DUS DEAS 455

DRAFT UGANDA STANDARD

First Edition
2016-mm-dd

Long lasting insecticide treated mosquito nets — Specification

Reference number
DUS DEAS 455: 2016

Compliance with this standard does not, of itself confer immunity from legal obligations

A Uganda Standard does not purport to include all necessary provisions of a contract. Users are responsible for its correct application

The Executive Director
Uganda National Bureau of Standards
P.O. Box 6329
Kampala
Uganda
Tel: 256 417 333 250/1/2/3
Fax: 256 414 286 123
E-mail: info@unbs.go.ug
Web: www.unbs.go.ug

National foreword

Uganda National Bureau of Standards (UNBS) is a parastatal under the Ministry of Trade, Industry and Cooperatives established under Cap 327, of the Laws of Uganda, as amended. UNBS is mandated to co-ordinate the elaboration of standards and is

(a) a member of International Organisation for Standardisation (ISO) and

(b) a contact point for the WHO/FAO Codex Alimentarius Commission on Food Standards, and

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The work of preparing Uganda Standards is carried out through Technical Committees. A Technical Committee is established to deliberate on standards in a given field or area and consists of representatives of consumers, traders, academicians, manufacturers, government and other stakeholders.

Draft Uganda Standards adopted by the Technical Committee are widely circulated to stakeholders and the general public for comments. The committee reviews the comments before recommending the draft standards for approval and declaration as Uganda Standards by the National Standards Council.

This Draft Uganda Standard, DUS DEAS 455: 2016, *Long lasting insecticide treated mosquito nets — Specification,* is identical with and has been reproduced from a Draft East African Standard, DEAS 455: 2016*, Long lasting insecticide treated mosquito nets — Specification,* and is being proposed for adoption as a Uganda Standard.

This standard cancels and replaces US 307:2014, *Mosquito nets — Specification,* which has been technically revised.

This standard was developed by the Subcommittee on Textiles and textile products (SC 1) under the Textiles, leather, paper and related products' Standards Technical Committee (UNBS/TC 7).

Wherever the words, "East African Standard" appear, they should be replaced by "Uganda Standard."

DEAS 455: 2016

ICS 59.080.30

DRAFT EAST AFRICAN STANDARD

Long lasting insecticide treated mosquito nets — Specification

EAST AFRICAN COMMUNITY

Second Edition 2016

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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.

In order to achieve this objective, the Community established an East African Standards Committee mandated to develop and issue East African Standards.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. 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 procedures of the Community.

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.

EAS 455 was prepared by Technical Committee EAS/TC 061, *Textiles and Textile Products*.

This second edition cancels and replaces the first edition (EAS 455:2008), which has been technically revised.

Introduction

This East African Standard specifies the requirements of two types of mosquito nets namely treated and untreated nets.

Mosquito nets which are available in the market can be categorised according to their sizes. In determining the quality of this widely used customer item, more emphasis should be given not only to the netting fabric but also to stitching and other attachments. In that case shape, size and dimensions are very important. This East African Standard which outlines requirements for the manufacture and workmanship of mosquito nets will be the guidance for manufacturers and will protect the buyers.

For the purpose of deciding whether a particular requirement of this East African 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.

The number of significant places retained in the rounded off value shall be the same as that of the specified value in this East African Standard.

In the preparation of this Standard assistance was drawn from the following publications:

US 307: Mosquito Nets – Specifications

KS 1305-1, 2 Specification for mosquito netting.

Approved specifications published by World Health Organization on long l (WHO).

Long lasting insecticide treated mosquito nets — Specification

1 Scope

This Draft East African Standard prescribes the requirements and test methods for treated Long Lasting Insecticidal nets (LLIN).

2 Normative references

The following referenced documents are indispensable for the application of this standard. 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 numerical values.

EAS 237, Textiles — Determination of colour fastness — Colour fastness to washing — Test 3.

EAS 258, Textiles — Determination of length and width of fabrics.

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 139, Textiles — Standard atmospheres for conditioning and testing

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 16663-1*: Fishing nets -- Method of test for the determination of mesh size -- Part 1: Opening of mesh*

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

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

ISO 1833-23 *Textiles — Quantitative chemical analysis — Part 23: Mixtures of polyethylene and polypropylene (method using cyclohexanone)*

ISO 1833-24 *Mixtures of polyester and some other fibres(method using phenol and tetrachloroethane)*

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

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

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 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 8388: *Knitted fabrics -- Types -- Vocabulary*

ISO/TR 11827: *Textiles -- Composition testing -- Identification of fibres*

ISO 6938: *Textiles -- Natural fibres -- Generic names and definitions*

ISO 2076: *Textiles -- Man-made fibres -- Generic names*

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 within the yarn or wound around the fibres

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

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

3.3
active ingredients
biologically active substance that forms part of the formulation mixture that are incorporated in LLIN as approved by World Health Organization or any other internationally recognized body

3.4
top ring
used in conical nets and made of non-corrosive, anti-rust and anti-buckling material, fixed to the roof of the net

3.5
height
the dimension measured along a vertical seam from the top to the bottom edge of the net

3.6 circumference
the perimeter of the net at its bottom edge

4 Requirements

4.1 Mono-treated nets

4.1.1 Physical characteristics

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

Table 1 — Physical characteristics for Long lasting insecticidal mosquito nets

Parameter	Specification			Test method
	Polypropylene	Polyester	Polyethylene	
Fibre	100% **Polypropylene**	100 % polyester	100 % polyethylene	ISO 1833-1,2,5,11,16,24, 25 ISO 11827
Liner density (Denier), min.	100			ISO 2060
Filaments	Mono/Multi-filament	Multi-filament	Mono/multi-filament	
Mesh count, holes/cm^2,	20 – 25	20 – 25	≥ 8	ISO 139, ISO 16663-1
Weight, g/m^2,	≥30	≥36	40±10	ISO 3801
Bursting strength at 7.3 cm^2, Kpa, min	450	350	250	ISO 13938-1 or ISO 13938-2
Dimensional stability, max.	Threshold of 5 %			ISO 5077 ISO 6330 ISO 3759
Colour fastness to: • Light • Washing	4 or better 4 or better			ISO 105-B01, B02 EAS 237
Seam	Sewn using 100% polyester thread			

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 specified therein.

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

SL NO	Ingredient*	Specification, g/kg	Test Method
i	Deltamethrin	1.05 – 2.8	A
ii	Permethrin	17 – 23	B
iii	Alpha – cypermethrin	3.75 - 8.4	C
* 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 the active ingredient requirement shall be as follows:

- Permethrin as 20 g/kg ± 25 %; and
- Piperonyl butoxide (PBO) as 10 g/kg ± 25 %.

4.2.2 Combination nets constructed with more than one fibre shall comply with the requirements of Table 3.

Table 3 — Specification for combination nets

Parameter	Requirement		Test method
	Roof	Sides	
Fibre composition	Polyethylene	Polyester	ISO 1833-1,2,5,11,16,24,25 ISO 11827
Linear density (Denier), min.	100	75 with strengthened 70 cm lower border or 100 without border	ISO 2060
Mesh count, holes/cm²	20 – 25	20 – 25	ISO 139, ISO 16663-1
Weight, g/m²	40 ± 5	35 ± 5	ISO 3801
Bursting strength, kPa, min.	400	250 for 75 Denier and 350 for 100 Denier	ISO 13938 -1
Shrinkage/expansion (%) max.	5	5	ISO 5077 ISO 6330
Colour fastness to: • Light	4 or better		ISO 105 B01, B02

• Washing	4 or better		EAS 237
Active ingredient*, g/kg	PBO: 4 ± 25 %.	Deltamethrin: 2.8 ± 25 %.	Annex D Annex 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 4 and Table 5 for rectangular and conical nets respectively or in other sizes, as the purchaser may require.

Table 4 — Sizes and dimensions for rectangular net

No	Net size	Width, min., cm	Length, min., cm	Height, min., cm
1	X-small	70	120	150
2	Small	100	180	170
3	Medium	130	180	170
4	Large	160	180	170
5	X- Large	190	180	170
	Test method	**Annex E**		

Table 5 — Sizes and dimensions for conical nets

No.	Net size	Height, min., cm	Circumference, min., cm	Top ring diameter, min., cm
1	Dome shaped cover net	50	230	-
2	X small	120	300	40
3	Single fitted conical	180	850	56
4	Double fitted conical	220	1050	56
5	Extra double fitted	250	1250	65
	Test method	**Annex E**		

.4.4 Manufacture and workmanship

4.4.1 Construction

4.4.1.1 Reinforcement at Bottom:

The bottom edge of the walls shall be stitched and reinforced. The hem shall be firm and capable of withstanding the normal conditions of use.

NOTE A sheeting border is sometimes required 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 lock stitch and the number of stitches per decimetre shall be 30 to 38. The stitching shall be made by using spun polyester 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 metal rings or fabric loops.

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

4.4.2.3 Conical nets shall be equipped with a loop/non-rusting ring with sufficient strength.

4.4.3 Top support ring

Conical nets shall have a top support ring made of non-rusting metal and which has sufficient strength to withdraw the net when hanged at the required position.

4.4.4 Defects

The mosquito nets shall be free from defective holes (holes having diameter more than the normal hole size of the netting), stitching defects, stains and observable defects.

5 Packing and labelling

5.1 Packing

For bulk packaging, the material shall be leakproof, sufficiently large to wrap and strap completely the mosquito net bundles.

For individual nets, the requirements for packaging materials shall comply with the respective Partner States relevant legislation.

5.2 Labelling

5.2.1 Outside packaging

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

a) type of netting fibre;

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

c) country of origin;

d) colour;

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

f) lot or batch number;

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

h) care instructions in accordance with ISO 3758

5.2.2 Tag

The following information shall be legibly and indelibly marked on a permanent tag attached to individual nets

a) brand name or registered trademark;

b) type of netting fibre;

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

d) country of origin;

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

f) lot or batch number;

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

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

i) care instructions in accordance with ISO 3758

7 Sampling

7.1 Lot

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

7.2 Scale of sampling and testing

The number of pieces to be selected from a lot shall be in accordance with table 6. Samples shall be tested from each lot for ascertaining conformity to the requirements of this East African Standard.

Table 6 — 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 net. 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)

Weight 0.03 g(to the nearest of 0.01 mg) of Deltamethrin neat standard, quantitatively transfer to 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 1.3mL/min, isocratic

A.4.1.3 Guard Column Superguard 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 - 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 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 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 c.a 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

$$\text{Deltamethrin content (g/kg)} = \frac{a}{w}$$

where

a is the Deltamethrin reading from the analysis in milligrams; 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:

$$\text{Deltamethrin content (mg/m}^2\text{)} = \text{Deltamethrin content (g/kg)} \times D$$

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

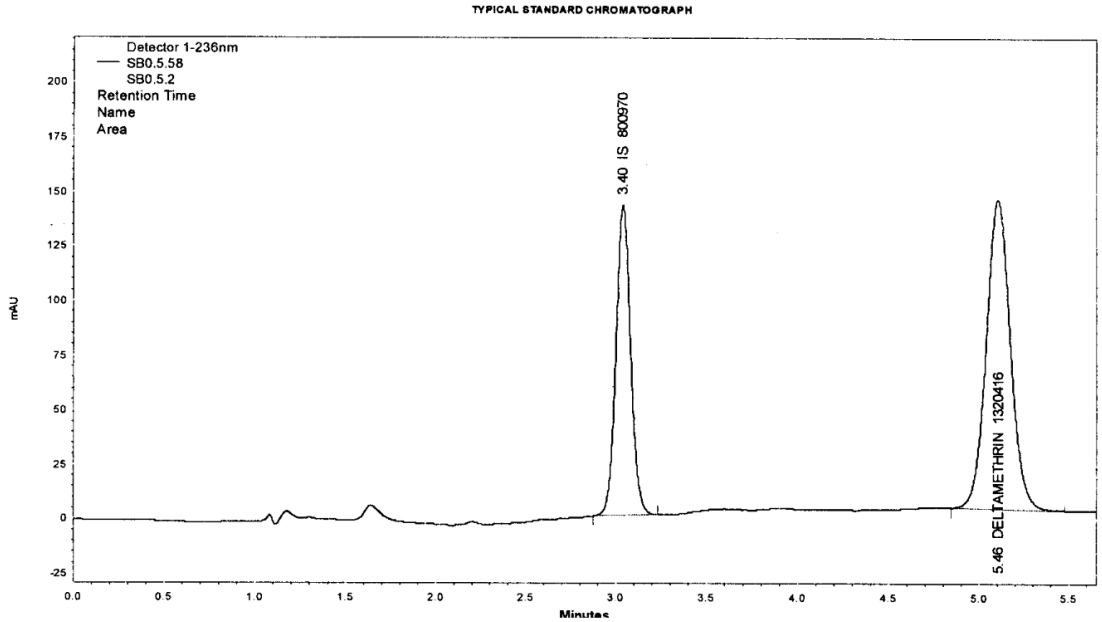

Figure 1 — Chromatogram of calibration solution

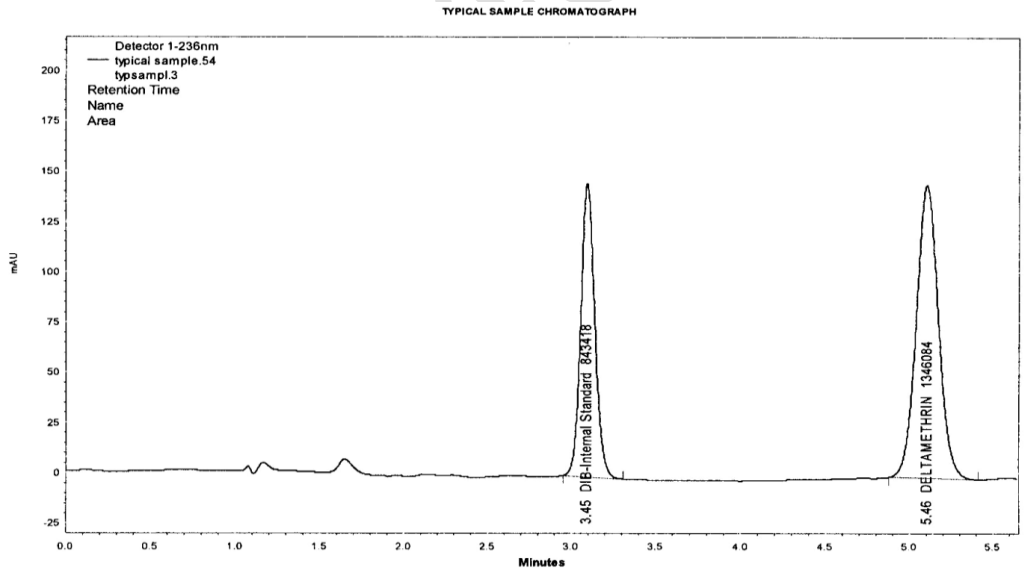

Figure 2 — Chromatogram of sample

Annex B
(normative)

Determination of Permethrin content in long lasting insecticide treated mosquito nets

B.1 Sample preparation

B.1.1 General

Take at least 100 g.

B.1.2 Identity tests

B.1.2.1 GLC, equipped with a split/splitless and a flame ionization detector. Capillary column fused silica, 30 m x 0.25 (i.d) mm, film thickness:0.25 µm, coated with cross linked dimethyl (DB-1 or equivalent) and electric integrator or data system

B.1.2.2 Infrared. Extract the sample with suitable solvent. Filter and evaporate the solvent. Proceed as for 331/TC/m/2.2

B.1.2.3 Permethrin. The content of permethrin in test samples are determined by capillary GC using flame ionization detection and triphenyl phosphate as internal standard, and trans-isomer ratio is calculated from the chromatogram obtained

B.2 Reagents

B.2.1 Heptane analytical grade

B.2.2 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.

B.2.3 Calibration solution. Homogenise the permethrin working standard. When the permethrin working standard is waxy solid or partly waxy solid homogenize it by warming it to melting and stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) 72 mg - 88 mg of permethrin working standards into a vial or stoppered flask (200 mL). Add by pipette internal standard solution (10 mL) and dissolve completely. Add by measuring cylinder heptanes (90 mL) and mix well (solutions C_A and C_B)

B.3 Procedure

B.3.1 Preparation of sample solution

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

B.3.2 Calculation of permethrin content

B.3.2.1 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 bracketed sample injections.

$$f_i = \frac{I_r \times s \times P}{H_s \times 2}$$

$$\text{Content of permethrin, g/kg} = \frac{f \times H_w}{I_q \times w}$$

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.

B.3.2.2 To determine precision,

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

B.4 Determination of surface concentration and release index

B.4.1 Reagents

To prepare the internal standard solution for calibration solution, dissolve triphenyl phosphate (0.1 g) in acetone (200 mL) to prepare a stock solution. Transfer by pipette the stock solution (5 mL) to a volumetric flask (50 mL). Make up to volume with acetone and mix well. Ensure that a sufficient quantity of this solution is prepared for all calibration standard to be analysed.

To prepare the calibration solution, homogenise the permethrin working standard. When the permethrin working standard is always solid or partly solid homogenise it by warming it to melting and by stirring. Prepare calibration solutions in duplicate. Weigh (to the nearest 0.1 mg) 90 mg - 110 mg (s mg) of permethrin working standard into a volumetric flask (100 mL) and make up to volume with acetone and mix well. Transfer by pipette this solution (1 mL to a volumetric flask (20 mL), make up to volume with acetone and mix well. Transfer by pipette this solution (5 mL) to a vial (20 mL), add by pipette internal standard solution for calibration solution (5 mL) and mix well (Solution C_A and C_B).

B.4.2 Apparatus as for 331/TC/m/3 except:

B.4.2.1 **Constant temperature oven** capable of controlling temperature with the range of ±2 ˚C is recommended.

B.4.2.2 **Rotary evaporator**

B.4.3 Procedure as for 331/TC/m/3 except:

B.4.3.1 Gas chromatographic conditions (typical)

B.4.3.2 Injection system

B.4.3.3 Slit flow approximately 10mL/min

B.4.4 Linearity check

Check the linearity of the detector response by injecting 1 µL of solution s with permethrin concentrations 0.1, 1 and 2.5 times that of the calibration solution before conducting analysis.

B.4.5 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 2.0 %. Then inject a 1-µL portion of the second solution. The response factor for this solution should not deviate by more than 2.0 % from that for the first calibration solution, otherwise prepare new calibration solutions.

B.4.6 Preparation of sample solution

Clean scissors and tweezers with acetone before use. Prepare sample solutions in tripricate for each sample (see Note 1) Cut ca .5 cm x 5 cm net samples with the scissors. Weigh accurately, to the nearest 0.1 mg, of each sample (w mg). Transfer it with the tweezers into a vial (20 mL). Add by pipette internal standard solution for sample solution (10 mL). Cap the vial and shake the solution by hand for 1 min (see Note 2). Take out ht netting with the tweezers and discard the solution.

Let the netting dry at room temperature for ca. 10 min. Transfer it into a vial (20 mL) with the tweezers. Cap the vial and place it in a temperature-controlled oven set at 70 ^{O}C. Heat the sample for 2 h (see Note 3). After heating, remove the vial from the oven and let it equilibrate to room temperature. Add by pipette internal standard solution for sample solution (10 mL) into the vial. Cap the vial tweezers. Let the netting dry at room temperature for ca. 10 min. Transfer the sample solution from the vial into the same round-bottom flask. Evaporate the solution in vacuo to dryness. Add by pipette acetone (2 mL) into the flask to dissolve the residue (solutions for “surface concentration at pot-wash 1”, S_{A1},S_{BI} and S_{C1}).

Transfer the dried netting into a vial (20 mL) with the tweezers. Repeat heating, internal standard solution adding, evaporation and dissolving procedures as above (solutions for surface concentration at post -wash 2”, S_{A2}, S_{B2} and S_{C2}). Let the netting dry at room temperature for ca. 10 min. Transfer the dried netting into a vial (20 mL) with the tweezers. Repeat heating, internal standard solution adding, evaporation and dissolving procedures as above. (Solutions for surface concentration at post-wash 3”, S_{A3},S_{B3}, and S_{C3})

NOTE 1 Analytical error is larger than average content determinations. Triplicate determinations, therefore, are recommended.

NOTE 2 The shaking speed is about 30 times per 10 s

NOTE 3 Put vials in a covered cardboard box while heating so that the vials are not exposed to direct streams of warm air.

B.4.7 Determination

Inject in duplicate 1 µL portions of each sample solution bracketing them by injections if the calibration solutions as follows; calibration solution CA, sample solution S_{A1}, sample solution S_{A1}, calibration solution CB, sample solution S_{B1}, sample solution S_{B1}, calibration solution CA and so on measure the relevant peak areas.

B.4.8 Calculation of surface concentration

Calculate the mean value of each pair of response factors bracketing the two injections of a sample and use this value for calculating the surface concentration of the bracketed sample injections.

$$f_i = \frac{I_r \times s \times P}{H_s}$$

$$\text{Surface concentration, µg/g} = \frac{f \times H_w}{I_q \times w \times 2}$$

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 of permethrin working standard taken,

w is the mass of sample taken, and

P is the purity of permenthrin working standard.

B.4.9 Calculation of release index

Calculate the mean value of the two injections of sample solutions SA3, SB3, and SC3 by the equations described in B.9.1, and the release index for each piece of the netting.

$$\text{Release index} = \frac{C}{B}$$

Where

B is the mean value of surface concentration at post-wash 2 (µg/g)

C is the mean value of surface concentration at post-wash 3 (µg/g)

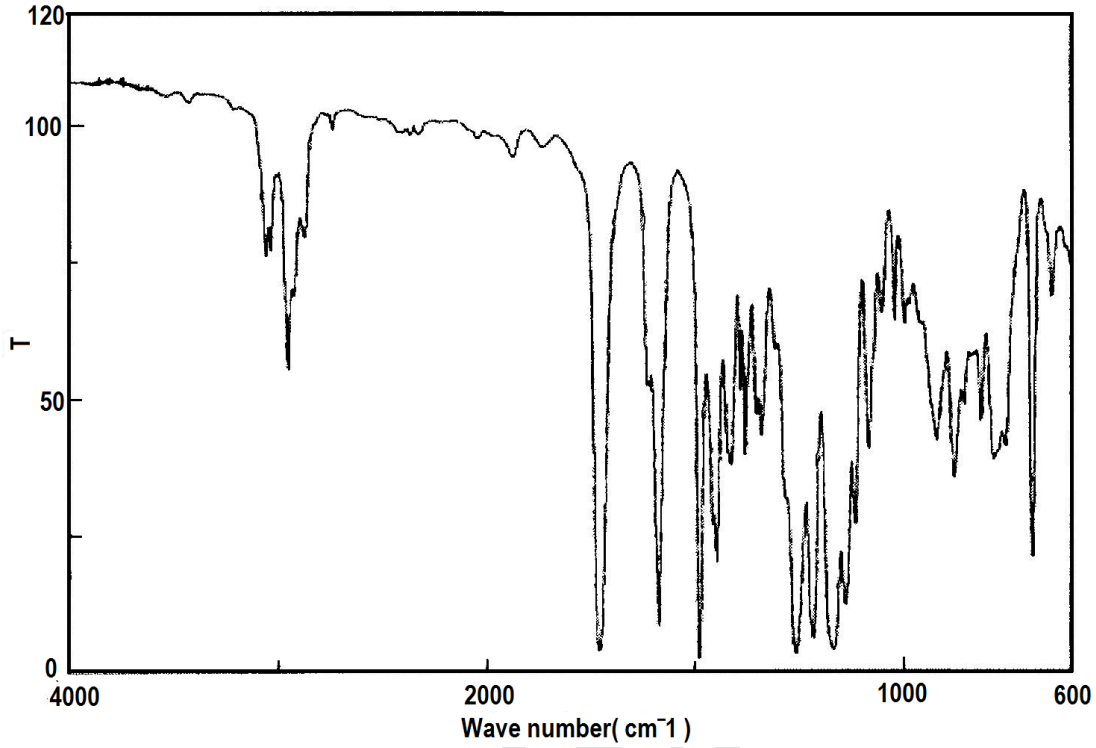

Figure 1 — Infrared spectrum of permethrin

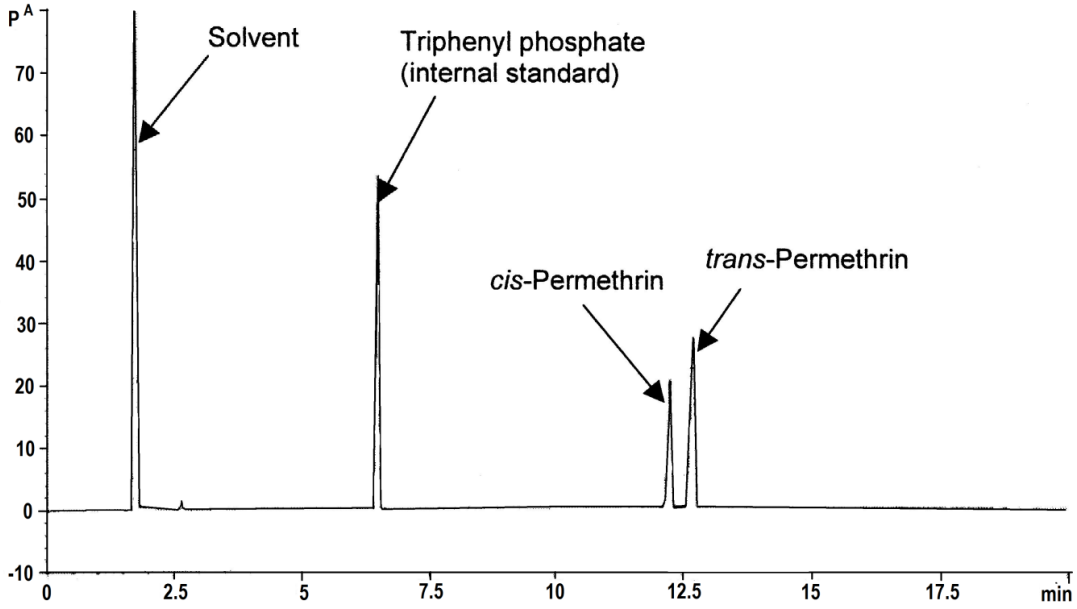

Figure 2 — Example of gas chromatogram of permethrin TC

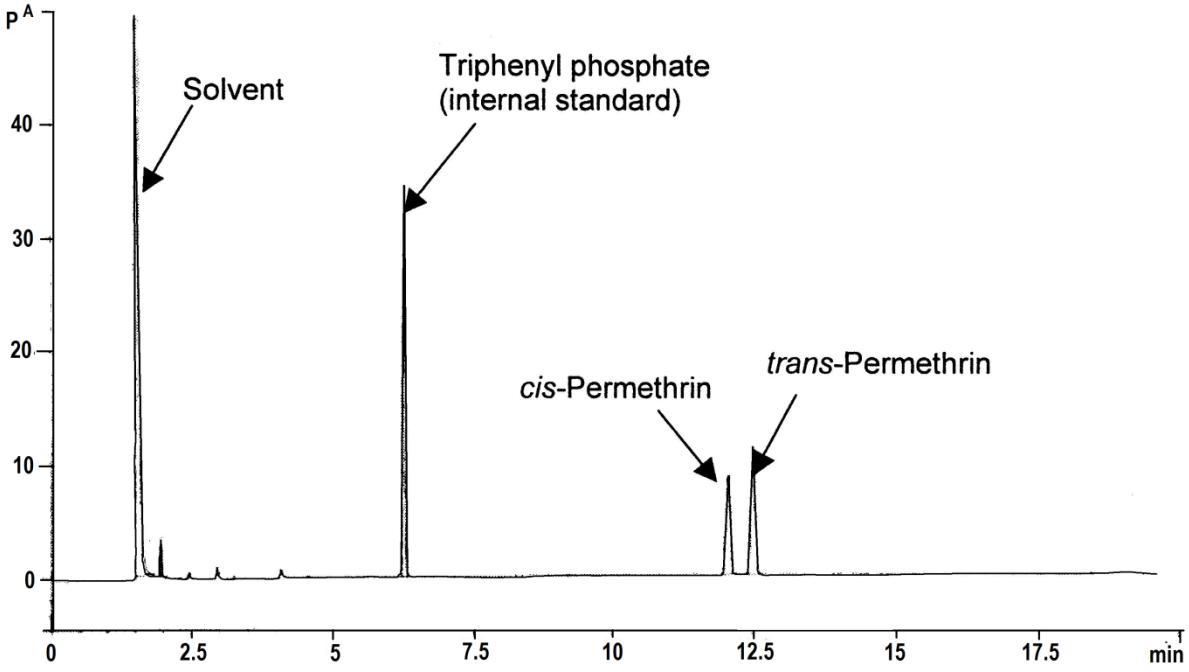

Figure 3 — Example of gas chromatogram of permethrin LN

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 - 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 atleast 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 (25ml). 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.5.3.1 Capillary column fused silica (for example, DB-1) 30mx 0.32 mm, film thickness of 0.25 µm

C.5.3.2 Electronic data evaluation system (for example, HP ChemStation)

C.5.3.3 Ultrasonic bath

C.5.3.4 Electronic balance

C.5.3.5 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 (1L).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 (note: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 100ml ethyl acetate each by shaking twice for 30 s. Both portions of ethyl acetate are combined together in a flask (250 mL). 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

$$\text{Concentration} = \frac{f \times H_w}{l_q \times w}$$

Where

f is the response factor

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

Lq is 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 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

a) Xylene

b) Piperonyl butoxide standard of known purity. Store below 0°C.

c) 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.

d) 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 5 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 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) Sprit 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

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 – 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 minutes. 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.

Concentration = R/w (g/kg)

Where:

R = Piperonyl butoxide reading from the analysis in mg

w = mass of sample taken in g

Annex E
(normative)

Measurement of mosquito net dimensions

D.1 Rectangular nets: Length, width and height

D.1.1 Apparatus

a) Flat table

b) Measuring tape or steel rule

D.1.2 Conditioning

Condition the net samples in accordance with ISO 139.

D.1.3 Procedure

Lay the conditioned net sample on a flat table (D.1.1.2), and take measurements of height, width and diameter.

D.1.4 Calculation

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

D.1.5 Report

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

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

D.2.1 Apparatus

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

b) Measuring tape or steel rule

c) Twine, of measuring at least 10 m

d) Felt pen marker

D.2.2 Conditioning

Condition the net sample in accordance with ISO 139.

D 2.3 Procedure

D.2.3.1 Top ring diameter

D.2.3.1.1 Procedure

Place the top portion of the net sample on a flat table (D.1.1.a) and put the twine (D.2.1 c) around the circumference of the top ring of the net sample, identifying the two ends with a marker (D 2.1 d) which represent the dimension of the top ring. Using a measuring tape (D.2.1 b) 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.

D.2.3.1.2 Calculation

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

D.2.3.1.3 Report

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

D.2.3.2 Bottom circumference

D.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.

D.2.3.2.2 Calculation

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

Calculate the bottom circumference as N x 2.

D.2.3.2.3 Report

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

D.2.3.3 Conical net height

D.2.3.3.1 Procedure

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

D.2.3.3.2 Report

Report the least measurement taken as the conical net height.

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