

TECHNICAL PERMETHRIN

Specification WHO/SIT/28
Approved 25 September 1989

1. Specification

1.1 Material

The material shall consist of a mixture of the 1RS, 3RS (cis) and 1RS, 3SR (trans) diastereoisomers of 3 - phenoxybenzyl - 3 (2,2- dichlorovinyl) - 2,2 - dimethyl-cyclopropanecarboxylate in a nominal 1RS, 3RS/1RS, 3SR (cis/trans) ratio within the range 25:75-50:50. It shall be in the form of a yellow-to-brown solid of low melting-point or a liquid, free from extraneous impurities or added modifying agents.

1.2 Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1), shall comply with the requirements of section 1.1 and with the following requirements.

1.2.1 *Total permethrin content (g/kg basis)*

The total permethrin content shall be declared (not less than 925 g/kg) and, when determined by the method described in section 2.1, the content obtained shall not differ from that declared by more than ± 25 g.

1.2.2 *1RS,3RS/1RS,3SR isomer ratio*

The 1RS,3RS/1RS,3SR (cis/trans) isomer ratio shall be declared, and determined by the method described in section 2.1. The minimum 1RS,3RS isomer content, expressed as a percentage of total isomers (permethrin content), shall be 25% and the maximum 50% with a corresponding maximum of 75% and minimum of 50% of 1RS,3SR isomer.

For a declared actual ratio up to 40/60, the permitted tolerance shall be $\pm 15\%$ and for a declared ratio above 40/60, the permitted tolerance shall be $\pm 10\%$.

1.2.3 *Acidity*

The acidity as determined by the method described in WHO/M/3, shall not be higher than 1.5 g/kg, calculated as H_2SO_4 . In the event of a dispute, the electrometric determination shall be the referee method.

1.2.4 *Material/insoluble in acetone*

The material, insoluble in acetone as determined by the method described in WHO/M/21.R1, shall not be higher than 1 g/kg.

1.2.5 *Water content*

The water content, determined by the method described in WHO/M/7.R1, shall not be greater than 1 g/kg.

1.3 Packing and marking of packages

The technical permethrin shall be packed in suitable clean containers, as specified in the order.

All packages shall bear, durably and legibly marked on the container the following:

Manufacturer's name
Technical permethrin to specification WHO/SIT/28
Actual 1RS,3RS/1RS,3SR (cis/trans) isomer ratio of the batch
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Permethrin is a pyrethroid that acts predominantly on the central nervous system; high dosages have been found to lead to tonic seizures in experimental animals. A high concentration in air may be an irritant to eyes, skin and mucous membranes. It may be hazardous if swallowed. Avoid skin contact; wear protective gloves, clean protective clothing and a face-mask when handling the material. Wash hands and exposed skin thoroughly after handling.

Keep containers out of the reach of children and well away from foodstuffs and animal feed and their containers.

Permethrin is toxic to aquatic wildlife. Avoid accidental contamination of water.

If poisoning occurs, call a physician. Treatment is symptomatic.

2. Methods of determining chemical and physical properties

2.1 Permethrin content

2.1.1 Outline of method

The sample is dissolved in 4-methylpentan-2-one containing *n*-octacosane as internal standard. Separation is carried out by gas-liquid chromatography on a column of Chromosorb W-HP coated with silicone OV 210. The permethrin, isolated as two isomer peaks, is determined by comparison with calibration solutions.

2.1.2 Special apparatus

1. *Gas-liquid chromatograph.* Capable of operating over the range 100 to 300°C, fitted with a flame ionization detector, injection port heater and on-column injection system and equipped with a suitable recorder or electronic integrator.
2. *Chromatographic column.* Glass column 1 m long, 4 mm internal diameter packed with 3% silicone OV 210 on Chromosorb W-HP (100-120 mesh).

Before use condition a freshly prepared column by purging with nitrogen overnight at 275°C. During this operation the column must not be connected to the detector to avoid contamination by any initial "bleed" of the stationary phase.

2.1.3 Special reagents

Permethrin working standard. Analytical grade of known permethrin content (minimum 940 g/kg) with a ratio of cis- to trans-isomer content, preferably similar to that of the sample being analyzed¹.

4-Methylpentan-2-one (methyl isobutyl ketone; MIBK).

Internal standard, n-octacosane. Select for use a batch which, when chromatographed under the conditions given below for the determination of permethrin, gives no peak with a similar retention time to either of the cis- or trans-isomer peaks.

¹ Certified standards of cis-permethrin (purity 99.1% m/m) and trans-permethrin (purity 99.5% m/m) are available from the Office of Reference Materials, National Physical Laboratory, Department of Trade and Industry, Teddington, Middlesex, TW11 OLW, United Kingdom, or any other sources of reference standards. These materials should be used to calibrate the working standard.

2.1.4 Preparation of standard solutions

Internal standard solution. Dissolve 1 g *n*-octacosane in 1 litre MIBK. As the reagent dissolves slowly in the solvent, it may be necessary to use an ultrasonic bath or to warm the solution. (Before use, allow the solution to return to room temperature).

Permethrin calibration solution. Crystallization may occur at ambient temperature, so the analytical standard must be homogenized before use. Warm the bottle at between 40 and 50°C until no crystals remain and then shake thoroughly. Weigh in duplicate (to the nearest 0.1 mg) about 0.1 g (M_A and M_B) of homogeneous standard into separate 100 ml conical flasks. Add 25.0 ml of *n*-octacosane internal standard solution and shake to dissolve the permethrin. (Solutions C_A and C_B). Prepare a solution of about 0.1 g of homogeneous standard in 25 ml of solvent MIBK (solution C_O).

2.1.5 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.

Temperatures

Oven	Use any set temperature between 190-220°C and control to $\pm 0.5^\circ\text{C}$ throughout the analyses.
Injection port	260°C
Detector	250°C

Gas flow rates

Hydrogen and air	Optimally set up as recommended for the detector by the manufacturer.
Carrier gas:	nitrogen (oxygen - free, i.e. containing less than 10 mg/l: 50 ml.min ⁻¹).

Approximate retention times

Permethrin <u>cis</u> -isomer peak	7.9 min
Permethrin <u>trans</u> -isomer peak	9.3 min
Internal standard peak	4.2 min

2.1.6 Sample preparation

Sampling. Homogenize the bulk material by heating to about 50°C and mixing thoroughly until no crystals remain before taking at least 25g as a sub-sample for analysis.

Preparation of the sample solutions. Homogenize the material by the method given here above for sampling.

Weigh (to the nearest 0.1 mg) in duplicate sufficient sample (w g) to contain 0.1 g of permethrin into 100 ml conical flasks. Add to each flask 25.0 ml of n -octacosane internal standard solution from the same pipette as used to prepare the permethrin calibration solution and shake the flasks thoroughly to dissolve the permethrin. (Solutions S_A and S_B). Prepare a solution without internal standard by dissolving about 0.1 g of permethrin in 25 ml of solvent MIBK (solution S_O).

2.1.7 *Equilibration of the system*

Inject at least 3 x 1.5 ml of one of the permethrin calibration solution C to equilibrate the system and use the data from these chromatograms to set the integrator parameters if one is being used and also to assess the stability of the system.

Inject 1.5 ml portions of the internal standard solution, and C_O and S_O solutions and check whether there are any interfering peaks from impurities. If there are, make any necessary corrections.

2.1.8 *Analysis of sample*

Carry out injections of 1.5 ml of the permethrin calibration solutions C_A and C_B and sample solutions S_A and S_B in the following sequence and record either the integrated areas of the peaks or measure by triangulation from the product of $EL \times JK^2$ (height x base).

Injection sequence: $C_{A1}, S_{A1}, S_{A2}, C_{B1}, C_{A2}, S_{B1}, S_{B2}, C_{B2}$.

Calculate the relative response factors (f_1, f_2 , etc.) for the pair of permethrin calibration injections which bracket the sample injections, e.g. use C_{A1} and C_{B1} for sample injection S_{A1}, S_{A2} etc., and obtain the mean response factor f

$$\text{Relative response factor} = \frac{H_s}{I_r \times M \times P}$$

Where H_s = Total area of the permethrin cis-isomer and trans-isomer peaks from the permethrin calibration solution.

I_r = Area of n -octacosane peak of the permethrin calibration solution.

M = Mass of permethrin analytical standard in the permethrin calibration solution(g).

P = Purity of the permethrin analytical standard (g/kg).

² If triangulation is used as a method of measurement of peak areas, it is essential that the peaks from the permethrin and the n -octacosane in the "calibration" and in the "sample chromatogram" should be of similar height. The recorder chart speed (25 mm/min), attenuation and sample size should be initially adjusted so that the peak heights are about 3/4 of the chart width and not less than 180 mm. The distance between the intercepts of the tangents on the base-line should be not less than 30 mm. The base-line should be parallel to, or coincide with, the electrical base-line.

The mass of internal standard is common to both permethrin calibration and sample solution and has therefore been omitted.

Successive measurements of the response factors should agree to within $\pm 0.5\%$ of their mean value. If not repeat the analysis.

2.1.9 Calculation

Calculate the permethrin content for each sample injection, e.g. S_{A1} , by the following equation:

$$\text{Total permethrin content (g/kg)} = \frac{H_m}{f \times I_q \times w}$$

Where f = mean relative response factor.

H_m = area (X) of the permethrin cis-isomer + area (Y) of the trans-isomer peaks in the sample solution.

I_q = areas of the n-octacosane peak, in the sample solution

w = mass of sample (g).

The cis-isomer: trans-isomer ratio is X/Y.

Where X is the area of the permethrin cis-isomer peak in the sample solution.

Y is the area of the permethrin trans-isomer peak in the sample solution.

Take the mean of the four values corresponding to the four injections S_{A1} , S_{A2} , S_{B1} , S_{B2} .

Calculate the total permethrin content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Permethrin
S_{A1}	C_{A1} and C_{B1}	Q%] U%
S_{A2}	C_{A1} and C_{B1}	R%]
S_{B1}	C_{A2} and C_{B2}	S%] V%
S_{B2}	C_{A2} and C_{B2}	T%]

Q and R, S and T should agree to within $\pm 0.5\%$ of their respective mean values (U and V). U and V should agree to within $\pm 1\%$ of their mean value. Take the mean of the two values U and V as the total permethrin content.

PERMETHRIN

EMULSIFIABLE CONCENTRATE

Specification WHO/SIF/50
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of technical permethrin dissolved in suitable solvents, with other necessary formulants added. It shall be in the form of a stable liquid free from suspended matter and sediment. The technical permethrin used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/28.

1.2 Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1), shall comply with the requirements of section 1.1 and with the following requirements.

1.2.1 *Total permethrin content (g/kg basis)*

The total permethrin content, determined by the method described in section, 2.1, shall not differ from the nominal content by more than the following amounts:

<i>Nominal content</i>	<i>Tolerance permitted</i>
Less than 100/ g/kg	± 10% of the nominal content
100-250 g/kg	± 6% of the nominal content
250-500 g/kg	± 5% of the nominal content

Higher nominal contents are not currently available. The average content of all samples taken shall not be lower than the nominal content.

1.2.2 *1RS,3Rs/1Rs,3SR isomer ratio*

The 1RS,3Rs/1Rs,3SR (cis/trans) isomer ratio shall be declared and determined by the method described in section 2.1. The minimum 1Rs,3Rs isomer content, expressed a maximum 50% with a corresponding maximum of 75% and minimum of 50% of 1RS,3SR isomer.

For a declared actual ratio up to 40/60 the permitted tolerance shall be ±15%, and for a declared ratio above 40/60, the permitted tolerance shall be ±10%.

1.2.3 *Water content*

The water content, determined by the method WHO/M/7.R1 shall not be higher than 3 g/kg.

1.2.4 *Acidity*

The acidity as determined by the method described in WHO/M/3, shall not be higher than 1.5 g/kg, calculated as H₂SO₄. In the event of a dispute, the electrometric determination shall be the referee method.

1.2.5 *Cold test*

No separation of solid or oily material shall occur when the concentrate is tested as described in method WHO/M/23.

1.2.6 *Flash point*

The flashpoint of the product, determined by the method WHO/M/10.R1 shall not be lower than 38°C and shall comply with all national and/or international transport regulations.

1.2.7 *Stability of the emulsion*

In standard soft water. Any separation, including creaming/oiling at the top and oiling/sedimentation at the bottom, of 100 ml of emulsion prepared in standard soft water with 5 ml of concentrate shall not exceed 2 ml when tested as described in WHO/M/13.R3.

In standard hard water. Any separation including creaming/oiling at the top and oiling/sedimentation at the bottom, of 100 ml of emulsion prepared in standard hard water with 5 ml of concentrate shall not exceed 2 ml when tested as described in WHO/M/13.R3.

1.2.8 *Heat stability*

The concentrate, after treatment as described in section 2.3, shall comply with the requirements of sections 1.2.1, 1.2.4 and 1.2.7 of this specification.

1.3 Packing and marking of packages

The permethrin emulsifiable concentrate shall be packed in suitable clean containers, as specified in the order.

All packages shall bear, durably and legibly marked on the container the following:

Manufacturer's name
Permethrin emulsifiable concentrate to specification WHO/SIT/50
Permethrin ... g/kg
Actual 1RS,3RS/1RS,3SR (cis/trans) isomer ratio of the batch
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Permethrin is a pyrethroid that acts predominantly on the central nervous system; high dosages have been found to lead to tonic seizures in experimental animals. A high concentration in air may be an irritant to eyes, skin and mucous membranes. It may be hazardous if swallowed. Do not inhale spray mist. Avoid skin contact; wear protective gloves, clean protective clothing and a face-mask when handling this concentrate. Wash hands and exposed skin thoroughly after handling.

Keep containers out of the reach of children and well away from foodstuffs and animal feed and their containers.

Permethrin is toxic to aquatic wildlife. Avoid accidental contamination of water. If poisoning occurs, call a physician. Treatment is symptomatic.

2. Methods of determining chemical and physical properties

2.1 Permethrin content

2.1.1 *Outline of method*

The sample is diluted with 4-methylpentan-2-one containing n -octacosane as internal standard. Separation is carried out by gas-liquid chromatography on a column of Chromosorb W-HP coated with silicone OV 210. The permethrin, isolated as two isomer peaks, is determined by comparison with calibration solutions.

2.1.2 *Special apparatus*

1. *Gas-liquid chromatograph.* Capable of operating over the range 100 to 300°C, fitted with a flame ionization detector, injection port heater and on-column injection system and equipped with a suitable recorder or electronic integrator.

2. *Chromatographic column.* Glass column 1 m long, 4 mm internal diameter packed with 3% silicone OV 210 on Chromosorb W-HP (100-120 mesh).

Before use condition a freshly prepared column by purging with nitrogen overnight at 275°C. During this operation the column must not be connected to the detector to avoid contamination by any initial "bleed" of the stationary phase.

2.1.3 *Special reagents*

Permethrin working standard. Analytical grade of known permethrin content (minimum 940 g/kg) with a ratio of cis- to trans-isomer content, preferably similar to that of the sample being analyzed¹.

4-Methylpentan-2-one (methyl isobutyl ketone; MIBK).

Internal standard, n-octacosane. Select for use a batch which, when chromatographed under the conditions given below for the determination of permethrin, gives no peak with a similar retention time to either of the cis- or trans-isomer peaks.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Dissolve 1 g n-octacosane in 1 litre MIBK. As the reagent dissolves slowly in the solvent, it may be necessary to use an ultrasonic bath or to warm the solution. (Before use, allow the solution to return to room temperature).

Permethrin calibration solution. Crystallization may occur at ambient temperature, so the analytical standard must be homogenized before use. Warm the bottle at between 40 and 50°C until no crystals remain and then shake thoroughly. Weigh in duplicate (to the nearest 0.1 mg) about 0.1 g (M_A and M_B g) of homogeneous standard into separate 100 ml conical flasks. Add 25.0 ml of n-octacosane internal standard solution and shake to dissolve the permethrin. (Solutions C_A and C_B). Prepare a solution of about 0.1 g of homogeneous standard in 25 ml of solvent MIBK (solution C_O).

2.1.5 *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.

¹ Certified standards of cis-permethrin (purity 99.1% m/m) and trans-permethrin (purity 99.5% m/m) are available from the Office of Reference Materials, National Physical Laboratory, Department of Trade and Industry, Teddington, Middlesex, TW11 OLW, United Kingdom, or any other sources of reference standards. These materials should be used to calibrate the working standard.

Temperatures

Oven	Use any set temperature between 190-220 ⁰ C and control to $\pm 0.5^{\circ}\text{C}$ throughout the analyses.
Injection port	260 ⁰ C
Detector	250 ⁰ C

Gas flow rates

Hydrogen and air	Optimally set up as recommended for the detector by the manufacturer.
Carrier gas:	nitrogen (oxygen - free, i.e. containing less than 10 mg/l: 50 ml.min ⁻¹).

Approximate retention times

Permethrin <u>cis</u> -isomer peak	7.9 min
Permethrin <u>trans</u> -isomer peak	9.3 min
Internal standard peak	4.2 min

2.1.6 *Sample preparation*

Sampling. Homogenize the bulk material by heating to about 50⁰C and mixing thoroughly until no crystals remain before taking at least 25g as a sub-sample for analysis.

Preparation of the sample solutions. Homogenize the material by the method given here above for sampling.

Weigh (to the nearest 0.1 mg) in duplicate sufficient sample (w g) to contain 0.1 g of permethrin into 100 ml conical flasks. Add to each flask 25.0 ml of n-octacosane internal standard solution from the same pipette as used to prepare the permethrin calibration solution and shake the flasks thoroughly to dissolve the permethrin. (Solutions S_A and S_B). Prepare a solution without internal standard by dissolving about 0.1 g of permethrin in 25 ml of solvent MIBK (solution S_O).

2.1.7 *Equilibration of the system*

Inject at least 3 x 1.5 ml of one of the permethrin calibration solution C to equilibrate the system and use the data from these chromatograms to set the integrator parameters if one is being used and also to assess the stability of the system.

Inject 1.5 ml portions of the internal standard solution, and C_O and S_O solutions and check whether there are any interfering peaks from impurities. If there are, make any necessary corrections.

2.1.8 Analysis of sample

Carry out injections of 1.5 ml of the permethrin calibration solutions C_A and C_B and sample solutions S_A and S_B in the following sequence and record either the integrated areas of the peaks or measure by triangulation from the product of $EL \times JK^2$ (height \times base).

Injection sequence: $C_{A1}, S_{A1}, S_{A2}, C_{B1}, C_{A2}, S_{B1}, S_{B2}, C_{B2}$.

Calculate the relative response factors (f_1, f_2 , etc.) for the pair of permethrin calibration injections which bracket the sample injections, e.g. use C_{A1} and C_{B1} for sample injection S_{A1}, S_{A2} etc., and obtain the mean response factor f

$$\text{Relative response factor} = \frac{H_s}{I_r \times M \times P}$$

Where H_s = Total area of the permethrin cis-isomer and trans-isomer peaks from the permethrin calibration solution.

I_r = Area of n-octacosane peak of the permethrin calibration solution.

M = Mass of permethrin analytical standard in the permethrin calibration solution(g).

P = Purity of the permethrin analytical standard (g/kg).

The mass of internal standard is common to both permethrin calibration and sample solution and has therefore been omitted. Successive measurements of the response factors should agree to within $\pm 0.5\%$ of their mean value. If not repeat the analysis.

2.1.9 Calculation

Calculate the permethrin content for each sample injection, e.g. S_{A1} , by the following equation:

$$\text{Total permethrin content (g/kg)} = \frac{H_m}{f \times I_q \times W}$$

Where f = mean relative response factor.

H_m = area (X) of the permethrin cis-isomer + area (Y) of the trans-isomer peaks in the sample solution.

I_q = areas of the n-octacosane peak, in the sample solution

W = mass of sample (g).

² If triangulation is used as a method of measurement of peak areas, it is essential that the peaks from the permethrin and the n-octacosane in the "calibration" and in the "sample chromatogram" should be of similar height. The recorder chart speed (25 mm/min), attenuation and sample size should be initially adjusted so that the peak heights are about 3/4 of the chart width and not less than 180 mm. The distance between the intercepts of the tangents on the base-line should be not less than 30 mm. The base-line should be parallel to, or coincide with, the electrical base-line.

The cis-isomer: trans-isomer ratio is X/Y. X is the area of the permethrin cis-isomer peak in the sample solution. Y is the area of the permethrin trans-isomer peak in the sample solution.

Take the mean of the four values corresponding to the four injections S_{A1} , S_{A2} , S_{B1} , S_{B2} . Calculate the total permethrin content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Permethrin
S_{A1}	C_{A1} and C_{B1}	Q%] U%
S_{A2}	C_{A1} and C_{B1}	R%]
S_{B1}	C_{A2} and C_{B2}	S%] V%
S_{B2}	C_{A2} and C_{B2}	T%]

Q and R, S and T should agree to within $\pm 0.5\%$ of their respective mean values (U and V). U and V should agree to within $\pm 1\%$ of their mean value. Take the mean of the two values U and V as the total permethrin content.

2.2 Heat stability

Keep 100 ml of the sample for 3 days at a temperature of $54 \pm 2^{\circ}\text{C}$ in a glass container sealed to avoid loss of volatile solvent, and then cool to room temperature.