						
Test for overall effect: Z = 0.24 (P = 0.81)						
Test for subgroup differences: Chi² = 0.30, df = 1 (P = 0.59), I² = 0%						



Footnotes

(1) Pitoa, Permanet 3.0, Low resistance

(2) Malanville, Permanet 3.0, Low resistance

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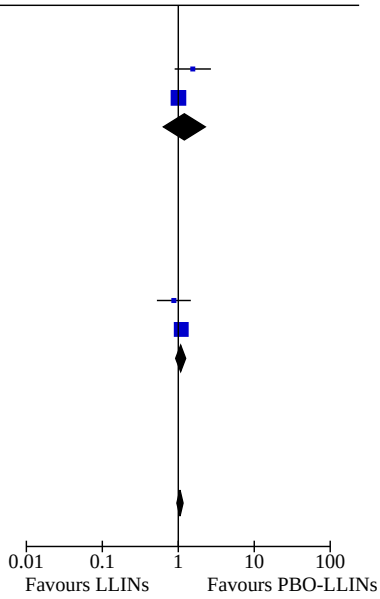
Analysis 2.10. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 10: Mosquito mortality (susceptible) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.10.1 Unwashed						
Moore 2016 (1)	23	161	22	239	2.7%	1.55 [0.90 , 2.69]
Tungu 2010 (2)	223	233	300	315	48.8%	1.00 [0.97 , 1.04]
Subtotal (95% CI)		**394**		**554**	**51.5%**	**1.20 [0.64 , 2.26]**
Total events:	246		322			
Heterogeneity: Tau² = 0.18; Chi² = 5.47, df = 1 (P = 0.02); I² = 82%						
Test for overall effect: Z = 0.57 (P = 0.57)						
2.10.2 Washed						
Moore 2016 (3)	23	186	28	198	3.0%	0.87 [0.52 , 1.46]
Tungu 2010 (2)	271	285	260	299	45.5%	1.09 [1.04 , 1.15]
Subtotal (95% CI)		**471**		**497**	**48.5%**	**1.07 [0.92 , 1.25]**
Total events:	294		288			
Heterogeneity: Tau² = 0.01; Chi² = 1.16, df = 1 (P = 0.28); I² = 14%						
Test for overall effect: Z = 0.93 (P = 0.35)						
Total (95% CI)		**865**		**1051**	**100.0%**	**1.05 [0.96 , 1.15]**
Total events:	540		610			
Heterogeneity: Tau² = 0.00; Chi² = 11.29, df = 3 (P = 0.01); I² = 73%						
Test for overall effect: Z = 1.08 (P = 0.28)						
Test for subgroup differences: Chi² = 0.11, df = 1 (P = 0.74), I² = 0%						



Footnotes

(1) Ifakara, Veeralin, Susceptible, *An arabiensis*. The population was resistant to deltamethrin and permethrin.

(2) Zeneti, Permanet 3.0, Susceptible

(3) Ifakara, Veeralin, Susceptible, *An. arabiensis*. The population was resistant to deltamethrin and permethrin.

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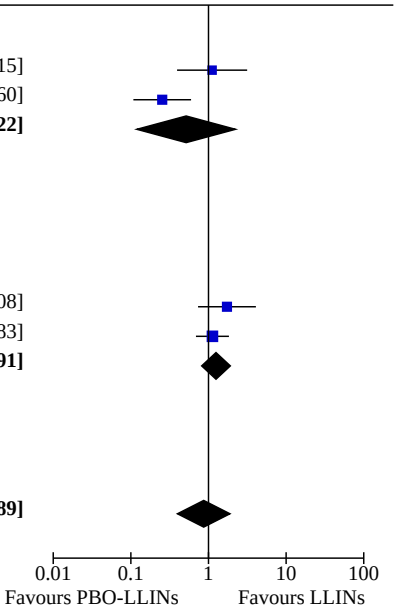
Analysis 2.11. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 11: Mosquito blood-feeding success (susceptible) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.11.1 Unwashed						
Moore 2016 (1)	6	161	8	239	21.3%	1.11 [0.39 , 3.15]
Tungu 2010 (2)	6	233	32	315	24.3%	0.25 [0.11 , 0.60]
Subtotal (95% CI)		**394**		**554**	**45.6%**	**0.52 [0.12 , 2.22]**
Total events:	12		40			
Heterogeneity: Tau² = 0.88; Chi² = 4.73, df = 1 (P = 0.03); I² = 79%						
Test for overall effect: Z = 0.89 (P = 0.37)						
2.11.2 Washed						
Moore 2016 (3)	13	186	8	198	24.2%	1.73 [0.73 , 4.08]
Tungu 2010 (2)	30	285	28	299	30.2%	1.12 [0.69 , 1.83]
Subtotal (95% CI)		**471**		**497**	**54.4%**	**1.25 [0.82 , 1.91]**
Total events:	43		36			
Heterogeneity: Tau² = 0.00; Chi² = 0.73, df = 1 (P = 0.39); I² = 0%						
Test for overall effect: Z = 1.03 (P = 0.30)						
Total (95% CI)		**865**		**1051**	**100.0%**	**0.87 [0.40 , 1.89]**
Total events:	55		76			
Heterogeneity: Tau² = 0.46; Chi² = 11.86, df = 3 (P = 0.008); I² = 75%						
Test for overall effect: Z = 0.36 (P = 0.72)						
Test for subgroup differences: Chi² = 1.30, df = 1 (P = 0.25), I² = 23.0%						



Footnotes

(1) Ifakara, Veeralin, Susceptible, *An arabiensis.* The population was resistant to deltamethrin and permethrin.

(2) Zeneti, Permanet 3.0, Susceptible

(3) Ifakara, Veeralin, Susceptible, *An. arabiensis.* The population was resistant to deltamethrin and permethrin.

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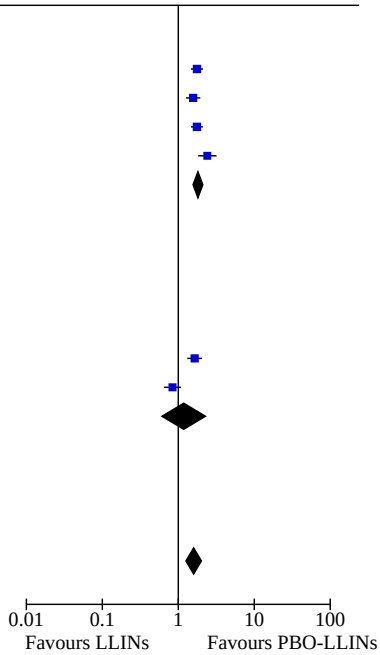
Analysis 2.12. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 12: Mosquito mortality (high resistance/Permanet) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.12.1 Not Washed						
Corbel 2010 (1)	181	232	73	165	17.6%	1.76 [1.47 , 2.12]
Koudou 2011 (2)	117	214	78	224	16.9%	1.57 [1.26 , 1.95]
Toé 2018 (3)	125	199	116	325	17.6%	1.76 [1.47 , 2.11]
Toé 2018 (4)	116	249	52	269	15.4%	2.41 [1.83 , 3.18]
Subtotal (95% CI)		**894**		**983**	**67.4%**	**1.81 [1.56 , 2.10]**
Total events:	539		319			
Heterogeneity: Tau² = 0.01; Chi² = 5.95, df = 3 (P = 0.11); I² = 50%						
Test for overall effect: Z = 7.88 (P < 0.00001)						
2.12.2 Washed						
Corbel 2010 (1)	119	241	79	263	16.7%	1.64 [1.31 , 2.06]
Koudou 2011 (1)	72	224	77	201	15.9%	0.84 [0.65 , 1.09]
Subtotal (95% CI)		**465**		**464**	**32.6%**	**1.18 [0.61 , 2.28]**
Total events:	191		156			
Heterogeneity: Tau² = 0.21; Chi² = 14.81, df = 1 (P = 0.0001); I² = 93%						
Test for overall effect: Z = 0.49 (P = 0.63)						
Total (95% CI)		**1359**		**1447**	**100.0%**	**1.59 [1.26 , 2.01]**
Total events:	730		475			
Heterogeneity: Tau² = 0.07; Chi² = 34.38, df = 5 (P < 0.00001); I² = 85%						
Test for overall effect: Z = 3.90 (P < 0.0001)						
Test for subgroup differences: Chi² = 1.57, df = 1 (P = 0.21), I² = 36.2%						



Footnotes

(1) Vallée du Kou, Permanet 3.0, High resistance
(2) Yaokoffikro, Permanet 3.0, High resistance
(3) Vallee du Kou 5, Permanet 3.0, High resistance
(4) Tengrela, Permanet 3.0, High resistance

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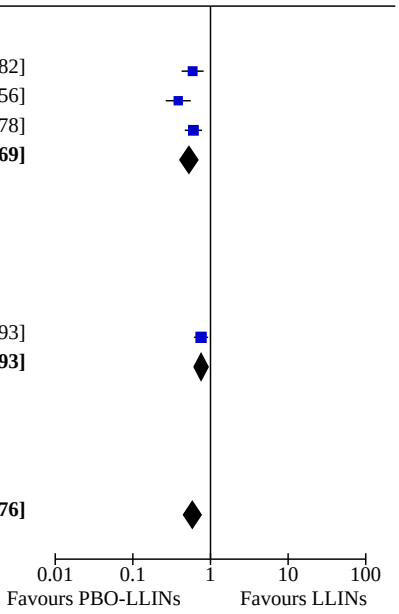
Analysis 2.13. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 13: Mosquito blood-feeding success (high resistance/Permanet) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.13.1 Unwashed						
Corbel 2010 (1)	48	232	58	165	23.1%	0.59 [0.42 , 0.82]
Toé 2018 (2)	28	199	119	325	20.8%	0.38 [0.26 , 0.56]
Toé 2018 (3)	63	249	113	269	26.8%	0.60 [0.47 , 0.78]
Subtotal (95% CI)		**680**		**759**	**70.7%**	**0.53 [0.40 , 0.69]**
Total events:	139		290			
Heterogeneity: Tau² = 0.03; Chi² = 4.29, df = 2 (P = 0.12); I² = 53%						
Test for overall effect: Z = 4.72 (P < 0.00001)						
2.13.2 Washed						
Corbel 2010 (1)	88	241	127	263	29.3%	0.76 [0.61 , 0.93]
Subtotal (95% CI)		**241**		**263**	**29.3%**	**0.76 [0.61 , 0.93]**
Total events:	88		127			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.63 (P = 0.009)						
Total (95% CI)		**921**		**1022**	**100.0%**	**0.58 [0.45 , 0.76]**
Total events:	227		417			
Heterogeneity: Tau² = 0.05; Chi² = 10.34, df = 3 (P = 0.02); I² = 71%						
Test for overall effect: Z = 4.04 (P < 0.0001)						
Test for subgroup differences: Chi² = 4.40, df = 1 (P = 0.04), I² = 77.2%						



Footnotes

(1) Vallée du Kou, Permanet 3.0, High resistance
(2) Vallee du Kou 5, Permanet 3.0, High resistance
(3) Tengrela, Permanet 3.0, High resistance

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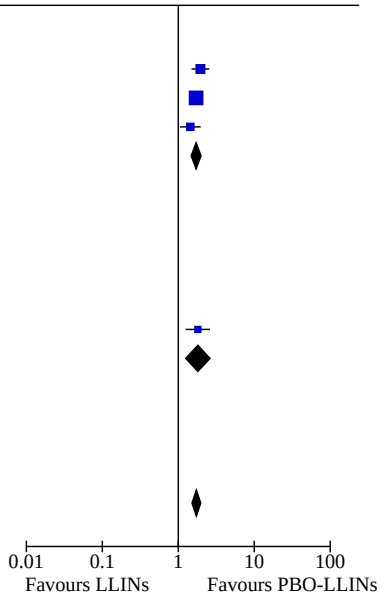
Analysis 2.14. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 14: Mosquito mortality (high resistance/Olyset) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.14.1 Unwashed						
Pennetier 2013 (1)	53	66	39	95	24.4%	1.96 [1.49 , 2.56]
Toé 2018 (2)	146	272	97	310	44.8%	1.72 [1.41 , 2.09]
Toé 2018 (3)	62	221	57	293	17.8%	1.44 [1.05 , 1.97]
Subtotal (95% CI)		**559**		**698**	**87.0%**	**1.72 [1.48 , 1.99]**
Total events:	261		193			
Heterogeneity: Tau² = 0.00; Chi² = 2.17, df = 2 (P = 0.34); I² = 8%						
Test for overall effect: Z = 7.08 (P < 0.00001)						
2.14.2 Washed						
Pennetier 2013	64	96	21	57	13.0%	1.81 [1.25 , 2.61]
Subtotal (95% CI)		**96**		**57**	**13.0%**	**1.81 [1.25 , 2.61]**
Total events:	64		21			
Heterogeneity: Not applicable						
Test for overall effect: Z = 3.16 (P = 0.002)						
Total (95% CI)		**655**		**755**	**100.0%**	**1.73 [1.51 , 1.97]**
Total events:	325		214			
Heterogeneity: Tau² = 0.00; Chi² = 2.22, df = 3 (P = 0.53); I² = 0%						
Test for overall effect: Z = 8.08 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.07, df = 1 (P = 0.79), I² = 0%						



Footnotes

(1) Malanaville, Olyset Plus, High resistance
(2) Vallee du Kou 5, Olyset Plus, High resistance
(3) Tengrela, Olyset Plus, High resistance

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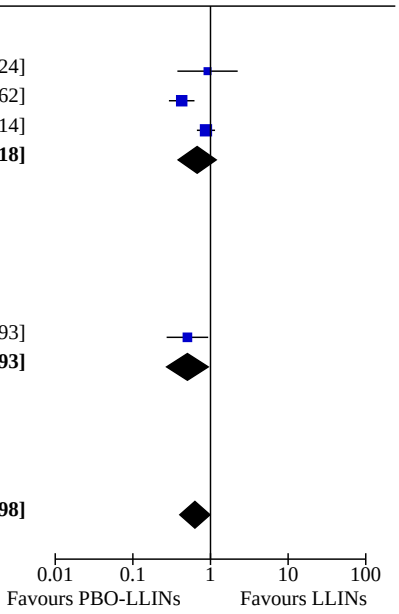
Analysis 2.15. Comparison 2: Commercial pyrethroid-PBO nets versus commercial LLINs: hut trials, Outcome 15: Mosquito blood-feeding success (high resistance/Olyset) hut/night (adjusted ICC 0.1)

Study or Subgroup	PBO-LLINs Events	PBO-LLINs Total	LLINs Events	LLINs Total	Weight	Risk Ratio M-H, Random, 95% CI
2.15.1 Unwashed						
Pennetier 2013 (1)	7	66	11	95	15.0%	0.92 [0.37 , 2.24]
Toé 2018 (2)	31	272	83	310	29.7%	0.43 [0.29 , 0.62]
Toé 2018 (3)	62	221	94	293	33.2%	0.87 [0.67 , 1.14]
Subtotal (95% CI)		**559**		**698**	**78.0%**	**0.67 [0.38 , 1.18]**
Total events:	100		188			
Heterogeneity: Tau² = 0.18; Chi² = 9.75, df = 2 (P = 0.008); I² = 79%						
Test for overall effect: Z = 1.38 (P = 0.17)						
2.15.2 Washed						
Pennetier 2013 (1)	12	96	29	117	22.0%	0.50 [0.27 , 0.93]
Subtotal (95% CI)		**96**		**117**	**22.0%**	**0.50 [0.27 , 0.93]**
Total events:	12		29			
Heterogeneity: Not applicable						
Test for overall effect: Z = 2.18 (P = 0.03)						
Total (95% CI)		**655**		**815**	**100.0%**	**0.63 [0.40 , 0.98]**
Total events:	112		217			
Heterogeneity: Tau² = 0.14; Chi² = 10.72, df = 3 (P = 0.01); I² = 72%						
Test for overall effect: Z = 2.03 (P = 0.04)						
Test for subgroup differences: Chi² = 0.46, df = 1 (P = 0.50), I² = 0%						



Footnotes
(1) Malanville, Olyset Plus, High resistance
(2) Vallee du Kou 5, Olyset Plus, High resistance
(3) Tengrela, Olyset Plus, High resistance

ADDITIONAL TABLES

Table 1. World Health Organization Pesticide Evaluation Scheme (WHOPES) classification

WHOPES Phase	Definition
WHOPES Phase I. Laboratory bioassays	Cone bioassays: these studies are conducted in the laboratory setting and use standard WHO protocols (WHO 2013, Section 2.2.1), when 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 are maintained on a sugar solution diet while entomological outcomes (mosquitoes knocked down 1 hour post exposure, and mosquito mortality 24 hours post exposure) are measured. Tunnel tests: these studies are conducted in the laboratory setting and use standard WHO protocols (WHO 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 9 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 they are 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.

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Table 1. World Health Organization Pesticide Evaluation Scheme (WHOPES) classification *(Continued)*

	Wire-ball bioassays: these studies are conducted in the laboratory setting, where mosquitoes are introduced into a wire-ball frame that has been covered with the intervention or control LLIN. Mosquitoes are exposed for 3 minutes, after which they are transferred to a holding container, and entomological outcomes (mosquitoes knocked down 1 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 trials 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 1 person or animal per hut. Huts are designed to resemble local housing based on a West or East African design (WHO 2013; Section 3.3.1-2). However these trials 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 are 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 live mosquitoes are collected each morning from inside the net, inside the hut, and inside the exit traps. They are then scored as blood-fed or non-blood-fed, and as 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 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 data available on insecticide resistance status of local malaria vectors. Households are assigned as conventional LLINs or PBO-LLINs. Randomization can be done at the household or village level. Adult mosquitoes are collected from study houses, and mosquito density is measured. An indication of malaria transmission is measured at the study sites by recording infections in mosquitoes, parasite prevalence, or malaria incidence.

LLIN: long-lasting insecticidal nets; PBO: piperonyl butoxide; WHOPES: World Health Organization Pesticide Evaluation Scheme.

Table 2. World Health Organization (WHO)-recommended long-lasting insecticidal nets (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
Interceptor G2	Alpha-cypermethrin and chlorfenapyr incorporated into polyester	Interim
LifeNet	Deltamethrin incorporated into polypropylene	Interim
MAGNet	Alpha-cypermethrin incorporated into polyethylene	Full
MiraNet	Alpha-cypermethrin incorporated into polyethylene	Interim
Olyset Net	Permethrin incorporated into polyethylene	Full
Olyset Plus	Permethrin (20 g/kg) and PBO (10 g/kg) incorporated into polyethylene	Interim
Panda Net 2.0	Deltamethrin incorporated into polyethylene	Interim

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Table 2. World Health Organization (WHO)-recommended long-lasting insecticidal nets (LLINs) *(Continued)*

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

LLIN: long-lasting insecticidal net; PBO: piperonyl butoxide; WHO: World Health Organization.

Table 3. World Health Organization (WHO)-recommended insecticide products for treatment of mosquito nets for malaria vector control

Insecticide	Formulation	Dosage[a]
Alpha-cypermethrin	SC 10%	20 to 40
Cyfluthrin	EW 5%	50
Deltamethrin	SC 1% WT 25% WT 25% + binder[b]	15 to 25
Etofenprox	EW 10%	200
Lambda-cyhalothrin	CS 2.5%	10 to 15
Permethrin	EC 10%	200 to 500

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

Table 4. Definition of resistance level

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

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

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Table 5. Stratification of resistance level

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

[a]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?	↓	—	↓	
Intervention				
Did the study include a long-lasting insecticidal net (LLIN) or insecticide-treated net (ITN)?	↓	—	↓	State net LLIN or ITN
Was the intervention net either of the following? 1. A piperonyl butoxide (PBO) LLIN that received a minimum of interim World Health Organization (WHO) approval.	↓	—	↓	State net type
Was the control net either of the following? 1. A pyrethroid LLIN of the same fabric impregnated with the same insecticide and dose as the intervention net. 2. A pyrethroid LLIN impregnated with the same insecticide at any dose.	↓	—	↓	State which objective study meets
Study design				
Was the study one of the following? 1. Experimental hut study 2. Village trial	↓	—	↓	State study type
For experimental hut study and village trial. Was the study conducted in Africa?	↓	—	↓	State country
Outcome				
Did the study include at least 1 of the following outcome measures? 1. Mortality	↓	—	↓	

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

2. Blood feeding
3. Sporozoite rate
4. Not passed through the net
5. Deterrence
6. Exophily
7. Mosquito density
8. Parity rate

Decision				
Is the study eligible for inclusion?	—	—	↓	State reason(s) for exclusion
	Discuss with authors			

ITN: insecticide-treated net; LLIN: long-lasting insecticidal net; PBO: piperonyl butoxide; WHO: World Health Organization.

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Table 7. Experimental hut trials: deterrence data

Study ID	Locality	Net type	Net washed	Total number in ITN hut	Total number in UTN hut	Deterrence (%) reported	Deterrence (%) calculated
Bayili 2017	Vallée du Kou	DawaPlus 2.0	No	1548	1848	16.23	16.23
Bayili 2017	Vallée du Kou	DawaPlus 2.0	Yes	2155	1848	0	-16.61
Bayili 2017	Vallée du Kou	DawaPlus 3.0	No	1365	1848	26.13	26.14
Bayili 2017	Vallée du Kou	DawaPlus 3.0	Yes	1981	1848	0	-7.20
Bayili 2017	Vallée du Kou	DawaPlus 4.0	No	846	1848	54.22	54.22
Bayili 2017	Vallée du Kou	DawaPlus 4.0	Yes	1646	1848	10.93	10.93
Corbel 2010	Malanville	Permanet 2.0	Yes	195	285	31.58	31.58
Corbel 2010	Malanville	Permanet 3.0	Yes	210	285	26.32	26.32
Corbel 2010	Malanville	Permanet 2.0	No	243	285	14.74	14.74
Corbel 2010	Malanville	Permanet 3.0	No	214	285	24.91	24.91
Corbel 2010	Pitoa	Permanet 2.0	Yes	310	401	22.69	22.69
Corbel 2010	Pitoa	Permanet 3.0	Yes	163	401	59.35	59.35
Corbel 2010	Pitoa	Permanet 2.0	No	105	401	73.82	73.82
Corbel 2010	Pitoa	Permanet 3.0	No	146	401	63.59	63.59
Corbel 2010	Vallée du Kou	Permanet 2.0	Yes	788	908	13.22	13.22
Corbel 2010	Vallée du Kou	Permanet 3.0	Yes	724	908	20.26	20.26
Corbel 2010	Vallée du Kou	Permanet 2.0	No	329	908	63.77	63.77
Corbel 2010	Vallée du Kou	Permanet 3.0	No	463	908	49.01	49.01
Koudou 2011	Yaokoffikro	Permanet 3.0	No	303	796	62.1	61.93

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Table 7. Experimental hut trials: deterrence data *(Continued)*

Koudou 2011	Yaokoffikro	Permanet 2.0	No	317	796	60.4	60.18
Koudou 2011	Yaokoffikro	Permanet 3.0	Yes	313	796	60.1	60.68
Koudou 2011	Yaokoffikro	Permanet 2.0	Yes	281	796	64.4	64.70
Menze 2020	Mibellon	PermaNet 2.0	No	237	390	39.2	39.2
Menze 2020	Mibellon	PermaNet 3.0	No	153	390	60.8	60.8
Menze 2020	Mibellon	Olyset Net	No	176	390	54.9	54.9
Menze 2020	Mibellon	Olyset Plus	No	199	390	49	49
Moore 2016	Ifakara	Veeralin LN	No	722	810	11	10.86
Moore 2016	Ifakara	Veeralin LN	Yes	727	810	10	10.25
Moore 2016	Ifakara	MAGNet LN	No	1070	810	0	-32.10
Moore 2016	Ifakara	MAGNet LN	Yes	773	810	5	4.57
Moore 2016	Ifakara	Veeralin LN	No	89	170	48	47.65
Moore 2016	Ifakara	Veeralin LN	Yes	85	170	50	50.00
Moore 2016	Ifakara	MAGNet LN	No	114	170	33	32.94
Moore 2016	Ifakara	MAGNet LN	Yes	103	170	39	39.41
N'Guessan 2010	Akron	Permanet 3.0	No	128	185	31	30.81
N'Guessan 2010	Akron	Permanet 3.0	Yes	155	185	NR	16.22
N'Guessan 2010	Akron	Permanet 2.0	No	114	185	38	38.38
N'Guessan 2010	Akron	Permanet 2.0	Yes	174	185	NR	5.95
Pennetier 2013	Malanville	Olyset Plus	No	67	69	NR	2.90
Pennetier 2013	Malanville	Olyset Plus	Yes	101	69	NR	-46.38

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Table 7. Experimental hut trials: deterrence data *(Continued)*

Pennetier 2013	Malanville	Olyset Net	No	96	69	NR	-39.13
Pennetier 2013	Malanville	Olyset Net	Yes	124	69	NR	-79.71
Toé 2018	Tengrela	Olyset Net	No	923	480	-92.29	-92.29
Toé 2018	Tengrela	Olyset Plus	No	695	480	-44.79	-44.79
Toé 2018	Tengrela	Permanet 2.0	No	858	480	-78.75	-78.75
Toé 2018	Tengrela	Permanet 3.0	No	794	480	-65.42	-65.42
Toé 2018	VK5	Olyset Net	No	1458	1095	-33.15	-33.15
Toé 2018	VK5	Olyset Plus	No	1278	1095	-16.71	-16.71
Toé 2018	VK5	Permanet 2.0	No	1075	1095	1.83	1.83
Toé 2018	VK5	Permanet 3.0	No	657	1095	40	40.00
Tungu 2010	Zeneti	PermaNet 3.0	No	425	723	41	41.22
Tungu 2010	Zeneti	PermaNet 2.0	No	574	723	21	20.61
Tungu 2010	Zeneti	PermaNet 3.0	Yes	558	723	23	22.82
Tungu 2010	Zeneti	PermaNet 2.0	Yes	586	723	19	18.95

ITN: insecticide-treated net; LLIN: long-lasting insecticidal net; NR: not reported; PBO: piperonyl butoxide; UTN: untreated net; WHO: World Health Organization.

Table 8. Village trials: mosquito density data

Study ID	Net type	Species	Density measurement	Collection method	Baseline density	Post-intervention density	Reduction (%)
Awolola 2014	Untreated	*An gambiae* s.l.	Mean number caught per house	WT, IRC	16.2	17.1	-5.56
Awolola 2014	PermaNet 2.0	*An gambiae* s.l.	Mean number caught per house	WT, IRC	21.3	7.2	66.20
Awolola 2014	PermaNet 3.0	*An gambiae* s.l.	Mean number caught per house	WT, IRC	20.1	1.4	93.03

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Table 8. Village trials: mosquito density data *(Continued)*

Cisse 2017	PermaNet 2.0	*An gambiae* s.l.	Resting density per room per day	IRC	-	1.92	-
Cisse 2017	PermaNet 3.0	*An gambiae* s.l.	Resting density per room per day	IRC	-	3.05	-
Cisse 2017	Olyset	*An gambiae* s.l.	Resting density per room per day	IRC	-	3.21	-
Cisse 2017	Olyset Plus	*An gambiae* s.l.	Resting density per room per day	IRC	-	3.7	-
Mzilahowa 2014	Olyset	*An gambiae*	Mean number caught per catch	PSC	-	0.10	-
Mzilahowa 2014	Olset Plus	*An gambiae*	Mean number caught per catch	PSC	-	0.12	-
Mzilahowa 2014	PermaNet 2.0	*An gambiae*	Mean number caught per catch	PSC	-	0.13	-
Mzilahowa 2014	PermaNet 3.0	*An gambiae*	Mean number caught per catch	PSC	-	0.09	-
Mzilahowa 2014	Olyset	*An funestus*	Mean number caught per catch	PSC	-	0.08	-
Mzilahowa 2014	Olyset Plus	*An funestus*	Mean number caught per catch	PSC	-	0.16	-
Mzilahowa 2014	PermaNet 2.0	*An funestus*	Mean number caught per catch	PSC	-	0.27	-
Mzilahowa 2014	PermaNet 3.0	*An funestus*	Mean number caught per catch	PSC	-	0.13	-
Mzilahowa 2014	Olyset	*An gambiae*	Mean number caught per catch	LT	-	1.23	-
Mzilahowa 2014	Olset Plus	*An gambiae*	Mean number caught per catch	LT	-	0.27	-
Mzilahowa 2014	PermaNet 2.0	*An gambiae*	Mean number caught per catch	LT	-	0.96	-
Mzilahowa 2014	PermaNet 3.0	*An gambiae*	Mean number caught per catch	LT	-	1.44	-
Mzilahowa 2014	Olyset	*An funestus*	Mean number caught per catch	LT	-	2.02	-
Mzilahowa 2014	Olset Plus	*An funestus*	Mean number caught per catch	LT	-	2.1	-
Mzilahowa 2014	PermaNet 2.0	*An funestus*	Mean number caught per catch	LT	-	5.76	-
Mzilahowa 2014	PermaNet 3.0	*An funestus*	Mean number caught per catch	LT	-	3.76	-
Protopopoff 2018	Olyset (2015)	*Anopheles* species	Mean number caught per house per night	LT	-	2.61	-

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Table 8. Village trials: mosquito density data *(Continued)*

Protopopoff 2018	Olyset Plus (2015)	*Anopheles* species	Mean number caught per house per night	LT	-	1.85	-
Protopopoff 2018	Olyset (2016)	*Anopheles* species	Mean number caught per house per night	LT	-	3.60	-
Protopopoff 2018	Olyset Plus (2016)	*Anopheles* species	Mean number caught per house per night	LT	-	2.68	-
Staedke 2020	Permanet 2.0 (6 months)	*An gambiae* s.l.	Mean density per house	IRC	0.3	0.67	
Staedke 2020	Permanet 3.0 (6 months)	*An gambiae* s.l.	Mean density per house	IRC	0.8	0.17	78.75
Staedke 2020	Olyset (6 months)	*An gambiae* s.l.	Mean density per house	IRC	0.3	0.81	
Staedke 2020	Olyset Plus (6 months)	*An gambiae* s.l.	Mean density per house	IRC	0.1	0.16	
Staedke 2020	Permanet 2.0 (12 months)	*An gambiae* s.l.	Mean density per house	IRC	0.3	1.35	
Staedke 2020	Permanet 3.0 (12 months)	*An gambiae* s.l.	Mean density per house	IRC	0.8	0.52	35
Staedke 2020	Olyset (12 months)	*An gambiae* s.l.	Mean density per house	IRC	0.3	1.1	
Staedke 2020	Olyset Plus (12 months)	*An gambiae* s.l.	Mean density per house	IRC	0.1	0.23	
Staedke 2020	Permanet 2.0 (18 months)	*An gambiae* s.l.	Mean density per house	IRC	0.3	1.65	
Staedke 2020	Permanet 3.0 (18 months)	*An gambiae* s.l.	Mean density per house	IRC	0.8	1.57	
Staedke 2020	Olyset (18 months)	*An gambiae* s.l.	Mean density per house	IRC	0.3	0.66	
Staedke 2020	Olyset Plus (18 months)	*An gambiae* s.l.	Mean density per house	IRC	0.1	0.19	

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Table 8. Village trials: mosquito density data *(Continued)*

Stiles-Ocran 2013	No intervention	*An gambiae* s.s.	Mean number caught per village	IRC	230	79	65.65
Stiles-Ocran 2013	Permanet 2.0	*An gambiae* s.s.	Mean number caught per village	IRC	39	36	7.69
Stiles-Ocran 2013	Permanet 2.0	*An gambiae* s.s.	Mean number caught per village	IRC	82	45	45.12
Stiles-Ocran 2013	Permanet 3.0	*An gambiae* s.s.	Mean number caught per village	IRC	77	12	84.42
Stiles-Ocran 2013	Permanet 3.0	*An gambiae* s.s.	Mean number caught per village	IRC	178	15	91.57
Stiles-Ocran 2013	No intervention	*An gambiae* s.s.	Mean number caught per person per night per village	Indoor & outdoor HLC	415	72	82.65
Stiles-Ocran 2013	Permanet 2.0	*An gambiae* s.s.	Mean number caught per person per night per village	Indoor & outdoor HLC	33	31	6.06
Stiles-Ocran 2013	Permanet 2.0	*An gambiae* s.s.	Mean number caught per person per night per village	Indoor & outdoor HLC	79	64	18.99
Stiles-Ocran 2013	Permanet 3.0	*An gambiae* s.s.	Mean number caught per person per night per village	Indoor & outdoor HLC	98	19	80.61
Stiles-Ocran 2013	Permanet 3.0	*An gambiae* s.s.	Mean number caught per person per night per village	Indoor & outdoor HLC	156	36	76.92

An funestus: *Anopheles funestus*; *An gambiae*: *Anopheles gambiae*; HLC: human landing catch; IRC: indoor resting catch; LT: light trap; PSC: pyrethrum spray catch; WT: window trap.

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APPENDICES

Appendix 1. Detailed search strategies

The Cochrane Library

Description:

#1 piperonyl butoxide

#2 MeSH descriptor: [Piperonyl Butoxide] explode all trees

#3 #1 or #2

#4 Net* or bednet* or hammock* or curtain* or ITN* or LLIN* or "Insecticide-Treated Bednet*" or "Insecticide-Treated net*"

#5 Olyset* or PermaNet* or Veeralin

#6 DawaPlus* or Tsara* or Duranet*

#7 MeSH descriptor: [Insecticide-Treated Bednets] explode all trees

#8 #4 or #5 or #6 or #7

#9 #3 and #8

MEDLINE (PubMed)

	Query
#1	Search "Piperonyl Butoxide"[Mesh]
#2	Search piperonyl butoxide or PBO Field: Title/Abstract
#3	Search ("Piperonyl Butoxide"[MESH]) OR #2
#4	Search Net* OR bednet* OR curtain* OR ITN* OR LLIN* or "Insecticide-Treated Bednet*" or "Insecticide-Treated net*" Field: Title/Abstract
#5	Search "Olyset* or Permanet* or Veeralin Field: Title/Abstract
#6	Search DawaPlus* or Tsara* or Duranet* Field: Title/Abstract
#7	Search "Insecticide-Treated Bednets" [MESH]
#8	Search (((#4) OR #) OR #6) OR #7
#9	Search (#8) AND (#3)

Embase (OVID)

1 piperonyl butoxide/

2 piperonyl butoxide.tw.

3 1 or 2

4 PBO.tw.

5 3 or 4

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6 (Net* or bednet* or hammock* or curtain* or ITN* or LLIN* or "Insecticide-Treated Bednet*" or "Insecticide-Treated net*").mp.

7 (Olyset* or Permanet* or Veeralin).mp.

8 (DawaPlus* or Tsara* or Duranet*).mp.

9 insecticide treated net/

10 6 or 7 or 98 or 9

11 5 and 10

Web of Science™ Core Collection

Set	
# 5	#3 AND #4 Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years
# 4	#1 OR #2 Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years
# 3	**TOPIC:** (Net* OR bednet* OR ITN* OR LLIN* or “Insecticide-Treated Bednet*” or “Insecticide-Treated net*”) *OR* **TOPIC:** (Olyset* or PermaNet* or Veeralin) *OR* **TOPIC:** (DawaPlus*or Tsara* or Duranet*) Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years
# 2	**TOPIC:** (PBO) *NOT* **TOPIC:** (placebo) Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years
# 1	**TOPIC:** ("Piperonyl Butoxide") Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH Timespan=All years

CABI: CAB Abstracts®

Set	
# 3	#2 AND #1 Indexes=CAB Abstracts Timespan=All years
# 2	**TOPIC:** (Net* OR bednet* OR hammock* OR curtain* OR ITN* OR LLIN* or “Insecticide-Treated Bednet*” or “Insecticide-Treated net*”) *OR* **TOPIC:** (Olyset* or PermaNet* or Veeralin) Indexes=CAB Abstracts Timespan=All years
# 1	**TOPIC:** (PBO or "Piperonyl Butoxide") Indexes=CAB Abstracts Timespan=All years

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ClinicalTrials.gov and WHO ICTRP

piperonyl butoxide and malaria

Appendix 2. Study characteristics extraction form

Table 2.1 Trial characteristics of the included experimental hut trials

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

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

Table 2.2 Trial characteristics of the included village trials

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Trial **ID**	Trial-**name**	Trial-**location**	**Mosquito species (strain/origin)**	**Resistance level**	**Resistance status**	Trial-**start/end date**	**Intervention**	**Net washed**	**Net holed**	**Measured outcome**						
										M	**BF**	**SR**	**MD**	**PR**	**PP**	**CMC**

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

Appendix 3. Data extraction form

Table 3.1 Data extracted from experimental hut trials

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Trial ID	Trial name	Net type	Net washed	Net holed	Mosquito species	Resistance level	Resistance status	Total mosquitoes	Dead	Mosquito mortality (%)	BF	BF (%)	BFI	Number of mosquitoes deterred	Deterrence (%)	Exit trap	Exophily (%)	Total number of people (N)

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

Table 3.2 Data extracted from village trials

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Trial ID	Trial name	Net type	Net washed	Net holed	Mosquito species	Resistance level	Resistance status	Total mosquitoes	Dead	Mosquito mortality (%)	BF	BF (%)	BFI	Sporozoite (%)	Mosquito density (%)	Parity (%)	Total number of people (N)	PP (%)	CMC (%)

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

Appendix 4. 'Risk of bias' assessment form

Table 4.2 'Risk of bias' assessment for experimental hut trials

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Trial **ID**	Trial-**name**	**Comparability of mosquitoes in LLIN and LLIN + PBO 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**

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

Table 4.3 'Risk of bias' assessment for village trials

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Trial **ID**	Trial **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**

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

Appendix 5. 'Risk of bias' assessment: experimental hut trials

'Risk of bias' component	Low	Unclear	High
Mosquito group comparability	Huts accessible to the same mosquito population	No or unclear information reported	Huts not accessible to the same mosquito population
Collectors blinded	Outcomes assessed blinded	No or unclear information reported If outcomes assessed were not blinded, but this is unlikely to influence the results, we will judge this to be low risk	Outcomes assessed not blinded, and this is likely to influence the results If outcomes assessed were not blinded, but this is unlikely to influence the results, we will judge this to be low risk
Sleepers blinded	Outcomes assessed blinded	No or unclear information reported If outcomes assessed were not blinded, but this is unlikely to influence the results, we will judge this to be low risk	Outcomes assessed not blinded, and this is likely to influence the results If outcomes assessed were not blinded, but this is unlikely to influence the results, we will judge this to be low risk
Sleeper bias	Sleepers were rotated between huts according to a Latin square design	No or unclear information reported	Sleepers not rotated between huts
Treatment allocation	Treatments randomized Treatments not randomized; however equal attractiveness demonstrated	No or unclear information reported	Treatments not randomized, and equal attractiveness not demonstrated
Treatment rotation	Treatments rotated through huts according to a Latin square design	No or unclear information reported	Treatments not rotated
Standardized hut design	Huts of West or East African design	No or unclear information reported	Huts of non-standardized design
Cleaning	Huts cleaned between treatments	No or unclear information reported	Huts not cleaned between treatments
Incomplete outcome data addressed	No or low missing data; reason for missing data is unlikely to be related to the true outcome	No or unclear information reported	High missing data; reason for missing data is likely to be related to the true outcome
Raw data reported	Raw data reported	No or unclear information reported	Raw data not reported
Conflicting interests	No conflict of interest stated	No or unclear information reported	Conflict of interest stated

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Appendix 6. 'Risk of bias' assessment: village trials

'Risk of bias' component	Low	Unclear	High
Recruitment bias	No participants recruited after clusters randomized	No or unclear information reported Recruitment bias not applicable to trial design, as it is related to human participants	Paricipants recruited to trial after clusters randomized
Mosquito group comparability	Mosquito populations comparable	No or unclear information reported	Mosquito populations comparable
Collectors blinded	Outcomes assessed blinded	No or unclear information reported Outcomes assessed not blinded, but this is unlikely to influence the results	Outcomes assessed not blinded, and this is likely to influence the results
Household blinded	Outcomes assessed blinded	No or unclear information reported If outcomes assessed were not blinded, but this is unlikely to influence the results, we will judge this to be low risk	Outcomes assessed not blinded, and this is likely to influence the results If outcomes assessed were not blinded, but this is unlikely to influence the results, we will judge this to be low risk
Treatment allocation	Treatments randomized	No or unclear information reported	Treatments not randomized
Allocation concealment	Allocation concealment procedures were adhered to	No or unclear information reported Allocation concealment procedures were not adhered to; however this is unlikely to affect the results	Allocation procedures were not adhered to, and this is likely to have affected the results
Incomplete outcome data addressed	No or low missing data; reason for missing data is unlikely to be related to the true outcome	No or unclear information reported	High missing data; reason for missing data is likely to be related to the true outcome
Raw data reported	Raw data reported	No or unclear information reported	Raw data not reported
Clusters lost to follow-up	No complete clusters lost from trial	No or unclear information as to whether clusters were lost from trial	At least 1 cluster lost from trial
Selective reporting	No selective reporting; all measured outcomes reported in results	No or unclear information on whether all measured outcomes were reported in results	Selective reporting; not all measured outcomes were reported in results
Correct statistical methods; adjusted for clustering	Clustering was taken into account and statistical methods adjusted for clustering	No or unclear information as to whether clustering was taken into account for statistical methods	Trial did not take clustering into account for statistical methods
Conflicting interests	No conflict of interest stated	No or unclear information reported	Conflict of interest stated

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Appendix 7. Prespecified changes for review update 2021

Protocol section	Protocol changes
Background and research question	We will update any references and background information
Inclusion criteria	We propose to remove objective 1 (evaluate whether adding PBO to pyrethroid LLINs increases the epidemiological and entomological effectiveness of the nets' and focus instead on comparing pyrethroid-PBO nets with their non-PBO equivalent (objective 2). As a result, laboratory studies will be excluded. We make this decision as we only identified two studies meeting the inclusion criteria for objective 1 in Gleave 2018, both of which were laboratory assays; results from these cannot readily be translated into public health outcomes.
Methods	We will subgroup our analysis on epidemiological data by follow-up time. We will update the search strategy terms as one brand of bednet has changed name, and we will perform a new search to identify all possible trials.

This table was approved by the CIDG editorial team on 26 Oct 2020.

WHAT'S NEW

Date	Event	Description
30 June 2021	Amended	The author team corrected minor spelling problems in the abstract and summary of findings tables. They also corrected raw participant numbers in the summary of findings tables for moderate- and low-resistance settings. These edits do not alter the review findings or outcomes.

HISTORY

Protocol first published: Issue 8, 2017
Review first published: Issue 11, 2018

Date	Event	Description
21 June 2021	Amended	The author team made minor edits to Summary of findings 1. Under 'Patient or population' they added "adults and children living in malaria-endemic areas". The corrections to parasite prevalence numbers reported do not impact the odds ratios reported, or review findings or interpretation.
24 May 2021	New citation required and conclusions have changed	This is an update of the first Cochrane Review of pyrethroid-PBO nets (Gleave 2018). The date of search is 25 September 2020.
24 May 2021	New search has been performed	The prespecified changes to the protocol (before the review update commenced) are given in Appendix 7. We excluded studies using only laboratory assays from this review update due to the challenges in extrapolating public health value from laboratory

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Date	Event	Description
		bioassays alone. We amended the search strategy including different search terms due to a bed net brand name change. A new search was undertaken to capture all relevant trials for this update.
6 June 2019	Amended	Abstract amended. Authors' conclusions section: changed from "reduce mosquito mortality and blood feeding rates" to "increase mosquito mortality and reduce blood feeding rates"

CONTRIBUTIONS OF AUTHORS

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

KG, NL, and LC conducted trial screening, data extraction, and analysis.

MC and LC provided statistical support.

KG, NL, LC, and HR wrote the final manuscripts, and all review authors approved the final manuscript.

HR is the guarantor of the review.

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 its External Scientific Advisory Committee (ESAC) meetings.
MC has no known conflicts of interest.
LC 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 bed net manufacturers through her own research and her previous role on 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

- Foreign, Commonwealth and Development Office (FCDO), UK

  Project number 300342-104
- World Health Organization (WHO), Switzerland

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

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Previously, PBO-nets were classified as PBO-LLINs; however as the durability of PBO on nets has not been classified as long-lasting, these were subsequently referred to as pyrethroid-PBO nets. As a result of this, our review title changed from ‘Piperonyl butoxide (PBO) combined with pyrethroids in long-lasting insecticidal nets (LLINs) to prevent malaria in Africa' to ‘Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa'.

We added Leslie Choi as a review author.

Additional criteria for assessing the risk of bias of village trials were added. These are in line with the Cochrane ‘Risk of bias’ tool (Higgins 2017), as well as the five additional criteria listed in Section 16.3.2 of the *Cochrane Handbook for Systematic Reviews of Interventions* that relate specifically to cluster-randomized trials (Higgins 2011).

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The published protocol stated all stratified analysis factors under subgroup analysis (Gleave 2017). We have corrected this to state that subgroup analysis was performed only on whether nets were unwashed or washed.

Differences between review (2018) and review update (2021)

The prespecified changes to the protocol (before the review update commenced) are given in Appendix 7. In brief, the published review included laboratory bioassay studies (n = 2) (Gleave 2018). We excluded studies using only laboratory assays from this review update due to the challenges in extrapolating public health value from laboratory bioassays alone. We amended the search strategy including different search terms due to a bed net brand name change. A new search was undertaken to capture all relevant trials for this update.

INDEX TERMS

Medical Subject Headings (MeSH)

Africa [epidemiology]; Culicidae; Drug Combinations; Feeding Behavior; Insecticide Resistance [*drug effects]; *Insecticide-Treated Bednets; Malaria [epidemiology] [*prevention & control]; Mortality; Mosquito Control [*methods]; *Pesticide Synergists; *Piperonyl Butoxide; *Pyrethrins; Randomized Controlled Trials as Topic

MeSH check words

Animals; Humans

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