Supplementary Information

Consistent non-inferiority of second-in-class pyrethroid–PBO nets across diverse experimental hut and ambient chamber assays in Côte d'Ivoire and Tanzania

Supplementary File 1: Extended Methods

Insecticide susceptibility test

A subset of *An. gambiae s.l.* collected at the larval stage and reared until adults emerged were tested to confirm pyrethroid-resistance of wild population from M'Bé (Côte d'Ivoire) and Lupiro (Tanzania) by using WHO tube assays [1] and papers treated with diagnostic concentration of 0.05% alpha-cypermethrin, 0.05% deltamethrin and 0.75% permethrin for one hour either with or without pre-exposure to 4% PBO paper for one hour. Four replicates of 25 unfed *An. gambiae s.l.* females (3-5 days old) were tested. The number of mosquitoes knocked down after exposure to the pyrethroids/PBO was recorded after 1 h exposure period; then mosquitoes were transferred to the observation tube and provided access to 10% sugar solution and held for 24 hours before scoring mortality. Exposure of susceptible *An. gambiae* (Kisumu strain in Côte d'Ivoire and Ifakara strain in Tanzania) to pyrethroid-treated papers/PBO was conducted to check the quality of treated papers.

The WHO insecticide resistance monitoring guideline [1] was used to define resistance status. A test population was classified as susceptible if 24-hour mortality was ≥98%, resistant if mortality was <90%, and indicating potential resistance if mortality fell between 90% and 97%.

Washing protocol

Nets were washed individually in aluminium bowls following the WHO guidelines [2]. Nets were washed in 10 litres of tap water (maximum hardness 5 dH) containing 2 g/L Savon de Marseille soap. Each net was agitated for 3 min, soaked for 4 min, and agitated for 3 min more, at 20 rotations per minute. Rinsing used 20 litres of clean water per net. Nets were dried horizontally in shade and stored in labelled plastic bags at 27 ± 2°C. Washing intervals differed by product regeneration time: 7 days for Olyset® Net, 5 days for PermaNet® 3.0 and VEERALIN®, 3 days for Tsara Boost®,

and 2 days for Olyset® Plus. When washing was completed, each net was labelled with unique identifier and stored at 27 ± 2°C for a minimum of seven days before testing.

Net pieces preparation for quality checks (Cone bioassay and chemical analysis)

Prior to wash, one piece (30 cm × 30 cm Côte d'Ivoire; 25 cm x 25 cm in Tanzania) netting was cut from each of the five positions from four nets of each test (Olyset® Plus, Permanet® 3.0, TSARA® Boost and VEERALIN®) and a reference net (Olyset® Net). After the washings are completed and before field trials, five net pieces, one from each of the five positions, were taken from two additional nets from Olyset® Net, Olyset® Plus, TSARA Boost® and VEERALIN®. From the two additional PermaNet® 3.0, one piece was taken from the side panels and three pieces from the roof panel. At the end of the experimental hut trial, from each arm, two of the three replicate nets used in the huts was randomly chosen for the analysis.

WHO cone bioassay

The bioefficacy of all treated nets was assessed by WHO cone bioassay using local susceptible and resistant strains: *An. gambiae* Kisumu (susceptible) and wild resistant *An. gambiae s.l.* (M'Bé site) in Côte d'Ivoire, alongside *An. gambiae* Ifakara (susceptible) and Kingani (resistant) strains in Tanzania [2]. Thirty-three (33) nets (all three nets per comparison arm) were bio-assayed before and after washing 20 times, and after field trial. The washed nets were left the necessary number of days (RTs) between the 20th wash and the 2nd bioassays to ensure that the LNs regenerated fully before the assays. For each net, 5 cones were placed on 5 sections (roof and 4 sides). Five, 3-5 day-old female mosquitoes were introduced into each cone for a 3 min exposure to nets. Bioassays were replicated 4 times per net to ensure that at least 100 mosquitoes are tested per net. Mosquitoes were then transferred to a plastic cup and provided access to 10% sugar solution. Knocked down mosquitoes were scored 60 minutes after the end of the exposure period and mortality was recorded 24 hours post holding.

Chemical analysis

To determine the pyrethroids (alpha-cypermethrin, deltamethrin and permethrin) and PBO content in the treatment arms, chemical analysis of LLIN samples was done, prior to and after washing 20 times, and at the end of field trial. All netting samples for chemical analysis were wrapped in labelled aluminium foils, put in sealed bags and stored at +4°C before shipping them away to

Gembloux (WHO collaborating centre), Belgium, for chemical analysis. All net samples from each net were analysed to provide an average target concentration of the insecticide and PBO within each treatment net.

Perceived side effects

In the first experimental round (9 nights in Cote d'Ivoire and 6 nights in Tanzania), structured questionnaires adapted from WHO guidelines [2] were administered to all volunteers each morning upon waking to record perceived and observed adverse effects. Interviews of volunteers at both sites showed no negative side effects such as nose irritation, itching of face and hands, or dizziness. Volunteers did not perceive any odour from the nets.

Reference

1. WHO. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd ed. World Health Organization, Geneva (2026)
2. WHO. Guidelines for laboratory and field testing of long-lasting insecticidal nets. World Health Organization, Geneva. (2013).

Supplementary Table S1. Mortality (24-hour) of wild adult *An. gambiae* s.l. from M'Be (Côte d'Ivoire) and *An. arabiensis* from Lupiro (Tanzania) and laboratory-reared *An. arabiensis* after exposure to pyrethroids at discriminating concentration in WHO tube tests with, and without, prior PBO exposure

Insecticides and synergist	***An. gambiae* s.l. (M'Be, Côte d'Ivoire)**	***An. arabiensis* (Lupiro, Tanzania)**	**Lab-reared *An. arabiensis* (Kingani, Tanzania)**
Alphacypermethrin 0.05%	7	21	5
PBO 4% + Alphacypermethrin 0.05%	34	96	100
Deltamethrin 0.05 %	9	37	34
PBO 4% + Deltamethrin 0.05 %	22	100	100
Permethrin 0.75%	6	4	3
PBO 4% + Permethrin 0.75%	25	98	100

Supplementary Table S2. Environmental conditions of the studies. Median (IQR) temperature and relative humidity across New Ifakara Hut (NIEH) in Tanzania, West African Hut (WAH) in Côte d'Ivoire), and I-ACT in Tanzania). Conditions were within acceptable ranges for all three platforms

Location	**Temp (°C) WAEH**	**%RH WAEH**	**Temp (°C) NIEH**	**%RH NIEH**	**Temp (°C) I-ACT**	**%RH I-ACT**
Hut/I-ACT indoors	27 (24-30)	82 (76-89)	27 (26-29)	72 (61-80)	26 (25-27)	62 (55-69)
Net storage room	27 (26-29)	77 (66-85)	26 (25-28)	74 (65-83)	27 (25-29)	81 (73-87)
Mosquito holding (semi-field)	26 (25-26)	82 (76-89)	28 (27-29)	69 (63-75)	26 (25-26)	78 (73-83)
Insectary (lab-reared)	26 (25-27)	84 (77-89)	27 (26-28)	80 (74-85)	27 (26-28)	80 (74-85)
Laboratory testing	28 (27-29)	74 (72-77)	27 (26-29)	75 (67-81)	25 (24-26)	61 (50-73)
Mosquito holding (bioassay)	28 (27-29)	74 (64-82)	27 (26-29)	75 (67-81)	26 (25-26)	78 (73-83)

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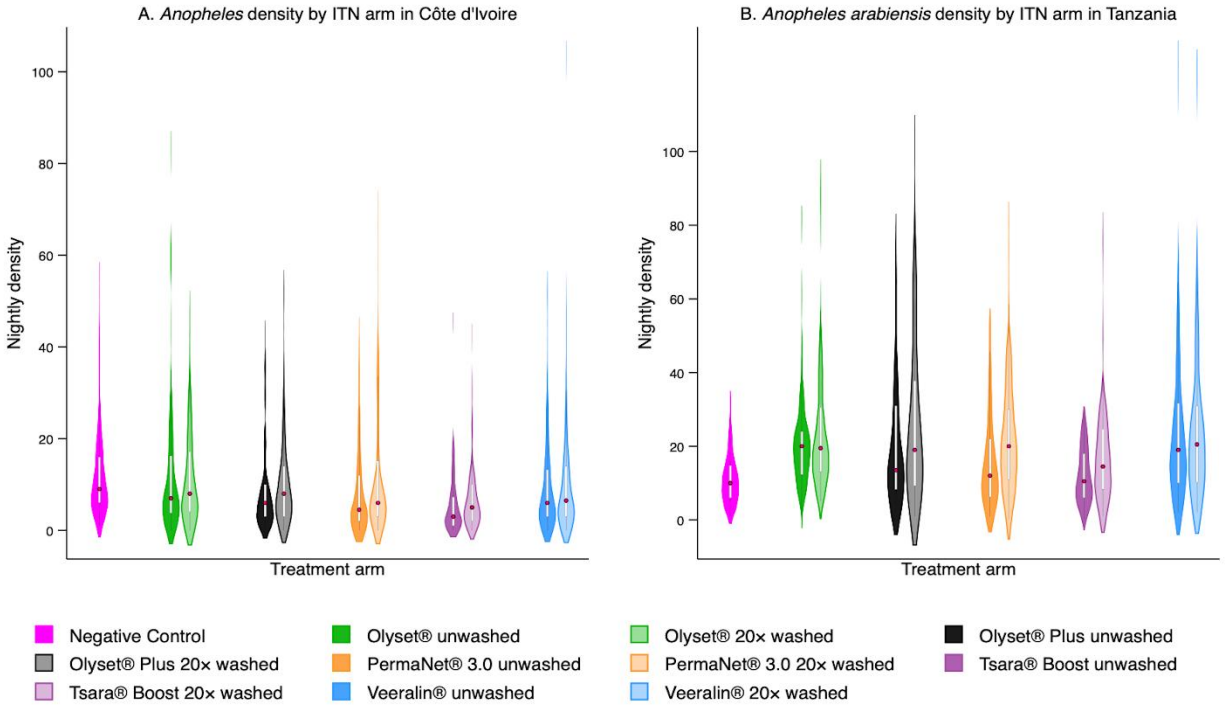
Supplementary Figure S1. Violin plot of the distribution of numbers of female *Anopheles* mosquitoes collected per hut-night in West African-type experimental hut in M'Bé, Côte d'Ivoire (**A**) and New-Ifakara-type experimental huts in Lupiro, Tanzania (**B**). Filled full-colour violins: unwashed nets; filled faint-colour violins: 20× washed nets.



Supplementary Table S3. Post-hoc power of the studies on the primary outcome of mortality. Based on 1000 simulations using GLMM parameters estimated from the completed studies. Shown is mortality, model parameters and simulated power for comparisons with Olyset® Plus across the three assays

a. Observed 24-hour mortality

Assay	Net type	Unwashed (%)	20× washed (%)	Pooled* (%)
West African huts	Olyset® Plus	18.4	13.4	15.9
	PermaNet® 3.0	27.0	10.5	18.5
	Tsara Boost®	23.1	11.4	17.1
	Veeralin®	26.9	19.0	22.8
Ifakara huts	Olyset® Plus	42.8	33.3	38.2
	PermaNet® 3.0	53.4	36.3	44.9
	Tsara Boost®	42.3	40.7	41.5
	Veeralin®	55.7	39.3	47.5
I-ACT	Olyset® Plus	96.9	82.3	89.6
	PermaNet® 3.0	99.5	94.8	97.2
	Tsara Boost®	98.9	96.9	97.9
	Veeralin®	98.7	96.0	97.4

* Combined data for unwashed and washed nets of each net type

b. Parameters used in the power simulations

Assay	Parameter	Unwashed	20× washed	Pooled*
West African huts	Mean mosquitoes per observation	6	7	6
	Overall variability	0.6560	0.6560	0.7052
	Volunteer dispersion	0.1954	0.1954	0.1728
	Hut dispersion	0.1628	0.1628	0.1743
	Day dispersion	0.2752	0.2752	0.2834
	Mosquito dispersion	0.9438	0.9438	0.9492
Ifakara huts	Mean mosquitoes per observation	13	19	15
	Overall variability	1.0970	1.0970	1.1490
	Volunteer dispersion	0.1817	0.1817	0.1740
	Hut dispersion	0.3797	0.3797	0.3962
	Day dispersion	0.4649	0.4649	0.4668
	Mosquito dispersion	0.6924	0.6924	0.7018
I-ACT	Mean mosquitoes per observation	15	15	15
	Overall variability	1.0060	1.0060	1.1870
	Volunteer dispersion	0.09774	0.09774	0.1317
	Hut dispersion	0	0	0
	Day dispersion	0.69532	0.69532	0.6833
	Mosquito dispersion	0.00007534	0.00007534	0.00005135

* Combined data for unwashed and washed nets of each net type

c. Simulated power for comparisons with Olyset® Plus

Assay	ITN	Unwashed	20× washed	Pooled*
West African huts	PermaNet® 3.0	100 (96.4–100)	84 (75.3–90.6)	99 (94.6–100)
	TSARA® Boost	100 (96.4–100)	83 (74.2–89.8)	98 (93.0–99.8)
	VEERALIN®	100 (96.4–100)	100 (96.4–100)	100 (96.4–100)
Ifakara huts	PermaNet® 3.0	100 (96.4–100)	97 (91.5–99.4)	99 (94.6–100)
	TSARA® Boost	80 (70.8–87.3)	100 (96.4–100)	99 (94.6–100)
	VEERALIN®	100 (96.4–100)	100 (96.4–100)	99 (94.6–100)
I-ACT	PermaNet® 3.0	100 (96.4–100)	100 (96.4–100)	100 (96.4–100)
	TSARA® Boost	100 (96.4–100)	100 (96.4–100)	100 (96.4–100)
	VEERALIN®	100 (96.4–100)	100 (96.4–100)	100 (96.4–100)

* Combined data for unwashed and washed nets of each type

Mortality values are percentages. Power estimates were obtained from 1,000 simulations; values in parentheses are 95% simulation intervals. “Pooled” combines the unwashed and 20-times-washed conditions. IACT, Ifakara Ambient Chamber Test.

Supplementary Table S4. Superiority analysis of pyrethroid-PBO products over standard-pyrethroid ITNs on 24-hour mortality (M24)

a. West African experimental huts (M'Bé, Côte d'Ivoire)

Net type	N	n dead	% M24 (95% CI)	OR (95% CI)	P-value	Conclusion
Unwashed						
Olyset®	1451	109	8.4(6.2-10.7)	1 (reference)	-	Reference
Olyset® Plus	1090	199	18.4(14.6-22.3)	2.56(1.97-3.32)	<0.0001	Superior
PermaNet® 3.0	1049	217	27.0(21.4-32.6)	3.16(2.44-4.09)	<0.0001	Superior
Tsara® Boost	707	162	23.1(17.9-28.2)	3.78(2.87-4.98)	<0.0001	Superior
Veeralin®	1211	243	26.9(21.8-31.9)	2.99(2.32-3.85)	<0.0001	Superior
20 washes						
Olyset®	1482	108	6.3(4.1-8.6)	1 (reference)	-	Reference
Olyset® Plus	1292	166	13.4(10.2-16.7)	1.95(1.50-1.5-2)	<0.0001	Superior
PermaNet® 3.0	1494	173	10.5(7.9-13)	1.60(1.23-2.08)	<0.0001	Superior
Tsara® Boost	894	100	11.4(8.0-14.9)	1.69(1.25-2.27)	<0.0001	Superior
Veeralin®	1347	219	19(14.7-23.4)	2.68(2.08-3.46)	<0.0001	Superior

b. Ifakara experimental huts (Tanzania)

Product	N	n dead	% M24 (95% CI)	OR (95% CI)	P-value	Conclusion
Unwashed						
Olyset®	1514	598	35.2 (29.4-41.1)	1 (reference)	-	Reference
Olyset® Plus	1489	719	42.8 (36.5-49.1)	1.60 (1.36-1.87)	<0.0001	Superior
PermaNet® 3.0	1170	737	53.4 (46.4-60.5)	2.69 (2.27-3.19)	<0.0001	Superior
Tsara® Boost	869	443	42.3 (35.7-48.9)	1.71 (1.43-2.05)	<0.0001	Superior
Veeralin®	1710	1031	55.7 (50.0-61.4)	2.51 (2.15-2.93)	<0.0001	Superior
20 washes						
Olyset®	1687	528	30.5 (25.6-35.5)	1 (reference)	-	Reference
Olyset® Plus	1929	676	33.3 (28.4-38.6)	1.34 (1.15-1.55)	<0.0001	Superior
PermaNet® 3.0	1609	669	36.3 (30.2-42.3)	1.55 (1.33-1.81)	<0.0001	Superior
Tsara® Boost	1294	539	40.7 (34.4-46.9)	1.71 (1.46-2.01)	<0.0001	Superior
Veeralin®	1780	774	39.3 (33.4-45.1)	1.69 (1.45-1.96)	<0.0001	Superior

c. Ifakara-Ambient Chamber Test (I-ACT, Tanzania)

Product	N	n dead	% M24 (95% CI)	OR (95% CI)	P-value	Conclusion
Unwashed						
Olyset®	787	533	67.7 (62.3-73.2)	1 (reference)	-	Reference
Olyset® Plus	794	769	96.9 (95.5-98.2)	6.23 (4.72-8.23)	<0.0001	Superior
PermaNet® 3.0	791	787	99.5 (98.9-100)	8.78 (6.45-11.97)	<0.0001	Superior
Tsara® Boost	790	781	98.9 (98.2-99.5)	8.04 (5.96-10.86)	<0.0001	Superior
Veeralin®	794	784	98.7 (97.7-99.7)	7.97 (5.91-10.74)	<0.0001	Superior
20 washes						
Olyset®	789	538	68.2 (62.6-73.8)	1 (reference)	-	Reference
Olyset® Plus	790	650	82.3 (78.0-86.5)	1.98 (1.59-2.47)	<0.0001	Superior
PermaNet® 3.0	793	752	94.8 (92.6-97.1)	4.89 (3.76-6.36)	<0.0001	Superior
Tsara® Boost	791	766	96.9 (94.9-98.8)	6.04 (4.57-7.97)	<0.0001	Superior
Veeralin®	792	761	96.0 (94.2-97.8)	5.57 (4.24-7.30)	<0.0001	Superior

Odds ratios for the effect of second-in-class ITNs compared to the PBO positive control, Olyset® Plus, were estimated using logistic regression, adjusting for volunteer and day as fixed effects, hut as fixed effect in M'Bé or random effect in Tanzania. For I-ACT, hut was not included in the model. For superiority on mortality outcome, OR must be higher than 1.00 and p-value<0.05. N = Number of mosquitoes collected, n dead = Number of mosquitoes dead, OR = Odds Ratio; CI = Confidence interval.

Supplementary Table S5. Non-inferiority analysis of second-in-class pyrethroid-PBO products over Olyset® Plus ITNs on 24-hour mortality (M24)

a. West African experimental huts (M'Bé, Côte d'Ivoire)

Product	N	n dead	% M24 (95% CI)	Delta	OR (95% CI)	NI conclusion
Unwashed						
Olyset® Plus	1090	199	18.4 (14.6–22.3)		1 (reference)	Reference
PermaNet® 3.0	1049	217	27.0 (21.4–32.6)	0.56	1.24 (0.98–1.56)	Non-inferior
Tsara® Boost	707	162	23.1 (17.9–28.2)	0.56	1.48 (1.15–1.90)	Superior
Veeralin®	1211	243	26.9 (21.8–31.9)	0.56	1.17 (0.94–1.46)	Non-inferior
20 washes						
Olyset® Plus	1292	166	13.4 (10.2–16.7)		1 (reference)	Reference
PermaNet® 3.0	1494	173	10.5 (7.9–13.0)	0.43	0.82 (0.64–1.04)	Non-inferior
Tsara® Boost	894	100	11.4 (8.0–14.9)	0.43	0.86 (0.65–1.14)	Non-inferior
Veeralin®	1347	219	19.0 (14.7–23.4)	0.43	1.37 (1.09–1.74)	Superior

b. Ifakara experimental huts (Tanzania)

Product	N	n dead	% M24 (95% CI)	Delta	OR (95% CI)	NI conclusion
Unwashed						
Olyset® Plus	1489	719	42.8 (36.5–49.1)		1 (reference)	Reference
PermaNet® 3.0	1170	737	53.4 (46.4–60.5)	0.75	1.69 (1.42–2.00)	Superior
Tsara® Boost	869	443	42.3 (35.7–48.9)	0.75	1.07 (0.90–1.28)	Non-inferior
Veeralin®	1710	1031	55.7 (50.0–61.4)	0.75	1.57 (1.35–1.83)	Superior
20 washes						
Olyset® Plus	1929	676	33.3 (28.4–38.6)		1 (reference)	Reference
PermaNet® 3.0	1609	669	36.3 (30.2–42.3)	0.71	1.16 (1.00–1.34)	Non-inferior
Tsara® Boost	1294	539	40.7 (34.4–46.9)	0.71	1.28 (1.10–1.49)	Superior
Veeralin®	1780	774	39.3 (33.4–45.1)	0.71	1.26 (1.09–1.45)	Superior

c. Ifakara-Ambient Chamber Test (I-ACT, Tanzania)

Product	N	n dead	% M24 (95% CI)	Delta	OR (95% CI)	NI conclusion
Unwashed						
Olyset® Plus	794	769	96.9 (95.5–98.2)		1 (reference)	Reference
PermaNet® 3.0	791	787	99.5 (98.9–100)	0.28	1.41 (0.98–2.02)	Non-inferior
Tsara® Boost	790	781	98.9 (98.2–99.5)	0.28	1.29 (0.91–1.84)	Non-inferior
Veeralin®	794	784	98.7 (97.7–99.7)	0.28	1.28 (0.90–1.82)	Non-inferior
20 washes						
Olyset® Plus	790	650	82.3 (78.0–86.5)		1 (reference)	Reference
PermaNet® 3.0	793	752	94.8 (92.6–97.1)	0.66	2.47 (1.88–3.24)	Superior
Tsara® Boost	791	766	96.9 (94.9–98.8)	0.66	3.05 (2.29–4.07)	Superior
Veeralin®	792	761	96.0 (94.2–97.8)	0.66	2.81 (2.12–3.73)	Superior

Odds ratios for the effect of second-in-class ITNs compared to the PBO positive control, Olyset® Plus, were estimated using logistic regression, adjusting for volunteer and day as fixed effects, hut as fixed effect in M'Bé or random effect in Tanzania. For I-ACT, hut was not included in the model. Lower bound of the confidence interval of Odds ratio must fall above Delta to be deemed non-inferior. Lower bound of the confidence interval of OR must be above both Delta and 1 to be superior. N = Number of mosquitoes collected, n dead = Number of mosquitoes dead, OR = Odds Ratio; CI = Confidence interval. Delta represents the non-inferiority margin.

Supplementary Table S6. Superiority analysis of pyrethroid-PBO products over standard-pyrethroid ITNs on blood-feeding

a. West African experimental huts (M'Bé, Côte d'Ivoire

Product	N	n fed	% fed (95% CI)	OR (95% CI)	P-value	Conclusion
Unwashed						
Olyset®	1451	663	45.9 (41-50.8)	1 (reference)		Reference
Olyset® Plus	1090	183	16.1 (12.3-19.9)	0.21 (0.18-0.26)	<0.0001	Superior
PermaNet® 3.0	1049	300	27.5 (22.1-32.9)	0.42 (0.35-0.51)	<0.0001	Superior
Tsara® Boost	707	206	27 (21.4-32.6)	0.43 (0.35-0.52)	<0.0001	Superior
Veeralin®	1211	411	30.6 (25.4-35.7)	0.54 (0.45-0.64)	<0.0001	Superior
20 washes						
Olyset®	1482	737	46 (41-51.1)	1 (reference)		Reference
Olyset® Plus	1292	383	24.2 (20-28.4)	0.41 (0.35-0.49)	<0.0001	Superior
PermaNet® 3.0	1494	503	33.9 (28.9-38.9)	0.51 (0.44-0.60)	<0.0001	Superior
Tsara® Boost	894	302	31.9 (26.6-37.1)	0.51 (0.42-0.61)	<0.0001	Superior
Veeralin®	1347	329	24.1 (19.6-28.5)	0.33 (0.28-0.39)	<0.0001	Superior

b. Ifakara experimental huts (Tanzania)

Product	N	n fed	% fed (95% CI)	OR (95% CI)	P-value	Conclusion
Unwashes						
Olyset®	1514	65	5.1 (3.3-6.9)	1 (reference)		Reference
Olyset® Plus	1489	31	3.5 (1.2-5.9)	0.50 (0.32-0.78)	0.002	Superior
PermaNet® 3.0	1170	32	4.7 (1.9-7.5)	0.67 (0.43-1.03)	0.070	Not superior
Tsara® Boost	869	48	5.3 (3.3-7.3)	1.27 (0.86-1.88)	0.226	Not superior
Veeralin®	1710	91	6.5 (4.1-8.9)	1.40 (1.00-1.96)	0.048	Not superior
20 washes						
Olyset®	1687	100	7.3 (5.4-9.2)	1 (reference)		Reference
Olyset® Plus	1929	84	6.9 (3.6-10.3)	0.69 (0.50-0.93)	0.016	Superior
PermaNet® 3.0	1609	77	5.7 (3.8-7.6)	0.76 (0.55-1.04)	0.083	Not superior
Tsara® Boost	1294	76	7.1 (3.7-10.4)	0.89 (0.65-1.22)	0.482	Not superior
Veeralin®	1780	123	9.6 (6.1-13.1)	1.12 (0.84-1.49)	0.430	Not superior

c. Ifakara-Ambient Chamber Test (I-ACT, Tanzania)

Product	N	n fed	% fed (95% CI)	OR (95% CI)	P-value	Conclusion
Unwashed						
Olyset®	787	207	26.3 (21.4-31.1)	1 (reference)		Reference
Olyset® Plus	794	22	2.8 (1.6-4.0)	0.07 (0.05-0.11)	<0.0001	Superior
PermaNet® 3.0	791	54	6.8 (4.7-9.0)	0.18 (0.13-0.25)	<0.0001	Superior
Tsara® Boost	790	51	6.5 (4.5-8.5)	0.17 (0.13-0.24)	<0.0001	Superior
Veeralin®	794	50	6.3 (3.9-8.6)	0.17 (0.12-0.24)	<0.0001	Superior
20 washes						
Olyset®	789	250	31.7 (26.2-37.2)	1 (reference)		Reference
Olyset® Plus	790	65	8.3 (4.8-11.8)	0.18 (0.13-0.24)	<0.0001	Superior
PermaNet® 3.0	793	52	6.6 (3.8-9.4)	0.13 (0.10-0.19)	<0.0001	Superior
Tsara® Boost	791	62	7.8 (4.6-11.0)	0.17 (0.12-0.23)	<0.0001	Superior
Veeralin®	792	66	8.4 (5.6-11.1)	0.18 (0.13-0.24)	<0.0001	Superior

Odds ratios for the effect of second-in-class ITNs compared to the PBO positive control, Olyset® Plus, were estimated using logistic regression, adjusting for volunteer and day as fixed effects, hut as fixed effect in M'Bé or random effect in Tanzania. For I-ACT, hut was not included in the model. For superiority on blood-feeding outcome, OR must be lower than 1.00 and p-value <0.05. N = Number of mosquitoes collected, n fed = Number of mosquitoes fed, OR = Odds Ratio; CI = Confidence interval.

Supplementary Table S7. Non-inferiority analysis of second-in-class pyrethroid-PBO products over first-in-class Olyset Plus ITNs on blood feeding

a. West African experimental huts (M'Bé, Côte d'Ivoire)

Product	N	n fed	% fed (95% CI)	Delta	OR (95% CI)	Conclusion
Unwashed						
Olyset® Plus	1090	183	16.1 (12.3-19.9)		1 (reference)	Reference
PermaNet® 3.0	1049	300	27.5 (22.1-32.9)	1.57	1.97 (1.58-2.46)	Inconclusive
Tsara® Boost	707	206	27.0 (21.4-32.6)	1.57	1.99 (1.56-2.53)	Inconclusive
Veeralin®	1211	411	30.6 (25.4-35.7)	1.57	2.52 (2.04-3.11)	Inconclusive
20 washes						
Olyset® Plus	1292	383	24.2 (20.0-28.4)		1 (reference)	Reference
PermaNet® 3.0	1494	503	33.9 (28.9-38.9)	1.42	1.24 (1.04-1.48)	Inconclusive
Tsara® Boost	894	302	31.9 (26.6-37.1)	1.42	1.23 (1.01-1.50)	Inconclusive
Veeralin®	1347	329	24.1 (19.6-28.5)	1.42	0.81 (0.67-0.97)	Non-inferior

b. Ifakara experimental huts (Tanzania)

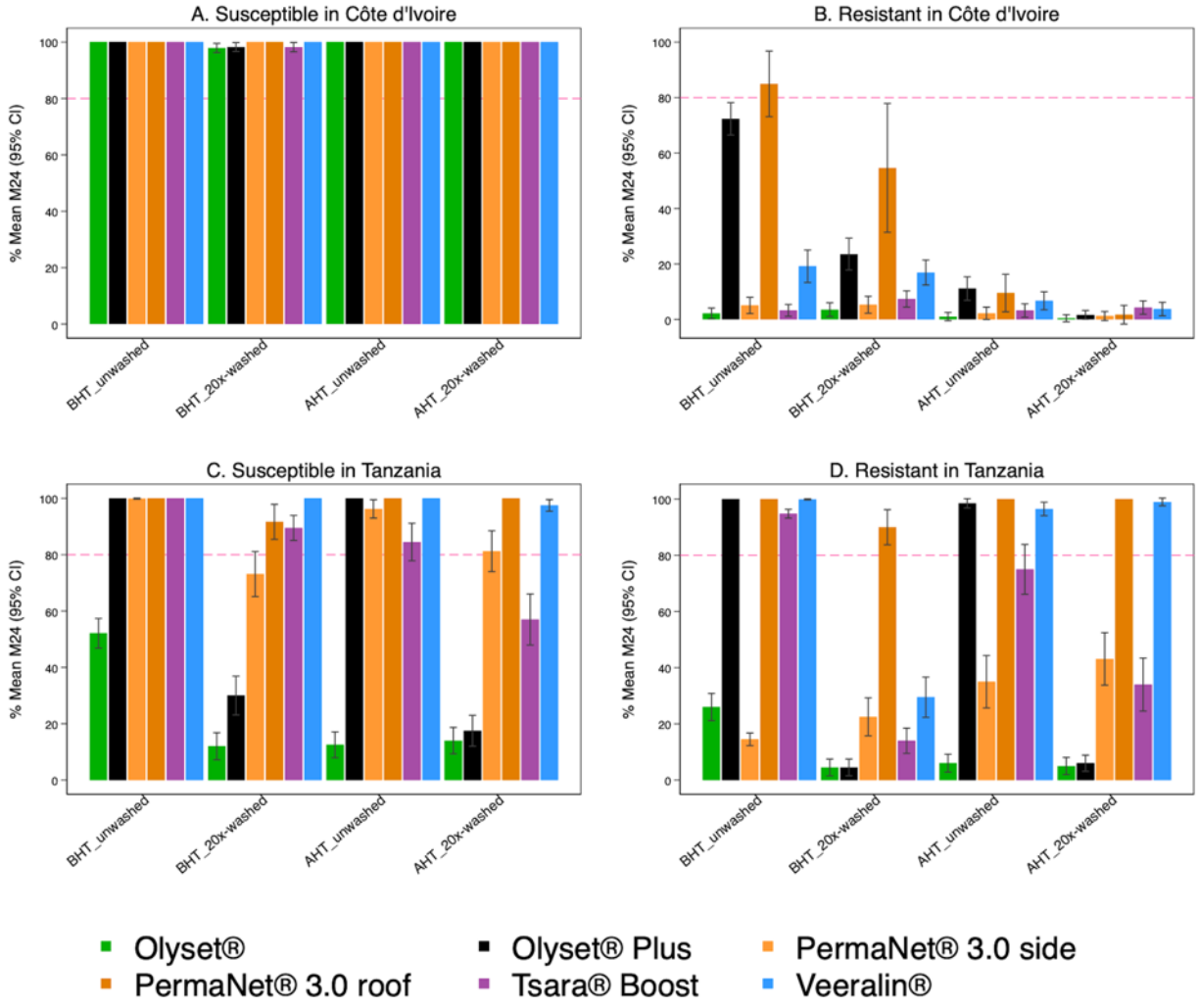
Product	N	n fed	% fed (95% CI)	Delta	OR (95% CI)	Conclusion
Unwashed						
Olyset® Plus	1489	31	3.5 (1.2-5.9)		1 (reference)	Reference
PermaNet® 3.0	1170	32	4.7 (1.9-7.5)	2.97	1.33 (0.80-2.20)	Non-inferior
Tsara® Boost	869	48	5.3 (3.3-7.3)	2.97	2.53 (1.58-4.03)	Inconclusive
Veeralin®	1710	91	6.5 (4.1-8.9)	2.97	2.79 (1.83-4.24)	Inconclusive
20 washes						
Olyset® Plus	1929	84	6.9 (3.6-10.3)		1 (reference)	Reference
PermaNet® 3.0	1609	77	5.7 (3.8-7.6)	2.16	1.10 (0.80-1.53)	Non-inferior
Tsara® Boost	1294	76	7.1 (3.7-10.4)	2.16	1.30 (0.94-1.81)	Non-inferior
Veeralin®	1780	123	9.6 (6.1-13.1)	2.16	1.63 (1.21-2.20)	Inconclusive

c. Ifakara-Ambient chamber test (I-ACT, Tanzania)

Product	N	n fed	% fed (95% CI)	Delta	OR (95% CI)	Conclusion
Unwashed						
Olyset® Plus	794	22	2.8 (1.6-4.0)		1 (reference)	Reference
PermaNet® 3.0	791	54	6.8 (4.7-9.0)	3.59	2.56 (1.53-4.27)	Inconclusive
Tsara® Boost	790	51	6.5 (4.5-8.5)	3.59	2.44 (1.46-4.10)	Inconclusive
Veeralin®	794	50	6.3 (3.9-8.6)	3.59	2.39 (1.42-4.00)	Inconclusive
20 washes						
Olyset® Plus	790	65	8.3 (4.8-11.8)		1 (reference)	Reference
PermaNet® 3.0	793	52	6.6 (3.8-9.4)	2.03	0.75 (0.51-1.11)	Non-inferior
Tsara® Boost	791	62	7.8 (4.6-11.0)	2.03	0.93 (0.65-1.35)	Non-inferior
Veeralin®	792	66	8.4 (5.6-11.1)	2.03	1.10 (0.70-1.46)	Non-inferior

Odds ratios for the effect of second-in-class ITNs compared to the PBO positive control, Olyset® Plus, were estimated using logistic regression, adjusting for volunteer and day as fixed effects, hut as fixed effect in M'Bé or random effect in Tanzania. For I-ACT, hut was not included in the model. Upper bound of the confidence interval of OR must lie below Delta to be deemed non-inferior. N = Number of mosquitoes collected, n fed = Number of mosquitoes fed, OR = Odds Ratio; CI = Confidence interval. Delta represents the non-inferiority margin.

Supplementary Figure S3. WHO cone bioassay mortality (%) of susceptible *Anopheles gambiae* s.l. Kisumu (**A)** and Ifakara (**C**) strains, and wild pyrethroid resistant *Anopheles gambiae* s.l. from M'Bé, Côte d'Ivoire (**B**) and Lupiro, Tanzania (**D**). Mean 24-h mortality of mosquitoes exposed for 3 min to unwashed and 20 times washed nets before (BHT) and after (AHT) hut trial. Bars represent mean mortality with error bars indicating 95% confidence intervals. The dashed pink line represents 80% WHO threshold for mosquito mortality



Supplementary Table S8a: Mean concentration of pyrethroids and retention in treatment nets used in M'Bé experimental huts (WAEH), Côte d'Ivoire

Net type	a.i. target dose and tolerance limit (g/kg)	Period	Status	Mean a.i. content (g/kg)	RSD (%)*	a.i. retention (%)+
Olyset®	Permethrin 20.0 (15 – 25)	BHT	UW	19.5	0.3	
			20x W	17.0	0.5	87
		AHT	UW	18.89	0.2	
			20x W	17.19	0.1	91
Olyset® Plus	Permethrin 20.0 (15 – 25)	BHT	UW	20.10	1.4	
			20x W	14.94	0.2	74
		AHT	UW	16.75	13.1	
			20x W	15.14	0.4	90
PermaNet® 3.0 sides	Deltamethrin 2.1 (1.6 – 2.6)	BHT	UW	2.07	2.7	
			20x W	0.23	7.2	11
		AHT	UW	2.07	3.3	
			20x W	0.20	2.9	10
PermaNet® 3.0 roof	Deltamethrin 4.0 (3.0 – 5.0)	BHT	UW	4.02	1.6	
			20x W	2.76	1.0	69
		AHT	UW	3.36	1.2	
			20x W	2.71	0.1	81
Tsara® Boost	Deltamethrin 3.0 (2.25 – 3.75)	BHT	UW	3.09	5.5	
			20x W	2.61	2.8	85
		AHT	UW	2.65	3.7	
			20x W	2.65	10.0	100
Veeralin®	Alpha-cypermethrin 6.0 (4.5 – 7.5)	BHT	UW	6.53	2.3	
			20x W	5.82	0.5	89
		AHT	UW	6.06	0.7	
			20x W	5.64	2.7	93

a.i.: active ingredient; BHT: Before hut trial; AHT: After hut trial; UW: unwashed net; 20x W: washed net; * RSD Relative standard deviation for a.i. variation between net samples; + a.i. retention relative to content before washing

Supplementary Table S8b: Mean concentration of pyrethroids and retention in treatment nets used in Ifakara experimental huts (NIEH), Tanzania

Net type	a.i. target dose and tolerance limit (g/kg)	Period	Status	Mean a.i. content (g/kg)	RSD (%)*	a.i. retention (%)+
Olyset®	Permethrin 20.0 (15 – 25)	BHT	UW	19.5	0.3	
			20x W	17.90	0.5	92
		AHT	UW	19.64	0.3	
			20x W	17.65	1.4	90
Olyset® Plus	Permethrin 20.0 (15 – 25)	BHT	UW	20.10	1.4	
			20x W	14.58	13.5	73
		AHT	UW	19.24	1.7	
			20x W	15.55	1.1	81
PermaNet® 3.0 sides	Deltamethrin 2.1 (1.6 – 2.6)	BHT	UW	2.07	2.7	
			20x W	0.44	14.5	21
		AHT	UW	1.89	4.6	
			20x W	0.65	36.7	34
PermaNet® 3.0 roof	Deltamethrin 4.0 (3.0 – 5.0)	BHT	UW	4.02	1.6	
			20x W	3.31	0.1	82
		AHT	UW	3.78	0.9	
			20x W	3.10	11.3	82
Tsara® Boost	Deltamethrin 3.0 (2.25 – 3.75)	BHT	UW	3.09	5.5	
			20x W	2.39	2.7	77
		AHT (++)	UW	*NA*	*NA*	
			20x W	*NA*	*NA*	*NA*
Veeralin®	Alpha-cypermethrin 6.0 (4.5 – 7.5)	BHT	UW	6.53	2.3	
			20x W	5.88	3.0	90
		AHT	UW	6.16	0.8	
			20x W	5.80	0.0	94

a.i.: active ingredient; BHT: Before hut trial; AHT: After hut trial; UW: unwashed net; 20x W: washed net; * RSD Relative standard deviation for a.i. variation between net samples; + a.i. retention relative to content before washing;
(++): the analysis of the deltamethrin content in the unwashed and washed nets at the end of the field trial showed abnormal values, therefore they were not be valided

Supplementary Table S8c: Piperonyl butoxide (PBO) content and retention in treatment nets used in M'Bé (Côte d'Ivoire) and Ifakara (Tanzania) experimental huts

	Product	PBO target dose and tolerance limit (g/kg)	Period	Status	Mean a.i. content (g/kg)	RSD (%)*	a.i. retention (%)+
M'Bé huts (Côte d'Ivoire)	Olyset® Plus	10.0 (7.5 – 12.5)	BHT	UW	9.44	3.2	
				20x W	5.11	0.5	54
			AHT	UW	7.55	9.3	
				20x W	5.08	2.0	67
	PermaNet® 3.0 roof	25.0 (18.8 – 31.2)	BHT	UW	26.74	1.2	
				20x W	14.11	0.2	53
			AHT	UW	18.51	0.6	
				20x W	11.99	1.1	65
	Tsara® Boost	11.0 (8.25 – 13.75)	BHT	UW	12.35	2.9	
				20x W	7.84	0.6	64
			AHT	UW	12.63	9.7	
				20x W	6.64	12.2	53
	Veeralin®	2.2 (1.7 – 2.8)	BHT	UW	2.78	0.8	
				20x W	2.01	0.9	72
			AHT	UW	2.44	0.4	
				20x W	1.99	6.9	82
Ifakara huts (Tanzania)	Olyset® Plus	10.0 (7.5 – 12.5)	BHT	UW	9.44	3.2	
				20x W	4.80	24.8	51
			AHT	UW	8.22	1.1	
				20x W	5.14	3.0	63
	PermaNet® 3.0 roof	25.0 (18.8 – 31.2)	BHT	UW	26.74	1.2	
				20x W	13.78	1.2	52
			AHT	UW	18.65	4.8	
				20x W	13.02	3.3	70
	Tsara® Boost	11.0 (8.25 – 13.75)	BHT	UW	12.35	2.9	
				20x W	7.07	2.6	57
			AHT	UW	12.40	8.5	
				20x W	7.02	4.3	57
	Veeralin®	2.2 (1.7 – 2.8)	BHT	UW	2.78	0.8	
				20x W	2.12	2.7	76
			AHT	UW	2.53	0.8	
				20x W	2.06	2.8	82

a.i.: active ingredient; BHT: Before hut trial; AHT: After hut trial; UW: unwashed net; 20x W: washed net; * RSD Relative standard deviation for a.i. variation between net samples; + a.i. retention relative to content before washing