

PERMETHRIN-INCORPORATED MOSQUITO NET (NI)

Interim Specification WHO/IS/NI/331/2002

1 Description

The material shall consist of netting, formed from high density polyethylene mono-filament fibres, incorporating technical permethrin complying with WHO specification WHO/SIT/28, together with any necessary stabilizers, plasticizers and other formulants. The material shall be suitable for use as a long lasting insecticidal mosquito net.

2 Active ingredient

2.1 Identity tests (Note 1)

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

2.2 Total permethrin content

The permethrin content shall be 20g/kg and, when determined by the method described in Note 1, the average content shall not differ by more than ± 3 g/kg.

2.3 1RS,3RS (*cis*) isomer percentage

The 1RS/3RS (*cis*-) isomer percentage, when determined by the method described in Note 1, shall be in the range 35% to 45%.

2.4 Permethrin initial surface concentration

The initial surface amount of permethrin on the yarn, determined by the method described in Note 2, shall be not less than 500 μ g/g of netting.

2.5 Permethrin release index (bleeding speed index)

The permethrin release index from the yarn, when determined by the method described in Note 2, shall be within the range 0.1 to 0.3.

3 Physical properties

3.1 Fibre characteristics

The fibres shall be of high density polyethylene mono-filament (Note 3) with a melt index of 0.9-1.1 g/10 min (ISO 1833-1997).

3.2 Netting mesh size

The netting shall have a minimum of 56 complete holes/inch².

3.3 Dimensional stability of netting to washing

Dimensional stability (length and width): $\pm 10\%$ of initial dimension (ISO 5077-1984).

3.4 Mass per m² of net

Mass/m²: 50 ± 5 g/m² (ISO3801-1977).

3.5 Bursting strength

Minimum bursting strength: 350 Kpa (ISO 13938-1-1999).

4 Storage stability

4.1 Stability at elevated temperature (MT 46.3, CIPAC J, pp. 128-130)

After storage at $54 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ for 2 weeks, the total permethrin content (Note 1) shall not be lower than 95% relative to the determined average content found before storage (Note 5) and the netting must continue to comply with the clauses for isomer ratio (2.3), dimensional stability (3.3), and bursting strength (3.5). The initial surface concentration of permethrin (2.4) shall be not more than 2.5 times the value found before storage, whereas the permethrin release index (2.5) shall be not less than half the value found before storage (Note 2).

Note 1 Determination of total permethrin content and 1RS/3RS (*cis*) isomer percentage

Outline of method

Permethrin is extracted from the sample with xylene and diethylhexyl adipate is added as an internal standard. Determination is carried out by gas-liquid chromatography with a flame ionization detector, using a capillary column coated with 1 μm film of 50% diphenyl and 50% dimethylpolysiloxane. The *cis*-isomer has the shorter retention time.

Identity test

Using the method described below, the relative retention times (i.e. relative to the internal standard) of the *cis*- and *trans*-permethrin peaks obtained from the sample should agree within $\pm 1\%$ of those obtained from an authentic standard of permethrin.

Apparatus

Gas-liquid chromatograph. The instrument should be designed for operation in the range 100 to 300°C and equipped with a hydrogen flame-ionization detector, an injection port heater, an on-column injection system, and a suitable recorder or electronic integrator.

Chromatographic column. Capillary column, 30 m x 0.53 mm with a 1 μm film thickness 50% diphenyl- and 50% dimethylpolysiloxane, or equivalent, stationary phase. Before use, condition new columns by purging with helium overnight at about 250°C. During this operation the column must not be connected to the detector to avoid potential contamination by "bleed" of the stationary phase.

Automatic digital integrator or chromatographic data system compatible with the gas chromatograph.

Solvent extraction apparatus. 250 ml borosilicate twin-necked round-bottom glass flask with thermometer to fit, reflux condenser and heating mantle.

Reagents

Permethrin standard. Analytical standard of known purity.

Internal standard. Analytical reagent grade diethylhexyl adipate [otherwise known as bis(2-ethylhexyl)adipate or hexanedioic acid, bis(2-ethylhexyl) ester] (Taoka Chemical Co.,Ltd., 2-11, Nishimikuni 4-chome, Yodogawa-ku, Osaka 〒532-0006, Japan, or equivalent).

Xylene. Analytical reagent grade(mixed isomers).

Preparation of calibration solutions

Internal standard solution. Weigh approximately 100 mg (to the nearest 0.1 mg) of diethylhexyl adipate into 50 ml volumetric flask, dissolve and dilute to volume with xylene. Store under refrigeration, remove from refrigerator and allow to reach room temperature naturally before using.

Permethrin calibration solutions. Weigh approximately 100 and 200 mg (to the nearest 0.1mg) of permethrin standard into separate 50 ml volumetric flasks, dissolve and dilute to volume with xylene. Pipette accurately 10 ml of each solution in separate 25 ml volumetric flasks. Add exactly 10 ml of the internal standard solution and make to volume with xylene to make the calibration solutions.

Label the calibration solutions A and B. Solution A is the working calibration solution for gas-liquid chromatography. Solution B is used to check for possible weighing errors in the preparation of the calibration solution. The supply of the solutions A and B can be replenished from time to time.

Equilibration of the gas chromatographic system

Inject at least 3 x 1 µl of permethrin calibration solution A, to equilibrate the system. Use the data from these chromatograms to set integration parameters if necessary and to assess the stability of the system. The response ratios (total peak area of cis- and trans-permethrin/peak area of the internal standard) of three consecutive injections must agree to within 2%.

Operating conditions for gas-liquid chromatography

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

	Temperature		Gas pressures
Column oven	220°C	Helium (carrier)	140 kPa
Injector	270°C	Hydrogen	60 kPa
Detector	280°C	Air	50 kPa

Adjust the column oven temperature if required to obtain retention time windows for cis- and trans-permethrin (approximately 21.0 and 22.4 min) and diethylhexyl adipate (approximately 3.9 min), but the column temperature must not exceed 260°C.

Sampling

Cut a piece of netting, at least 50 g, from the centre of a net.

Extraction of permethrin from netting

Cut the net with scissors to produce 2 to 3 mm lengths of yarn. Weigh (to the nearest 1 mg) 2,000 mg of sample into a 250 ml flask. Add about 50 ml of xylene and exactly 2 ml of the internal standard solution. Fit the reflux condenser and heat the mixture at

75°C for 60 min to extract the permethrin. Cool the extract to room temperature and an aliquot of it can be used directly as the sample solution.

Determination of permethrin content and cis isomers percentage

Inject duplicate aliquots of 1 µl of calibration solution A. Calculate the response ratios by dividing the total area of cis- and trans-permethrin peak by the area of the internal standard peak. The duplicate response ratios should agree to within 2%. Calculate the average response ratio obtained from calibration solution A. Inject duplicate aliquots of 1 µl of each of the sample solutions. The duplicate response ratios should agree to within 2%. Calculate the average response ratio obtained with the sample solution. To determine the cis-isomer ratio, record the area of the cis-isomer peak and the total area of cis- and trans-isomer peaks produced by injection of the sample extract.

Calculations

For each injection, the response ratio, r , is given by the equation:

$$r = \frac{\text{Total area of cis- and trans-permethrin peaks}}{\text{Area of internal standard peak}}$$

The total permethrin content is given by the equation:

$$\text{Permethrin content (g/kg)} = \frac{r_2 \times m_1 \times P}{r_1 \times m_2}$$

Where:

r_1 = average response ratio for the calibration solution A;

r_2 = average response ratio for the sample solution;

m_1 = mass (mg) of permethrin standard in the calibration solution A;

m_2 = mass (mg) of sample taken;

P = purity of permethrin standard (g/kg).

The 1RS/3RS (cis) isomer content is given by the equation:

$$\text{1RS/3RS(cis) isomer content (\%)} = \frac{A_{cis}}{A_{total}} \times 100$$

Where:

A_{cis} = Area of cis-permethrin peak in the sample solution

A_{total} = Total area of cis- and trans-permethrin peaks in the sample solution

Note 2 Determination of the initial surface concentration and the release index (bleeding speed index) of permethrin

Outline of method

A weighed piece of netting is rinsed with acetone for 1 minute, to remove the permethrin from the surface of the yarn, and the initial surface concentration is determined from the solution obtained. The initial surface concentration is determined before and after storage at elevated temperature (clause 4.1) and the method of calculation of both parameters is similar.

The rinsed net is then heated at 70 °C for 2 hr to accelerate permethrin migration from

the core of the yarn to the surface. The heated net is rinsed again with acetone, to remove the migrated permethrin from the surface of the yarn. The net is subjected to a total of three successive cycles of heating and rinsing. The release index of permethrin is determined as the concentration found in the final (fourth) rinse obtained after the last (third) heating cycle, **before or after** storage as appropriate, divided by the concentration found in the initial surface concentration rinse **before** storage.

Apparatus

Gas-liquid chromatograph. The instrument should be designed for operation in the range 100 to 300°C and equipped with a hydrogen flame-ionization detector, an injection port heater, an on-column injection system, and a suitable recorder or electronic integrator.

Chromatographic column. Capillary column, 30 m x 0.53 mm with a 1 µm film thickness 50% diphenyl- and 50% dimethylpolysiloxane, or equivalent, stationary phase. Before use, condition new columns by purging with helium overnight at about 250°C. During this operation the column must not be connected to the detector to avoid potential contamination by "bleed" of the stationary phase.

Automatic digital integrator or chromatographic data system compatible with the gas chromatograph.

Solvent extraction apparatus. 100 ml screw-capped glass vials.

Oven. Operating at 70°C.

Reagents

Permethrin standard. Analytical standard of known purity.

Internal standard. Analytical reagent grade of diethylhexyl adipate [otherwise known as bis(2-ethylhexyl)adipate or hexanedioic acid, bis(2-ethylhexyl) ester].

Acetone. Analytical reagent grade.

Preparation of calibration solutions

Internal standard solution 1. Weigh 100 mg (to the nearest 0.1 mg) of diethylhexyl adipate into a 50 ml volumetric flask, dissolve and dilute to volume with acetone. Store in a refrigerator. Remove from the refrigerator and allow to reach room temperature naturally before using.

Internal standard solution 2. Pipette accurately 3 ml of internal standard solution 1 into a 20 ml volumetric flask and dilute to volume with acetone.

Permethrin calibration solutions A and B. Weigh approximately 100 and 200 mg (to the nearest 0.1 mg) of permethrin standard into each of two separate 50 ml volumetric flasks, dissolve and dilute to volume with acetone. Pipette accurately 3 ml of each solution into two separate 20 ml volumetric flasks. Add exactly 3 ml of internal standard solution 1 to each flask and make to volume with acetone to prepare calibration solutions A and B. Solution A is the working calibration solution for gas-liquid chromatography. Solution B is used to check for possible weighing error in the preparation of solution A.

Equilibration of the system

See Note 1, equilibration of the system.

Operational conditions for gas-liquid chromatography

See Note 1, operational conditions.

Sampling and extraction of permethrin

Cut the net to provide a piece of approximately 11 x 11 cm (approximately 0.6 g), put in a 100 ml screw-capped vial, add by pipette 50 ml of acetone and fit the vial cap securely.

Shake the vial vigorously for 1 minute, to remove permethrin initially present on the surface area of yarn, and carefully remove the rinsed net from acetone solution to a clean glass beaker or watch glass. Evaporate the acetone extract to dryness and add 3 ml of internal standard solution 2 to dissolve the residue and thus prepare sample solution 1.

Air-dry the rinsed net, then transfer it to an oven at 70 °C for 2 hours, to accelerate migration of permethrin from inner part of yarn to surface area. Put the heated net in a new 100 ml screw vial and add 50 mL of acetone. Shake the vial vigorously for 1 minute, remove the rinsed net and prepare the extract, as above. Carry out the heating/rinsing cycle a total of 3 times on the same piece of net. Evaporate the acetone extract derived from the final rinse (only) to dryness and add 3 ml of internal standard solution 2 to dissolve the residue and prepare sample solution 2.

Analysis of extracts

See Note 1: Determination of permethrin content and cis percentage.

Calculations

For each injection the response ratio r is given by the equation:

$$r = \frac{\text{Total area of cis- and trans-permethrin peaks}}{\text{Area of internal standard peak}}$$

The permethrin content (μg) is given by the equation:

$$\text{Permethrin content } (\mu\text{g}) = \frac{r_2 \times m_1 \times P}{r_1}$$

Where:

r_1 = average response ratio for the calibration solution A;

r_2 = average response ratio for the sample solution 1 or 2;

m = mass ($\cdot\text{g}$) of permethrin standard in the calibration solution A;

P = purity of permethrin standard (%).

The **initial surface concentration** of permethrin on the yarn is given by the equation:

$$\text{Surface amount } (\cdot\text{g/g}) = \frac{\text{Permethrin } (\cdot\text{g}) \text{ from sample solution 1}}{\text{Mass(g) of sample}}$$

The **release index** of permethrin from the yarn (bleeding speed index) is given by the

equation:

$$\text{Release index} = \frac{\text{Surface amount of permethrin}(\cdot\text{g/g}) \text{ from sample solution 2}}{\text{Surface amount of permethrin}(\cdot\text{g/g}) \text{ from sample solution 1}}$$

Where:

sample solution 1 is that produced **before** storage at elevated temperature;
sample solution 2 is that produced **before or after** storage at elevated temperature,
as appropriate.

Note 3 The linear density (mass per unit length) of the monofilament fibre should be $210 \pm 15\%$ decitex (g/10,000 m yarn, ISO 2060 - 1995). Compliance with this criterion ensures that the insecticidal activity of the netting persists for the expected length of time. The decitex value cannot be measured in the manufactured netting but may be measured during production of the fibre, on specific request.