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ICS 59.080.30

DRAFT EAST AFRICAN STANDARD

Long lasting insecticide treated mosquito nets — Specification

EAST AFRICAN COMMUNITY

2021

Third Edition

Copyright notice

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Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the Principles and procedures for development of East African Standards.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

The committee responsible for this document is Technical Committee EASC/TC 061, Textiles, textile products and accessories.

Attention is drawn to the possibility that some of the elements of this document may be subject of patent rights. EAC shall not be held responsible for identifying any or all such patent rights.

This third edition cancels and replaces the second edition EAS 455:2019, which has been technically revised.

The main changes compared to the previous edition are as follows:

— reduction of the minimum linear density for the 100 % polyester mono-treated nets from 100D to 75D

— expression of the linear density requirement as a minimum and not a range

— expression of mesh count as a minimum and not a range

— adjusting the mesh count specifications to cater for nets made out of 150D

— adjusting the bursting strength requirement to cater for nets made out of 75D

— alignment of the active ingredients in combination nets as per WHO interim specifications

Long lasting insecticide treated mosquito nets —Specification

1 Scope

This Draft East African Standard specifies requirements, sampling and test methods for treated Long Lasting Insecticidal Nets (LLIN).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EAS 124, *Rounding off number values*

ISO 105-B01, *Textiles — Tests for colour fastness — Part B01: Colour fastness to light: Daylight*

ISO 105-B02, *Textiles -- Tests for colour fastness — Part B02: Colour fastness to artificial light: Xenon arc fading lamp test*

ISO 105-C10, *Textiles — Tests for colour fastness — Part C10: Colour fastness to washing with soap or soap and soda*

ISO 139, *Standard atmospheres for conditioning and testing*

ISO 1833-1, *Textiles — Quantitative chemical analysis — Part 1: General principles of testing*

ISO 1833-2, *Textiles — Quantitative chemical analysis — Part 2: Ternary fibre mixtures*

ISO 1833-5, *Textiles — Quantitative chemical analysis — Part 5: Mixtures of viscose, cupro or modal and cotton fibres (method using sodium zincate)*

ISO 1833-11, *Textiles — Quantitative chemical analysis — Part 11: Mixtures of cellulose and Polyester fibres (method using sulphuric acid)*

ISO 1833-16, *Textiles — Quantitative chemical analysis — Part 16: Mixtures of polypropylene fibres and certain other fibres (method using xylene)*

ISO 1833-24, *Textiles — Quantitative chemical analysis — Part 24: Mixtures of polyester and some other fibres (method using phenol and tetrachloroethane)*

ISO 1833-25, *Textiles — Quantitative chemical analysis — Part 25: Mixtures of polyester and certain other fibres (method using trichloro acetic acid and chloroform)*

ISO 2060, *Textiles — Yarn from packages — Determination of linear density (mass per unit length) by the Skein method*

ISO 2076, *Textiles — Man-made fibres — Generic names*

ISO 3758, *Textiles — Care labelling code using symbols*

ISO 3759, *Textiles — Preparation, marking and measuring of fabric specimens and garments in tests for determination of dimensional change*

ISO 3801, *Textiles — Woven fabrics — Determination of mass per unit length and mass per unit area*

ISO 5077, *Textiles — Determination of dimensional change in washing and drying*

ISO 6330, *Textiles — Domestic washing and drying procedures for textile testing*

ISO 6938, *Textiles — Natural fibres — Generic names and definitions*

ISO 8388, *Knitted fabrics — Types — Vocabulary*

ISO 8499, *Knitted fabrics — Description of defects — Vocabulary*

ISO 13938 *(all parts), Textiles — Bursting properties of fabrics*

ISO 16373-1, *Textiles — Dyestuffs — Part 1: General principles of testing coloured textiles for dyestuff identification*

ISO 16373-2, *Textiles — Dyestuffs — Part 2: General method for the determination of extractable dyestuffs including allergenic and carcinogenic dyestuffs (method using pyridine-water)*

ISO 16373-3, *Textiles — Dyestuffs — Part 3: Method for determination of certain carcinogenic dyestuffs (method using triethylamine/methanol)*

ISO/TR 11827, *Textiles — Composition testing — Identification of fibres*

3 Terms and definitions

For the purposes of this Standard, the following terms and definitions and those in ISO 6938 and ISO 2076 shall apply

3.1
Long Lasting Insecticidal Nets (LLIN)
factory treated mosquito net made with a netting material that has insecticide incorporated in the yarn or coated on the net

3.2
mono-treated net
finished net treated with a single active ingredient

3.3
combination net
finished net treated with two or more active ingredients

3.4
active ingredients
biologically active substances that form part of the formulation mixture that are incorporated or coated in LLIN as approved by World Health Organization or any other internationally recognized body

3.5
top ring
used in conical nets and made of non-corrosive, anti-rust and anti-buckling material, fixed to the roof of the net

3.6
height
dimension measured along a vertical seam from the top to the bottom edge of the net

3.7
circumference
perimeter of the net at its bottom edge

4 Requirements

4.1 Mono-treated nets

4.1.1 Physical characteristics

4.1.1.1 For long lasting insecticidal mosquito nets, the type of filaments for the fabrics (determined through visual examination) shall be as follows:

a) polypropylene: mono/multi-filament

b) polyester: multi-filament

c) polyethylene: mono/multi-filament

4.1.1.2 Long lasting insecticidal mosquito nets shall conform to the physical characteristics specified in Table 1 when tested in accordance with the methods prescribed therein.

Table 1 — Physical characteristics for long lasting insecticidal mosquito nets

Parameter		Requirement			Test method
		Polyester net	Polypropylene net	Polyethylene net	
Fibre composition		100 % polyester	100 % polypropylene	100 % polyethylene	ISO 1833-1 ISO 1833-2 ISO 1833-16 ISO 1833-24 ISO/TR 11827
Linear density (Denier), min.		75	100	100	ISO 2060
Mesh count, holes/cm^2, min.		24 for 75D and 100D and. 10.6 for 150D	20	8	Annex F
Mass per unit area, g/m^2, min.		28.8	35	28.8	ISO 3801
Bursting strength at 7.3 cm^2, kPa, min.		250 for 75D 350 for 100D and 380 for 150D	350	250	ISO 13938-1 ISO 13938-2
Dimensional stability after 1 wash at 30 °C normal, %, max.	Shrinkage	10	10	10	ISO 5077 ISO 6330 ISO 3759
	Expansion	5	5	10	

Colour fastness (if coloured) to:	Light	4 or better	ISO 105-B01, ISO 105-B02 ISO 105-C10
	Washing	4 or better	
Seam		Sewn using 100 % polyester or100% polypropylene filament thread	ISO 1833-24 ISO 1833-16

4.1.2 Active ingredient

For mono-treated nets, the active ingredient shall comply with the requirements of Table 2 when tested in accordance with the methods prescribed therein.

Table 2 — Requirements for active ingredient for mono-treated nets

S/No.	Ingredient[a]	Requirement g/kg	Test method
I	Deltamethrin	1.05 – 3.50	Annex A
ii	Permethrin	15.00 – 25.00	Annex B
iii	Alpha –cypermethrin	3.38 – 5.63	Annex C

[a] In the event that an active ingredient other than those already mentioned above is used, it shall be as recommended by WHO (http://www.who.int/whopes/quality/) or any other internationally recognized body and their affiliated entities published specifications for public health pesticides for treated mosquito nets.

4.2 Combination nets

4.2.1 Combination nets with single fibre construction shall comply with the requirements of Table 1 and when the following two active ingredients are used, they shall comply with the requirements of Table 3 when tested in accordance with the methods prescribed therein.

Table 3 — Requirements for active ingredient for combination nets

S/No.	Ingredient[a]	Requirement	Test method
i	Permethrin	20 g/kg ± 25 %	Annex B
ii	Piperonyl butoxide (PBO)	10 g/kg ± 25 %	Annex D

[a] In the event that an active ingredient other than those already mentioned above is used, it shall be as recommended by WHO (http://www.who.int/whopes/quality/) or any other internationally recognized body and their affiliated entities published specifications for public health pesticides for treated mosquito nets.

4.2.2 Combination nets constructed with more than one fibre shall comply with the requirements of Table 4 when tested in accordance with the methods prescribed therein

Table 4 — Requirements for combination nets

Parameter		Requirement		Test method
		Roof	Sides	
Fibre composition		Polyethylene	Polyester	ISO 1833-1 ISO 1833-2 ISO 1833-16 ISO 1833-24 ISO 1833-25 ISO/TR 11827
Linear density (Denier), min.		100	75 with strengthened 70 cm lower border or 100 without border	ISO 2060
Mesh count, holes/cm^2, min.		15.5	24 for 75D and 100D and 10.6 for 150D	Annex F
Mass per unit areat, g/m^2 , min.		28.8	28.8	ISO 3801
Bursting strength at 7.3 cm^2, kPa, min.		300	250 for 75 Denier and 350 for 100 Denier	ISO 13938 -1 ISO 13938-2
Dimensional stability after 1 wash at 30 °C normal, %, max.	Shrinkage	10	10	ISO 5077 ISO 6330
	Expansion	5	5	
Colour fastness (if coloured) to:	Light	4 or better		ISO 105-B01 ISO 105-B02 ISO 105-C10
	Washing	4 or better		
Active ingredient[a], g/kg		PBO: 25.0 ± 25% DM: 4.0g ± 25%	Deltamethrin: 2.8 ± 25 % for 75D and 2.1 ± 25 % for 100D and 150D	Annex D Annex A

[a] As per WHO (http://www.who.int/whopes/quality/) or any other internationally recognized body and their affiliated entities published specifications for public health pesticides for treated mosquito nets.

4.3 Shapes, sizes and dimensions

4.3.1 Shape

The mosquito net shall be rectangular or conical in shape unless otherwise agreed between the purchaser and the supplier.

4.3.2 Sizes and dimensions

The nets shall be supplied in sizes and dimensions as specified in Table 5 and Table 6 for rectangular and conical nets respectively or in other sizes, as the purchaser may require

Table 5 — Sizes and dimensions for rectangular net

S/No.	Net size	Width, min. cm	Length, min. cm	Height, min cm	Test method
i	X-small	70	120	150	Annex E
ii	Small	100	180	170	
iii	Medium	130	180	170	
iv	Large	160	180	170	
v	X- Large	190	180	170	

Table 6 — Sizes and dimensions for conical nets

S/No.	Net size	Height, cm, min.	Bottom circumference, cm, min.	Centre circumference, cm, min.	Top ring diameter, cm, min.	Test method
i	Dome shaped cover net	50	230	-	-	Annex E
ii	X small	120	300	170	40	
iii	Single fitted conical	180	850	470	56	
iv	Double fitted conical	220	1 050	550	56	
v	Extra double fitted	250	1 250	655	65	

4.4 Manufacture and workmanship

4.4.1 Construction

4.4.1.1 Reinforcement at bottom

The bottom edge of the walls shall be over-lock stitched or reinforced (self-edge finish or self-binding selvedge). The hem shall be firm and capable of withstanding the normal conditions of use.

NOTE A sheeting border may be used to improve the lifespan of nets.

4.4.1.2 Seams and stitching

When visually examined, the seams shall be of even tension throughout and loose ends securely fastened off. The net seams shall be made with overlock stitch and the number of stitches per decimetre shall be 27 to 40. The stitching shall be made by using polyester or polypropylene multifilament sewing thread of matching shade.

4.4.1.3 Knit type

The net shall be in any one of the warp knitted constructions, for example raschel and tricot as specified in ISO 8388.

4.4.2 Net attachments or tying tapes

4.4.2.1 Rectangular nets shall be equipped with non-rusting rings or fabric loops.

4.4.2.2 Conical nets shall be equipped with a loop and non-rusting rings.

4.4.2.3 Each ring/loop shall have a corresponding string for suspensions.

4.4.3 Top support ring

Conical nets shall have a top support ring made of non-rusting material which can securely hold the net when hanged at the required position.

4.4.4 Defects

The mosquito net shall appear clean, be free from visible extraneous matter, visible damage (such as splitting or tearing) and visible manufacturing defects (such as poorly made seams) as described in ISO 8499

5 Restricted Colorants

The mosquito nets (if coloured) shall be free from listed amines and carcinogenic dyestuffs specified in ISO 16373, 1, 2 & 3. Dyestuff classes are identified in accordance with ISO 16373

6 Packaging

Mosquito nets shall be packaged in suitable materials so as to prevent soiling and damage during transportation and storage.

7 Labelling

7.1 Outside packaging

The following information shall be legibly and indelibly marked on the outside packaging of individual nets:

a) fibre composition;

b) linear density;

c) mass per unit area;

d) net size and dimensions in centimetres (height, width and length for rectangular nets or diameter and height for conical nets)[see Annex F];

e) country of origin;

f) colour;

g) name and physical address of the manufacturer and/or importer/distributor;

h) lot or batch number;

i) name and amount (in grams per kilogram) of insecticide incorporated in the net; and

j) care instructions in accordance with ISO 3758.

7.2 Tag

The following information shall be legibly and indelibly marked on a permanent tag attached to individual nets: brand name or registered trademark;

a) fibre composition;

b) linear density;

c) mass per unit area;

d) net size and dimensions in centimetres (height, width and length for rectangular nets or diameter and height for conical nets;

e) country of origin;

f) name of the manufacturer and/or importer/distributor;

g) lot or batch number;

h) name and amount (in grams per kilogram) of insecticide incorporated or coated in the net;

i) the efficacy of the chemical indicated in terms of the number of washes; and

j) care instructions in accordance with ISO 3758.

8 Sampling

8.1 Lot

In any consignment all the pieces of mosquito nets delivered to a consignee against the same dispatch note shall constitute a lot.

8.2 Scale of sampling and testing

The number of pieces to be selected from a lot shall be in accordance with Table 7. Samples shall be tested from each lot for ascertaining conformity to the requirements of this standard.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with EAS 124.

Table 7 — Sampling plan

Number of pieces in a lot	Number of pieces to be sampled
Up to 8	2
9 to 15	3

16 to 25	4
26 to 50	5
51 and above	7

Annex A
(normative)

Determination of Deltamethrin content in long lasting insecticide treated mosquito nets by High Performance Liquid Chromatography

A.1 Sampling

A sample is defined as one finished bed net taken randomly from a batch of bed nets. Sub-sample by cutting 18 pieces of size 100 cm^2 randomly from the whole net. Cut 18 pieces into half, one portion is used for Deltamethrin content analysis and the other is used for washing test if required.

The portion used for Deltamethrin content analysis is cut into pieces of less than 2 cm x 2 cm each. Mix well. The sample shall be separately packed in aluminium foil and kept out of direct sunlight at room temperature or lower.

A.2 Identification tests

Use the HPLC method below. The retention time of Deltamethrin in the sample solution should not deviate by more than 15 s from that of the calibration solution if column oven is available.

A.3 Active ingredient

A.3.1 Outline of method

The sample is extracted in a mixture of iso-octane and 1,4-dioxane.

The Deltamethrin content is determined by normal phase high performance liquid chromatography using dipropyl phthalate as internal standard and detection at 236 nm.

A.3.2 Scope

This method is used for Deltamethrin determination in the net sample, before and after washing.

A.3.3 Reagents

A.3.3.1 **Iso octane**, HPLC grade

A.3.3.2 **1,4 Dioxan**, HPLC grade. Add 0.15 % (v/v) water before use.

A.3.3.3 **Deltamethrin**, neat standard

A.3.3.4 **Dipropyl phthalate**

A.3.3.5 **Water,** HPLC grade or high

A.3.3.6 **Extraction solvent (ES):** iso octane + 1,4 dioxane = 80 + 20

A.3.3.7 **Mobile Phase (MP):** iso octane + 1,4 dioxane = 95 + 5

A.3.3.8 Internal standard solution (IS), 0.5 mg/ml of dipropyl phthalate in extraction solvent

A.3.3.9 Deltamethrin standard solution, 0.6 mg/ml (DS). Weigh 0.03 g (to the nearest of 0.01 mg) of Deltamethrin neat standard, quantitatively transfer to a 50-ml volumetric flask, dissolve completely with extraction solvent, keep regulated by water bath at 20 ˚C, fill up to mark with extraction solvent.

A.3.4 Apparatus

A.3.4.1 Shaker

A.3.4.2 Ultrasonic bath

A.3.4.3 HPLC, equipment with pump, auto-injector, column oven (optional) and UV detector, Guard Column (Supelguard Si). Analytical Column Supelco Si 5 µm, 150 x 4.6 or Lichrosorb Si60, 5 µm, 150 x 4.6

A.4 Procedure

A.4.1 Operating conditions

A.4.1.1 Mobile Phase: iso octan + 1,4 dioxane = 95 + 5

A.4.1.2 Flow rate: 1.3 ml/min, isocratic

A.4.1.3 Guard Column: Supelguard Si

A.4.1.4 Analytical Column: Licherosorb Si 60, 5 µm, 150 x 4.6 40 °C if column oven is available, or room temperature

A.4.1.5 Inject volume: 5 µl

A.4.1.6 Wavelength: 236 nm

A.4.1.7 Run time: 6 min to 8 min

A.4.2 Preparation of calibration curve

A.4.2.1 Into a series of clean 20-ml PTFE liner screw cap vials, add accordingly as table below. Filter through a 0.45-µm syringe filter before use.

Code	IS, ml	DS, ml	ES, ml	Delta, mg	Total volume, ml
C1	1	0.5	13.5	0.30	15 ml
C2	1	0.7	13.2 ml	0.42	15 ml
C3	1	0.9	12.8 ml	0.54	15 ml
C4	1	1.1	12.4 ml	0.66	15 ml
C5	1	1.3	12.1 ml	0.78	15 ml

A.4.2.2 All standards should be kept in the refrigerator (at a temperature of 2 to 8°C) if not in use in well-tightened paraffin film sealed cap.

A.4.3 Calibration

Daily calibrate HPLC system with full series of 05 standards, beginning with the lowest level standard. The correlation coefficient should be less than 0.99 over this range.

A.4.4 Preparation of sample

Weigh (to the nearest 0.1 mg) into an Erlenmeyer or a screw cap neutral glass bottle (50-ml) sufficient sample to contain about 0.5 mg of Deltamethrin. For 75D, 100D and 150D netting sample, suitable weight (*w* g) is 0.3 g, 0.4 g and 0.6 g respectively. Add by pipette 1.0 ml internal standard solution. Add 14 ml extract solvent. Replace the cap closely.

Put the bottles into the ultrasonic bath, setting temperature 80 °C, running time 15 min. Vigorously shake the bottle using the shaker in 30 min at room temperature, speed of shaking is at level of 150 - 200 beats per minute.

Using a syringe membrane filter with pore size of 0.45 µm or finer, filter 1 ml of extract solution into clean amber. The sample shall be injected within 24 h since extraction; for longer waiting time the vial should be kept in a refrigerator.

A.4.5 Determination

The Deltamethrin content, expressed as grams per kilogram, shall be calculated as follows:

$$\frac{a}{w}$$

Where;

a is the Deltamethrin reading, in milligrams, from the analysis; and

w is the mass, in grams, of sample taken.

NOTE Conventional unit of active ingredient content (Deltamethrin) for netting is milligrams per square metre. The conversion from grams per kilogram (g/kg) to milligrams per square metre (mg/m^2) is made as follows:

Deltamethrin content (mg/m^2) = Deltamethrin content (g/kg) x D

Where;

D is the weight per square metre of typical polyester netting:

- 75 Denier netting, D = 30 g/m^2
- 100 Denier netting, D = 40 g/m^2
- 150 Denier netting, D = 60g/m^2

A.4.6 Method validation summary

A.4.6.1 Precision

Analyze 05 replicates RSD is 1.8 %.

A.4.6.2 Accuracy

Recovery of 04 Laboratory Synthetic samples is in the range of 97 % to 101 %.

Averaged at 99.3 %.

A.4.6.3 Linearity

Over the range of Deltamethrin from 0.30 mg to 0.78 mg, the correlation coefficient R^2 is observed not less than 0.995.

Determination of release /Retention Index

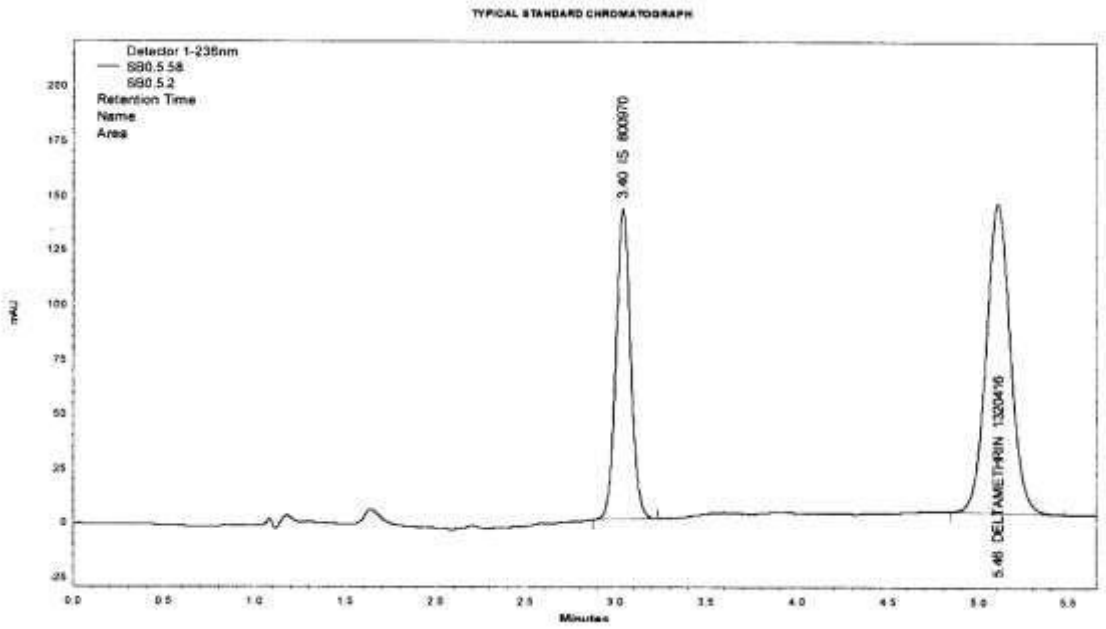

Figure A.1 — Chromatogram of calibration solution

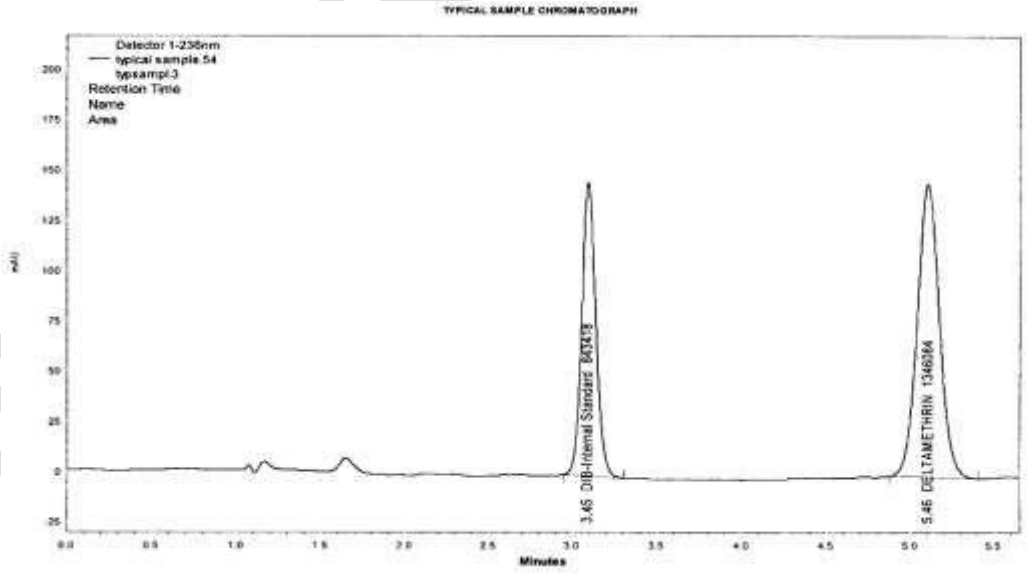

Figure A.2 — Chromatogram of sample

Annex B
(normative)

Determination of Permethrin content in long lasting insecticide treated mosquito nets

B.1 Sampling

Take at least 500 g.

B.2 Identity tests

B.2.1 GLC. Use the GLC method below. The retention times of *cis*- and *trans*-permethrin should not deviate by more than 1% from those of the permethrin standard and the intensities of the permethrin isomers should give the same pattern as in the standard (Fig. B.1).

B.2.2 Infrared. Extract the sample with a suitable solvent. Filter and evaporate the solvent. Prepare a film between NaCl plates and scan from 4000 to 400 cm^{-1}. The spectrum produced from the sample should not differ significantly from that of the standard (Fig. B.2)

B.3 Permethrin

The content of permethrin (sum of *cis*- and *trans*-isomers) is determined by capillary GC using flame ionisation detection and triphenyl phosphate as internal standard. The *trans*-isomer fraction is calculated from the chromatogram obtained.

B.3.1 Reagents

B.3.1.1 *Heptane*

B.3.1.2 *Acetone*

B.3.1.3 *Permethrin working standard* technical product of certified purity. Store refrigerated.

B.3.1.4 *Triphenyl phosphate* internal standard. Must not show peaks with the same retention times as *cis*- and *trans*-permethrin.

B.3.1.5 *Internal standard solution* Dissolve triphenyl phosphate (1.0g) in heptane (150ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.

B.3.1.6 *Calibration solution* Homogenise the permethrin working standard. When the permethrin is waxy solid or partly waxy solid homogenise it by warming it to melting and by stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1mg) 72 to 88 mg (*s* mg) of permethrin standard into a vial or stoppered flask (200ml). Add by pipette internal standard solution (10.0 ml) and dissolve. Add by measuring cylinder heptane (90ml) and mix well (solutions C_A and C_B).

B.3.2 Apparatus

B.3.2.1 *Gas chromatograph* equipped with a split/split less injection and a flame ionisation detector

B.3.2.2 *Capillary column* fused silica, 30 m x 0.25 mm (i.d.), film thickness: 0.25 µm, coated with crosslinked dimethyl polysiloxane (DB-1 or equivalent)

B.3.2.3 *Electric integrator or data system*

B.3.3 Procedure

B.3.3.1 Gas chromatographic conditions (typical)

B.3.3.1.1 ***Colum****n* fused silica, 30 m x 0.25 mm (i.d.), film thickness: 0.25 µm, coated with cross linked dimethyl polysiloxane (DB-1 or equivalent)

B.3.3.1.2 ***Injection system***

Injector split injection

Split flow approximately 100 ml/min

Injection volume 1µm

B.3.3.1.3 ***Detector*** flame ionisation

B.3.3.1.4 ***Temperatures***

Column oven 240℃

B.3.3.1.5 **Injection port** 265℃

B.3.3.1.6 ***Detector*** 265℃

B.3.3.1.7 ***Carrier gas*** helium, 30 cm/s

B.3.3.1.8 ***Retention times***

triphenyl phosphate: about 6.5 min

cis-permethrin: about 12.4 min

trans-permethrin: about 12.9 min

B.3.3.2 ***Linearity check*** Check the linearity of the detector response by injecting 1 µl of solutions with permethrin concentrations 0.5, 1 and 2 times that of the calibration solution before conducting analysis.

B.3.3.3 ***System equilibration*** Prepare two calibration solutions. Inject 1 µl portions of the first one until the response factors obtained for two consecutive injections differ by less than 1.0%. Then inject a 1 µl portion of the second solution. The response factor for this solution should not deviate by more than 1.0% from that for the first calibration solution, otherwise prepare new calibration solutions.

B.3.3.4 ***Preparation of sample solution*** Clean a pair of scissors with acetone before use. Cut the sample with scissors into 5 – 10 mm squares. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1mg) sufficient sample to contain 36 to 44 mg (w mg) of permethrin into a vial or stoppered flask (100 ml). Add by pipette internal standard solution (5.0 ml) and by measuring cylinder heptane (45 ml). Place the vial or stoppered flask in a water bath (85-90℃) for 45 min. Shake the vial or stoppered flask once or twice during the extraction. Filter a portion of each sample solution through a filter paper prior to analysis (solutions S_A and S_B).

B.3.3.5 ***Determination*** Inject in duplicate 1 µl portions of each sample solution bracketing them by injections of the calibration solutions as follows; calibration solution C_A, sample solution S_A, sample solution

S_A, calibration solution C_B, sample solution S_B, sample solution S_B, calibration solution C_A, and so on. Measure the relevant peak areas.

B.3.3.6 ***Calculation of permethrin content*** Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the permethrin contents of the

$$f_{1=}\frac{h \times S \times P}{H_{s \times 2}}$$

$$Content\ of\ permethrin = \frac{f \times H_w}{l_{q \times w}} g/Kg$$

where;

f_i is the individual response factor;

f is the mean response factor;

H_S is the total peak area of permethrin (cis-permethrin + trans-permethrin) in the calibration solution;

H_W is the total peak area of permethrin (cis-permethrin + trans -permethrin) in the sample solution;

I_r is the peak area of the internal standard in the calibration solution;

I_q is the peak area of the internal standard in the sample solution;

s is the mass, in milligrams, of permethrin working standard in the calibration solution;

w is the mass, in milligrams, of sample taken; and

P is the purity, in grams per kilograms, of permethrin working standard.

Repeatability, **r** = 1.6 g/kg at 20.3 g/kg active ingredient content

1.3 g/kg at 20.0 g/kg active ingredient content

0.9 g/kg at 18.7 g/kg active ingredient content

Reproducibility, R = 1.9 g/kg at 20.3 g/kg active ingredient content

1.5 g/kg at 20.0 g/kg active ingredient content

1.5 g/kg at 18.7 g/kg active ingredient content

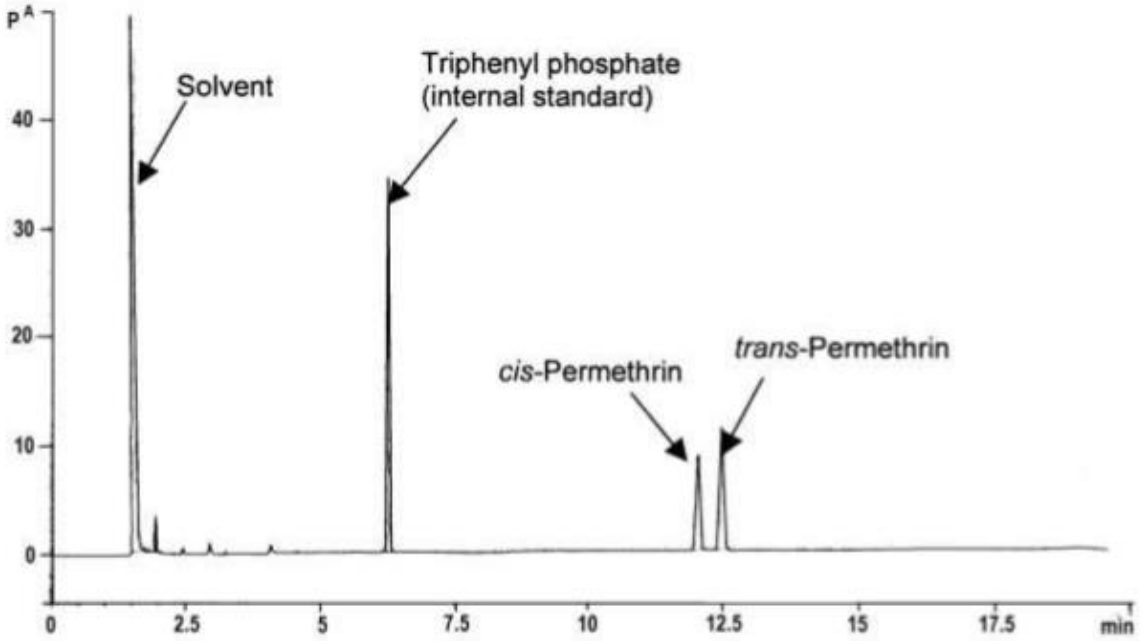

Figure B.1 — Example of gas chromatogram of permethrin LN

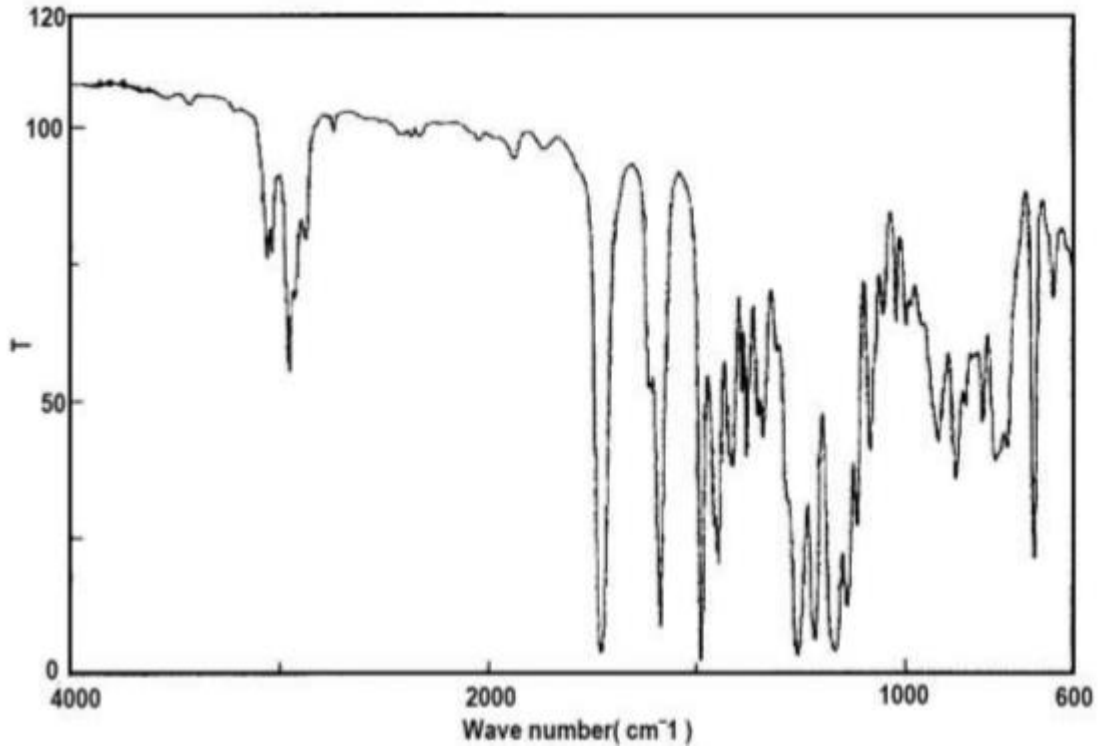

Figure B.2 — Infrared spectrum of permethrin

Annex C
(normative)

Determination of Alphacypermethrin content in long lasting insecticide treated mosquito nets

C.1 Sampling

Take net specimens.

C.2 Identity test

GLC capable of operating over a range 100 ºC to 300 ºC, fitted with a flame ionization detector

C.3 Alphacypermethrin

Alphacypermethrin 454/TC/(M)3 is dissolved in tetrahydrofuran and determined by capillary gas chromatography in split injection mode using flame ionization and internal standardization.

C.4 Reagents

C.4.1 Tetrahydrofuran

C.4.2 Aphacypermethrin standard of known purity, Di(2-ethylhexyl)phthalate(dioctyl phthalate, DOP), internal standard, purity at least 980 g/kg and giving no peaks with similar retention times to alphcypermethrin.

C.4.3 Citric acid 5 % solution, dissolve citric acid (25 g) in water (500 ml).

C.4.4 Internal standard solution, dissolve dioctyl phthalate (5.0 g) intetrahydrofuran (500 ml). Ensure sufficient quantity of this solution is prepared for all samples and calibration solutions to be analysed.

C.5 Calibration solutions

C.5.1 Preparation of calibration solutions

Weigh 50 mg alpha cypermethrin (to the nearest 0.1 mg) in a volumetric flask (25-ml). Fill to the mark with THF. Place the flask in an ultrasonic bath for 10 min. After temperature equilibrium pipette 1.50 ml, 4.50 ml of this solution into three volumetric flaks (50 ml). Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone) and fill up each to the mark with THF.

These solutions are used as calibration solutions A (C_A), B (C_B) and C (C_C). Transfer 200 µl out of each flask into separate GC vials. Add one drop of citric acid in each case and seal the vials. Place the vials into the sample tray (cooled down to 15 ºC) of the GC apparatus.

NOTE Citric acid is added to stop epimerization of alphacypermethrin in solution.

C.5.2 Description of calibration solutions

The calibration solutions are described as follows:

- C_A: concentration of approximately 3.0 mg alphacypermethrin in 50 ml THF;
- C_B: concentration of approximately 6.0 mg alphacypermethrin in 50 ml THF; and
- C_C: concentration of approximately 9.0 mg alphacypermethrin in 50 ml THF.

C.5.3 Apparatus

C.5.3.1 Gas chromatograph with flame ionization detector (for example, HP6890 Plus)

C.5.3.2 Automatic sample injector (for example, HP series 7683) equipped with a sample which is cooled down to 15 ºC

C.5.3.3 Capillary column fused silica (for example, DB-1) 30 m x 0.32 mm, film thickness of 0.25 µm

C.5.3.4 Electronic data evaluation system (for example, HP ChemStation)

C.5.3.5 Ultrasonic bath

C.5.3.6 Electronic balance

C.5.3.7 Laboratory glassware

C.6 Procedure

C.6.1 Operating conditions

As per alphacypermethrin 454/TC/(M)3

C.6.2 Preparation of the samples

C.6.2.1 Determination via the water/detergent extraction process

C.6.2.1.1 First step: Quantification of the adherent contents of alphacypermethrin on surface fibres

a) Procedure done by manufacturer

Place 2 g impregnated net in a beaker (one-litre). Add soap (commercially available bar soap or as flakes, 2g/l) and water (500 ml). The washing is done for 10 min at 30 ºC on a shaker with 155 movements/min. Directly after the washing, the wash liquor is acidified with 10 ml/l acetic acid 30% in order to prevent hydrolysis of the extracted alphacypermethrin (there is no difference in hydrolysis in neutral water compared to wash liquor after 10 min at 30 ºC). Transfer 150 ml of the washing liquid into a separation funnel (500-ml). The extraction procedure is done twice with 100 ml ethyl acetate each by shaking twice for 30 s. Both portions of ethyl acetate are combined together in a flask (250ml). Ethyl acetate is evaporated in a rotary evaporator. The flask is stoppered and sent to the analytical laboratory.

b) Procedure done by analytical laboratory

Fill up the flask with THF (50 ml). Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone). Transfer 200 µl of sample out of the flask into a separate GC vial. Add one drop of citric acid into the vial.

C.6.2.1.2 Second step: Quantification of the total contents of alphacypermethrin

Weigh (to the nearest 0.1 mg) 1 g of the impregnated net into a volumetric flask (100 ml) and add THF (50 ml). Place this flask in a refluxing apparatus. Heat up the flask in the apparatus and reflux at approximately 90 ºC (oil bath temperature). Sampling will be done after 5 min refluxing. Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone). Transfer 200 µl of the sample into separate GC vial. Add one drop of citric acid into the vial.

C.6.2.2 Determination via the n-hexane extraction process

C.6.2.2.1 First step: Quantification of the adherent contents of alphacypermethrin on the surface of the fibers

Weigh (to the nearest adherent drops of n-hexane) out of the flask into another volumetric flask (100-ml) and add THF (50 ml). Place this flask in a refluxing apparatus. Heat up the flask in the apparatus and reflux at approximately 90 ºC. Sampling will be done after 5 min refluxing. Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone). Transfer 200 µl of the sample into a separate GC vial. Add one drop of citric acid into the vial.

C.6.2.2.2 Second step: Quantification of the total contents of alpha-cypermethrin

Transfer the net (without adherent drops of n-hexane) out of the flask in to another volumetric flask (100-ml) and add THF (50 ml). Place this flask in a refluxing apparatus. Heat up the flask in the apparatus and reflux at approximately 90 ºC. Sampling will be done after 5 min refluxing. Add 0.5 ml of internal standard solution (dioctyl phthalate, 1 % in acetone). Transfer 200 µl of the same into a separate GC vial. Add one drop of citric acid into the vial.

C.6.2.3 System calibration

As for alphacypermethrin 454/TC/(M3)

C.6.2.4 Determination

Each calibration solution C_i and each sample solution S_J is injected twice. The following sequence is advised:

C_A , $C_{A,}$, C_B, C_B,C_C, $C_{C,}$ S_A, S_A,S_B, S_B,S_C,$S_{C,...}$

C.6.2.5 Calculation

As for alphacypermethrin 454/TC/(M)3 and see test of linearity

$$Concentration = \frac{f \times H_w}{l_{q \times w}}$$

Where;

f is the response factor

H_w is the total peak area of alphacypermethrin (cis +ll) in the sample solution

L_q s the peak area of internal standard in the sample solution

w is the mass of sample taken

C.6.2.6 Surface concentration and release index

a) Determination

The determination of alphacypermethrin content is done via the n-hexane extraction process from fibres.

Each calibration solution Cj and each sample solution Sj ai are injected twice. The following sequence is advised: C_A, C_A,C_B,C_B,C_B,C_C,C_C,S_{A1},S_{A1},S_{A2},S_{A2},S_{A3},S_{A3},S_{B1},S_{B1},...

- S_A is the first rinse; S_A mean value of S_{A1}, SA_2, S_{A3}
- S_B is the second rinse; S_B mean value of S_{B1}, S_{B2}, S_{B3}
- S_c is the third rinse; S_C mean value of S_{C1}, S_{C2}, S_{C3}

where $_{1,2,3}$ are net samples

b) Calculation of the release index

Calculate the mean value of sample solutions S_C and S_B by the equations described in C.6.2.5 and the release index for each piece of netting.

$$Release\ index = 1 - (S_c / S_B)$$

Where;

S_B refers to second rinse; S_B mean S_{B1}, S_{B2}, S_{B3}

S_C refers to third rinse; S_C mean value of S_{C1}, S_{C2}, S_{C3}

Annex D
(normative)

Determination of Piperonyl butoxide in polyethylene matrix in long lasting insecticide treated mosquito nets by Gas Chromatography

D.1 Scope

The method is suitable for the determination of piperonyl butoxide in polyethylene matrix.

D.2 Outline of method

The sample is extracted by refluxing with xylene. The piperonyl butoxide content is determined by capillary gas chromatography using flame ionisation detection and internal standard.

D.3 Reagents

D.3.1 Xylene

D.3.2 Piperonyl butoxide standard of known purity. Store below 0 °C.

D.3.3 Octadecane internal standard: Internal standard solution. Weigh (to the nearest 0.1 mg) into a volumetric flask (50 ml) octadecane (0.4 g). Fill to the mark with xylene and mix well.

Calibration solutions: Allow piperonyl butoxide to equilibrate to ambient temperature. Then weigh (to the nearest 0.1 mg) into a volumetric flask (50 ml) 0.25 g. Fill to the mark with xylene and mix well. To pipette 0.50 ml, 1.50 ml, 2.00 ml, 3.00 ml and 4.00 ml of this solution into five volumetric flasks (25 ml). Add 2 ml of internal standard solution and fill up each to the mark with xylene and mix well.

i. Five solutions are used as calibration solutions A (CA), B (CB), C (CC), D (CD), E (CE). Transfer 200 ml out of each flask into separate GC vials. Place the vial into the sample tray of GC apparatus.

ii. Description of the calibration solutions:

- CA: concentration of approximately 2.5 mg piperonyl butoxide in 25 ml xylene
- CB: concentration of approximately 7.5 mg piperonyl butoxide in 25 ml xylene
- CC: concentration of approximately 10.0 mg piperonyl butoxide in 25 ml xylene
- CD: concentration of approximately 15.0 mg piperonyl butoxide in 25 ml xylene
- CE: concentration of approximately 20.0 mg piperonyl butoxide in 25 ml xylene

D.4 Apparatus

D.4.1 Gas chromatograph capable of operating over the range 180 °C to 250 °C fitted with a flame ionisation detector, a split injector, and an auto sampler

D.4.2 Capillary column fused silica, 30 m x 0.32 mm (i.d.) with 100% methyl polysiloxane, cross-linked, surface bonded stationary phase and 0.25 µm film thickness (Durabond-1 or equivalent).

D.4.3 Electronic integrator or data system.

D.5 Operating Procedure

D.5.1 Operating conditions (typical)

a) Column: Fused silica, 30 m x 0.32 mm (i.d.) with 100 % methyl polysiloxane, cross-linked, surface bonded stationary phase and 0.25 µm film thickness (Durabond-1 or equivalent);

b) Injection system: Injector split injection;

c) Injector temperature 250 °C;

d) Split ratio 20:1;

e) Purge flow 1 ml/min;

f) Injection volume 1 µl;

g) Detector system: Type; Flame ionisation;

h) Temperature 300 °C;

i) Oven temperatures: Initial 180 °C;

j) Program;

- 180 °C hold for 11 min
- 200 °C at 10 °C/min, hold for 8 min
- 210 °C at 10 °C/min, hold for 18 min
- 245 °C at 30 °C/min, hold for 4 min
- Total run time 45 min

k) Gas flow rates;

- Helium (carrier) linear velocity: 39 cm/min at 180 °C
- Helium (make up) 30 ml/min
- Hydrogen 40 ml/min
- Air 400 ml/min

l) Total flow 35 ml/min; and

m) Retention time

- octadecane: about 6 min
- Piperonyl butoxide: about 23 min

D.5.2 Preparation of sample

Cut the sample with scissors into 1 cm to 2 cm squares and thoroughly mixed. Weigh (to the nearest 0.1 mg) sufficient sample to contain 12.5 mg of piperonyl butoxide into a 250-ml reflux flask. Add 23 ml xylene and 2 ml internal standard solution. Reflux the sample about 30 min. Cool down the sample to room temperature. Filter the solution through a 0.45-µm Teflon filter membrane. Transfer into a separate GC vial.

D.5.3 System equilibration

Inject into the gas chromatograph a 1-µl portion of the sample solution to condition the column and to check for the appropriate flow rates and integration events.

D.5.4 Determination

Inject in duplicate into the gas chromatograph 1-µl portions of the calibration and sample solutions in the following sequence; CA, CA, CB, CB, CC, CC, CD, CD, CE, CE, S1, S1, S2, S2, etc.

D.6 Calculation

The piperonyl butoxide content, shall be calculated as follows:

Concentration = R/w (g/kg)

Where;

R is the Piperonyl butoxide reading, in milligrams, from the analysis, and

w is the mass, in grams, of sample taken.

Annex E
(normative)

Measurement of mosquito net dimensions

E.1 Rectangular nets: Length, width and height

E.1.1 Apparatus

E.1.1.1 Flat table

E.1.1.2 Measuring tape or steel rule

E.1.2 Conditioning

Condition the net samples in accordance with ISO 139.

E.1.3 Procedure

Lay the conditioned net sample on a flat table (E.1.1.1), and take measurements of height, width and diameter.

E.1.4 Calculation

If more than one net sample is tested, take the average measurement for each dimension.

E.1.5 Report

Report the value of the net dimension as the average calculated in E.1.4 in centimetres.

E.2 Circular nets: Top ring diameter, bottom circumference and conical height

E.2.1 Apparatus

E.2.1.1 Hook supported at a vertical distance of at least more than the height of the net sample to be tested

E.2.1.2 Measuring tape or steel rule

E.2.1.3 Twine, of measuring at least 10 m

E.2.1.4 Felt pen marker

E.2.2 Conditioning

Condition the net sample in accordance with ISO 139.

E 2.3 Procedure

E.2.3.1 Top ring diameter

E.2.3.1.1 Procedure

Place the top portion of the net sample on a flat table (E.1.1.1) and put the twine (E.2.1.3) around the circumference of the top ring of the net sample, identifying the two ends with a marker (E 2.1.4) which represent the dimension of the top ring. Using a measuring tape (E.2.1.2) determine the top ring circumference (s) of the net as the distance between the two points marked on the twines. Repeat the test on each of the other net samples.

E.2.3.1.2 Calculation

Take the average of the individual measurements as the top circumference of the conical nets.

E.2.3.1.3 Report

Report the top ring circumference as the value(S) calculated in E.2.3.1.2 in centimetres.

E.2.3.2 Bottom circumference

E.2.3.2.1 Procedure

Lay the bottom part of the net on a flat table removing any curls and take the measurement (N) from one end of the flattened net to the other using a twine. Repeat the procedure for other net samples.

E.2.3.2.2 Calculation

Take the average of the measurements (N) taken in E.2.3.2.1.

Calculate the bottom circumference as N x 2.

E.2.3.2.3 Report

Report the value of bottom circumference of the net as the value (N x 2) calculated in E.2.3.2.2 in centimetres.

E.2.3.3 Conical net height

E.2.3.3.1 Procedure

Hang the net with the loop from a hook. Take measurements of height along all the vertical seams.

E.2.3.3.2 Report

Report the least measurement taken as the conical net height.

Annex F
(normative)

Determination of mesh count

F.1 Principle

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 scanning/photocopying the fabric. Indirect methods may ease counting and provide a permanent record. Before counting, the fabric should be conditioned according to ISO 139 (4 h, 20 ºC, 65 % relative humidity).

F.2 Procedure

Use a template to define the square of netting, taking care not to stretch or distort the fabric. The template should be a rigid sheet, 1 mm - 2 mm thick, in or on which an accurately calibrated (±1 % in each dimension) square (for example, 2 cm x 2 cm or 5 cm x 5 cm) has been cut or marked. If a template is not available and a ruler shall be used, great care is required to ensure that the area counted is square. If possible, at least one edge of the square to be counted should be aligned with a row of complete holes in the fabric. Count replicate squares in pieces selected and calculate the average and note the lowest value

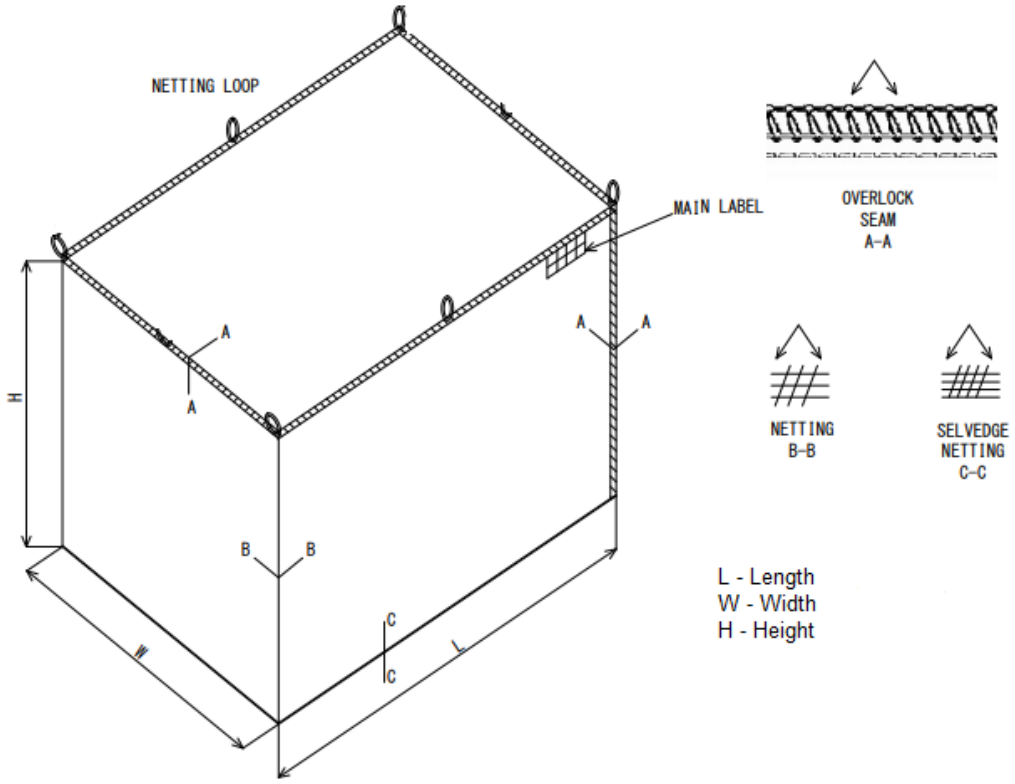

Figure 1 — Typical illustration of a rectangular mosquito net

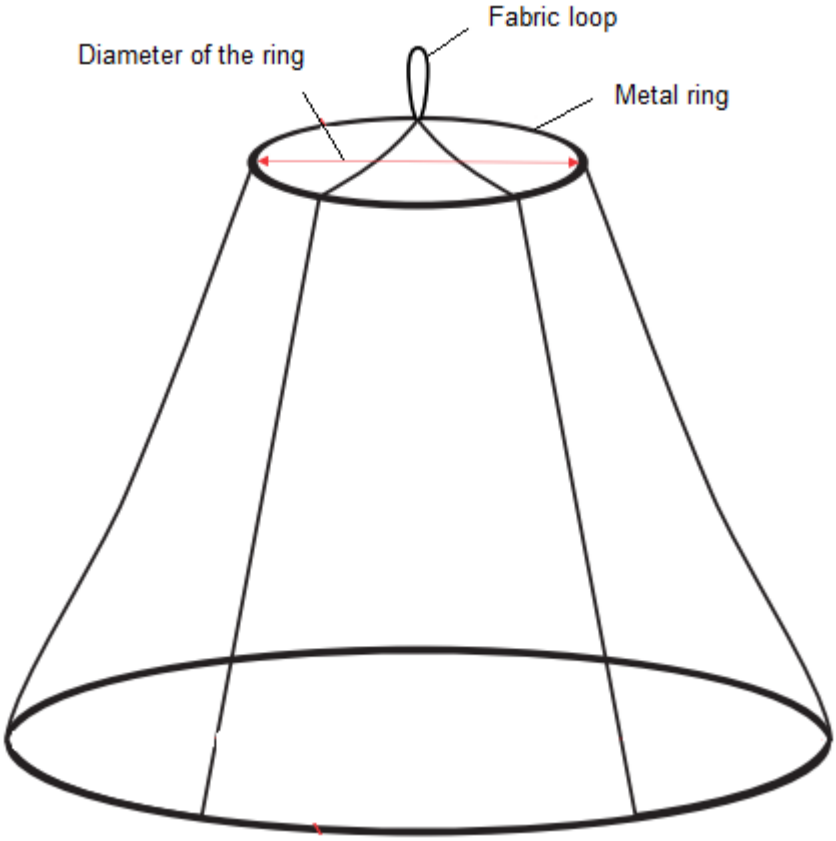

Figure 2 — Typical illustration of a conical mosquito net

Bibliography

[1] EAS 455:2019, *Long lasting insecticide treated mosquito nets — Specification*

[2] *WHO Interim specification 333/LN/1 (July 2019)*

[3] *WHO Interim specification 333/LN/2 (January 2019)*

[4] *WHO Interim specification 333+33/LN/1 (NETTING) (April 2019)*

[5] WHO Interim specification 333+33/LN/1 (NET) (April 2019)

[6] WHO Interim specification 333+33/LN/2 (NETTING) (January 2019)

[7] WHO Interim specification 333+33/LN/2 (NET) (January 2019)

