

**WHO SPECIFICATIONS AND EVALUATIONS  
FOR PUBLIC HEALTH PESTICIDES**

**ALPHA-CYPERMETHRIN + PYRIPROXYFEN**

**LONG-LASTING (INCORPORATED INTO  
FILAMENTS) INSECTICIDAL NET**

A racemic mixture of:

(*S*)- $\alpha$ -cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dichlorovinyl)-  
2,2-dimethylcyclopropane-carboxylate and  
(*R*)- $\alpha$ -cyano-3-phenoxybenzyl-(1*S*,3*S*)-3-(2,2-dichlorovinyl)-  
2,2-dimethylcyclopropane-carboxylate

+

4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether



**World Health  
Organization**

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## Disclaimer<sup>1</sup>

WHO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

WHO disclaims any and all liability for any injury, death, loss, damage or other prejudice of any kind that may be arise as a result of, or in connection with, the manufacture, sale, transportation, storage, handling, preparation and/or use of pesticides which are found, or are claimed, to have been manufactured to comply with these specifications.

Additionally, WHO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

WHO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, WHO does not in any way warrant or represent that any pesticide claimed to comply with a WHO specification actually does so.

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<sup>1</sup> This disclaimer applies to all specifications published by WHO.

## INTRODUCTION

WHO establishes and publishes specifications\* for technical material and related formulations of public health pesticides with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 2002, the development of WHO specifications follows the **New Procedure**, described in the Manual for Development and Use of FAO and WHO Specifications for Pesticides. This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by WHO and the experts of the “FAO/WHO Joint Meeting on Pesticide Specifications” (JMPS).

WHO specifications now only apply to products for which the technical materials have been evaluated. Consequently, from the year 2002 onwards the publication of WHO specifications under the **New Procedure** has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

**Part One:** The Specification of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the above-mentioned manual.

**Part Two:** The Evaluation Report(s) of the pesticide, reflecting the evaluation of the data package carried out by WHO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the above-mentioned manual and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

WHO specifications under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. WHO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

**Specifications bear the date (month and year) of publication of the current version. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.**

\* Footnote: The publications are available on the Internet under (<http://www.who.int/pq-vector-control/prequalified-lists/en/>).

**PART ONE**  
**SPECIFICATIONS**

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**ALPHA-CYPERMETHRIN + PYRIPROXYFEN**

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# WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

## ALPHA-CYPERMETHRIN

### INFORMATION

#### Common name

alpha-cypermethrin (E-ISO, BSI), alpha-cyperméthrine (F-ISO)

#### Synonyms

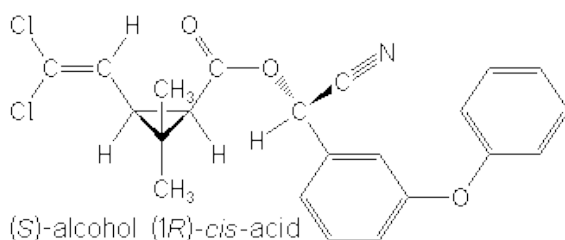
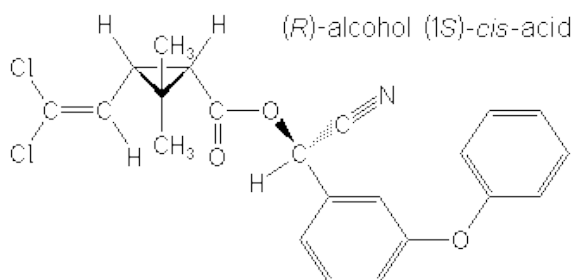
alphamethrin (rejected common name), alfoxylate

#### Chemical names

**IUPAC** a racemic mixture of: (*S*)- $\alpha$ -cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (*R*)- $\alpha$ -cyano-3-phenoxybenzyl-(1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

**CA:** [1 $\alpha$ (*S*\*),3 $\alpha$ ]-( $\pm$ )-cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate

#### Structural formula



#### Empirical formula

$C_{22}H_{19}Cl_2NO_3$

#### Relative molecular mass

416.3

*CAS Registry number*

67375-30-8

*CIPAC number*

454

*Identity tests*

GC retention time, IR spectrum.

# WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

## PYRIPROXYFEN

### INFORMATION

*ISO common name*

pyriproxyfen (BSI, E-ISO)

*Synonyms*

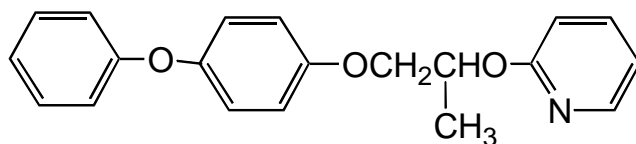
none

*Chemical names*

*IUPAC* 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether

*CA* 2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine

*Structural formula*



*Empirical formula*

$C_{20}H_{19}NO_3$

*Relative molecular mass*

321.37

*CAS Registry number*

95737-68-1

*CIPAC number*

715

*Identity tests*

HPLC retention time, IR spectrum.



## WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

### ALPHA-CYPERMETHRIN + PIRIPROXYFEN LONG-LASTING (INCORPORATED INTO FILAMENTS) INSECTICIDAL NET

WHO specification 454+715/LN (November 2018\*)

*This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (454+715/2018). It should be applicable to relevant products of this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers, irrespective of the source of TC. The evaluation report (454+715/2018), given in PART TWO, forms an integral part of this publication.*

#### 1 Description

The product shall be in the form of netting made with a 4-lock stitch warp knitting pattern (Note 1), consisting of 120 or 150 denier (Note 2) monofilament fibres made from a blend of High Density Polyethylene (HDPE) and Linear Low Density Polyethylene (LLDPE), incorporating technical alpha-cypermethrin complying with the requirements of WHO specification 454/TC (January 2013) and technical pyriproxyfen complying with the requirements of WHO specification 715/TC (October 2017), together with any other necessary formulants. The product shall appear clean and shall be free from visible extraneous matter (Note 3), visible damage (such as splitting or tearing) and visible manufacturing defects (such as poorly made seams or a knit structure that is either not uniform or too loose to remain uniform in use), and shall be suitable for use as an insecticidal net with long-lasting activity (Note 4).

#### 2 Active ingredients

- 2.1 **Identity tests** (CIPAC/5043, extension of 454/LN/M/2, CIPAC Handbook M, p. 40, 2009 for alpha-cypermethrin; CIPAC/4887, extension of 715/TC/M/2 and 715/LN/M/2, CIPAC Handbook O, p. 143, 2017 and for pyriproxyfen) (Notes 5 & 6)

The active ingredients shall each comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

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\* This specification is applicable to long-lasting (incorporated into filaments) insecticidal nettings and nets produced by Disease Control Technologies, LLC and commercialised under the trade name of Royal Guard™. The subject of the extension of specifications for LN has been discussed by the JMPS in 2009. The 2009 Meeting agreed that - in contrast to other formulations - an extension of a specification to nominally similar LN of other manufacturers was not possible with the data currently available and that the manufacturer and the product should be named in a footnote or in the specification.

Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: <http://www.who.int/pq-vector-control/prequalified-lists/en/>

2.2 **Alpha-cypermethrin content** (CIPAC/5043, extension of 454/LN/M/3.2, CIPAC Handbook M, p. 41, 2009) (Notes 5 & 7)

The alpha-cypermethrin content shall be declared (5.5 g/kg for 120 denier yarn and 5.0 g/kg for 150 denier yarn) and, when determined, the average measured content shall not differ from that declared by more than  $\pm 25\%$ .

2.3 **Pyriproxyfen content** (CIPAC/4887, extension of 715/TC/M/3 and 715/LN/M/3, CIPAC Handbook O, p. 143, 2017) (Notes 6 & 7).

The pyriproxyfen content shall be declared (5.5 g/kg for 120 denier yarn and 5.0 g/kg for 150 denier yarn) and, when determined, the average measured content shall not differ from that declared by more than  $\pm 25\%$ .

2.4 **Alpha-cypermethrin wash resistance index** (MT 195, CIPAC Handbook O, p. 205, 2017) (Note 8)

The wash resistance index of alpha-cypermethrin from the netting, when determined, shall be within the range 93% to 101%.

2.5 **Pyriproxyfen wash resistance index** (MT 195, CIPAC Handbook O, p. 205, 2017) (Note 8)

The wash resistance index of pyriproxyfen from the netting, when determined, shall be within the range 88% to 99%.

3 **Physical properties** (Notes 7 & 14)

3.1 **Fabric weight (mass per m<sup>2</sup>)** (ISO 3801 / EN 12127)

The mass per unit area shall be declared (40 g/m<sup>2</sup> for 120 denier yarn and 45 g/m<sup>2</sup> for 150 denier yarn), and when determined, shall not differ from that declared by more than  $\pm 5$  g/m<sup>2</sup>.

3.2 **Netting mesh size**

When counted by the method given in Note 9, the average number of complete holes/cm<sup>2</sup> shall be not less than 20 holes/cm<sup>2</sup> and the lowest value shall be not less than 18 holes/cm<sup>2</sup>.

3.3 **Dimensional stability of netting to washing** (Note 10)

Not more than 5% shrinkage/expansion in both directions.

3.4 **Bursting strength** (ISO 13938:2) (Note 11)

The bursting strength of the fabric shall be declared (not less than 400 kPa for 120 denier yarn, not less than 450 kPa for 150 denier yarn) and, when determined, the average shall be not less than that declared.

If seams are present, their average bursting strength shall be not less than the measured average for the fabric.

### 3.5 Flammability (EN 1102) (Note 12)

Tested according to EN 1102 the following requirements should be achieved\*:

After removing the ignition source the following fire phenomena should not occur:

- ignition
- propagation of the flame or glow.
- flaming debris
- ignition of the filter paper

\*Fulfilling the requirements above the flame speed rate is 0 mm/s, i.e., no flame or glow achieves first and third marker threads.

Formation of holes is allowed provided that the burnt or melted width and length of the holes does not exceed 50 mm and 150 mm, respectively.

## 4 Storage stability

### 4.1 Stability at elevated temperature (MT 46.3.4, CIPAC Handbook O, p. 176, 2017)

After storage at  $54 \pm 2^\circ\text{C}$  for 2 weeks, the determined average active ingredients content (measured individually) shall not be lower than 95%, relative to the determined average content found before storage (Note 13) and the product shall continue to comply with the clauses for:

- wash resistance index (2.4 and 2.5);
- dimensional stability (3.3);
- bursting strength (3.4).

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**Note 1** The specification applies to manufactured nets and bulk netting, which may be rectangular or conical in design.

The knitting pattern of this netting material is specified to as a 4-lock stitch warp knit structure with a lock in each of the 4 corners of a hole - see figure 1 hereafter.

**Note 2** The linear density (denier) of the fibres cannot be measured in the netting or the manufactured bed net but it should be identified on the packaging.

**Note 3** Occasional short lengths of loose thread present in made up nets are not considered to be extraneous matter.

**Note 4** Long-lasting insecticidal netting is expected to retain its insecticidal activity during its life span and through a number of washes (public health products).

**Note 5** The extension of the scope (CIPAC/5043) of CIPAC methods 454/LN/M/2 & 3.2 for identification and determination of alpha-cypermethrin content in incorporated into polyethylene LN containing both alpha-cypermethrin and pyriproxyfen, with the modification of having di-cyclohexyl phthalate as internal standard was accepted as full CIPAC method in 2017, but the methods are not yet published in a Handbook. Prior to their publication in the next Handbook, copies of the methods may be obtained through the CIPAC website, <https://www.cipac.org/index.php/methods-publications/pre-published-methods>

**Note 6** The extension of the scope (CIPAC/4887) of CIPAC methods 715/TC/M/2 & 3 for identification and determination of pyriproxyfen in incorporated into polyethylene LN containing both permethrin and pyriproxyfen was confirmed in 2016 (CIPAC/5043) to be applicable for identification and determination of pyriproxyfen in incorporated into

polyethylene LN containing both alpha-cypermethrin and pyriproxyfen. The CIPAC methods 715/LN/M/2 & 3 for identification and determination of pyriproxyfen in incorporated into polyethylene LN containing both permethrin and pyriproxyfen are published in Handbook O, p. 143, 2017.

**Note 7** Samples should be taken according to Figure 2 (see after the Notes) or on a convenient diagonal across the width of bulk material. Samples must be sufficiently large to conduct all tests required and representative of the net or netting. Except where seams are to be tested, do not test material within 10 cm of seams or knit selvedge.

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

**Note 8** The content of alpha-cypermethrin in the net pieces before and after washing should be determined by the method CIPAC/5043, extension of the scope of CIPAC method 454/LN/M/3.2.

The content of pyriproxyfen in the net pieces before and after washing should be determined by the method CIPAC/4887, extension of method 715/TC/M/3, now published as CIPAC method 715/LN/M/3 in Handbook O, p. 143, 2017.

Wash resistance index values higher than 100% and up to 101% are acceptable due to the uncertainty of measurement of the CIPAC method MT 195.

**Note 9** In the absence of a simple or standard method to determine the size of holes, which may have complex shapes, in highly flexible fabrics, mesh size is determined by counting the number of holes in a square of the fabric. Counting may be done directly on the fabric or indirectly by taking a picture/photocopy of the fabric. Indirect methods may ease counting and provide a permanent record. The number of holes per measured area is converted in holes/cm<sup>2</sup>. Before counting, the fabric should be conditioned according to ISO 139 (4 h, 20°C, 65% relative humidity).

Use a template to define the square of netting, taking care not to stretch or distort the fabric. The template should be a 1-2 mm thick rigid sheet, in/on which an accurately calibrated ( $\pm 1\%$  in each dimension) square (e.g. 1 x 1 in or 5 x 5 cm) has been cut/marked. If a template is not available and a ruler must be used, great care is required to ensure that the area counted is square. Where practicable, one edge of the square to be counted should be aligned with a row of complete holes in the fabric. Incomplete holes  $\geq \frac{1}{2}$  are counted as complete holes, whereas those  $< \frac{1}{2}$  are not counted. Count 5 replicate squares selected according to Note 6, calculate the average and note the lowest value.

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

**Note 10** Method of preparation, marking and measuring: ISO 3759. Method of washing: ISO 6330. Method of calculation: ISO 5077. Size of test portions: 500 mm x 500 mm; mark off 350 mm x 350 mm within each test portion. Test a total of 4 replicate portions, 2 washed in each of 2 separate loads. Type of washing machine: ISO type A (front loading). Washing programme: 30°C Mild programme. Fill the washer with fabrics and ballast Type III (polyester ballast) up to 2 kg (according to the ISO 6330 standard). Drying: flat drying.

**Note 11** Test method: ISO 13938 part 2 with conditioning of the fabric as specified in the ISO standard. The declared bursting strength, and testing for compliance with it, should be based on tests of 7.3 cm<sup>2</sup> areas of fabric. Five replicate tests should be conducted on samples taken at approximately equal distances on a diagonal across the netting, taking no sample within 10 cm of a border or seam. In made up rectangular nets, the "diagonal" may correspond to figure 1. The average of the 5 measurements is calculated.

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

Note 12 Flammability test according to EN 1102, using the surface ignition method (position the burner perpendicular to the surface of the specimen).

The following shall be reported: the after flame time, the afterglow time, the maximum burnt or damage width and length, whether or not flame reaches vertical edge of the specimen, whether or not a hole is burnt or melted in the specimen, whether or not any flaming debris falls below the bottom edge of the specimen and ignition of the filter paper.

Definitions according to ISO 4880:1997 (not included in EN 1102:1995 and EN ISO 6941:1995):

- Ignition: initiation of combustion.
- Combustion: exothermic reaction of a combustible substance with an oxidizer, accompanied by flames and/or glowing and/or emission of smoke.

Procedure for measuring burnt or damage width and length dimensions of each specimen:

Remove the specimen from the specimen holder and place it on a flat horizontal surface. Place a rule on top of the test specimen along the line of maximum damage and parallel with the length side of the test specimen. Measure the maximum length in millimetres from the lowest point of burnt or damage to the end of the hole. To measure the burnt or damage width, proceed in the same way but with the ruler parallel to the width side of the test specimen. Proceed in the same way for the other 5 specimens.

Note 13 Samples of the product taken before and after the storage stability test should be analyzed concurrently after the test in order to reduce the analytical error.

Note 14 Normative references for physical tests:

Currently the following standards are the latest versions of the documents to be used for physical tests. The updated version of the standard should always be used when available.

ISO 139:2005/Amd.1:2011 Textiles - Standard atmospheres for conditioning and testing.- Textiles - Standard atmospheres for conditioning and testing.

ISO 3801:1977 - Textiles - Woven fabrics - Determination of mass per unit length and mass per unit area.

EN 12127:1997 - Textiles - Fabrics - Determination of mass per unit area using small samples.

ISO 3759:2011 - Textiles - Preparation, marking and measuring of fabric specimens and garments in tests for determination of dimensional change.

ISO 6330:2012 - Textiles - Domestic washing and drying procedures for textile testing.

ISO 5077:2007 - Textiles - Determination of dimensional change in washing and drying.

ISO 13938-2:1999 - Textiles - Bursting properties of fabrics - Part 2: Pneumatic method for determination of bursting strength and bursting distension

EN 1102:1995 - Textiles and textile products. Burning behaviour. Curtains and drapes. Detailed procedure to determine the flame spread of vertically oriented specimens.

Figure 1 Knitting pattern of Royal Guard™.

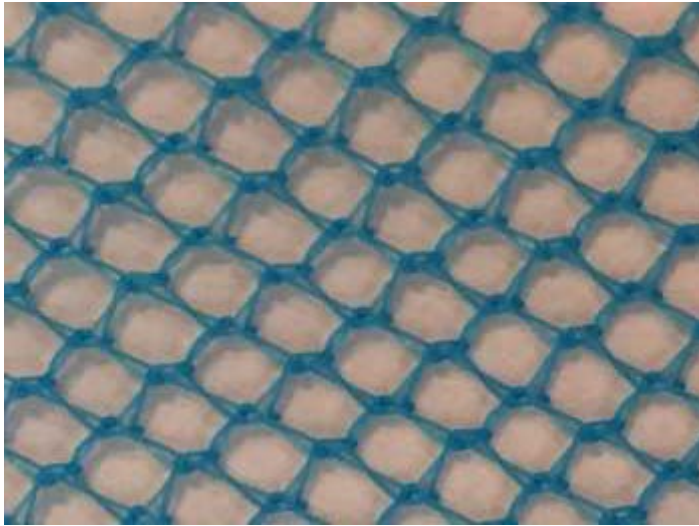
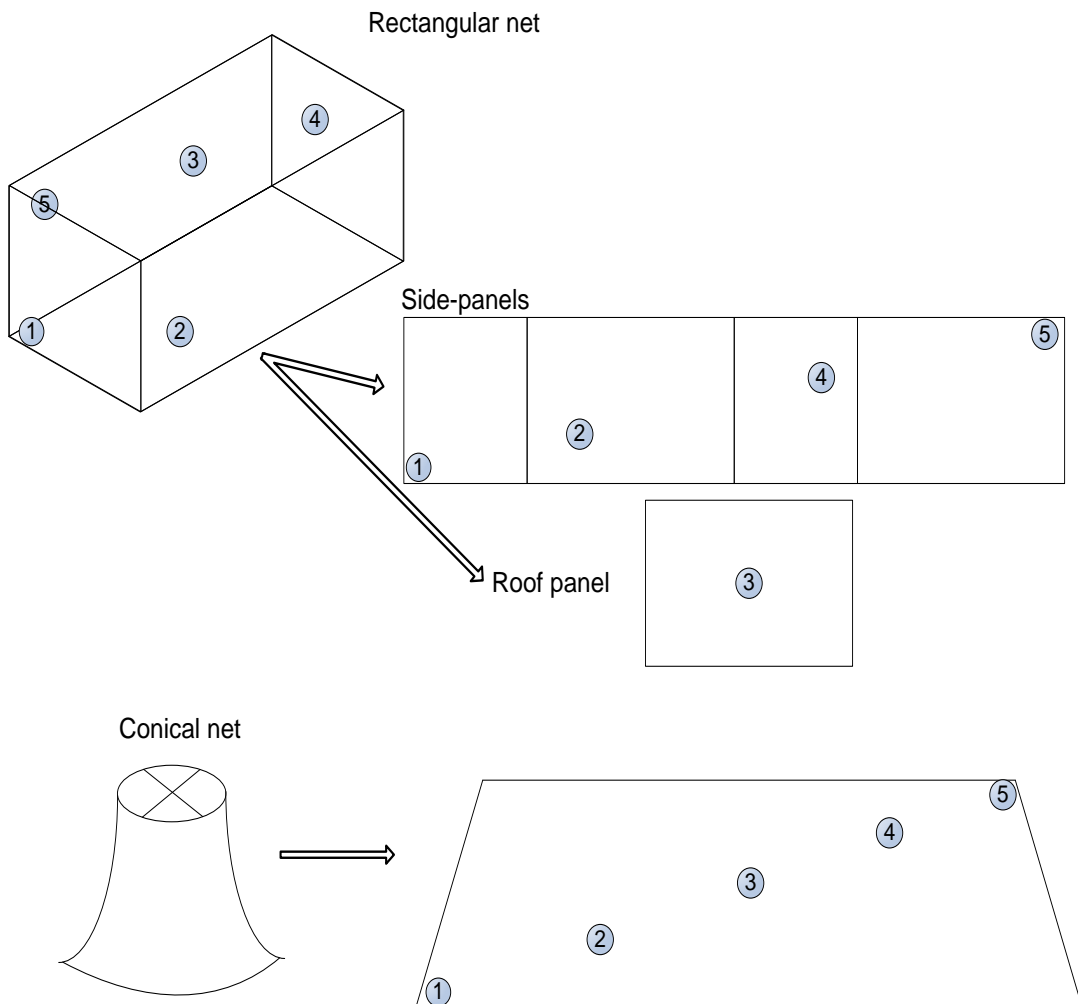


Figure 2 Recommended positions from which 5 pieces of netting should be taken from a made up bed net and combined to form a representative sample.



**PART TWO**  
**EVALUATION REPORTS**

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**ALPHA-CYPERMETHRIN + PIRIPROXYFEN**

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## WHO SPECIFICATIONS FOR PUBLIC HEALTH PESTICIDES

### **ALPHA-CYPERMETHRIN + PYRIPROXYFEN**

#### FAO/WHO EVALUATION REPORT 454+715/2018

#### **Recommendations**

The Meeting recommended the following:

The specification for alpha-cypermethrin + pyriproxyfen long-lasting (incorporated into filaments) insecticidal net, proposed by Disease Control Technologies, LLC, and as amended, should be adopted by WHO, subject to the successful assessment of the product by WHO PQT-VC.

#### **Appraisal**

A draft specification and supporting data for alpha-cypermethrin + pyriproxyfen long-lasting (incorporated into filaments) insecticidal net (LN), provided by Disease Control Technologies, LLC (USA), were considered by the Meeting for development of a new WHO specification. The data and test reports provided by the manufacturer to support this specification were generated by the manufacturer as well as by independent laboratories. The proposed specification was largely in agreement with the LN specification guideline of the FAO/WHO Manual on pesticides specifications (FAO/WHO 2016).

The LN under consideration (Royal Guard™) is a warp-knitted fabric in which alpha-cypermethrin and pyriproxyfen are incorporated into 120 or 150 denier monofilament fibres made from a blend of High Density Polyethylene (HDPE) and Linear Low Density Polyethylene (LLDPE).

The manufacturer provided written confirmations that the active ingredients (alpha-cypermethrin and pyriproxyfen) incorporated into the LN are from sources compliant with the existing WHO specifications 454/TC (January 2013) for alpha-cypermethrin and 715/TC (October 2017) for pyriproxyfen (Tagros Chemicals India Limited).

FAO/WHO specifications normally refer only to a single active ingredient. In this particular case where alpha-cypermethrin, a non-systemic insecticide with contact and stomach action, and pyriproxifen, an insect growth regulator, are co-formulated in order to offer an new product against malaria vectors, the Meeting agreed to develop a single specification for this formulation containing a mixture of two active ingredients.

#### Description

The Meeting agreed that the specification should be applied to netting, in bulk, and to finished bed nets, consisting of 120 or 150 denier monofilament fibres made from a proprietary blend of High Density Polyethylene (HDPE) and Linear Low Density Polyethylene (LLDPE). The manufacturer informed the Meeting that the knitting pattern is specified as a 4-lock stitch warp knit structure with a lock in each of the 4 corners of a hole. The Meeting agreed to reflect this property in the description clause and to include a figure of this knitting pattern. Nevertheless this property was not supported by a relevant physical test.



### Active ingredients content

The target doses of both alpha-cypermethrin and pyriproxyfen are 5.5 g/kg for 120 denier yarn and 5.0 g/kg for 150 denier yarn respectively with a tolerance of  $\pm 25\%$ . This difference in active ingredients content for the two fabric weights when expressed in g/kg was proposed by the manufacturer in order to keep the same active ingredient content of 225 mg/m<sup>2</sup>.

The manufacturer initially collaborated with an independent laboratory in order to develop a single extraction method followed by HPLC in order to determine both alpha-cypermethrin and pyriproxyfen content. The content of alpha-cypermethrin and pyriproxyfen was determined by reversed phase high performance liquid chromatography using UV detection at 254 nm (HPLC-DAD) and dicyclohexyl phthalate as internal standard after extraction by ultrasonication with heptane or xylene and solvent exchange to acetonitrile. Nevertheless, the Meeting did not accept this method because it does not allow to separate the cis I isomer from the cis II isomer (alpha-cypermethrin).

The manufacturer later confirmed that the CIPAC methods 454/LN/M/2 & 3.2 published in Handbook M are applicable for identification and determination of alpha-cypermethrin content in incorporated into polyethylene LN containing both alpha-cypermethrin and pyriproxyfen. The content of alpha-cypermethrin is determined by capillary gas chromatography using flame ionisation detection (GC-FID) and dioctyl phthalate as internal standard, after extraction by refluxing with xylene and 10% citric acid solution for 30 minutes. Citric acid is added to avoid the epimerization of alpha-cypermethrin during the extraction and in the inlet of the GC.

The manufacturer provided later a method based on the method CIPAC/4887 (which is an extension of the CIPAC method 715/TC/M/3) for determination of pyriproxyfen and alpha-cypermethrin. In this method, alpha-cypermethrin and pyriproxyfen are extracted in a water bath at 85-90°C for 45 minutes with heptane in presence of dicyclohexyl phthalate as internal standard and citric acid. Alpha-cypermethrin is determined by gas chromatography with flame ionisation detection (GC-FID), and pyriproxyfen by high performance liquid chromatography with UV diode array detection (HPLC-DAD) after solvent exchange to acetonitrile.

The extension of the scope (CIPAC/5043) of CIPAC methods 454/LN/M/2 & 3.2 for identification and determination of alpha-cypermethrin content in incorporated into polyethylene LN containing both alpha-cypermethrin and pyriproxyfen, with the modification of having di-cyclohexyl phthalate as internal standard was accepted as full CIPAC method in 2017.

The extension of the scope (CIPAC/4887) of CIPAC methods 715/TC/M/2 & 3 for identification and determination of pyriproxyfen in incorporated into polyethylene LN containing both permethrin and pyriproxyfen was confirmed in 2016 (CIPAC/5043) to be applicable for identification and determination of pyriproxyfen in incorporated into polyethylene LN containing both alpha-cypermethrin and pyriproxyfen. The CIPAC methods 715/LN/M/2 & 3 for identification and determination of pyriproxyfen in incorporated into polyethylene LN containing both permethrin and pyriproxyfen are published in Handbook O.

Data on 3 different batches of the 120 and 150 denier products showed that the average active ingredients content fully complies with the specified limits and that the

new proposed combined method provides similar results to those using the individual CIPAC methods.

The spatial variation data on 3 different batches of the 120 and 150 denier products measuring alpha-cypermethrin and pyriproxyfen content on 5 individual net pieces taken according to the Figure 2 of the specification showed an acceptable homogeneity of the active ingredients content within and between the nets (within-net RSD < 10% and between-net RSD < 5%).

The WHOPES Phase I testing and evaluation of Royal Guard™ showed that alpha-cypermethrin and pyriproxyfen content in the LN comply with the target doses ( $\pm 25\%$ ), and an acceptable homogeneity of the active ingredients between the nets. The within-net variation, expressed as the relative standard deviation (RSD) of the alpha-cypermethrin and pyriproxyfen content found on 5 nets pieces taken each from 4 different nets, ranged from 5.1% to 12.4% and from 4.2% to 14.0% respectively. The between-net variation, expressed as the relative standard deviation (RSD) of the alpha-cypermethrin and pyriproxyfen content found on 4 different nets, was 4.3% and 3.7% respectively (CRA-W 2017).

#### Active ingredients wash resistance index

An adequate amount of the active ingredients must be present at the surface of the LN, for efficacy reasons, whereas the majority must reside within the LN, to avoid excessive losses during washing and to provide a reservoir from which the surface is replenished with active ingredients. The wash resistance index is determined by analyzing separate washed and unwashed pieces of the same fabric.

Where the active ingredient and synergist are incorporated into filaments, rapid loss of molecules is not likely to occur during washing but, if re-equilibration to the surface is too slow, the product may be ineffective for an unacceptable period of time after washing. Alternatively, if the re-equilibration is too rapid, the surface concentration could become higher than expected, leading to higher losses of active ingredient and synergist during washing and possibly increased user exposure to the active ingredient.

The method MT 195 for determination of wash resistance index of LN was adopted as full CIPAC method in 2013 and is now published in the Handbook O. This method is a further standardization of the WHO washing method published in the “WHO Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets”, document WHO/CDS/WHOPES/GCDPP/2005.11 (WHO 2005). Briefly, the wash resistance index is determined by analyzing net samples in triplicate representing wash points 0 and 4 for total active ingredient content and calculating the average wash resistance index per wash using the equation for a free migration stage behaviour. A wash resistance index per wash of 95% indicates that at least 95% of the insecticide present in samples washed 1 to 3 times is still present after an additional wash step. The wash resistance index applies to the average obtained from triplicate tests performed on net pieces removed from the same net or batch of netting (CIPAC 2017).

A net like Royal Guard™ containing two active ingredients (a pyrethroid and an insect growth regulator) poses additional complexity in matching the wash resistances of the two pesticides in order to maintain the ratio of the two actives in a similar range. The results of MT 195 show a reasonable agreement between the two wash resistance ranges.

The manufacturer provided data on one batch of the 120 and 150 denier products unwashed and washed 4 times according to the CIPAC method MT 195. The wash resistance index ranged from 99% to 101% for alpha-cypermethrin and from 97% to 98% for pyriproxyfen. The manufacturer proposed to specify a range from 93% to 101% for alpha-cypermethrin and from 88% to 99% for pyriproxyfen, and this was agreed by the Meeting.

The WHOPEs Phase I testing results on alpha-cypermethrin and pyriproxyfen content and associated biological efficacy of Royal Guard™, 120 denier, washed up to 25 times (according to the WHO washing procedure) showed an exponential decay of the alpha-cypermethrin and pyriproxyfen content in function of the number of washes (free-migration stage behaviour). The overall alpha-cypermethrin and pyriproxyfen retention after 20 washes was 76.2% and 57.5% respectively, corresponding to an average retention index per wash of 98.7% and 97.8% respectively, as estimated by the exponential regression curve (CRA-W 2017), and this is in agreement with the specification tolerances.

#### Relevant impurities

There are no relevant impurities identified in the existing WHO specification 454/TC for alpha-cypermethrin (January 2013) neither in the existing WHO specification 715/TC for pyriproxyfen (October 2017).

#### Physical properties

Data provided by the manufacturer on one batch of the 120 and 150 denier products showed:

- an average and minimum number of complete holes / cm<sup>2</sup> in agreement with the specified minimum limits of 20 and 18, respectively.
- a bursting strength higher than the minimum limit of 400 kPa for 120 denier yarn and 450 kPa for 150 denier yarn.
- a dimensional stability to washing in agreement with the standard of maximum 5% shrinkage / expansion.
- That no fire phenomena occurred.

The fabric weight measured on 3 batches of the 120 and 150 denier products showed that they comply with the limits of 40 g/m<sup>2</sup> and 45 g/m<sup>2</sup> ± 5 g/m<sup>2</sup>, respectively for the for 120 and 150 denier yarns.

#### Storage stability

The manufacturer provided data on the 120 and 150 denier products showing that, after storage at 54°C for 2 weeks, the average alpha-cypermethrin and pyriproxyfen content (measure individually) are higher than 95% relative to the average content found before storage, and that the nets still comply with the limits set for dimensional stability to washing and bursting strength.

The wash resistance index after accelerated storage ranged from 94% to 95% for alpha-cypermethrin and from 90% to 98% for pyriproxyfen, and therefore still complies with the limits set before storage.

## ANNEX 1: REFERENCES

Study number	Author(s)	Year	Study title. Study identification number. Report identification number. GLP [if GLP]. Company conducting the study
	Butenhoff Andy	2015	Royal Guard™ LLIN Submission Dossier for WHOPES Evaluation Under a New Specification. Data package submitted to JMPS. Disease Control Technologies, LLC, October 22, 2015. Data package including several test reports from TÜV SÜD PSB Pte. Ltd., Singapore.
	Butenhoff Andy	2016	Replies from Disease Control Technologies on the evaluator's questions on the Royal Guard™ specification. Data package submitted to JMPS. Disease Control Technologies, LLC, April 07, 2016. Data package including several test reports from TÜV SÜD PSB Pte. Ltd., Singapore.
RE/17/U10/24571	CRA-W	2017	Determination of alpha-cypermethrin and pyriproxyfen in net samples from the WHOPES Phase I study on Royal Guard, 120 denier. Report RE/17/U10/24571 of the Walloon Agricultural Research Centre, Gembloux, Belgium for WHO, October 30, 2017.
	FAO/WHO	2016	Manual on development and use of FAO and WHO specifications for pesticides. Second revision of the 1 <sup>st</sup> edition. FAO, Rome and WHO, Geneva, March 2016 (internet publications).
	Shareen Chan & Wong Bee Hui	2016	Flammability of Royal Guard, 120 denier. Test report No. 7191140652-EEC16/09-CSL of TUV/SUD PSB Singapore for Disease Control Technologies, July 01, 2016.
	Shareen Chan & Wong Bee Hui	2016	Flammability of Royal Guard, 150 denier. Test report No. 7191140652-EEC16/10-CSL of TUV/SUD PSB Singapore for Disease Control Technologies, July 01, 2016.
	WHO	2005	Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. Document WHO/CDS/WHOPES/GCDPP/2005.11. WHO, Geneva, 2005.
	WHO	2015	Determination of fabric strength of long-lasting insecticidal nets. Report of a WHO consultation, Geneva, 20-22 August 2014. WHO/HTM/NTD/WHOPES/2015.1