

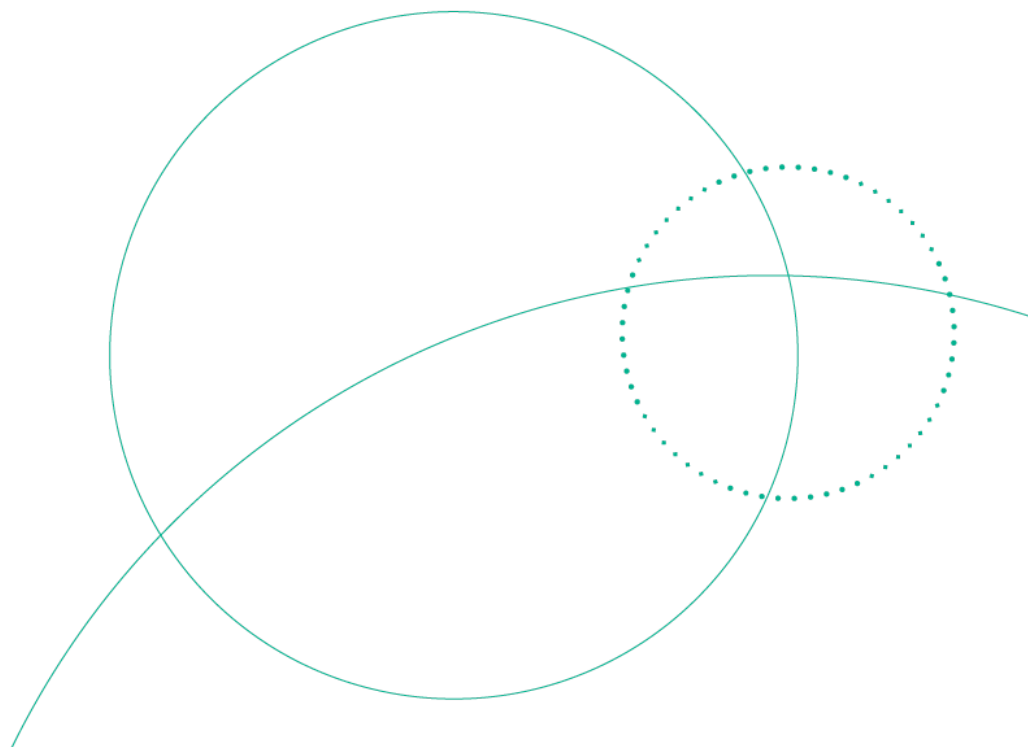
WHO Prequalification Programme / Vector Control Product Assessment

WHO Public Assessment Report: WHOPAR Part 3

Yorkool G3 LN
(Tianjin Yorkool)

021-003

Quality Assessment



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1 Chemical and physical data

1.1 Chemical and physical properties

Data on the chemical and physical properties of the active ingredient and the product Yorkool G3 LN were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP) and are considered complete. Product specific properties are summarized in Table 1. Numerical results are presented as: mean (range). These summary results are based on the analysis of batches: 20180074, 20180515, 20180516, 20200326, 20200415, 20200513, 20200518.

Complete results from the Studies TSNT01152359, 2440, 20199, and 21046 are available in Appendix 1.

Table 1. Chemical and physical properties for Yorkool G3 LN		
Data requirement	Test method ID	Result
Identification of deltamethrin and piperonyl butoxide (PBO)	Deltamethrin CIPAC (N) 333/LN/(M2)/3 Piperonyl butoxide CIPAC (N) 33/LN/(M)/3	The active ingredients each comply with an identity test
Deltamethrin mean content	CIPAC (N) 333/LN/(M2)/3	(3.07 -3.48 g/kg)* (2.965 - 3.52 g/kg)**
Accelerated storage deltamethrin retention		(97.1 -97.7%)**
R-alpha deltamethrin mean content	CIPAC (O) 333/TC	0.022 g/kg (~0.7% wrt Deltamethrin)
Piperonyl butoxide content	CIPAC (N) 33/LN/(M)/3	(11.55 -12.32 g/kg)* (11.04 - 12.562 g/kg)**
Accelerated storage piperonyl butoxide retention		(97.2 -99.1%)**
Wash resistance index (Deltamethrin)	CIPAC (O) MT 195	(97.08 -98.43%)**
Accelerated storage deltamethrin WRI		(96.59 -98.95%)**
Wash resistance index (piperonyl butoxide)	CIPAC (O) MT 195	(90.61 - 98.26%)**
Accelerated storage piperonyl butoxide WRI		(92.75 - 97.36%)**
Mesh size	See Appendix 2	20 (16-21) holes/cm ² **
Fabric weight	ISO 3801/EN 12127 EN 20139-1992	(37.7- 40.82 g/m ²)**
Dimensional stability of netting to washing	ISO3759-2011 / ISO5077-2007 / ISO6330-2012	Length: (-2.8% to -1.8%)** Width: (+0.3% to +2.4%)**
Accelerated storage dimensional stability		Length: (-2.1% to +0.3%)** Width: (-2% to +2.4%)**
Bursting strength	ISO 13938-2-1999 (30mm Diaphragm Diameter)	(440 - 497.9 kPa)**
Accelerated storage bursting strength		(411 - 482.4 kPa)**
Seam bursting strength	ISO 13938-2-1999 (30mm Diaphragm Diameter)	(≥ 445 - ≥ 565 kPa)**
Accelerated storage seam bursting strength		(≥ 416 - ≥ 541.1 kPa)**
Flammability (Batch 1 178 20)	EN 1102:2016	No ignition or propagation. Maximum hole length 100 mm, width 23 mm.

* range of means

** range of individual measurements in samples

No significant differences were recorded among the properties of the product kept at ambient temperature and after accelerated storage stability test conditions.

1.2 Manufacturing, composition and formulant information

Data on the manufacturing process and product composition for Yorkkool G3 LN have been provided and are adequate. A summary is presented in Table 2. Detailed information on the manufacturing process and product formulation is considered Confidential Business Information (CBI).

Table 2. Manufacturing process and product composition data submitted for Yorkkool G3 LN	
Description of starting material	Deltamethrin TC and piperonyl butoxide TC formulated as part of the production process as masterbatches. The sources of active ingredients are supported by a current evaluation report confirming compliance of the materials with the established WHO specification.
Declaration of product formulation	Included in the confidential business information.
Production / formulation process	The fabric is manufactured by the preparation of the deltamethrin and piperonyl butoxide masterbatches, the extrusion and warping of the filament, warp knitting, and heat setting. The finished product is manufactured by cutting and sewing of the fabric, addition of label tag, folding prior to packaging, packing, and baling.
Packaging	Nets may be packed individually in 52 cm × 40 cm plastic bags with printed labels or tied into bundles of five loose nets prior to baling with one adhesive label per bale. The sizes of the bags and bales may vary depending on the customer requirements.
Discussion of impurities	The <i>R</i> -alpha-diastereomer of deltamethrin was analysed and reported as a percentage of the total deltamethrin concentration. There are no relevant impurities of toxicological concern.
Certification of limits	Deltamethrin: 3.0 g/kg, acceptable limits 2.25-3.75 g/kg Piperonyl butoxide: 11.0 g/kg, acceptable limits 8.25-13.75 g/kg

1.3 Enforcement analytical method

Table 3. Details of the analytical method used to determine Deltamethrin and Piperonyl butoxide in Yorkkool G3 LN	
Quantification of deltamethrin and piperonyl butoxide	Deltamethrin: CIPAC (N) 333/LN/(M2)/3 Piperonyl butoxide: CIPAC (N) 33/LN/(M)/3

These methods are appropriate for the determination of the active ingredient content of the product.

2 Chemical and entomological fabric characterisation

Laboratory studies to characterize the availability of the active ingredient and synergist and the insecticidal effect of the fabric of Yorkool G3 LN on Anopheline mosquito species were submitted to WHO as part of the prequalification dossier. Sampled pieces of ITNs used in the biological laboratory studies were characterized for their deltamethrin and piperonyl butoxide content using HPLC and GC.

2.1 Laboratory studies

2.1.1 Entomological characterisation

Data on the wash regeneration and wash resistance properties of the Yorkool G3 LN product were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP). These summary results are based on ITNs drawn from batch 20180515.

One laboratory study was submitted to characterise the Yorkool G3 LN fabric. Bioavailability was evaluated in wash regeneration and wash resistance studies. The endpoint used to evaluate bioavailability was 24-hour control corrected mortality. Demonstration of bioavailability post-wash was used to determine the wash interval that was selected for use in the wash resistance study. Thresholds of $\geq 95\%$ knockdown and/or $\geq 80\%$ mortality in WHO cone tests were used to determine wash resistance.

The bioavailability of deltamethrin and piperonyl butoxide on the surface of the ITN was characterized using the insecticide susceptible test system *Anopheles gambiae* s.s. Ifakara strain and the pyrethroid resistant test system *Anopheles arabiensis* Kingani strain, with pyrethroid resistance mediated by the over-expression of cytochrome P450 enzymes and an observed mortality to the diagnostic dose of deltamethrin of 25%. WHO cone tests were the experimental method used in bioavailability studies.

The wash interval was determined to be one day. Using this wash interval, Yorkool G3 was wash resistant to 25 washes using insecticide susceptible and pyrethroid resistant mosquito test systems. These results are summarized in Tables 4-6.

Table 4. Wash regeneration study results for Yorkool G3 using the insecticide susceptible *An. gambiae* Ifakara test system and the pyrethroid resistant *An. arabiensis* Kingani strain in WHO cone tests to characterize the bioavailability of deltamethrin and PBO

Days post-wash	<i>An. gambiae</i> Ifakara			<i>An. arabiensis</i> Kingani		
	n	%KD (95% CI)	%M24 (95% CI)	n	%KD (95% CI)	%M24 (95% CI)
1	80	100	100	80	100	93.75 (83.7 – 100)
2	80	100	100	80	100	100
3	80	100	100	80	100	100
5	80	100	100	80	100	100
7	80	100	100	80	100	100

Table 5. Wash resistance study results for Yorkool G3 using the insecticide susceptible *An. gambiae* Ifakara test system and the pyrethroid resistant *An. arabiensis* Kingani strain in WHO cone tests to characterize the bioavailability of deltamethrin and PBO

Wash No.	<i>An. gambiae</i> Ifakara			<i>An. arabiensis</i> Kingani		
	n	%KD (95% CI)	%M24 (95% CI)	n	%KD (95% CI)	%M24 (95% CI)
0	80	100	100	80	100	100
1	80	100	100	80	100	100
3	80	100	100	80	100	100
5	80	100	100	80	100	100
10	80	100	100	80	100	96.3 (95.1-97.4)
15	80	100	100	80	100	88.8 (85.8-91.8)
20	80	100	100	80	100	87.5 (83.4-91.6)
25	80	100	100	80	100	86.3 (84.0-88.5)

Table 6. Wash resistance study results for Yorkool G3 using the insecticide susceptible *An. gambiae* Ifakara test system and the pyrethroid resistant *An. arabiensis* Kingani strain in WHO cone tests to characterize the bioavailability of deltamethrin and PBO in nets washed using a 'field' wash methodology

Wash No.	<i>An. gambiae</i> Ifakara			<i>An. arabiensis</i> Kingani		
	n	%KD (95% CI)	%M24 (95% CI)	n	%KD (95% CI)	%M24 (95% CI)
0	500	100	100	500	100	100
20	500	100	100	500	100	(88.2-93.0)

2.1.1.1 Chemical characterisation

Data on the deltamethrin and piperonyl butoxide content of sampled pieces of the Yorkool G3 LN product used in the entomological laboratory wash resistance study and the supplemental laboratory study were provided. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP). These summary results are based on ITNs drawn from batch 20180515. The results are summarized in Tables 7 and 8.

Table 7. AI content and retention of sampled pieces of Yorkool G3 used in the entomological wash resistance study (batch number 20180515)

Wash No.	Mean Deltamethrin content (g/kg)	RSD (%)	Deltamethrin retention	Deltamethrin retention per wash	Mean piperonyl butoxide content (g/kg)	RSD (%)	Piperonyl butoxide retention	Piperonyl butoxide retention per wash
0	3.42 (3.24-3.57)	2.65	-	-	11.53 (11.26-11.72)	1.24	-	-
1	3.39 (3.37-3.41)	0.55	99.12	(97.54-99.96)	10.44 (10.37-10.50)	0.54	90.55	(90.21-90.87)
3	3.31 (3.28-3.35)	0.99	96.78	(98.73-99.25)	9.59 (9.49-9.68)	0.83	83.17	(93.53-94.65)
5	3.16 (3.14-3.17)	0.47	92.40	(98.08-98.74)	8.39 (8.33-8.45)	0.61	72.77	(93.67-94.05)
10	3.09 (3.07-3.11)	0.61	90.35	(98.89-99.11)	7.56 (7.50-7.61)	0.62	65.57	(95.78-95.96)
15	3.04 (3.03-3.06)	0.53	88.89	(99.15-99.30)	7.31 (7.23-7.39)	0.98	63.40	(96.89-97.14)
20	2.94 (2.92-2.96)	0.58	85.96	(99.17-99.31)	6.42 (6.37-6.46)	0.69	55.68	(97.07-97.15)
25	2.77 (2.74-2.79)	0.84	80.99	(99.10-99.21)	5.59 (5.55-5.64)	0.69	48.48	(97.11-97.17)

The mean AI content presented in Table 7 was determined based on 20 net samples belonging to batch 20180515, indicating ranges to the AI content in parenthesis.

AI retention per wash in Table 7 (ranges in parenthesis) is calculated as:

- AI retention per wash = $100 \times \sqrt[n]{t_n/t_0}$ where:
 - t_n = total active ingredient content after n washing cycles
 - t_0 = total active ingredient content before washing
 - n = number of washes.

Table 8. AI content of sampled pieces of Yorkkool G3 used in the entomological supplementary laboratory study (batch number 20180515)

Wash No.	Mean deltamethrin content (g/kg)	Mean piperonyl butoxide content (g/kg)
0	3.42 (3.24-3.57)	11.53 (11.26-11.72)
20	2.98 (2.82-3.09)	6.32 (6.20-6.43)

The mean AI content presented in Table 8 was determined based on 20 net samples belonging to batch 20180515, indicating ranges to the AI content in parenthesis, at 0 washes and 20 washes.

2.2 Chemical and entomological fabric characterisation conclusions

The submitted laboratory studies characterize the fabric of Yorkkool G3 against two strains of *An. gambiae* complex mosquitoes. Following three washes intended to deplete the surface of the fabric of bioavailable insecticide, the laboratory results demonstrate that sufficient bioavailable insecticide to induce mortality in insecticide susceptible and pyrethroid resistant test systems was present one day after washing.

Wash resistance to 25 washes using standardised washing methods was demonstrated against two *An. gambiae* complex test systems, one which was insecticide susceptible and one pyrethroid resistant system carrying metabolic resistance mechanisms. Based on the submitted studies, Yorkkool G3 is wash resistant to 25 washes using a one-day wash interval against pyrethroid susceptible *An. gambiae* Ifakara and against pyrethroid resistant *An. arabiensis* Kingani which carries metabolic resistance mediated by the over-expression of cytochrome P450 enzymes.

3 Overall quality conclusions

Based on the studies and information provided, all data requirements for the prequalification assessment of product quality have been satisfied. These data have been relied upon to assess the formulation, manufacturing process, physical/chemical characteristics, biological regeneration time, and bioavailability using products prepared with a defined wash interval of the proposed product for the purpose of establishing the identity of the product and assuring that the product can be produced consistently.

The methods for assessing the physical/chemical properties of the product were CIPAC methods and/or validated methods.

The quality component of the dossier is considered complete, and the assessment of the submitted information on quality supports prequalification of the product.

Table 9. List of studies submitted to WHO as part of the prequalification dossier	
Studies that were relied upon for decision making	
Study number	Study title
BIT 030	Phase I equivalency evaluation of Yorkool G3 long-lasting insecticidal nets compared to Tsara Boost and Olyset Plus long-lasting insecticidal nets using laboratory reared strains of pyrethroid susceptible <i>Anopheles gambiae</i> s.s. and pyrethroid resistant <i>Anopheles arabiensis</i> in Tanzania.
BIT030	Chemical content analysis of Yorkool G3 LN at the Ifakara Health Institute (IHI) in Tanzania
TSNT01152359, 2440, 20199, and 21046	Yorkool G3 LN - Quality evaluation of deltamethrin and piperonyl butoxide long lasting (incorporated into filaments) insecticidal net
Studies that were not used to inform decision making	
None	

4 Manufacturing release specifications

4.1 Summary of manufacturing release specifications

Table 10. Summary of manufacturing release specifications			
Description			
The material shall be in the form of netting, consisting of 130 denier* knitted mono-filament polyethylene yarn, incorporating technical deltamethrin complying with the requirements of WHO specification 333/TC (current version) and with technical piperonyl butoxide complying with the requirements of WHO specification 33/TC (current version) together with any necessary other formulants.* The product shall appear clean and shall be free from visible extraneous matter,* visible damage (such as splitting or tearing) and visible manufacturing defects (such as poorly made seams or a weave that is either not uniform or too loose to remain uniform in use) and shall be suitable for use as an insecticidal net with long-lasting activity.*			
ID	Property	Method	Declared value
1*	Sampling Plan	See Appendix 2	
2*	Deltamethrin content	CIPAC (N) 333/LN/(M2)/3, p. 34 2012	3.0 g/kg ± 25%
3	Piperonyl butoxide content	CIPAC (N) 33/LN/(M)/3, p. 111 2012	11.0 g/kg ± 25%
4*	Deltamethrin wash resistance index	MT195, CIPAC Handbook O, p. 205, 2017	Within the range 93% to 100%
5*	Piperonyl butoxide wash resistance index	MT195, CIPAC Handbook O, p. 205, 2017	Within the range 90% to 100%
6	Fabric weight	ISO 3801 / EN 12127	40 g/m ² ± 10%
7*	Bursting strength – fabric	ISO 13938:2	Not less than 400kPa
8*	Bursting strength – seam	ISO 13938:2	Not less than the average bursting strength for fabric
9*	Netting mesh size	See Appendix 2	Average ≥ 17 holes/cm ² Min. 16 holes/cm ²

* Indicates that additional information is available in Appendix 2.

Manufacturers are expected to rely on the information above as part of a QC management plan and for validation of product quality when released. To the extent required, Certificates of Analysis to support the release of products should present results for the attributes identified in the above table.

4.2 Storage

Accelerated storage stability data were generated as per CIPAC MT 46.3. Test samples were stored for 14 days at 54°C. No significant differences were recorded among the properties of the product kept at ambient temperature and after accelerated storage stability test conditions.

Products should be stored and transported in appropriate conditions in accordance with the recommendations of the manufacturer.

Where products have been subjected to prolonged storage or adverse conditions during storage, analysis and testing are recommended to assess changes in characteristics and their suitability for use.

Appendix 1. Summary of available data considered in Module 3

Batches used to generate the physical/chemical data

Batch Number	Date	Formulation	Uses
20180074	05/2018	-	-
20180515	05/2018	-	In-use stability (laboratory), semi-field study
20180516	05/2018	-	Semi-field study
20200326	03/2020	-	Semi-field study
20200415	04/2020	-	Semi-field studies
20200513	05/2020	-	-
20200518	05/2020	-	Semi-field study

Product characteristics

Studies TSNT01152359, 2440, 20199, and 21046

Property	Batch ID	Test Method	Results
Deltamethrin mean content	20180516	CIPAC(N) 333/LN/(M2)/-	3.07 g/kg (RSD 2.426%)
	20200415		3.48 g/kg (RSD 0.96%)
	20200513		3.45 g/kg (RSD 0.73%)
Deltamethrin distribution (Numbers in parenthesis indicate percentage of target dose)	20180515	CIPAC(N) 333/LN/(M2)/-	3.02-3.33 g/kg (100.7-111.0%)
	20180516		2.965-3.188 g/kg (98.8-106.3%)
	20200326		3.01-3.16 g/kg (100.3-105.3%)
	20200415		3.43-3.52 g/kg (114.3-117.3%)
	20200513		3.42-3.48 g/kg (114.0-116.0%)
	20200518		3.01-3.16 g/kg (100.3-105.3%)
Piperonyl butoxide (PBO) mean content	20180516	CIPAC (N) 33/LN/(M)/-	12.32 g/kg (RSD 1.197%)
	20200415		11.56 g/kg (RSD 0.78%)
	20200513		11.55 g/kg (RSD 0.35%)
PBO distribution (Numbers in parenthesis indicate percentage of target dose)	20180515	CIPAC (N) 33/LN/(M)/-	11.95-12.23 g/kg (108.6-111.2%)
	20180516		12.059-12.562 g/kg (109.6-114.2%)
	20200326		11.04-12.05 g/kg (100.4-109.5%)
	20200415		11.44-11.64 g/kg (104.0-105.8%)
	20200513		11.51-11.61 g/kg (104.6-105.5%)
	20200518		11.05-12.05 g/kg (100.5-109.5%)
<i>R</i> -alpha deltamethrin content	20180516	CIPAC (O) 333/TC	0.022 g/kg (~0.7% wrt Deltamethrin)
	20200415		Nil
	20200513		Nil
Wash resistance index (Deltamethrin)	20180516	CIPAC (O) MT 195	98.43%
	20200415		97.16%
	20200513		97.08%
Wash resistance index (PBO)	20180516	CIPAC (O) MT 195	98.26%
	20200415		92.67%
	20200513		92.61%
Physical state of netting	20180515	OPPTS 830.6303	Clean with no visible defects
	20180516		
	20200326		
	20200415		
	20200513		
	20200518		
Colour	20180515	OPPTS 830.6302	Blue
	20180516		Light blue
	20200326		Blue
	20200415		Blue
	20200513		Blue
	20200513		Blue

Property	Batch ID	Test Method	Results
	20200518		Blue
Odour	20180516 20200415 20200513	OPPTS 830.6304	Mild plastic-like No specific odour No specific odour
Dimensional stability	20180074 20180515 20200326 20200415 20200513 20200518	ISO3759-2011 / ISO5077-2007 / ISO6330-2012	-2.8%, +0.3% -2.5%, +2.2% -1.8%, +2.1% -1.9%, +2.4% -1.9%, +2.1% -1.8%, +2.1%
Bursting strength	20180074 20180515 20200326 20200415 20200513 20200518	ISO 13938-2-1999 (30mm Diaphragm Diameter)	440 kPa 485 kPa 461 kPa 493.5 kPa 497.9 kPa 461 kPa
Seam bursting strength	20180074 20180515 20200326 20200415 20200513 20200518		445/506 kPa 515/555 kPa 512/535 kPa 565.1/592.6 kPa 551.8/565.9 kPa 512/535 kPa
Mesh size	20180074 20180515 20200326 20200415 20200513 20200518	ISO 139 (1973)	16-18 holes/cm ² (mean 17) 19 holes/cm ² 19 holes/cm ² 19-21 /cm ² (mean 20) 19-21 /cm ² (mean 20) 19 holes/cm ²
Fabric weight	20180074 20180515 20200326 20200415 20200513 20200518	ISO 3801/EN 12127 EN 20139-1992	37.7 g/m ² 38 g/m ² 39 g/m ² 40.82 g/m ² 40.75 g/m ² 39 g/m ²
Fabric weight (conditioned)	20180516		39.154 g/m ²
Fabric weight (oven-dry)	20180516		37.977 g/m ²
Flammability	20180074 20180515 20200326 20200513 20200415 20200518	EN 1102:2016	No ignition or propagation. Maximum hole length 100 mm, width 23 mm.

Storage stability

Studies TSNT01152359, 20199, and 21046:

Property	Batch ID	Before	After	Change
Deltamethrin content	20180516	3.07 g/kg	2.999 g/kg	-0.071 g/kg (-2.3%)
	20200415	3.48 g/kg	3.39 g/kg	-0.09 g/kg (-2.6%)
	20200513	3.45 g/kg	3.35 g/kg	-0.10 g/kg (-2.9%)
PBO content	20180516	12.32 g/kg	12.206 g/kg	-0.114 g/kg (-0.9%)
	20200415	11.56 g/kg	11.25 g/kg	-0.31 g/kg (-2.7%)
	20200513	11.55 g/kg	11.23 g/kg	-0.32 g/kg (-2.8%)
Wash resistance index (Deltamethrin)	20180516	98.43%	98.95%	+0.52%
	20200415	97.16%	96.72%	-0.44%
	20200513	97.08%	96.59%	-0.49%
Wash resistance index (PBO)	20180516	98.26%	97.36%	-0.90%
	20200415	92.67%	92.77%	+0.10%
	20200513	92.61%	92.75%	+0.14%
Dimensional stability	20180074	-2.8%, +0.3%	+0.3%, -2.0%	-
	20200415	-1.9%, +2.4%	-2.1%, +2.4%	-
	20200513	-1.9%, +2.1%	-2.1%, +2.2%	-
Bursting strength	20180074	440 kPa	411 kPa	-29 kPa
	20200415	493.5 kPa	481.7 kPa	-11.8 kPa
	20200513	497.9 kPa	482.4 kPa	-15.5 kPa
Seam bursting strength	20180074	≥ 445 kPa	≥ 416 kPa	-29 kPa
	20200415	≥ 565.1 kPa	≥ 541.1 kPa	-24.0 kPa
	20200513	≥ 551.8 kPa	≥ 531.2 kPa	-20.6 kPa

Appendix 2. Manufacturing release specifications: methods and notes

Description

- The linear density (denier) of the yarn cannot be measured in the netting or the manufactured bed net but it should be identified on the packaging.
- Two different master batches of polyethylene are used together for the yarn, one with deltamethrin and the other one with piperonyl butoxide. The yarn contained the active ingredient and synergist are used for the net by knitting.
- Occasional short lengths of loose thread present in the netting are not considered to be extraneous matter.

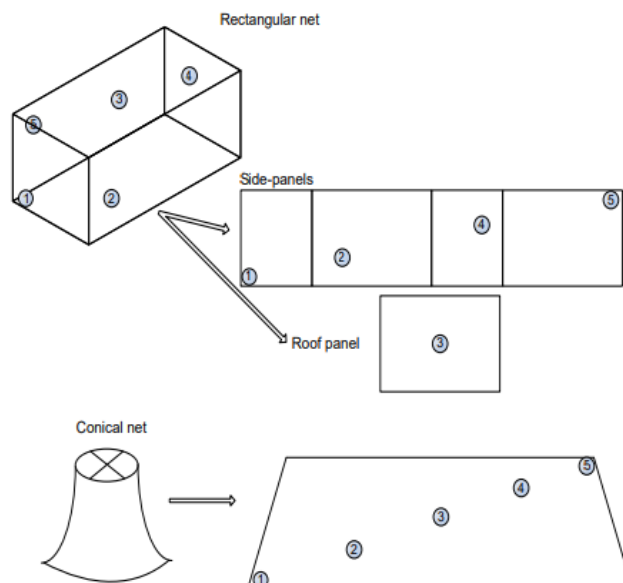
Long-lasting insecticidal netting is expected to retain its insecticidal activity during its lifespan and through a number of washes (public health products) or in worst-case expected climatic conditions (agricultural products)

Sampling Plan – Applicable to all attributes for which samples are to be taken from various parts of the constructed ITN.

- Sampling should be noted as Figure 1:

Figure 1 General method for sampling rectangular and conical nets

Recommended positions from which 5 pieces of netting should be taken from a made up bed net and combined to form a representative sample.



Samples should be taken according to Figure 1 or on a convenient diagonal across the width of bulk material. Samples must be sufficiently large to conduct all tests required and representative of the net or netting. Except where seams are to be tested, do not test material within 10 cm of seams or selvages. Where a final product is made from more than one type of netting, each type of netting should be sampled and tested separately.

Use sharp scissors, or equivalent, to minimize damage to the fibres and fabric and thus avoid any consequential bias in the results of certain tests. Roll up the strips or squares and place them in labelled, new, clean aluminium foil prior to analysis. Samples should be kept cool, avoiding heat sources (including direct sunlight) or freezing, and analyzed/tested with minimum delay. Representative portions (sub-samples) for testing should be taken as described in each test method.

For the purposes of chemical analysis, the analytical method and the number and size of test portions analyzed should be designed to provide results with a relative standard deviation (RSD) $\leq 5\%$ or as applicable in certain justifiable cases. Test portion and replication requirements for physical test methods are defined in the methods or Notes referenced.

Attribute 2: Deltamethrin content

For complete identification and good quantification, deltamethrin which is a single pyrethroid stereoisomer consisting of $[\alpha S, 1R, 3R]$ -isomer (also known as the S-isomer) must be separated from the $[\alpha R, 1R, 3R]$ -isomer (otherwise known as the R-isomer), which is not part of the active ingredient and not a relevant impurity. These diastereomers may be separated by non-chiral techniques as provided in the CIPAC method for deltamethrin.

Attributes 4 and 5: Deltamethrin and piperonyl butoxide wash resistance index

The content of deltamethrin in the net pieces before and after washing should be determined by the method 333/LN/(M2)/3, CIPAC Handbook N, p.34, 2012. The content of piperonyl butoxide in the net pieces before and after washing should be determined by the method 33/LN/(M)/3, CIPAC Handbook N, p.111, 2012.

Attributes 7 and 8: Bursting strength – fabric and bursting strength – seam

Test method: ISO 13938 part 2 with conditioning of the fabric as specified in the ISO standard. The declared bursting strength, and testing for compliance with it, should be based on tests of 7.3 cm² areas of fabric. Proposed specifications based on tests of 50 cm² area must be supported by data showing the suitability of the proposed value and its relationship to minimum of 250 kPa (which is based on 7.3 cm² area). Five replicate tests should be conducted on samples taken at approximately equal distances on a diagonal across the netting, taking no sample within 10 cm of a border or seam. In made up rectangular nets, the “diagonal” may correspond to figure 1. The average of the 5 measurements is calculated.

The method to test seam bursting strength is identical to that used to test the fabric, except that 5 replicate tests should be made, with the seam centred on the test head. Up to 5 seams may be tested but, if there are < 5 seams, replicate measurements should be made on 1 or more seams, to provide a total of 5 measurements.

Attribute 9: Mesh size

In the absence of a simple or standard method to determine the size of holes, which may have complex shapes, in highly flexible fabrics, mesh size is determined by counting the number of holes in a square of the fabric. Counting may be done directly on the fabric or indirectly by taking a picture/photocopy of the fabric. Indirect methods may ease counting and provide a permanent record. The number of holes per measured area is converted in holes/cm². Before counting, the fabric should be conditioned according to ISO 139 (4 h, 20°C, 65% relative humidity).

Use a template to define the square of netting, taking care not to stretch or distort the fabric. The template should be a 1-2 mm thick rigid sheet, in/on which an accurately calibrated ($\pm 1\%$ in each dimension) square (e.g., 1 x 1 in or 5 x 5 cm) has been cut/marked. If a template is not available and a ruler must be used, great care is required to ensure that the area counted is square. Where practicable, one edge of the square to be counted should be aligned with a row of complete holes in the fabric. Incomplete holes $\geq \frac{1}{2}$ are counted as complete holes, whereas those $< \frac{1}{2}$ are not counted. Count 5 replicate squares selected according to the sampling plan, calculate the average and note the lowest value.

Another suitable method is the use of a stereomicroscope with an image analyser software, where the number of holes in a defined area is counted. In case of discrepancy between the netting mesh size using stereomicroscopic method and direct or indirect counting method, the stereomicroscopic method shall be the referee method.