AFRICAN STANDARD

DARS 1601:2024

First Edition 2024

Long Lasting Insecticide Treated Mosquito Nets — Specification

Reference No. DARS 1601:2024(E)
ICS 55.080

Table of contents

DARS 1601:2024

Foreword

The African Organization for Standardization (ARSO) is an African intergovernmental organization established by the United Nations Economic Commission for Africa (UNECA) and the Organization of African Unity (AU) in 1977. One of the fundamental mandates of ARSO is to develop and harmonize African Standards (ARS) for the purpose of enhancing Africa's internal trading capacity, increase Africa's product and service competitiveness globally and uplift the welfare of African communities. The work of preparing African Standards is normally carried out through ARSO technical committees. Each Member State interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, Regional Economic Communities (RECs), governmental and non-governmental organizations, in liaison with ARSO, also take part in the work.

ARSO Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare ARSO Standards. Draft ARSO Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an ARSO Standard requires approval by at least 75 % of the member bodies casting a vote.

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

This African Standard was prepared by ARSO TC 053, *Textiles, textile products and accessories*

ARSO Central Secretariat
International House 3rd Floor
P. O. Box 57363 — 00200 City Square
NAIROBI, KENYA

Tel. +254-20-2224561, +254-20-3311641, +254-20-3311608

E-mail: arso@arso-oran.org
Web: www.arso-oran.org

Copyright notice

ARSO Central Secretariat
International House 3rd Floor
P.O. Box 57363 — 00200 City Square
NAIROBI, KENYA

Tel: +254-20-2224561, +254-20-3311641, +254-20-3311608

E-mail: arso@arso-oran.org
Web: www.arso-oran.org

DARS 1601:2024

Introduction

Malaria is a serious and sometimes fatal disease in Africa. More than 200 million children, and adults are affected yearly by malaria.

As there is need to eradicate malaria, long lasting insecticidal treated mosquito nets has been developed to protect and prevent malaria infections and reduce death rates.

Long Lasting Insecticide Treated Mosquito Nets — Specification

1 Scope

This committee Draft African Standard specifies requirements, sampling, and test methods for treated Long Lasting Insecticide 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.

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

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

ISO 1833-25, *Textiles — Quantitative chemical analysis — Part 25: Mixtures of polyester with certain other fibres (method using trichloroacetic 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 13938-1, *Textiles — Bursting properties of fabrics — Part 1: Hydraulic method for determination of bursting strength and bursting distension*

ISO 13938-2, *Textiles — Bursting properties of fabrics — Part 2: Pneumatic method for determination of bursting strength and bursting distension*

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 document, the terms and definitions given in ISO 6938, ISO 2076 and the following apply. ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1 Long Lasting Insecticidal Nets (LLIN)

factory treated mosquito nets 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 multi-treated 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

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 General requirements

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; and

c) polyethylene: mono/multi-filament.

4.1.2 Specific requirements

Long lasting insecticidal mosquito nets shall comply with the specific requirements given in Table 1 when tested in accordance with the test methods specified therein.

Table 1 — Specific requirements 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, D, min.		75		100	100	ISO 2060
Mesh count, holes/cm^2, min.		For 75D and 100D	24	20	8	Annex I
		For 150D	10.6			
Mass per unit area, g/m^2, min.		28.8		35	28.8	ISO 3801
Bursting strength at 7.3 cm^2, kPa, min.		For 75D	250	350	250	ISO 13938-1 ISO 13938-2
		For 100D	350			
		For 150D	380			
Dimensional stability after one 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

4.1.3 Active ingredients

Active ingredients used in mono-treated nets shall comply with the requirements given in Table 2 when tested in accordance with the test methods specified therein.

Table 2 — Requirements for active ingredients used in mono-treated nets

S/N	Active ingredient[a]	Requirement g/kg	Test method
I	Deltamethrin	1.05 – 3.50	Annex A, Annex B
ii	Permethrin	15.00 – 25.00	Annex C
iii	Alpha-cypermethrin	3.38 – 5.63	Annex E, Annex F
[a] In the event that an active ingredient other than those already mentioned above is used, it shall be as recommended by World Health Organisation or any other internationally recognized body and their affiliated entities published specifications for public health pesticides for treated mosquito nets.			

4.2 Multi-treated nets

4.2.1 multi-treated nets with single fibre construction shall comply with the requirements given in Table 1, when tested in accordance with the test methods specified therein.

4.2.2 multi-treated nets constructed with more than one fibre shall comply with the requirements given in Table 3 when tested in accordance with the methods prescribed therein.

Table 3 — Requirements for Multi-treated 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, D, min.	100	75 with strengthened 70 cm lower border or 100 without border		ISO 2060
Mesh count, holes/cm^2, min.	15.5	For 75 D and 100 D	24	Annex I
		For 150D	10.6	
Mass per unit area, g/m^2 , min.	28.8	28.8		ISO 3801

Bursting strength at 7.3 cm^2, kPa, min.		300	For 75 D	250	ISO 13938-1 ISO 13938-2
			For 100 D	350	
Dimensional stability after one 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		Piperonyl butoxide (PBO): 25.0 ± 25 %, Deltamethrin (DM): 4.0 ± 25 %	For 75D and	Deltamethrin: 2.8 ± 25 %	Annex G, Annex A, Annex B
			For 100D and 150D	Deltamethrin: 2.1 ± 25 %	
[a] As per World Health Organisation or any other internationally recognized body and their affiliated entities published specifications for public health pesticides for treated mosquito nets.					

4.2.3 Active ingredients used in multi-treated nets with single fibre construction shall comply with the requirements given in Table 4 when tested in accordance with the test methods specified therein.

Table 4 — Requirements for active ingredients used in multi-treated nets with single fibre construction

S/N	Active ingredient[a]	Requirement g/kg	Test method
i	Permethrin	20 ± 25 %	Annex D
ii	Piperonyl butoxide (PBO)	10 ± 25 %	Annex G
[a] In the event that an active ingredient other than those already mentioned above is used, it shall be as recommended by World Health Organisation 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

Long lasting insecticidal mosquito nets shall be rectangular or conical in shape unless otherwise agreed between the purchaser and the supplier.

4.3.2 Sizes and dimensions

Long lasting insecticidal mosquito 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/N	Net size	Width, min. cm	Length, min. cm	Height, min cm	Test method
i	X-small	70	120	150	Annex H
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/N	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 H
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. 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

Long lasting insecticidal mosquito nets 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

Long lasting insecticidal mosquito nets 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 fabric for Long lasting insecticidal mosquito nets (when coloured) shall be free from listed amines and carcinogenic dyestuffs specified in ISO 16373-1, ISO 16373-2, and ISO 16373-3. Dyestuff classes are identified in accordance with ISO 16373-1.

6 Packaging

Long lasting insecticidal 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 labelled 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);

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 labelled on a permanent tag attached to individual nets:

a) brand name or registered trademark;

b) fibre composition;

c) linear density;

d) mass per unit area;

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

b) country of origin;

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

d) lot or batch number;

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

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

g) care instructions in accordance with ISO 3758.

8 Sampling

8.1 Lot

In any consignment, all the pieces of long lasting insecticidal 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.

Table 7 — Sampling plan

Number of pieces in a lot	Number of pieces to be sampled
Up to 8	2
9 - 15	3
16 - 25	4
26 - 50	5
51 and above	7

Annex A
(normative)

Determination of deltamethrin content in impregnated long lasting insecticide treated mosquito nets by High Performance Liquid Chromatography (HPLC)

A.1 Sampling

A sample is defined as one finished bed net taken randomly from a batch of bed nets. Sub-sample by cutting five pieces of netting from the recommended positions shown in Figure A.1.

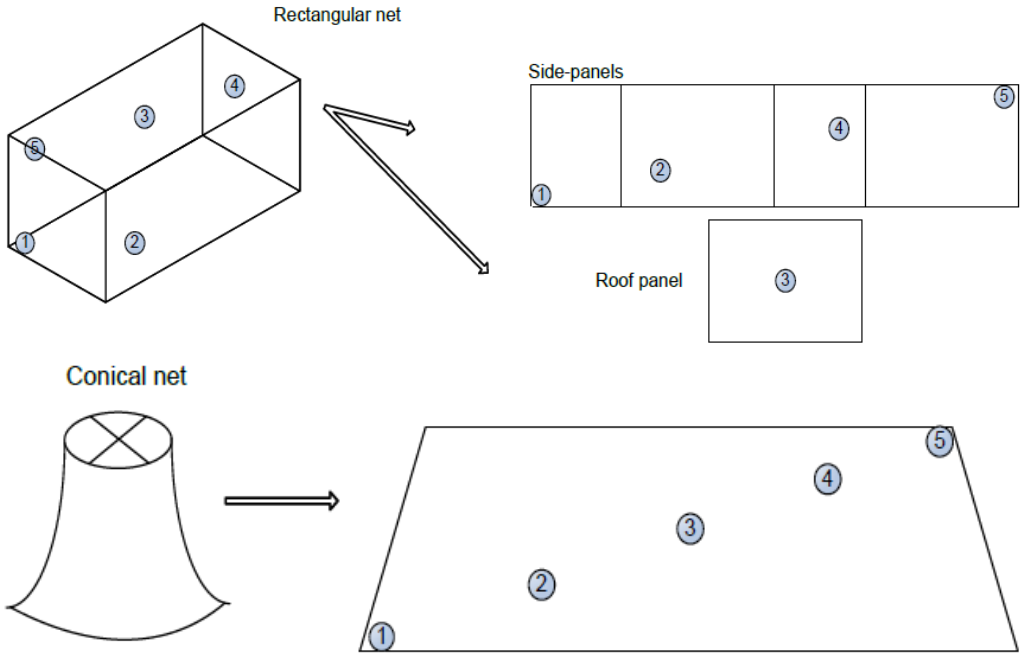

Figure A.1 — Recommended positions for cutting netting pieces

A.2 Identity test

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.

Chromatogram of sample solution and calibration solution are as shown in Figure A.2 and Figure A.3.

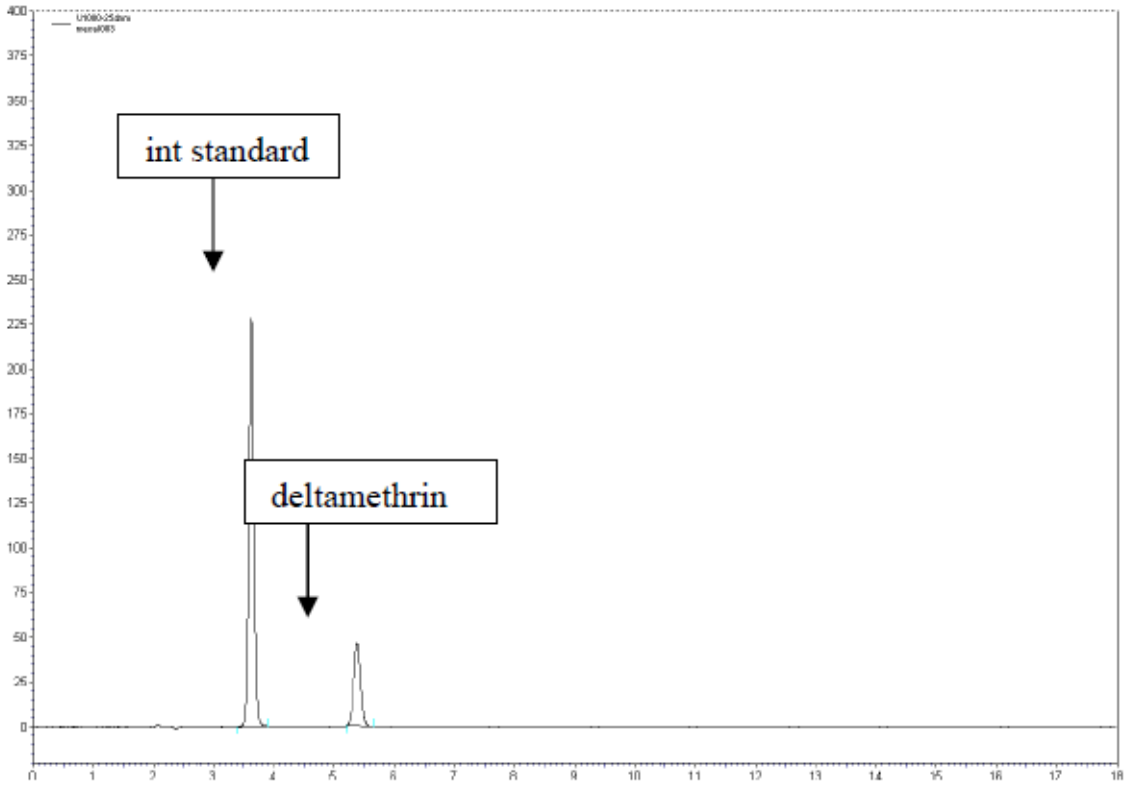

Figure A.2 — Chromatogram of a calibration solution

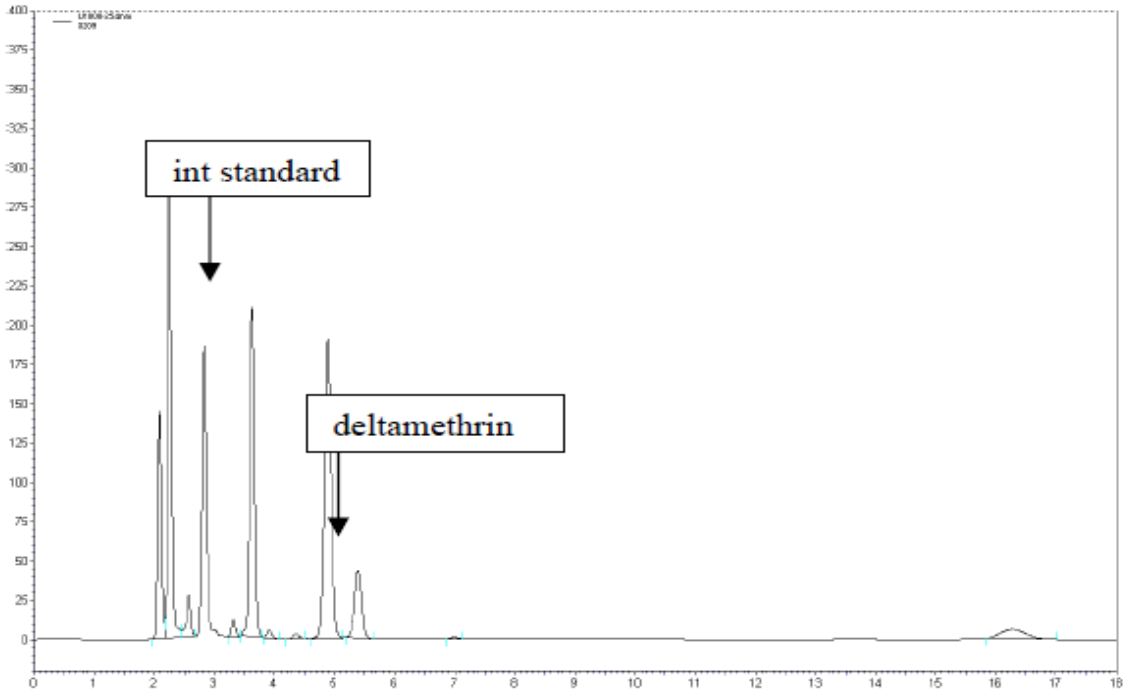

Figure A.3 — Chromatogram of sample solution

A.3 Active ingredient (deltamethrin)

A.3.1 Outline of method

The sample is extracted by refluxing with xylene. The solvent is evaporated and the residue is dissolved in mobile phase. The deltamethrin content is determined by normal phase high performance liquid chromatography using internal standardisation and detection at 254 nm.

WARNING — Safe handling precautions provided on xylene material safety data sheet are to be observed when handling xylene, one of the hazardous reagents.

A.3.2 Reagents

A.3.2.1 **1,4-Dioxane** (HPLC grade). Add 0.15 % (v/v) water before use.

A.3.2.2 **2,2,4-Trimethylpentane (isooctane),** HPLC grade.

A.3.2.3 **Water** (HPLC grade).

A.3.2.4 **Mobile phase isooctane – 1,4-dioxane** (+ 0.15 % water), 94 + 6 (v/v).

A.3.2.5 **Dibutyl phthalate internal standard.**

A.3.2.6 **Deltamethrin standard** of known purity.

A.3.2.7 **Internal standard solution**, prepare a solution of dibutyl phthalate in mobile phase at a concentration of 2.0 g/L.

A.3.2.8 **Deltamethrin stock solution (DS)**, weigh (to the nearest of 0.01 mg) 0.06 g deltamethrin standard (*s* mg) into a volumetric flask (25 ml), add mobile phase (20 ml) and dissolve. Place the flask in a water bath at 20 °C for 15 min and fill to the mark with mobile phase stored at 20 °C. Mix well

A.3.2.9 **Calibration solutions**, transfer using a graduated pipette 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml and 0.7 ml of the deltamethrin stock solution to 5 polytetrafluoroethylene (PTFE) lined screw cap vials (20 ml). Add by pipette internal standard solution (2.0 ml) to each vial and by graduated pipette respectively 17.7 ml, 17.6 ml, 17.5 ml, 17.4 ml, and 17.3 ml mobile phase (solutions C_1, C_2, C_3, C_4, and C_5) [see Table A.1]. Mix well. Filter the solutions through 0.45 µm syringe filters before use.

Table A.1 — Calibration solutions

Solution	**Internal (Int.) standard** ml	**Deltamethrin solution** ml	**Mobile phase** ml	**Deltamethrin** mg
C_1	2.00	0.3	17.7	0.012s
C_2	2.00	0.4	17.6	0.016s
C_3	2.00	0.5	17.5	0.020s
C_4	2.00	0.6	17.4	0.024s
C_5	2.00	0.7	17.3	0.028s

A.3.3 Apparatus

A.3.3.1 **High performance liquid chromatograph,** equipped with an automatic loop injector (20 µl) and a UV spectrophotometric detector capable of measuring at 254 nm.

A.3.3.2 **Column**, stainless steel, 150 mm x 4.6 mm (i.d.), packed with Lichrosorb Si60 5 µm, or Supelco Si, 5 µm, or equivalent.

A.3.3.3 **Guard column** Supel guard or equivalent.

A.3.3.4 **Hot plate with magnetic stirrer.**

A.3.3.5 **Reflux condenser.**

A.3.3.6 **Electronic integrator or data system.**

A.3.3.7 **Disposable syringe,** with 0.45 µm filter.

A.4 Procedure

A.4.1 Operating conditions (typical)

Column	150 x 4.6 mm (i.d.), Lichrosorb Si60, 5 µm, or Supelco Si, 5 µm
Mobile phase	isooctane – 1,4-dioxane (+ 0.15 % water), 94 + 6 (v/v)
Flow rate	1.5 ml/min
Column temperature	ambient
Injection volume	5 µl
Detector wavelength	254 nm
Retention time	deltamethrin: 5.5 min
	Internal standard: 3.7 min

A.4.2 Preparation of calibration curve

Inject 5 µl portions of calibration solutions C_1, C_2, C_3, C_4 and C_5 beginning with the solution with the lowest concentration. Prepare a calibration curve by plotting the deltamethrin to dibutyl phthalate peak area ratios versus the mass of the standards (mg). Using the method of least squares, calculate the equation for the straight line that best fits the experimental calibration data. The correlation coefficient should be 0.99 or better. Prepare the calibration curve daily with the full series of five standards.

A.4.3 Preparation of sample

Cut the sample into small pieces of less than 2 cm × 2 cm and homogenise. Weigh (to the nearest 0.1 mg) 0.3 g (w mg) of the sample into a reflux flask (100 ml). Add xylene (18 ml) and by pipette internal standard solution (2.0 ml). Attach the flask to the reflux condenser and reflux the sample for about 30 min while stirring. Cool the sample to room temperature and filter the solution through a 0.45 µm filter membrane. Take 0.5 ml of the filtered solution and evaporate the solvent using a stream of nitrogen. Dissolve the residue quantitatively in 0.5 ml of mobile phase.

A.4.4 Determination

Inject in duplicate 5-µl portions of the sample solution, determine the deltamethrin to internal standard response ratios and calculate the average (R).

A.4.5 Calculation

Deltamethrin content, expressed in grams per kilogram shall be calculated using the formula below:

$$\text{Deltamethrin content} = \frac{(R - b) \times P}{a \times w}$$

where

R is the average deltamethrin to dibutyl phthalate peak area ratio, in the sample;

a is the slope of calibration curve;

b is the intercept of calibration curve;

P is the purity, in grams per kilogram (g/kg), of the deltamethrin standard.

Annex B
(normative)

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

B.1 Sampling

Sampling shall be done in accordance with A.1.

B.2 Identity test

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.

Chromatogram of deltamethrin in an insecticidal net is as shown in Figure B.1.

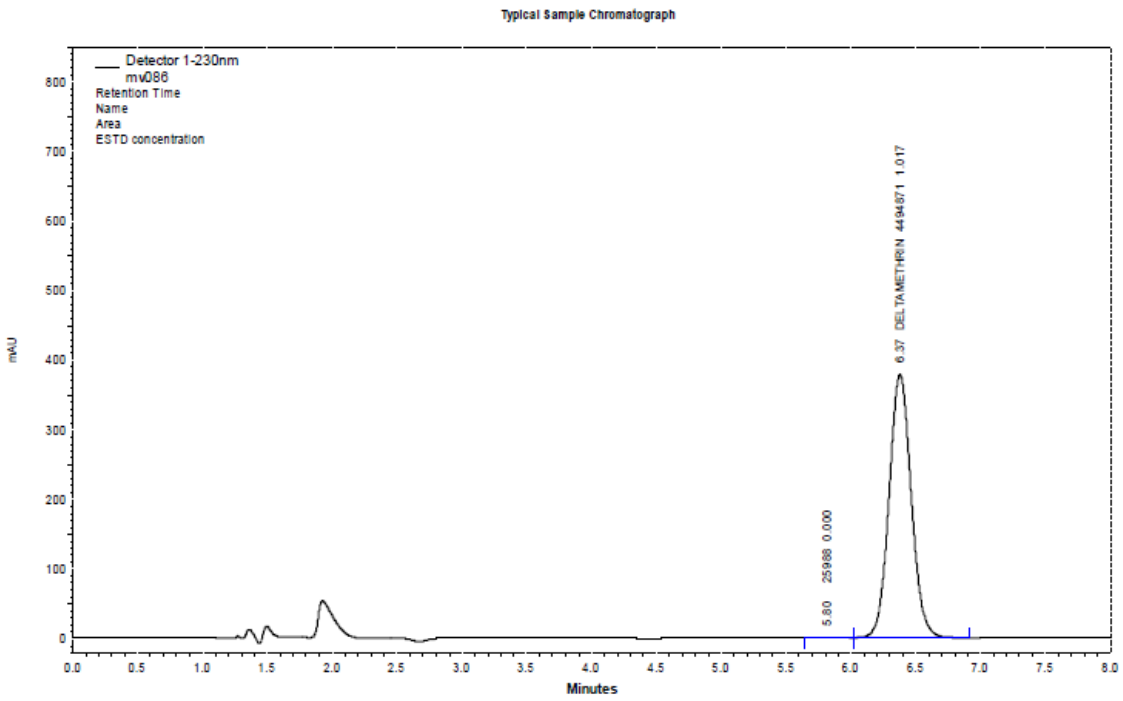

Figure B.1 — Chromatogram of deltamethrin in an insecticidal net

B.3 Active ingredient (deltamethrin)

B.3.1 Outline of method

The sample is extracted by refluxing with xylene. The solvent is evaporated and the residue is dissolved in mobile phase. The deltamethrin content is determined by normal phase high performance liquid chromatography using internal standardisation and detection at 254 nm.

WARNING — Safe handling precautions provided on xylene material safety data sheet are to be observed when handling xylene, one of the hazardous reagents.

B.3.2 Reagents

B.3.2.1 **1,4-Dioxane** (HPLC grade). Add 0.15 % (v/v) water before use.

B.3.2.2 **2,2,4-Trimethylpentane (isooctane),** HPLC grade.

B.3.2.3 **Water** (HPLC grade).

B.3.2.4 **Mobile phase isooctane – 1,4-dioxane** (+ 0.15 % water), 94 + 6 (v/v).

B.3.2.5 **Dibutyl phthalate internal standard.**

B.3.2.6 **Deltamethrin standard** of known purity.

B.3.2.7 **Internal standard solution**, prepare a solution of dibutyl phthalate in mobile phase at a concentration of 2.0 g/l.

B.3.2.8 **Deltamethrin stock solution (DS)**, weigh (to the nearest of 0.01 mg) 0.06 g deltamethrin standard (*s* mg) into a volumetric flask (25 ml), add mobile phase (20 ml) and dissolve. Place the flask in a water bath at 20 ^{0}C for 15 min and fill to the mark with mobile phase stored at 20 ^{0}C. Mix well.

B.3.2.9 **Calibration solution,** weigh (to the nearest 0.1 mg) in duplicate about 50 mg deltamethrin standard (*s* mg) into a volumetric flask (50 ml). Dissolve and fill to mark with solvent mixture. Transfer by pipette 1.0 ml of these solutions into separate volumetric flasks (50 ml) and dilute to the mark with solvent mixture. Mix well (solutions C_1 and C_2).

B.3.3 Apparatus

B.3.3.1 **High performance liquid chromatograph,** equipped with an automatic loop injector (20 µl) and a UV spectrophotometric detector capable of measuring at 254 nm.

B.3.3.2 **Column**, stainless steel, 150 x 4.6 mm (i.d.), packed with Lichrosorb Si60 5 µm, or Supelco Si, 5 µm, or equivalent.

B.3.3.3 **Guard column,** supel guard or equivalent.

B.3.3.4 **Hot plate with magnetic stirrer.**

B.3.3.5 **Reflux condenser.**

B.3.3.6 **Electronic integrator or data system.**

B.3.3.7 **Disposable syringe,** with 0.45 µm filter.

B.3.3.8 **Screw capped bottle,** of neutral glass with PTFE screw cap liner.

B.4 Procedure

B.4.1 Operating conditions (typical)

Column	150 x 4.6 mm (i.d.), Lichrosorb Si60, 5 µm, or Supelco Si, 5 µm
Mobile phase	isooctane – 1,4-dioxane (+ 0.15 % water), 94 + 6 (v/v)

Flow rate	1.5 ml/min
Column temperature	ambient
Injection volume	5 µl
Detector wavelength	254 nm
Retention time	deltamethrin: 5.5 min
	Internal standard: 3.7 min

B.4.2 Preparation of calibration curve

Inject 5-µl portions of calibration solutions C_1, C_2, beginning with the solution with the lowest concentration. Prepare a calibration curve by plotting the deltamethrin to dibutyl phthalate peak area ratios versus the mass of the standards (mg). Using the method of least squares, calculate the equation for the straight line that best fits the experimental calibration data. The correlation coefficient should be 0.99 or better.

B.4.3 Preparation of sample

B.4.3.1 Weigh (to the nearest 0.1 mg) sufficient sample to contain about 1 mg deltamethrin (*w* mg) into a well tightened screw capped neutral glass bottle (100 ml). Add by pipette solvent mixture (50.0 ml). Place the cap and tighten it well. Check for any loss of solvent during the extraction procedure by weighing the bottle before and after the extraction. Wipe out the bottle before weighing. The weight before and after the extraction should not differ by more than 0.05 g.

B.4.3.2 Place the bottle into an ultrasonic bath for 15 min. Then place the bottle in a shaker and shake at ambient temperature for 30 min at a frequency of 155 beats per min. Allow to cool to ambient temperature. Filter the solution through a 0.45-µm filter prior to injection.

B.4.4 Calculation

Deltamethrin content, expressed in grams per kilogram shall be calculated using the formula below:

$$\text{Deltamethrin content} = \frac{f \times H_w}{w \times 50}$$

Annex C
(normative)

Determination of permethrin content in long lasting insecticide treated mosquito nets

C.1 Sampling

Take at least 500 g.

C.2 Identity tests

C.2.1 Use the Gas liquid chromatography (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 (Figure C.1).

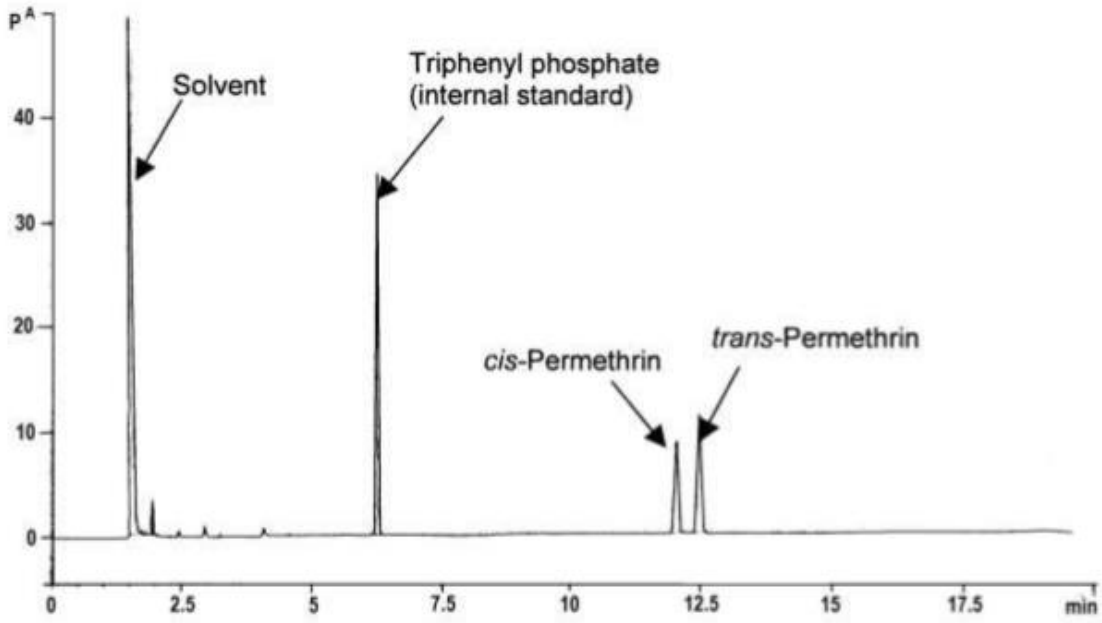

Figure C.1 — Example of gas chromatogram of permethrin LN

C.2.2 **Infrared,** extract the sample with a suitable solvent. Filter and evaporate the solvent. Prepare a film between NaCl plates and scan from 4 000 cm^{-1} to 400 cm^{-1}. The spectrum produced from the sample should not differ significantly from that of the standard (Figure C.2).

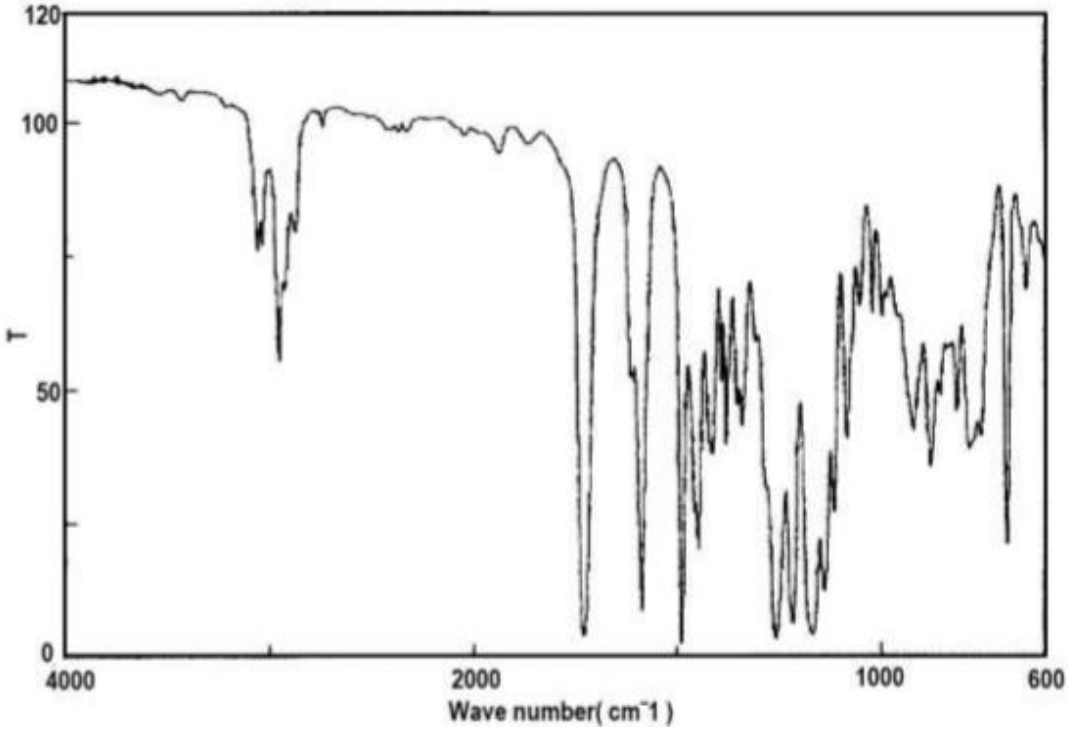

Figure C.2 — Infrared spectrum of permethrin

C.3 Permethrin

C.3.1 Outline of method

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

C.3.2 Reagents

C.3.2.1 Heptane.

C.3.2.2 Acetone.

C.3.2.3 Permethrin working standard, technical product of certified purity; store refrigerated.

C.3.2.4 Triphenyl phosphate internal standard, shall not show peaks with the same retention times as *cis*- and *trans*-permethrin.

C.3.2.5 Internal standard solution, dissolve triphenyl phosphate (1.0 g) in heptane (150 ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.

C.3.2.6 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 0.1 mg) 72 mg to 88 mg (*s* mg) of permethrin standard into a vial or stoppered flask (200 ml). Add by pipette internal standard solution (10.0 ml) and dissolve. Add by measuring cylinder heptane (90 ml) and mix well (solutions C_A and C_B).

C.3.3 Apparatus

C.3.3.1 **Gas chromatograph**, equipped with a split/split less injection and a flame ionisation detector.

C.3.3.2 **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).

C.3.3.3 **Electric integrator or data system.**

C.3.4 Procedure

C.3.4.1 Gas chromatographic conditions (typical)

C.3.4.1.1 Column, 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).

C.3.4.1.2 Injection system:

a) injector, split injection;

b) split flow, approximately 100 ml/min; and

c) injection volume, 1 µm.

C.3.4.1.3 Detector, flame ionisation

C.3.4.1.4 Temperatures, column oven, 240 °C

C.3.4.1.5 Injection port, 265 °C

C.3.4.1.6 Detector, 265 °C

C.3.4.1.7 Carrier gas, helium, 30 cm/s

C.3.4.1.8 Retention times:

a) triphenyl phosphate: about 6.5 min;

b) *cis*-permethrin: about 12.4 min; and

c) *trans*-permethrin: about 12.9 min.

C.3.4.2 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.

C.3.4.3 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.

C.3.4.4 Preparation of sample solution

Clean a pair of scissors with acetone before use. Cut the sample with scissors into 5 mm – 10 mm squares. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient

sample to contain 36 mg 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 °C - 90 °C) 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).

C.3.4.5 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.

C.3.4.6 Calculation of permethrin content

C.3.4.6.1 Calculate the mean value of each pair of response factors bracketing the two injections of a sample using the formula below:

$$f_1 = \frac{h \times S \times P}{H_S \times 2}$$

where

f_1 is the individual response factor;

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

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

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

C.3.4.6.2 Use the value obtained above for calculating the permethrin contents, expressed in grams per kilogram, using the formula below:

$$Content\ of\ permethrin = \frac{f \times H_w}{l_q \times w}$$

where

f is the mean response factor;

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;

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

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

Annex D
(normative)

Determination of permethrin content in incorporated insecticidal nets in the presence of piperonyl butoxide

D.1 Sampling

Take at least 500 g.

D.2 Identity tests

D.2.1 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 (Figure D.1).

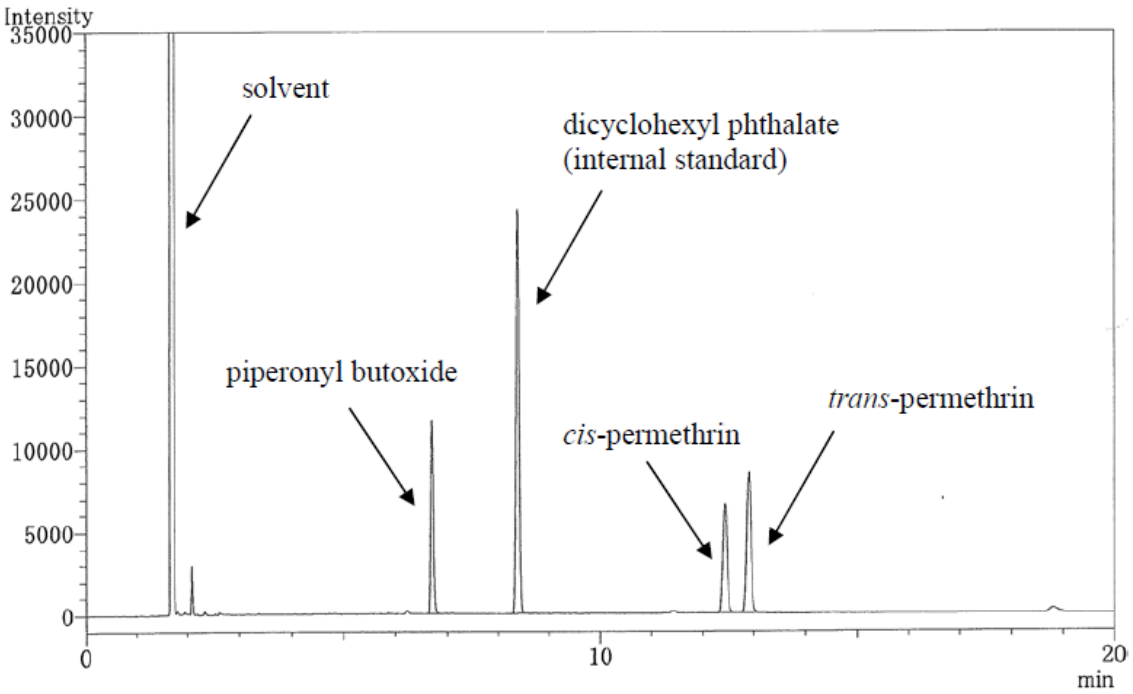

Figure D.1 — Gas chromatogram of permethrin/piperonyl butoxide LN

D.2.2 Use a Gas Chromatography-Mass Spectrometry (GC-MS) apparatus connected to a mass spectrometer with an electron impact ion source and separate the components by the GLC method below. Record the mass spectra of the peaks found at the retention times assigned to *cis*- and *trans*-permethrin. The mass spectra should match those found from the standard (Figures D.2 to D.5).

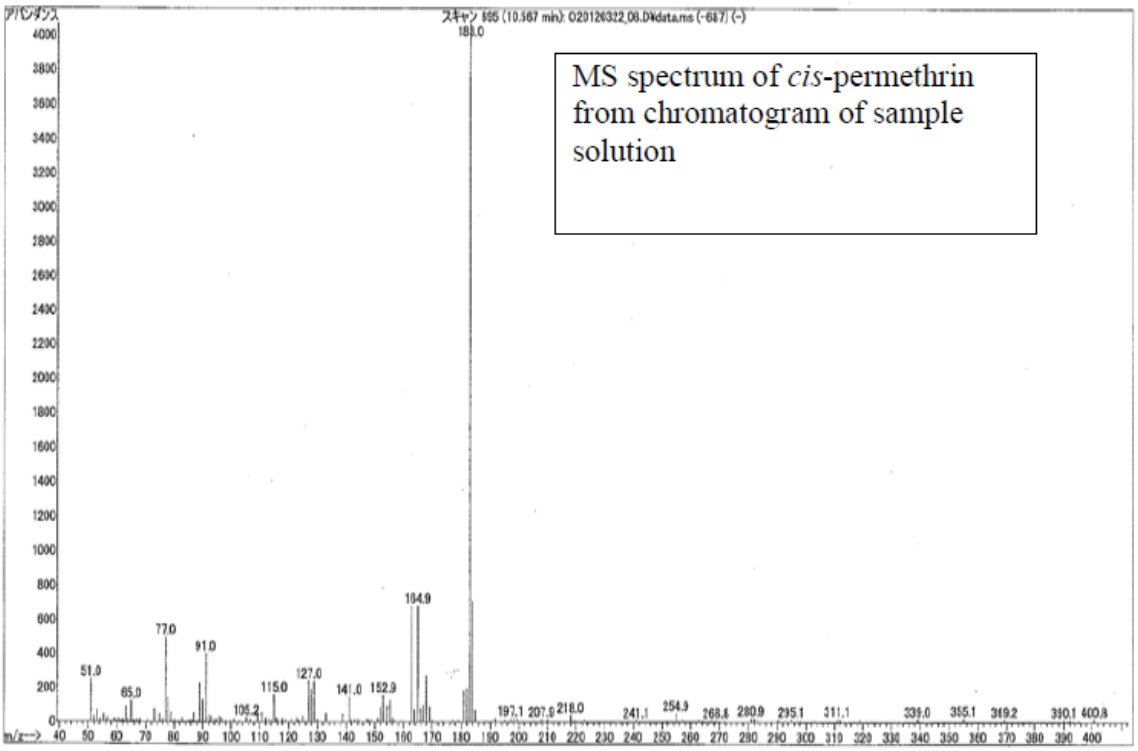

Figure D.2 — Mass spectrum of cis-permethrin from chromatogram of sample solution

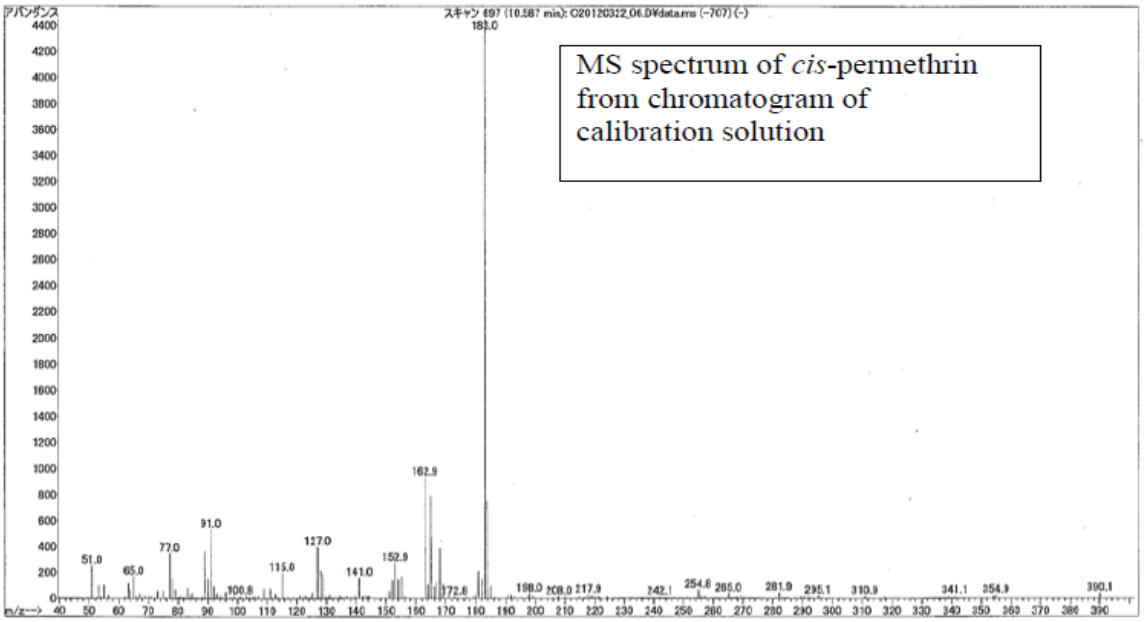

Figure D.3 — Mass spectrum of cis-permethrin from chromatogram of calibration solution

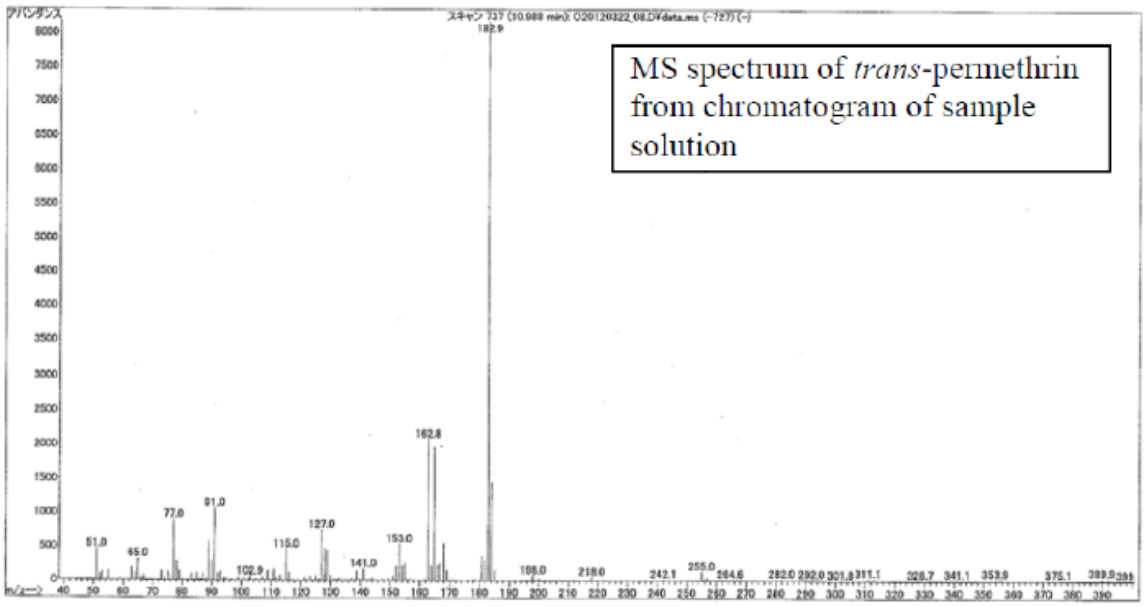

Figure D.4 — Mass spectrum of trans-permethrin from chromatogram of sample solution

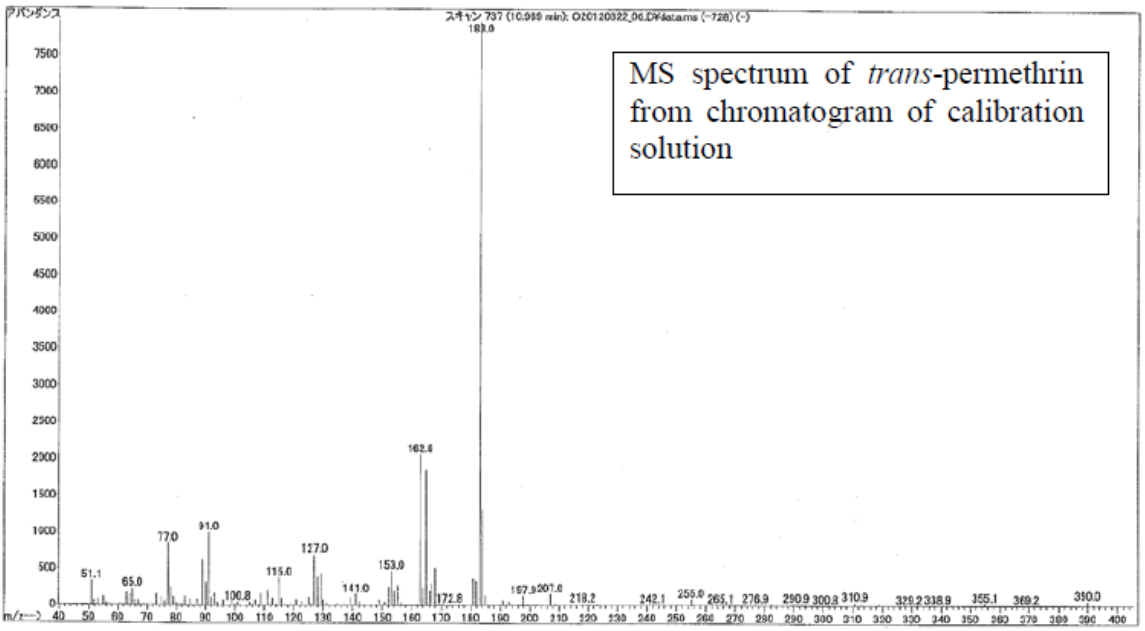

Figure D.5 — Mass spectrum of *trans*-permethrin from chromatogram of calibration solution

D.3 Permethrin

D.3.1 Outline of the method

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

D.3.2 Reagents

D.3.2.1 Heptane.

D.3.2.2 Permethrin standard technical product, of certified purity, store refrigerated.

D.3.2.3 Dicyclohexyl phthalate internal standard, shall not show peaks with the same retention times as *cis*-permethrin, *trans*- permethrin and piperonyl butoxide.

D.3.2.4 Internal standard solution, dissolve dicyclohexyl phthalate (0.73 g) in heptane (100 ml). Ensure that a sufficient quantity of this solution is prepared for all samples and calibration standards to be analysed.

D.3.2.5 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 0.1 mg) 72 mg to 88 mg (*s* mg) of permethrin standard into a vial or stoppered flask (200 ml). Add by pipette internal standard solution (10.0 ml) and dissolve. Add by measuring cylinder heptane (90 ml) and mix well (solutions C_A and C_B).

D.3.3 Apparatus

D.3.3.1 Gas chromatograph, equipped with a split/split less injection and a flame ionisation detector.

D.3.3.2 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).

D.3.3.3 Electric integrator or data system.

D.3.4 Procedure

D.3.4.1 Gas chromatographic conditions (typical)

D.3.4.1.1 Column, 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).

D.3.4.1.2 Injection system

a) Injector, split injection;

b) Split flow, approximately 100 ml/min; and

c) Injection volume, 1 µm.

D.3.4.1.3 Detector, flame ionisation

D.3.4.1.4 Temperatures

Column oven, 240 °C (use a short temperature program to remove formulants, if necessary)

D.3.4.1.5 Injection port, 265 °C

D.3.4.1.6 Detector, 265 °C

D.3.4.1.7 Carrier gas, helium, 30 cm/s

D.3.4.1.8 Retention times

a) dicyclohexyl phthalate: about 8.4 min;

b) *cis*-permethrin: about 12.4 min; and

c) *trans*-permethrin: about 12.9 min.

D.3.4.2 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.

D.3.4.3 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.

D.3.4.4 Preparation of sample solution

Clean a pair of scissors with acetone before use. Cut the sample with scissors into 5 mm – 10 mm squares. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 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 °C – 90 °C) 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).

D.3.4.5 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.

D.3.4.6 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 (expressed in grams per kilogram) of the bracketed sample injections. Calculate the sum of the cis- and trans- permethrin peak areas for each injection.

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

$$Content\ of\ permethrin = \frac{f \times H_w}{l_{q \times W}}$$

where

f_1 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.

D.3.4.7 Calculation of trans-isomer fraction percentage

$$\text{trans} - \text{isomer fraction percentage} = \frac{H_w t}{(H_w t + H_w C)} \times 100$$

where

$H_w t$ is the peak area of *trans*-permethrin in the sample solution; and

$H_w C$ is the peak area of *cis*-permethrin in the sample solution.

Annex E
(normative)

Determination of alphacypermethrin content in coated long lasting insecticide treated mosquito nets

E.1 Sampling

Take at least 100 g.

E.2 Identity tests

E.2.1 Use the GLC method below. The relative retention times of alpha-cypermethrin with respect to the internal standard for the sample solution should not deviate by more than 1 % from those of the calibration solution.

E.2.2 Infrared, prepare potassium bromide discs from the sample and from alpha-cypermethrin standard, using approximately 15 mg material and 300 mg potassium bromide. Scan the discs from 4 000 cm^{-1} to 400 cm^{-1}. The spectrum produced from the sample should not differ significantly from that of the standard.

E.3 Alphacypermethrin

E.3.1 Outline of method

Alphacypermethrin is dissolved in tetrahydrofuran and determined by capillary gas chromatography in split injection mode using flame ionization and internal standardization.

E.3.2 Reagents

E.3.2.1 Tetrahydrofuran.

E.3.2.2 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.

E.3.2.3 Citric acid 5 % solution, dissolve citric acid (25 g) in water (500 ml).

E.3.2.4 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.

E.3.2.5 Calibration solutions, weigh 50 mg alpha cypermethrin (to the nearest 0.1 mg) in a volumetric flask (25-ml). Fill to just below the mark with tetrahydrofuran and place the flask in an ultrasonic bath for 10 min. Allow to cool to room temperature and fill to the mark with tetrahydrofuran. Transfer by pipette 1.50 ml, 3.00 ml and 4.50 ml of this solution into separate volumetric flasks (50 ml). Add 0.5 ml of internal standard solution and fill each to the mark with tetrahydrofuran (solutions C_A, C_B and C_C).

Transfer 200 µl from 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 °C) of the GC apparatus.

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

E.3.3 Apparatus

E.3.3.1 Gas chromatograph, capable of operating over the range 100 °C to 300 °C, fitted with a flame ionisation detector, split/splitless injector and autosampler.

E.3.3.2 Capillary column fused silica 30 m x 0.25 mm with DB-1 of 0.25 µm film thickness (or equivalent dimethyl polysiloxane phase).

E.3.3.3 Electronic integrator or data system.

E.3.3.4 Reflux condenser.

E.3.4 Procedure

E.3.4.1 Operating conditions (typical)

Column	Fused silica, 30 m × 0.25 mm (i.d.) and 0.25 µmfilm thickness coated with dimethyl poly- siloxane packing
Injection system	
Injector	Split/splitless with fused silica liner containing a 1 cm plug of glass wool. It is important that the split liner is thoroughlydeactivated and conditioned before use, toensure no degradation of alpha-cypermethrinoccurs.
Split ratio	approximately 75-100 : 1
Split vent	approximately 75 ml/min
Injection volume	1 µl
Detector	Flame ionisation
Temperatures	
Column oven	225 ℃ to 235 ℃, isothermal
Injection port	260 ℃
Detector	300 ℃
Gas flow rates	
Helium (carrier gas)	approximately 0.8 ml/min (140 kPa at 230 ℃)Helium (make up) 60 ml/min or optimum for instrument
Septum purge	2 ml/min
Hydrogen	25 ml/min to 30 ml/min.) or as recommended for
Air	200 ml/min to 300 ml/min) the particular instrument
Retention times	alpha-cypermethrin cis I: approximately 27 min
	alpha-cypermethrin cis II: approximately 29 min
	DOP: approximately: 14 min

Purify all gases through molecular sieves. Purify the carrier gas further through an oxygen trap.

E.3.4.2 Preparation of the sample

Weigh samples in triplicate. Weigh (to the nearest 0.1 mg) 1 g of the impregnated net (*w* mg) into a round bottomed flask (100 ml) and add tetrahydrofuran (50 ml). Connect the flask to a reflux condenser. Heat the flask in an oil bath at approximately 90 °C for 5 min. Allow the flask to cool to room temperature and transfer the contents quantitatively to a volumetric flask (100 ml). Add internal standard solution (0.5 ml) and fill to the mark with tetrahydrofuran. Transfer 200 µl from each flask into separate GC vials (solutions S_1, S_2 and S_3). Add one drop of citric acid in each case and seal the vials. Place the vials into the sample tray (cooled to 15 °C) of the GC apparatus

E.3.4.3 Equilibration of the system

Inject a portion of one of the calibration solutions. Adjust the column oven temperature to obtain retention time windows for dioctyl phthalate 12.2 min to 14.9 min, for the cis II isomer 24.0 min to 29.7 min and for the cis I isomer 22.7 min to 27.7 min. In order to obtain adequate resolution the retention times of the cis I and the cis II peaks relative to the DOP peak should be not less than 1.9 and 2.0 respectively. Carry out alternate 1.0 µl injections of the calibration solutions C_A and C_B until the response factors consistently (at least two injections of each) differ by less than ± 1 % of the mean.

E.3.4.4 Determination

Inject into the gas chromatograph 1.0-µl portions of the calibration solutions C_A and C_B and the sample solutions S_A and S_B in the following sequence and record the alpha-cypermethrin integrated peak areas. Injection sequence: C_{A1}, S_{A1}, S_{A2}, C_{B1}, C_{A2}, S_{B1}, S_{B2}, C_{B2}.

Calculate the relative response factors (f_1, f_2,…) for the pair of calibration solutions which bracket the sample solutions for example, use C_{A1} and C_{B1} for sample injections S_{A1} and S_{A2}…, and obtain the mean response factor *f*. Repeat sample analysis if calibration response factors f_1 and f_2 differ by more than ±2 % of the mean *f*. Calculate for each sample injection for example, S_{A1} the alpha- cypermethrin cis II content (expressed in grams per kilogram).

E.3.4.5 Calculation

$$\mathrm{f} = \frac{I_r \times s \times P \times v}{H_s}$$

$$\text{Content of alpha} - \text{cypermethrin} = \frac{f \times H_w \times 2}{I_q \times w}$$

where

f is the average relative response factor;

H_s is the area of alpha-cypermethrin cis II peak in the calibration solution;

H_w is the area of alpha-cypermethrin cis II peak in the sample solution;

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

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

s is the mass, in milligrams, of alpha-cypermethrin standard taken for the calibration solution;

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

P is the purity, in grams per kilogram, of alpha-cypermethrin reference substance;

v is the dilution factor (0.06, 0.12, and 0.18 for calibration solutions C_A, C_B and C_C respectively).

Calculate the alpha-cypermethrin cis II content of the sample as the mean of the four determinations as follows:

Sample injection	Use response factorfrom	Content, g/kg	
S_{A1}	C_{A1} and C_{B1}	Q	)
			) X
S_{A2}	C_{A1} and C_{B1}	R	)
S_{B1}	C_{A2} and C_{B2}	S	)
			) Y
S_{B2}	C_{A2} and C_{B2}	T	)

Take the mean of the values X and Y as the alpha-cypermethrin content.

Repeatability *r* = 17 g/kg at 954 g/kg active ingredient content

Reproducibility *R* = 20 g/kg at 954 g/kg active ingredient content

Annex F
(normative)

Determination of Alphacypermethrin content in incorporated long lasting insecticide treated mosquito nets

F.1 Identity test

F.1.1 Use the GLC method below. The relative retention times of alpha-cypermethrin with respect to the internal standard for the sample solution should not deviate by more than 1 % from those of the calibration solution.

F.1.2 Infrared, prepare potassium bromide discs from the sample and from alpha-cypermethrin standard, using approximately 15 mg material and 300 mg potassium bromide. Scan the discs from 4 000 cm^{-1} to 400 cm^{-1}. The spectrum produced from the sample should not differ significantly from that of the standard.

F.2 Alphacypermethrin

F.2.1 Outline of method

The net sample is extracted with xylene.

F.2.2 Reagents

F.2.2.1 Tetrahydrofuran.

F.2.2.2 Aphacypermethrin standard of known purity. Store below 4 °C.

F.2.2.3 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.

F.2.2.4 Citric acid 5 % solution, dissolve citric acid (50 g) in tetrahydrofuran (500 ml).

F.2.2.5 Internal standard solution, dissolve dioctyl phthalate (50 mg) in tetrahydrofuran (1 000 ml). Ensure sufficient quantity of this solution is prepared for all samples and calibration solutions to be analysed.

F.2.2.6 Xylene (mixed isomers)

F.2.2.7 Calibration solutions, weigh (to the nearest 0.1 mg) 110 mg alpha-cypermethrin (*s* mg) into a volumetric flask (1 000 ml). Dissolve and fill to the mark with xylene. Transfer using a graduated pipette 40 ml, 60 ml and 80 ml of this solution into three separate volumetric flasks (100 ml). Fill to the mark with xylene and mix well (solutions C_1, C_2, C_3). Transfer by pipette (45.0 ml) of each solution to separate glass stoppered flasks (150 ml), add by pipette internal standard solution (50.0 ml) and citric acid 10 % solution (5 ml) to each flask. Mix well and fill to the mark with xylene (solutions C_A, C_B and C_C respectively).

F.2.3 Apparatus

F.2.3.1 Gas chromatograph, capable of operating over the range 100 °C to 300 °C, fitted with a flame ionisation detector, split/splitless injector and autosampler.

F.2.3.2 Capillary column fused silica 30 m x 0.25 mm with DB-1 of 0.25 µm film thickness (or equivalent dimethyl polysiloxane phase).

F.2.3.3 Reflux condenser.

F.2.4 Procedure

F.2.4.1 Operating conditions (typical)

Column	Fused silica, 30 m × 0.25 mm (i.d.) and 0.25 µmfilm thickness coated with dimethyl poly- siloxane packing.
Injection system	
Injector	Split/splitless with fused silica liner containing a 1 cm plug of glass wool. It is important that the split liner is thoroughly deactivated and conditioned before use, to ensure no degradation of alpha-cypermethrinoccurs.
Split ratio	approximately 75-100 : 1
Split vent	approximately 75 ml/min
Injection volume	1 µl
Detector	Flame ionisation
T*emperatures*	
Column oven	225 °C to 235 °C, isothermal
Injection port	260 °C
Detector	300 °C
Gas flow rates	
Helium (carrier gas)	approximately 0.8 ml/min (140 kPa at 230 ^{0}C)Helium (make up) 60 ml/min or optimum for instrument
Septum purge	2 ml/min
Hydrogen	25 ml/min to 30 ml/min or as recommended for
Air	200 ml/min to 300 ml/min the particular instrument
Retention times	alpha-cypermethrin cis I: approximately 27 min
	alpha-cypermethrin cis II: approximately29 min
	DOP: approximately: 14 min

Purify all gases through molecular sieves. Purify the carrier gas further through an oxygen trap.

F.2.4.2 Preparation of the sample.

Weigh (to the nearest 0.1 mg) 1 g (*w* mg) of the impregnated net into a 250 ml ground glass round bottomed flask. Add xylene (90 ml) and citric acid 10 % solution (10 ml). Attach the flask to a reflux condenser and reflux for 30 min. Cool to room temperature and stir. Add by pipette internal standard solution C_B (100.0 ml) and stir. Filter through a 0.45 µm filter before analysis (solution S). Transfer a quantity of the solution to a GC vial.

F.2.4.3 Equilibration of the system

Linearity check, check the linearity of the detector response by injecting in duplicate 1 µl of the calibration solutions C_A, C_B and C_C. Prepare a curve by plotting the alpha-cypermethrin to internal standard peak area ratio versus the mass of alpha-cypermethrin in the calibration solutions. Using the method of least squares calculate the equation for the straight line that best fits the experimental data. The correlation coefficient should be 0.999 or better.

Inject a portion of one of the calibration solutions. Adjust the column oven temperature to obtain retention time windows for dioctyl phthalate 12.2 min to 14.9 min, for the cis II isomer 24.0 min to 29.7 min and for the cis I isomer 22.7 min to 27.7 min. In order to obtain adequate resolution the retention times of the cis I and the cis II peaks relative to the DOP peak should be not less than 1.9 and 2.0 respectively. Carry out alternate 1.0 µl injections of the calibration solutions C_A and C_B until the response factors consistently (at least two injections of each) differ by less than ±1 % of the mean.

F.2.4.4 Determination

Inject into the gas chromatograph 1.0 µl portions of the calibration solutions C_A and C_B and the sample solutions S_A and S_B in the following sequence and record the alpha-cypermethrin integrated peak areas. Injection sequence: C_B, C_B, S_1, S_1, C_B, C_B, S_2, S_2,…..

Calculate the relative response factors (f_1, f_2,…) for the pair of calibration solutions which bracket the sample solutions for example, use C_{A1} and C_{B1} for sample injections S_{A1} and S_{A2} etc and obtain the mean response factor f. Repeat sample analysis if calibration response factors f_1 and f_2 differ by more than ±2 % of the mean f. Calculate for each sample injection for example, S_{A1} the alpha- cypermethrin cis II content (expressed in grams per kilogram).

F.2.4.5 Calculation

$$\mathrm{f} = \frac{I_r \times s \times P \times v}{H_s}$$

$$\text{Content of alpha} - \text{cypermethrin} = \frac{f \times H_w \times 2}{I_q \times w}$$

where

f is the mean response factor;

H_s is the peak area of alpha-cypermethrin in the calibration solution;

H_w is the peak area of alpha-cypermethrin 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 alpha-cypermethrin standard taken for the calibration solution;

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

P is the purity, in grams per kilogram, of alpha-cypermethrin reference substance;

v is the dilution factor (0.027 for calibration solution C_B).

Calculate the alpha-cypermethrin cis II content of the sample as the mean of the four determinations as follows:

Sample injection	Use response factorfrom	Content, g/kg	
S_{A1}	C_{A1} and C_{B1}	Q	)
			) X
S_{A2}	C_{A1} and C_{B1}	R	)
S_{B1}	C_{A2} and C_{B2}	S	)
			) Y
S_{B2}	C_{A2} and C_{B2}	T	)

Take the mean of the values X and Y as the alpha-cypermethrin content.

Repeatability r = 17 g/kg at 954 g/kg active ingredient content

Reproducibility R = 20 g/kg at 954 g/kg active ingredient content

Annex G
(normative)

Determination of Piperonyl butoxide in impregnated insecticidal nets in the presence of deltamethrin

G.1 Sampling

Sample in accordance with A.1.

G.2 Identity tests

GLC

GC-MS

G.3 Piperonyl butoxide

G.3.1 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.

WARNING — Safe handling precautions provided on xylene material safety data sheet are to be observed when handling xylene, one of the hazardous reagents.

G.3.2 Reagents

G.3.2.1 Xylene.

G.3.2.2 Piperonyl butoxide standard of known purity, store below 0 °C.

G.3.2.3 Octadecane internal standard.

G.3.2.4 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.

G.3.2.5 Calibration solutions, allow piperonyl butoxide to equilibrate to ambient temperature. Then weigh (to the nearest 0.1 mg) 0.25 g piperonyl butoxide (*s* mg) into a volumetric flask (50 ml). Fill to the mark with xylene and mix well. Transfer using a graduated pipette 0.50 ml, 1.50 ml, 2.00 ml, 3.00 ml and 4.00 ml of this solution to 5 volumetric flasks (25 ml). Add 2 ml of internal standard solution to each flask, fill each to the mark with xylene and mix well (solutions C_A, C_B, C_C, C_D and C_E). Filter the solutions through a 0.45 µm PTFE filter membrane and transfer 200 µl portions into GC vials.

G.3.3 Apparatus

G.3.3.1 Gas chromatograph, capable of operating over the range of 180 °C to 250 °C fitted with flame ionisation detection.

G.3.3.2 Capillary column fused silica, 30 m x 0.32 mm (i.d.) coated with 100% methyl polysiloxane, cross-linked, surface bonded stationary phase and 0.25 µm film thickness (TRI or equivalent).

G.3.3.3 Electronic integrator or data system.

G.3.3.4 Reflux condenser.

G.3.3.5 Disposable syringe, with 0.45 µm filter.

G.3.4 Procedure

G.3.4.1 Operating conditions (typical)

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)

Injection system

Injector	Split injection
Injector temperature	250 °C
Split ratio	0:1
Purge flow	1 ml/min
Injection volume	1 µl

Detection system

Type	Flame ionisation
Temperature	300 °C

Oven temperatures

Initial	180 °C
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

Gas flow rates

Helium (carrier)	linear velocity: 39 cm/min at 180 °C
Helium (make up)	30 ml/min
Hydrogen	40 ml/min
Air	400 400 ml/min
Total flow	35 ml/min

Retention times

Piperonyl butoxide	about 23 min

Octadecane	about 6 min

G.3.4.2 Preparation of sample

Cut the sample into small pieces of less than 2 cm × 2 cm and homogenise. Weigh (to the nearest 0.1 mg) about 0.5 g (*w* g) of the sample into a reflux flask (100 ml). Add xylene (23.0 ml) and by pipette, 2.0 ml of internal standard solution. Attach the flask to the reflux condenser and reflux the sample about 30 min while stirring. Cool the sample to room temperature and filter the solution through a 0.45-µm PTFE filter membrane. Transfer 200 µl of the sample to a GC vial.

G.3.4.3 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.

G.3.4.4 Determination and preparation of calibration curve

Inject in duplicate into the gas chromatograph 1-µl portions of the calibration and sample solutions in the following sequence; C_A, C_A, C_B, C_B, C_C, C_C, C_D, C_D, C_E, C_E, S_1, S_1, S_2, S_2, etc.

Prepare a curve by plotting the piperonyl butoxide to internal standard peak area ratios versus the mass of piperonyl butoxide in the calibration solutions (0.01*s*, 0.03*s*, 0.04*s*, 0.06*s*, and 0.08*s* mg respectively). Using the method of least squares, calculate the equation for the straight line that best fits the experimental piperonyl butoxide to internal standard response ratios of the sample solutions and calculate the average (*R)* for each sample.

G.3.5 Calculation

Piperonyl butoxide, expressed in grams per kilogram, shall be calculated using the formula below:

$$\text{Content of piperonly butoxide} = \frac{(R - b) \times P}{a \times w}$$

where

R is the average piperonyl butoxide to octadecane peak ratio in the sample;

a is the slope of calibration curve;

b is the intercept of calibration curve;

P is the purity, in grams per kilogram, of the piperonyl butoxide standard.

Illustrations for the Chromatogram are as shown in Figures G.1 to G.4.

NOTE A deltamethrin peak will not be visible on the chromatogram at the conditions given.

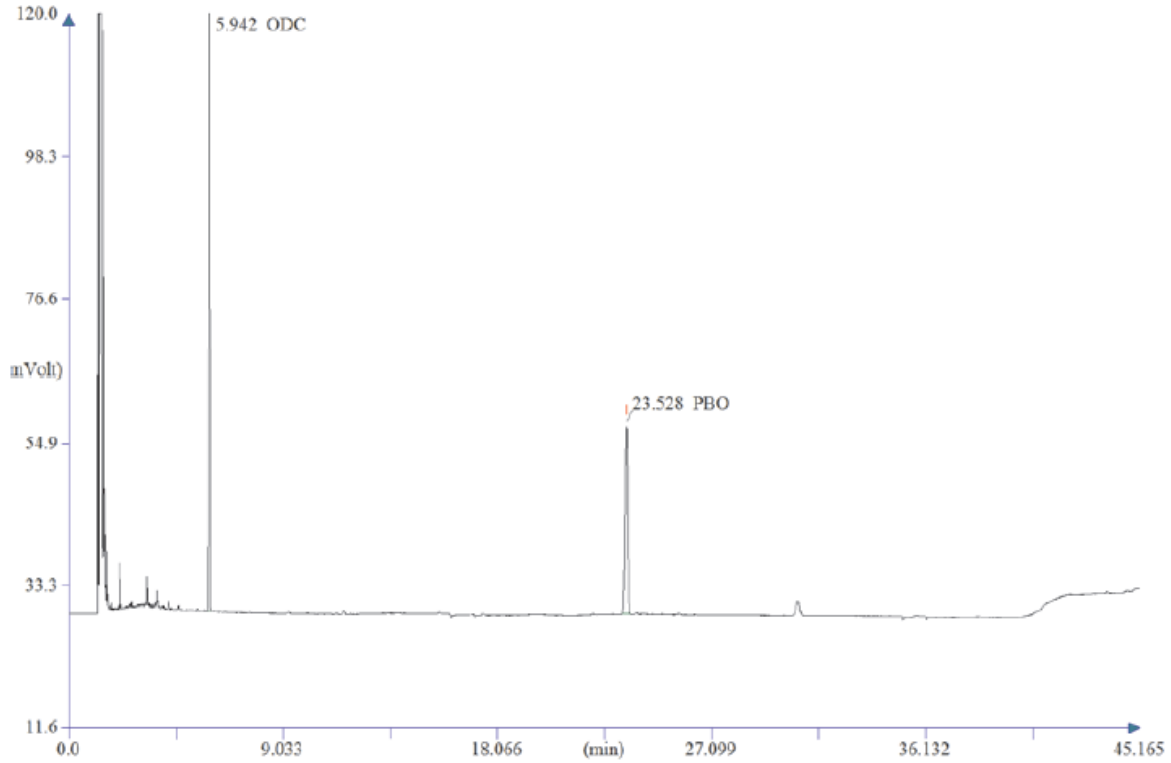

Figure G.1 — GC chromatogram of calibration solution

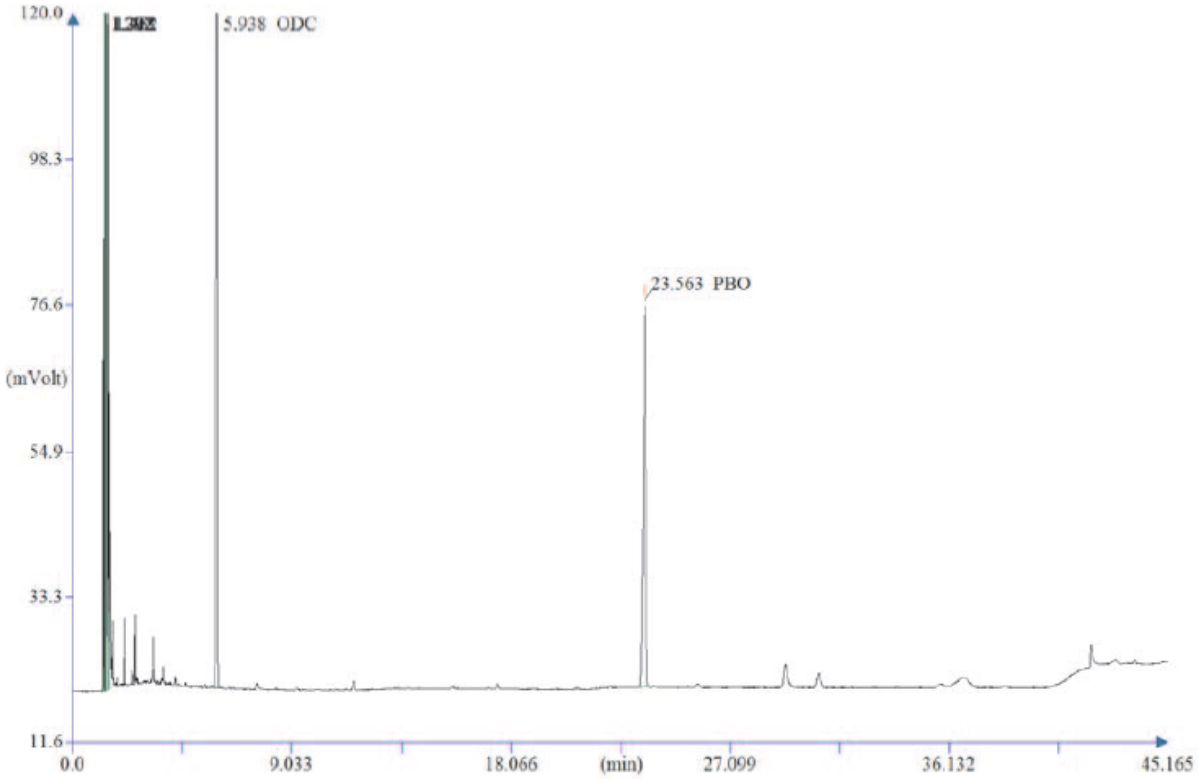

Figure G.2 — GC chromatogram of sample solution

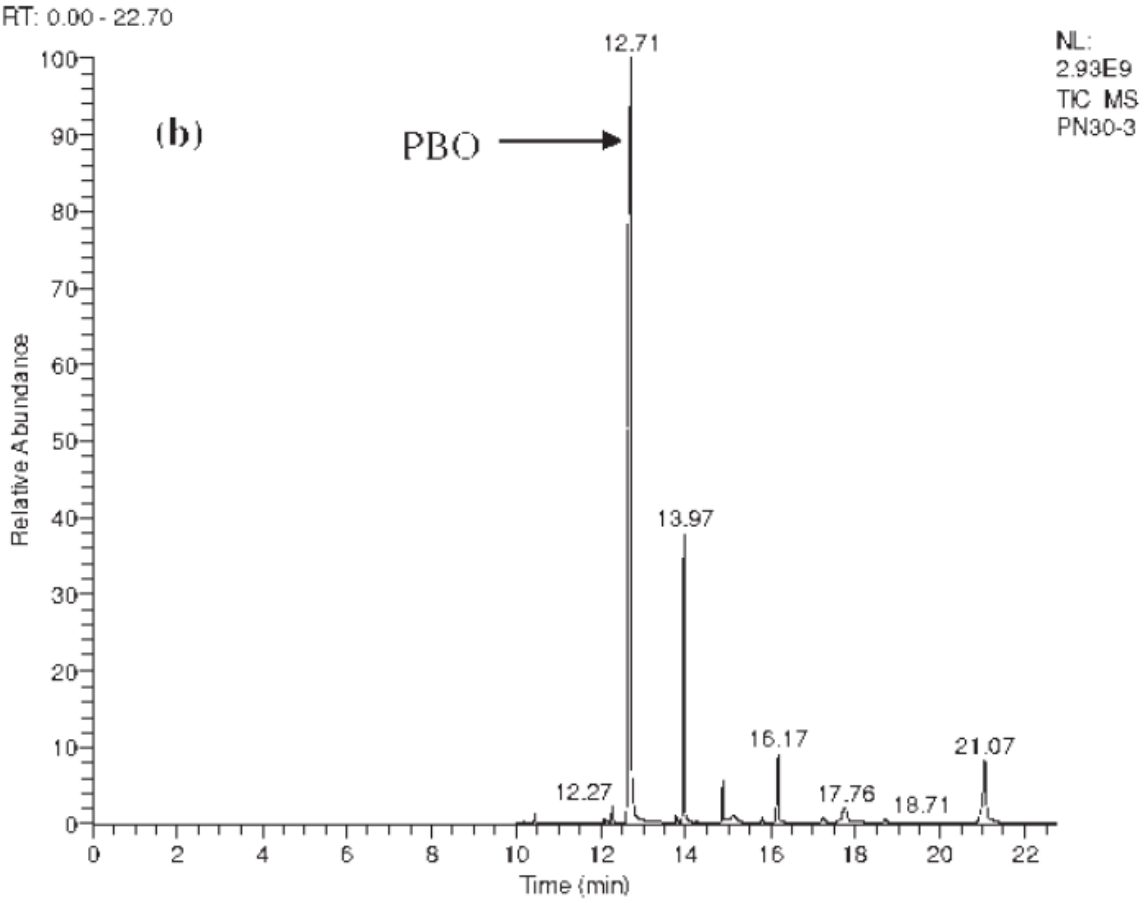

Figure G.3 — GC-MS chromatogram of sample solution

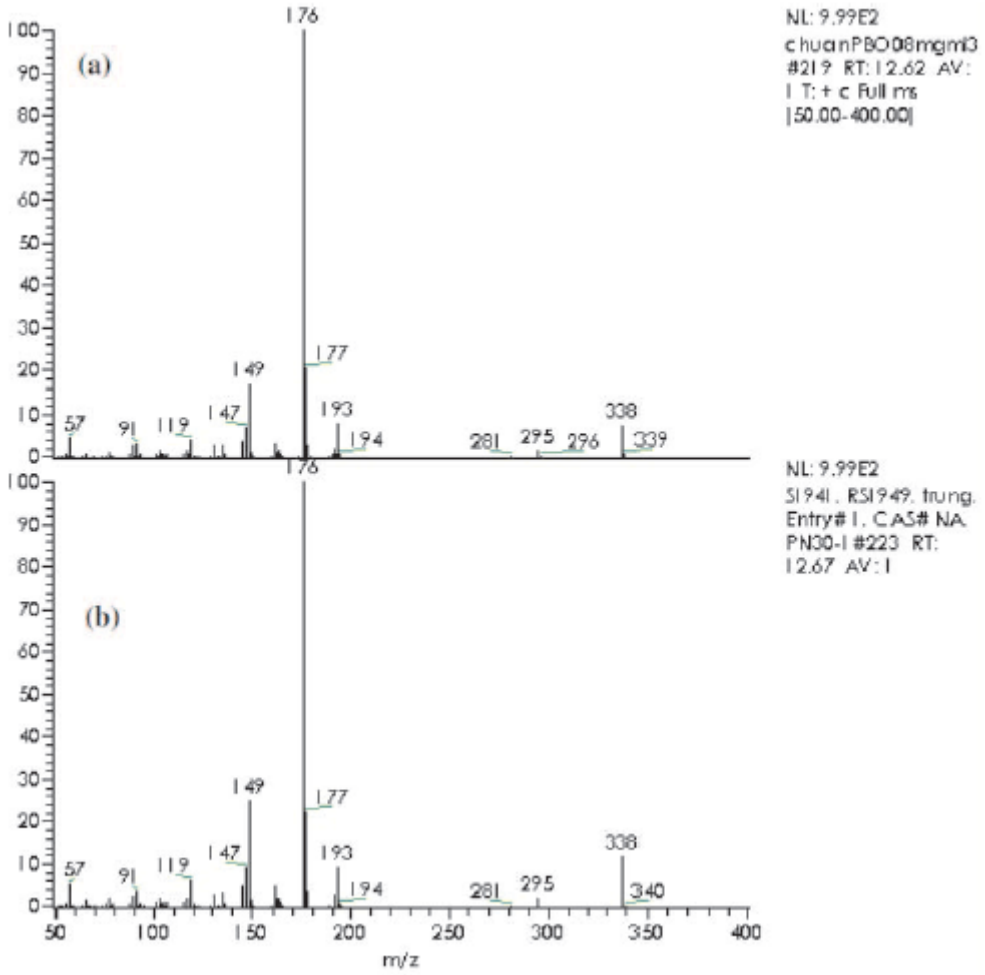

Figure G.4 — Full scan MS spectrum of piperonyl butoxide (a) in the standard solution and (b) in the sample solution

Annex H
(normative)

Measurement of mosquito net dimensions

H.1 Rectangular nets: Length, width and height

H.1.1 Apparatus

H.1.1.1 Flat table.

H.1.1.2 Measuring tape or steel rule.

H.1.2 Conditioning

Condition the net samples in accordance with ISO 139.

H.1.3 Procedure

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

H.1.4 Calculation

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

H.1.5 Report

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

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

H.2.1 Apparatus

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

H.2.1.2 Measuring tape or steel rule.

H.2.1.3 Twine, of measuring at least 10 m.

H.2.1.4 Felt pen marker.

H.2.2 Conditioning

Condition the net sample in accordance with ISO 139.

H.2.3 Procedure

H.2.3.1 Top ring diameter

H.2.3.1.1 Procedure

Place the top portion of the net sample on a flat table (see H.1.1.1) and put the twine (see H.2.1.3) around the circumference of the top ring of the net sample, identifying the two ends with a marker (see H 2.1.4) which represent the dimension of the top ring. Using a measuring tape (see H.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.

H.2.3.1.2 Calculation

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

H.2.3.1.3 Report

Report the top ring circumference as the value(s) calculated in H.2.3.1.2 in centimetres.

H.2.3.2 Bottom circumference

H.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.

H.2.3.2.2 Calculation

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

Calculate the bottom circumference as: *N* x 2.

H.2.3.2.3 Report

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

H.2.3.3 Conical net height

H.2.3.3.1 Procedure

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

H.2.3.3.2 Report

Report the least measurement taken as the conical net height.

Annex I
(normative)

Determination of mesh count

I.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 in accordance with ISO 139 (4 h, 20 ºC, 65 % relative humidity).

I.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.

Rectangular and conical mosquito nets are illustrated in Figure I.1 and Figure I.2

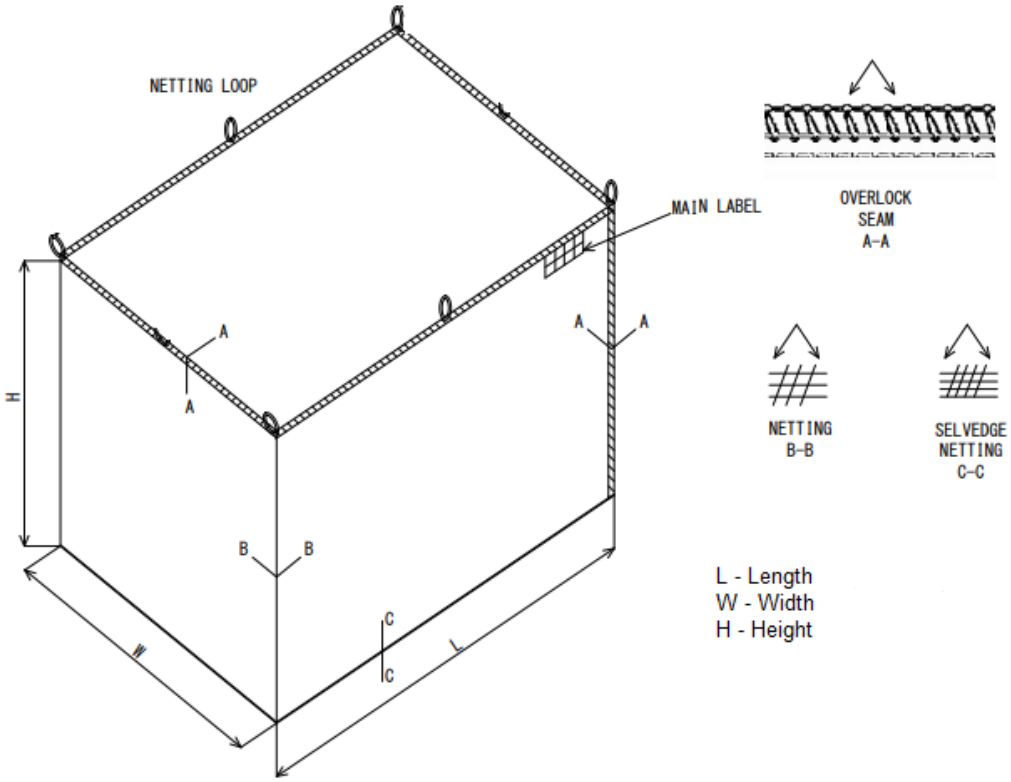

Figure I.1 — Typical illustration of a rectangular mosquito net

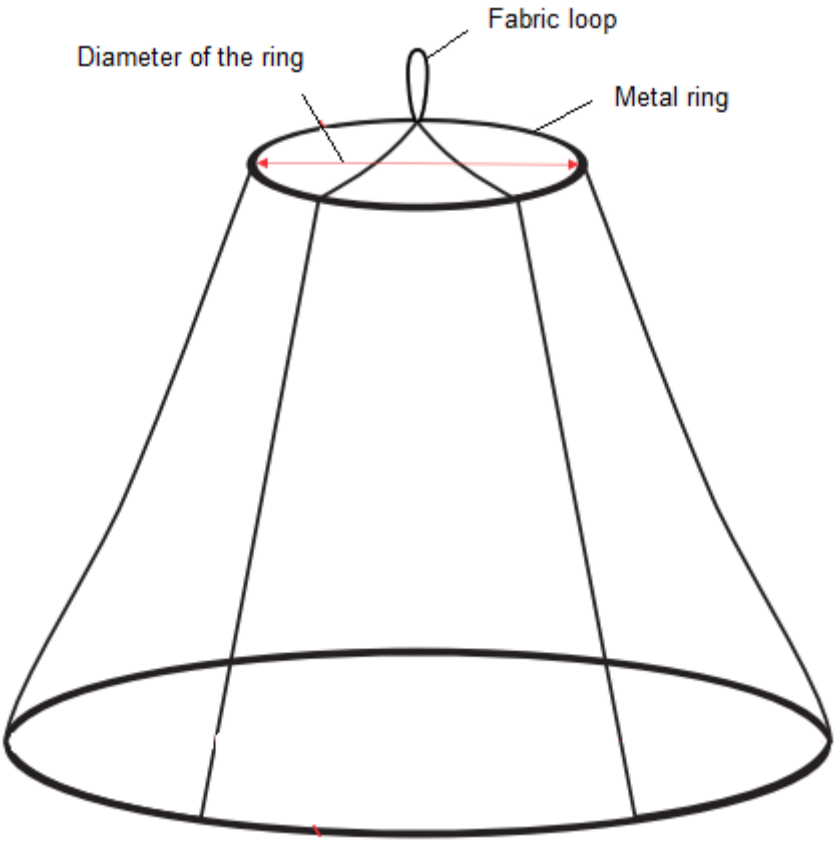

Figure I.2 — Typical illustration of a conical mosquito net

Annex I
(normative)

Determination of mesh count

I.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 in accordance with ISO 139 (4 h, 20 ºC, 65 % relative humidity).

I.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.
Rectangular and conical mosquito nets are illustrated in Figure I.1 and Figure I.2.

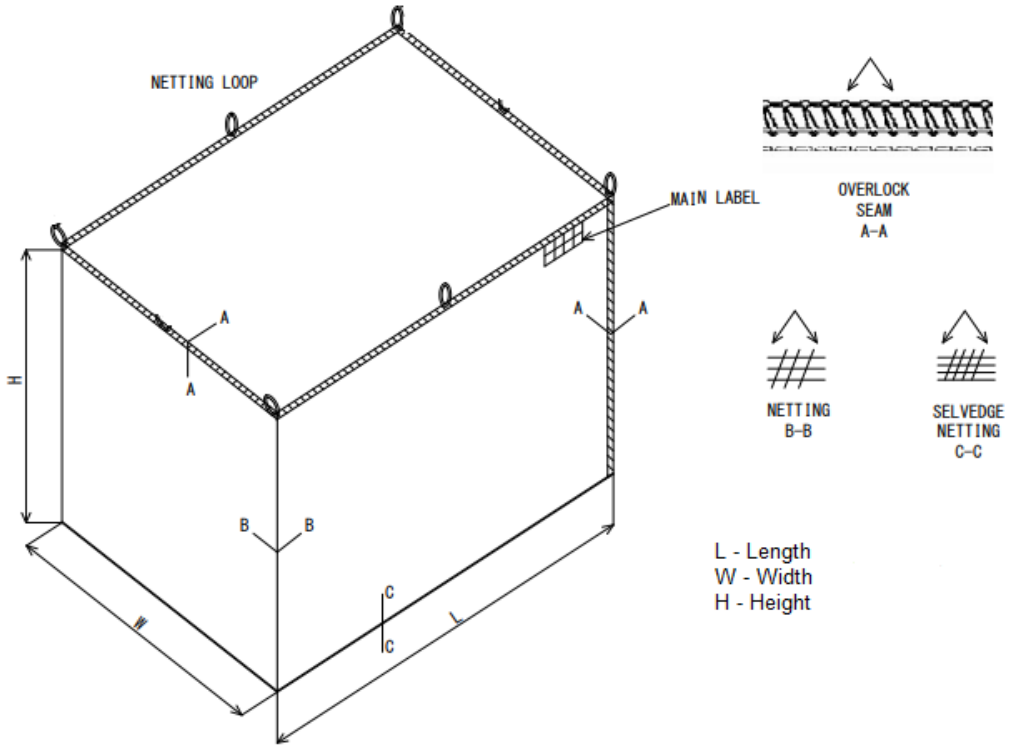

Figure I.1 — Typical illustration of a rectangular mosquito net

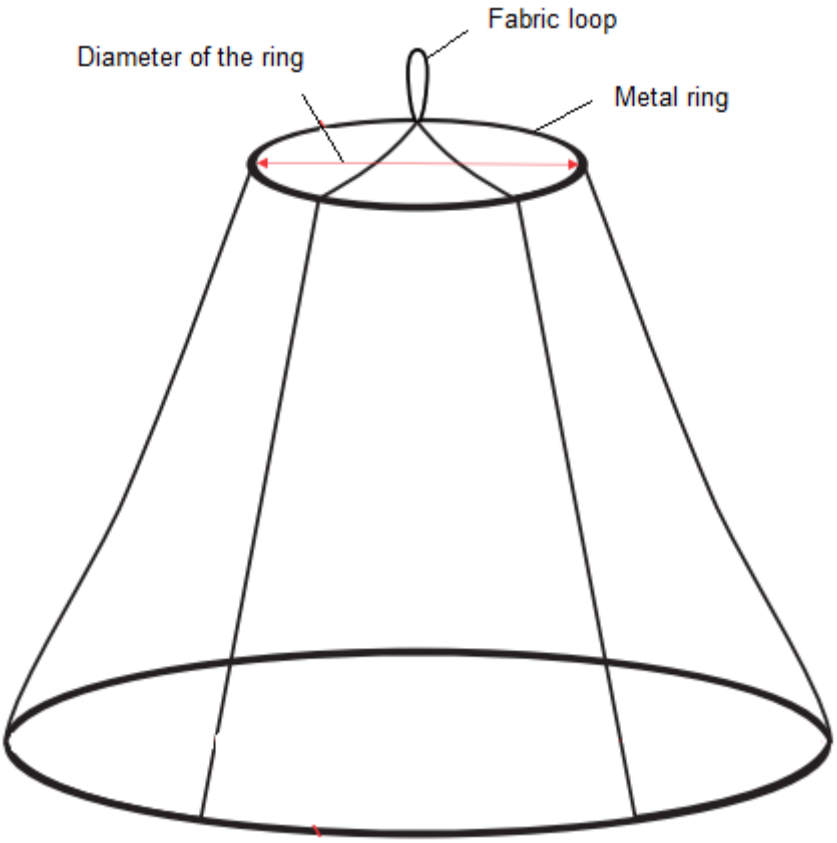

Figure I.2 — Typical illustration of a conical mosquito net

Bibliography

EAS 455: 2022, *Long lasting insecticide treated mosquito nets — Specification*