WHO/CTD/WHOPES/97.7
English only
Distr.: General

Interim specifications for pesticides used in public health

World Health Organization
Division of Control of tropical Diseases
WHO Pesticide Evaluation Scheme

CONTENTS

Preface

Specifications for pesticides which have been established by the WHO Expert Committee on Vector Biology and Control, Chemistry and Specifications of Pesticides have been published in a manual which is now its 7th edition[1] .

Between meetings of the Expert Committee, WHO issues interim specifications for those pesticides that have been shown to be of practical use in public health and for which analytical methods exist.

The Expert Committee recommended that, "should the need arise to strengthen an existing specification WHO should issue interim specifications that would supersede the existing specifications and recommend the use of those interim specifications in connection with the purchase of pesticides and their formulations".

The current publication includes interim specifications for lambda-cyhalothrin, etofenprox and brodifacoum. The WHO methods referred to in the specifications are those published in the manual mentioned above.

[1] Specifications for pesticides used in public health (WHO/CTD/WHOPES/97.1)

TECHNICAL LAMBDA-CYHALOTHRIN

Interim specification: WHO/IS/1.3021-1.rev1

1. Specification

1.1 Material

The material shall consist of lambda-cyhalothrin together with related manufacturing impurities and shall be in the form of a brown to green/brown solidified melt at ambient temperature, free from extraneous matter and 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 *Lambda-cyhalothrin content* (g/kg *basis)*

The lambda-cyhalothrin content shall be declared (not less than 830 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 ±20 g.

1.2.2 *Lambda-cyhalothrin cis A stereoisomer content (g/kg basis)*

α-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propanecarboxylate as a 1:1 mixture of (Z) - (1R, 3R), R-ester and (Z) - (lS, 3S), S-ester

The lambda-cyhalothrin cis A stereoisomer content, determined by the method described in section 2.2, shall not be higher than 100 g/kg.

1.2.3 *Lambda-cyhalothrin cis B' stereoisomer content (g/kg basis)*

α-cyano-3-phenoxybenzyl-3-(2-chloro-3,3 ,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate as a 1:1 mixture of (E)-(1R, 3R), S-ester and (E), (lS, 3S), R-ester

The lambda-cyhalothrin cis B' stereoisomer content, determined by the method described in section 2.2, shall not be higher than 10g/kg.

1.2.4 *Melting point*

The melting point of the material, determined by the method WHO/M/5R.1, shall not be lower than 43°C and shall not be depressed when mixed with an equal quantity of pure lambda-cyhalothrin.

1.2.5 *Water content*

The water content of the material, when determined by the Karl Fischer electrometric titration method (WHO/M/7R.1) shall not be higher than 5g/kg.

1.3 Packing and marking of packages

The technical lambda-cyhalothrin 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 lambda-cyhalothrin to specification WHO/IS/1.3021-1.rev1
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice.

Lambda-cyhalothrin is a pyrethroid that acts predominantly on the central nervous system; high dosages have been found to cause tonic seizures in experimental animals. A high concentration in air may be irritant and contact with the concentrated product may induce a temporary tingling sensation, particularly on the face. It may be hazardous if swallowed. Do not inhale spray mist. Avoid skin contact; wear protective gloves, clean protective clothing and a face mask (surgical type) when handling the material. Wash hands and exposed skin thoroughly after using.

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

Lambda-cyhalothrin 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 Lambda-cyhalothrin content

2.1.1 *Outline of method*

The sample is dissolved in dichloromethane containing hexacosane as internal standard. Separation is carried out by capillary gas chromatography, in the split injection mode using a dimethyl polysiloxane WCOT fused silica capillary column, with flame ionisation detector and preferably with an automatic injector. The lambda-cyhalothrin is determined by comparison with calibration solutions.

2.1.2 *Special apparatus*

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

Chromatographic column. 25m x 0.25mm i.d. and 0.12μm film thickness (or 50m x 0.32 mm i.d. and 0.12 μm film thickness) WCOT fused silica column coated with dimethyl polysiloxane (chemically bonded).

Injector system. Injector split/splitless in the split mode with fused silica liner. An automatic injector is strongly recommended to ensure the best analytical results.

It is important that the split liner is acid treated, thoroughly deactivated and conditioned before use, to ensure that lambda-cyhalothrin does not epimerise to the isomer during analysis.

Silanised glass wool packing alone should not be used in the liner.

Split ratio	100:1
Injection volume	1.0 μl using an autosampler

Detector system. Type flame ionisation (FID), range high sensitivity.

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

150ml conical flasks.

2.1.3 *Special reagents*

Dichloromethane

Hexacosane internal standard. Select for use a batch which, when chromatographed

under the conditions given below for the determination of lambda-cyhalothrin, gives no peak with a similar retention time to lambda-cyhalothrin, lambda-cyhalothrin cis A stereoisomer and lambda-cyhalothrin cis B' stereoisomer.

Lambda-cyhalothrin working standard, of known lambda-cyhalothrin content (minimum 990g/kg).

2.1.4 *Preparation of standard solutions*

Internal standard solutions

Dissolve hexacosane (2.0g) in dichloromethane (500ml) (Solution I). Ensure a sufficient quantity of this solution is prepared for all samples and calibration standards being analysed.

Lambda-cyhalothrin calibration solution

Weigh in duplicate (to the nearest 0.1mg) about 0.1g of lambda-cyhalothrin standard (M_A and M_B, g) into 150ml conical flasks. Add to each, 20.0ml of hexacosane solution I from a pipette and 30ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solutions C_A and C_B).

Prepare a solution without internal standard by dissolving about 0.1g of lambda-cyhalothrin standard in 50ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solution C_o).

2.1.5 *Operating conditions*

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

Temperatures

Column oven	210°C
Injector	300°C
Detector	300°C

Adjust the column oven temperature if required to obtain retention time windows for lambda-cyhalothrin (10.7-11.4 min) and hexacosane (12.2-13.0 min), but not exceeding 240°C.

Gas flow rates

Helium or hydrogen (carrier) 1ml min^{-1}

Nitrogen (make up)	)	flow rates as recommended
Hydrogen	)	for the gas chromatograph
Air	)	

All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

2.1.6 *Sample preparation*

Sampling

Homogenize the bulk material by heating to about 50°C and mix thoroughly before taking the sample. Take at least 25g. Re-homogenize before taking a sub-sample for analysis.

Preparation of the sample solutions

Warm the material to about 50°C and mix thoroughly. Weigh in duplicate (to the nearest 0.1mg) into 150ml conical flasks, sufficient sample (w, g) at 50°C to contain 0.1g of lambda-cyhalothrin. Allow the flask and sample to cool to room temperature before recording the final weight.

Add by pipette to each flask, 20.0ml of hexacosane solution I, 30ml of dichloromethane and shake thoroughly to dissolve the lambda-cyhalothrin. Dilute 5ml of each solution to 25ml with dichloromethane (Solutions S_A and S_B).

Prepare a solution without internal standard by dissolving about 0.1g of sample in 50ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solution S_o).

2.1.7 *Equilibration of the system*

Carry out 1.0µl injections of solutions I, C_o and S_o and check whether there are any interfering peaks from impurities. If there are, make any necessary corrections but do not use external calibration.

Inject calibration solutions C_A and C_B to equilibrate the system and use the data from these chromatograms to set the integration parameters. Calculate the response factors for these injections to check stability of the instrument. Response factors should not differ by more than ± 1% of the mean.

Where the detector sensitivity is shown to be low, the second dilution stage for calibration and sample solutions may be omitted.

2.1.8 *Analysis of sample*

Carry out 1.0µl injections of calibration solutions C_A and C_B and sample solutions S_A and S_B in the following sequence and record the integrated areas of the peaks.

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 calibration injections which bracket the sample injections, eg use C_{A1} and C_{B1} for sample injections S_{A1}, and S_{A2}, etc and obtain the mean response factor f.

Sample analysis should be repeated if calibration response factors f_1 and f_2 differ by more than ± 2% of the mean f.

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

H_s	=	area of lambda-cyhalothrin peak in the calibration solution
I_r	=	area of hexacosane peak in the calibration solution
M	=	mass of lambda-cyhalothrin analytical standard in the calibration solution (g)
P	=	purity of lambda-cyhalothrin standard (g/kg)

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

2.1.9 *Calculation*

Calculate the lambda-cyhalothrin content for each sample injection, eg S_{A1} by the following equation:

$$\textit{Lambda-cyhalothrin content (g/kg)} = \frac{Hw}{f \times I_q \times \underline{w}}$$

f	=	mean relative response factor
H_w	=	area of lambda-cyhalothrin peak in the sample solution
I_q	=	area of the hexacosane peak in the sample solution
$\underline{w}$	=	mass of sample (g)

Calculate the lambda-cyhalothrin content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Lambda-cyhalothrin	
S_{A1}	C_{A1} and C_{B1}	Q%	)
			) X%
S_{A2}	C_{A1} and C_{B1}	R%	)
S_{B1}	C_{A2} and C_{B2}	S%	)
			) Y%
S_{B2}	C_{A2} and C_{B2}	T%	)

Q and R, S and T should agree to within ± 0.5% of their mean values (X and Y). X and Y should agree to within ± 1% of their mean values.

Take the mean of the two values X and Y as the total lambda-cyhalothrin content.

2.2 Lambda-cyhalothrin cis A and cis B' stereoisomer content

2.2.1 *Procedure*

The cis A and cis B' stereoisomers are determined during the lambda-cyhalothrin chromatographic run as part of the same method.

Both peaks should be clearly separated from the lambda-cyhalothrin and internal standard peaks with retention times as follows:

Peak	Relative Retention time	Typical Retention time (min)
Cis A stereoisomer	0.94	10.5
Lambda-cyhalothrin	1.00	11.2
Cis B' stereoisomer	1.01	11.3
Internal standard	1.13	12.7

2.2.2 *Calculation*

Both impurities give the same chromatographic response as lambda-cyhalothrin and can be quantified using electronic integration to measure their peak area, and then ratioing against the lambda-cyhalothrin content of the sample, previously determined.

$$\underline{Cis}\ A\ content\ (g/kg) = \frac{Hw^{I} \times C}{Hw}$$

$$\underline{Cis}\ B'\ content\ (g/kg) = \frac{Hw^{II} \times C}{Hw}$$

where Hw^{1} = area of cis A stereoisomer peak in sample solution
Hw^{11} = area of cis B' stereoisomer peak in sample solution
Hw = area of lambda-cyhalothrin peak in sample solution
C = lambda-cyhalothrin content of sample in g/kg

LAMBDA-CYHALOTHRIN WATER DISPERSIBLE POWDER

Interim specification: WHO/IS/2.3021-1.rev1

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical lambda-cyhalothrin together with filler(s) and other necessary formulants and shall be in the form of a fine, free-flowing, whitish powder free from visible extraneous matter and hard lumps that wets out readily on stirring into water. The technical lambda-cyhalothrin used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/IS/1.3021-1.rev1.

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 *Lambda-cyhalothrin content (g/kg basis)*

The content of lambda-cyhalothrin determined by the method described in section 2.1, shall not differ from the nominal content by more than the following amount:

Nominal content	Tolerance permitted
Up to 25g/kg	± 15% of the nominal content
Above 25 up to 100 g/kg	± 10% 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 *pH of aqueous dispersion*

The pH of an aqueous dispersion, when tested by the method WHO/M/25, should lie in the range 5.5 to 9.0.

1.2.3 *Sieving after heat stability treatment*

Not less than 98% of the powder after the heat stability treatment (section 2.3) shall pass through a 75µm sieve when tested by the method described in WHO/M/4.R1.

1.2.4 *Suspensibility*

In standard hard water after heat stability treatment. When tested by the method described in section 2.2, a minimum of 50% of the lambda-cyhalothrin (0.2g/l) shall be in suspension 30 minutes after agitating a suspension containing 0.4g/l of lambda-cyhalothrin prepared in standard hard water from the powder subjected to the heat stability treatment described in section 2.3.

1.2.5 *Heat stability*

The powder after treatment as described in section 2.3 shall comply with the requirements of section 1.2.1 and 1.2.2 of this specification.

1.3 Packing and marking of packages

The lambda-cyhalothrin water dispersible powder shall be packed in suitable clean drums, as specified in the order. The drums shall contain a lining or bag of polyethylene or equivalent, with a nominal thickness of 0.1mm. The lining or bag shall be sealed after filling. All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name
Lambda-cyhalothrin water dispersible powder to specification:
WHO/IS/2.3021-1.rev1
Lambda-cyhalothrin…g/kg
Batch or reference number, and date of test
Net weight of contents
Date of formulation

and the following minimum cautionary notice

Lambda-cyhalothrin is a pyrethroid that acts predominantly on the central nervous system; high dosages have been found to cause tonic seizures in experimental animals. A high concentration in air may be irritant, and contact with the concentrated product may induce a temporary tingling sensation, particularly on the face. It may be hazardous if swallowed. Do not inhale spray mist. Avoid skin contact; wear protective gloves, clean protective clothing, and a face mask (surgical type) when handling the product. Wash hands and exposed skin thoroughly after using.

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

Lambda-cyhalothrin 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 Lambda-cyhalothrin content

2.1.1 *Outline of method*

The sample is dissolved in dichloromethane containing hexacosane as internal standard. Separation is carried out by capillary gas chromatography, in the split injection mode using a dimethyl polysiloxane WCOT fused silica capillary column, with flame ionisation detector and preferably with an automatic injector. The lambda-cyhalothrin is determined by comparison with calibration solutions.

2.1.2 *Special apparatus*

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

Chromatographic column. 25 x 0.25mm i.d. and 0.12 µm film thickness (or 50 m x 0.32 mm i.d. and 0.12 µm film thickness) WCOT fused silica column coated with dimethyl polysiloxane (chemically bonded).

Injector system. Injector split/splitless in the split mode with fused silica liner. An automatic injector is strongly recommended to ensure the best analytical results.

It is important that the split liner is acid treated, thoroughly deactivated and conditioned before use, to ensure that lambda-cyhalothrin does not epimerise to the isomer during analysis.

Silanised glass wool packing alone should not be used in the liner.

Split ratio	100:1
Injection volume	1.0µl using an autosampler

Detector system. Type flame ionisation (FID), range high sensitivity.

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

150ml conical flasks.

2.1.3 *Special reagents*

Dichloromethane

Hexacosane internal standard. Select for use a batch which, when chromatographed under the conditions given below for the determination of lambda-cyhalothrin, gives no peak with a similar retention time to lambda-cyhalothrin, lambda-cyhalothrin cis A stereoisomer and lambda-cyhalothrin cis B' stereoisomer.

Lambda-cyhalothrin working standard, of known lambda-cyhalothrin content (minimum 990 g/kg).

2.1.4 *Preparation of standard solutions*

Internal standard solution

Dissolve hexacosane (2.0g) in dichloromethane (500ml) (Solution I). Ensure a sufficient quantity of this solution is prepared for all samples and calibration standards being analysed.

Lambda-cyhalothrin calibration solution

Weigh in duplicate (to the nearest 0.1mg) about 0.1g of lambda-cyhalothrin standard (M_A and M_B, g) into 150ml conical flasks. Add to each, 20.0ml of hexacosane solution I from a pipette and 30ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solutions C_A and C_B).

Prepare a solution without internal standard by dissolving about 0.1g of lambda-cyhalothrin standard in 50ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solution C_o).

2.1.5 *Operating conditions*

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

Temperatures

Column oven	210°C
Injector	300°C
Detector	300°C

Adjust the column oven temperature if required to obtain retention time windows for lambda-cyhalothrin (10.7-11.4 min) and hexacosane (12.2-13.0 min), but not exceeding 240°C.

Gas flow rates

Helium or hydrogen (carrier) 1ml min^{-1}

Nitrogen (make up)	)	flow rates as recommended
Hydrogen	)	for the gas chromatograph
Air	)	

All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

2.1.6 *Sample preparation*

Sampling

Homogenize the bulk material before taking the sample. Take at least 500g. Rehomogenize before taking a sub-sample for analysis.

Preparation of sample solutions

Weigh in duplicate (to the nearest 0.1mg) into 150ml conical flasks, sufficient sample (w, g) to contain about 0.1g of lambda-cyhalothrin.

Add by pipette to each flask, 20.0ml of hexacosane solution I, 30ml of dichloromethane and shake thoroughly for 5-10 minutes (use an ultrasonic water bath if available) to dissolve the lambda-cyhalothrin. Allow the insoluble material to settle and filter the supernatant liquid through an appropriate filter-paper.

Dilute 5ml of each filtrate to 25ml with dichloromethane (solutions S_A and S_B).

Prepare a solution without internal standard by shaking s similar amount of sample with 50ml of dichloromethane. Filter and dilute 5ml of this filtrate to 25ml with dichloromethane (Solution S_o).

2.1.7 *Equilibration of the system*

Carry out 1.0µl injections of solutions I, C_o and S_o and check whether there are any interfering peaks from impurities. If there are, make any necessary corrections but do not use external calibration.

Inject calibration solutions C_A and C_B to equilibrate the system and use the data from these chromatograms to set the integration parameters. Calculate the response factors for these injections to check stability of the instrument. Response factors should not differ by more than ± 1% of the mean.

Where the detector sensitivity is shown to be low, the second dilution stage for calibration and sample solutions may be omitted.

2.1.8 *Analysis of sample*

Carry out 1.0µl injections of calibration solutions C_A and C_B and sample solutions S_A and S_B in the following sequence and record the integrated areas of the peaks.

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 calibration injections which bracket the sample injections, eg use C_{A1} and C_{B1} for sample injections S_{A1}, and S_{A2}, etc and obtain the mean response factor f.

Sample analysis should be repeated if calibration response factors f_1 and f_2 differ by more than ± 2% of the mean f.

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

H_s	=	area of lambda-cyhalothrin peak in the calibration solution
I_r	=	area of hexacosane peak in the calibration solution
M	=	mass of lambda-cyhalothrin analytical standard in the calibration solution (g)
P	=	purity of lambda-cyhalothrin standard (g/kg)

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

2.1.9 *Calculation*

Calculate the lambda-cyhalothrin content for each sample injection, eg S_{A1} by the following equation:

$$\textit{Lambda-cyhalothrin content (g/kg)} = \frac{H_w}{f \times I_q \times \underline{w}}$$

f	=	mean relative response factor
H_w	=	area of lambda-cyhalothrin peak in the sample solution
I_q	=	area of the hexacosane peak in the sample solution
$\underline{w}$	=	mass of sample (g)

Calculate the lambda-cyhalothrin content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Lambda-cyhalothrin	
S_{A1}	C_{A1} and C_{B1}	Q%	)
			) X%
S_{A2}	C_{A1} and C_{B1}	R%	)
S_{B1}	C_{A2} and C_{B2}	S%	)
			) Y%
S_{B2}	C_{A2} and C_{B2}	T%	)

Q and R, S and T should agree to within ± 0.5% of their mean values (X and Y). X and Y should agree to within ± 1% of their mean values.

Take the mean of the two values X and Y as the total lambda-cyhalothrin content.

2.2 Suspensibility after heat stability treatment

2.2.1 *Outline of method*

A suspension of known concentration of lambda-cyhalothrin in standard hard water is prepared, poured into a 250ml graduated cylinder, maintained at a constant temperature, and allowed to remain undisturbed for 30 minutes. The top 9/10ths are drawn off and the content of lambda-cyhalothrin in the bottom 1/10th is determined, so allowing to evaluate the active ingredient mass still in suspension after 30 minutes.

2.2.2 *Special apparatus*

A *250ml graduated cylinder* with a ground-glass stopper and a distance of 20.0-21.5cm between the bottom and the 250ml calibration mark.

A glass tube, about 40cm long and about 5mm in internal diameter, pointed at one end to an opening of 2-3mm, the other end being connected to a suitable source of suction.

2.2.3 *Special reagent*

Standard hard water. Dissolve 0.304g of anhydrous calcium chloride and 0.130g of magnesium chloride hexahydrate in distilled water and make up to one litre. This provides water with a hardness of 342 mg/l, calculated as calcium carbonate. Check the hardness by method WHO/M/26 and correct if appropriate.

2.2.4 *Procedure*

Weigh (to the nearest 1mg) into 100ml beaker an amount of the sample to form 250ml of a suspension containing 0.4g/l of lambda-cyhalothrin. Add a volume of water at 30 ± 2°C equal to at least twice the mass of the sample taken. Allow to stand for 30 seconds and then stir by hand for 30 seconds with a glass rod 4-6mm in diameter, at not more than four revolutions per second, making no deliberate attempt to break up any lumps. The immediately transfer the mixture quantitatively to the 250ml graduated cylinder, using water at 30 ± 2°C for rinsing, and again avoiding mechanical disintegration of lumps.

Immediately add sufficient water at 30 ± 2°C to bring the volume up to the 250ml mark. Insert the stopper and invert the cylinder end over end 30 times at the rate of one complete cycle every 2 seconds. This operation should be carried out as smoothly as possible, keeping the axis of rotation fixed. Allow the graduated cylinder to stand for 30 minutes in a water-bath at 30 ± 2°Cm taking care that the bath is free from vibrations.

Should excessive flocculation occur during the test, the material is unsatisfactory.

At the end of the 30 minutes settling period, insert the glass tube into the cylinder and, with a minimum of disturbance, withdraw nine-tenths, of the suspension (ie 225ml) by means of the suction tube in a period of 10-15 seconds. This is achieved by maintaining the tip of the glass tube just below the sinking surface of the suspension. Discard the suspension withdrawn.

2.2.5 *Determination of lambda-cyhalothrin in the retained one-tenth of the suspension*

Transfer the bottom one-tenth of suspension from the suspensibility test quantitatively to a 250ml glass-stoppered separating funnel. Use a maximum volume of 25ml of distilled water to rinse the 250ml graduated cylinder and combine the suspension and washings. Add 25ml of dichloromethane to the separating funnel, stopper, and shake for one minute. Formation of an emulsion at this stage may be overcome by adding 1g of sodium chloride crystals to the aqueous layer and re-shaking the contents of the funnel.

Run the separated dichloromethane layer through phase-separating paper (eg 1-PS available from Whatman) into a clean, dry 250ml round-bottom flask. Repeat the extraction with a further three 25ml aliquots of dichloromethane, combining all four extracts. Remove the dichloromethane under reduced pressure at 60 ± 2°C using a rotary evaporator and dissolve the residue in the 250ml flask in 10.0ml of hexacosane internal standard solution, and make up to 25ml with dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (solution S_A).

Determine the lambda-cyhalothrin content of the solution by gas chromatography as described in section 2.1.8 injecting duplicate 1.0µl aliquots of sample and calibration solutions.

2.2.6 *Calculation*

Mass of lambda-cyhalothrin (g) in the bottom one-tenth of suspension =

$$\frac{r_2 \times P \times m_1}{r_1 \times 2 \times 10^3}$$

where: r_1 = mean response ratio for the calibration solution
r_2 = mean response ratio for the sample solution
m_1 = mass (g) of lambda-cyhalothrin standard in the calibration solution
P = purity (g/kg) of lambda-cyhalothrin standard

$$\textit{Suspensibility \%} = \frac{10\,(b-a)}{9} \times \frac{100}{b} = \frac{111(b-a)}{b}$$

where: a = mass (g) of lambda-cyhalothrin in bottom one-tenth of suspension
b = mass (g) of lambda-cyhalothrin in the sample used in the suspensibility test (calculated from the mass of sample and its percentage lambda-cyhalothrin content).

2.3 Heat stability treatment

Fill a 50ml wide-mouthed glass bottle to within 1cm of the top with the sample. Seal the bottle with a phenolic plastic cap having a soft liner. Turn the cap firmly to ensure a tight seal and place the bottle in a forced-draught oven maintained at 54 ± 2°C for 3 days. At the end of the heating period, remove the bottle from the oven and allow it to come to room temperature before removing the cap.

After the completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

LAMBDA-CYHALOTHRIN EMULSIFIABLE CONCENTRATE

Interim specification: WHO/IS/3.3021-1.rev1

1. Specification

1.1 Description and ingredients

The material shall consist of technical lambda-cyhalothrin dissolved in suitable solvents with other necessary formulants added. It shall be in the form of a stable liquid free from visible suspended matter and sediments. The technical lambda-cyhalothrin used in the manufacture of the concentrate shall comply with the requirements of specification WHO/IS/1.3021-1.rev1.

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 the following requirements.

1.2.1 *Lambda-cyhalothrin content (g/kg basis)*

The content of lambda-cyhalothrin determined by the method described in section 2.1, shall not differ from the nominal content by more than the following amounts:

Nominal content	Tolerance permitted
Up to 100 g/kg	± 10% of the nominal content
Above 100 up to 250 g/kg	± 6% 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 *Water content*

The water content determined by the method described in the method WHO/M/7.R1 shall not be higher than 0.5 g/kg.

1.2.3 *Acidity or alkalinity*

The acidity or alkalinity of the emulsifiable concentrate, determined by the method described in WHO/M/3, shall not be higher than 0.5 g/kg calculated as H_2SO_4 or 0.5 g/kg calculated as NaOH.

1.2.4 *Cold test*

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

1.2.5 *Flash point*

The flash point of the product shall comply with all national and/or international transport regulations (see method WHO/M/10.R1.)

1.2.6 *Stability of the emulsion*

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

1.2.7 H*eat stability*

The emulsifiable concentrate, after treatment as described in section 2.3, shall comply with the requirements of section 1.2.1, 1.2.3, and 1.2.6 of this specification.

1.3 Packing and marking of packages

The lambda-cyhalothrin emulsifiable concentrate shall be packed in suitable, clean containers, as specified in the order. All packages shall bear, durably and legibly marked on the containers, the following:

Manufacturer's name
Lambda-cyhalothrin emulsifiable concentrate to specification
WHO/IS/3.3021-1.rev1
Lambda-cyhalothrin...g/kg
Batch or reference number, and date of test
Net weight of contents
Instructions for dilution
Date of formulation

and the following minimum cautionary notice:

Lambda-cyhalothrin is a pyrethroid that acts predominantly on the central nervous system; high dosages have been found to cause tonic seizures in experimental animals. A high concentration in air may be irritant and contact with the concentrated product may induce a temporary tingling sensation, particularly on the face. It may be hazardous if swallowed. Do not inhale spray mist. Avoid skin contact, wear protective gloves, clean protective clothing and a face mask (surgical type) when handling this concentrate. Wash hands and exposed skin thoroughly after using.

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

Lambda-cyhalothrin is toxic to aquatic wildlife. Avoid accidental contamination of water.

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

2. Method of determining chemical and physical properties

2.1 Lambda-cyhalothrin content

2.1.1 *Outline of method*

The sample is dissolved in dichloromethane containing hexacosane as internal standard. Separation is carried out by capillary gas chromatography, in the split injection mode using a dimethyl polysiloxane WCOT fused silica capillary column, with flame ionisation detector and preferably with an automatic injector. The lambda-cyhalothrin is determined by comparison with calibration solutions.

2.1.2 *Special apparatus*

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

Chromatographic column. 25 x 0.25mm i.d. and 0.12 µm film thickness (or 50 m x 0.32 mm i.d. and 0.12 µm film thickness) WCOT fused silica column coated with dimethyl polysiloxane (chemically bonded).

Injector system. Injector split/splitless in the split mode with fused silica liner. An automatic injector is strongly recommended to ensure the best analytical results.

It is important that the split liner is acid treated, thoroughly deactivated and conditioned before use, to ensure that lambda-cyhalothrin does not epimerise to the isomer during analysis.

Silanised glass wool packing alone should not be used in the liner.

Split ratio	100:1
Injection volume	1.0µl using an autosampler

Detector system. Type flame ionisation (FID), range high sensitivity.

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

150ml conical flasks.

2.1.3 *Special reagents*

Dichloromethane

Hexacosane internal standard. Select for use a batch which, when chromatographed under the conditions given below for the determination of lambda-cyhalothrin, gives no peak with a similar retention time to lambda-cyhalothrin, lambda-cyhalothrin cis A stereoisomer and lambda-cyhalothrin cis B' stereoisomer.

Lambda-cyhalothrin working standard, of known lambda-cyhalothrin content (minimum 990 g/kg).

2.1.4 *Preparation of standard solutions*

Internal standard solution

Dissolve hexacosane (2.0g) in dichloromethane (500ml) (Solution I). Ensure a sufficient quantity of this solution is prepared for all samples and calibration standards being analysed.

Lambda-cyhalothrin calibration solution

Weigh in duplicate (to the nearest 0.1mg) about 0.1g of lambda-cyhalothrin standard (M_A and M_B, g) into 150ml conical flasks. Add to each, 20.0ml of hexacosane solution I from a pipette and 30ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solutions C_A and C_B).

Prepare a solution without internal standard by dissolving about 0.1g of lambda-cyhalothrin standard in 50ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solution C_0).

2.1.5 *Operating conditions*

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

Temperatures

Column oven	210°C
Injector	300°C
Detector	300°C

Adjust the column oven temperature if required to obtain retention time windows for lambda-cyhalothrin (10.7-11.4 min) and hexacosane (12.2-13.0 min), but not exceeding 240°C.

Gas flow rates

Helium or hydrogen (carrier) 1ml min^{-1}

Nitrogen (make up)	)	flow rates as recommended
Hydrogen	)	for the gas chromatograph
Air	)	

All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

2.1.6 *Sample preparation*

Sampling

Homogenize the bulk material before taking the sample. Take at least 500g. Rehomogenize before taking a sub-sample for analysis.

Preparation of sample solutions

Weigh in duplicate (to the nearest 0.1mg) into 150ml conical flasks, sufficient sample (w, g) to contain about 0.1g of lambda-cyhalothrin.

Add by pipette to each flask, 20.0ml of hexacosane solution I, 30ml of dichloromethane and shake thoroughly to dissolve the lambda-cyhalothrin.

Dilute 5ml of each solution to 25ml with dichloromethane (Solutions S_A and S_B).

Prepare a solution without internal standard by shaking a similar amount of sample with 50ml of dichloromethane. Dilute 5ml of this solution to 25ml with dichloromethane (Solution S_o).

2.1.7 *Equilibration of the system*

Carry out 1.0µl injections of solutions I, C_o and S_o and check whether there are any interfering peaks from impurities. If there are, make any necessary corrections but do not use external calibration.

Inject calibration solutions C_A and C_B to equilibrate the system and use the data from these chromatograms to set the integration parameters. Calculate the response factors for these injections to check stability of the instrument. Response factors should not differ by more than ± 1% of the mean.

Where the detector sensitivity is shown to be low, the second dilution stage for calibration and sample solutions may be omitted.

2.1.8 *Analysis of sample*

Carry out 1.0µl injections of calibration solutions C_A and C_B and sample solutions S_A and S_B in the following sequence and record the integrated areas of the peaks.

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 calibration injections which bracket the sample injections, eg use C_{A1} and C_{B1} for sample injections S_{A1}, and S_{A2}, etc and obtain the mean response factor f.

Sample analysis should be repeated if calibration response factors f_1 and f_2 differ by more than ± 2% of the mean f.

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

H_s	=	area of lambda-cyhalothrin peak in the calibration solution
I_r	=	area of hexacosane peak in the calibration solution
M	=	mass of lambda-cyhalothrin analytical standard in the calibration solution (g)
P	=	purity of lambda-cyhalothrin standard (g/kg)

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

2.1.9 *Calculation*

Calculate the lambda-cyhalothrin content for each sample injection, eg S_{A1} by the following equation:

$$\textit{Lambda-cyhalothrin content (g/kg)} = \frac{H_w}{f \times I_q \times \underline{w}}$$

f	=	mean relative response factor
H_w	=	area of lambda-cyhalothrin peak in the sample solution
I_q	=	area of the hexacosane peak in the sample solution
$\underline{w}$	=	mass of sample (g)

Calculate the lambda-cyhalothrin content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Lambda-cyhalothrin	
S_{A1}	C_{A1} and C_{B1}	Q%	)
			) X%
S_{A2}	C_{A1} and C_{B1}	R%	)
S_{B1}	C_{A2} and C_{B2}	S%	)
			) Y%
S_{B2}	C_{A2} and C_{B2}	T%	)

Q and R, S and T should agree to within ± 0.5% of their mean values (X and Y). X and Y should agree to within ± 1% of their mean values.

Take the mean of the two values X and Y as the total lambda-cyhalothrin content.

2.2 Water content

Determine the water content by the Karl Fischer electrometric titration method WHO/M/7.R1 or by the Dean and Stark distillation method WHO/M/8.R1. The latter may not always be practicable owing to its unreliability at very low water contents. In the event of a dispute the Karl Fischer method shall be the referee.

2.3 Heat Stability Treatment

Keep 50ml of the sample for 3 days at a temperature of 54 ± 2°C in a glass container sealed to avoid loss of volatile solvent, and then cool to room temperature.

ETOFENPROX TECHNICAL

Interim specification WHO/IS/97.24.1

1. Specification

1.1 Material

The material shall consist of etofenprox together with related manufacturing impurities and shall be in the form of a white to yellow solidified melt at ambient temperature, free from extraneous matter 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 *Etofenprox content (g/kg basis)*

The etofenprox content shall be declared (not less than 990 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 5 g.

1.2.2 *Melting point*

The melting point of the material, determined by the method described in WHO/M/5, shall not be lower than 36 and shall not be depressed when mixed with an equal quantity of pure etofenprox.

1.2.3 *pH of aqueous dispersion*

The pH value of aqueous dispersion, determined by the method described in WHO/M/25, shall lie in the range 5 to 6.

1.2.4 *Material, insoluble in acetone*

The material insoluble in acetone, 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/7R1, shall not be higher than 1 g/kg.

1.3 Packing and marking of packages

The technical etofenprox 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 etofenprox to specification WHO/IS/97.24.1
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice.

Etofenprox is an insecticide with an action similar to the pyrethroids that act predominantly on the central nervous system. It may be hazardous if swallowed. Do not inhale spray mist. Avoid skin contact; wear protective gloves, clean protective clothing, and a face mask (surgical type) when handling the material. Wash hands and exposed skin thoroughly after using.

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

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

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

WHO has classified etofenprox as unlikely to present acute hazard in normal use.

2. Methods of determining chemical and physical properties

2.1 Etofenprox content

2.1.1 *Outline of method*

The sample is dissolved in cyclohexane containing di-cyclohexyl phthalate as internal standard. Separation is carried out by gas-liquid chromatography with a flame ionization detector on a column of Chromosorb W-HP coated with silicone AN-600. The etofenprox is determined by comparison with calibration solutions.

2.1.2 *Special apparatus*

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

Chromatographic column. Glass column 2 m long, 3 mm internal diameter packed with 5% silicone AN-600 on Chromosorb W-HP (60-80 mesh) or equivalent.

Injection volume. 1.0 µl.

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

Before use condition a freshly prepared column by purging with nitrogen overnight at 290 . 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*

Cyclohexane, p.a.

Internal standard. Di-cyclohexyl phthalate. Select for use a batch which, when chromatographed under the conditions given below for the determination of etofenprox, gives no peak with a similar retention time to etofenprox.

Etofenprox working standard. Analytical standard of known etofenprox content (minimum 999 g/kg).

2.1.4 *Preparation of standard solutions*

Internal standard solution

Dissolve 1 g di-cyclohexyl phthalate in 200 ml cyclohexane. 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. Keep the solution in thermostated bath if room temperature varies by more than 1.

Etofenprox calibration solution

Weigh in duplicate (to the nearest 0.1 mg) about 0.12 g of etofenprox standard (M_A and M_B, g) into separate 100 ml stoppered volumetric flasks. Add 20.0 ml of di-cyclohexyl phthalate internal standard solution, shake to dissolve the etofenrox and dilute to 100 ml with cyclohexane. (Solutions C_A and C_B). Keep the solution in thermostated bath if room temperature varies by more than 1.

Prepare a solution without internal standard by dissolving about 0.12 g of standard in 100 ml of cyclohexane. (Solution C_O).

2.1.5 *Operating conditions*

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

Temperatures

Column oven	230
Injector	270
Detector	270

Adjust the column oven temperature if required to obtain retention time windows for etofenprox (approximately 19 min) and di-cyclohexyl phthalate (approximately 9.5 min), but not exceeding 300 .

Gas flow rate

Nitrogen	50 ml min^{-1}
Hydrogen	40 ml min^{-1}
Air	500 ml min^{-1}

2.1.6 *Sample preparation*

Sampling

Homogenize the bulk material by heating to about 40 and mix thoroughly until no crystals remain before taking at least 100 g 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 about 0.12 g of etofenprox into 100 ml stoppered volumetric flasks. Add to each flask 20 ml of di-cyclohexyl phthalate internal standard solution by pipette, shake the flasks thoroughly to dissolve the etofenprox and dilute to 100 ml with cyclohexane. (Solution S_A and S_B).

Prepare a solution without internal standard by dissolving about 0.12 g of etofenprox in 100 ml of cyclohexane (Solution S_O).

2.1.7 *Equilibration of the system*

Inject at least 3 x 1.0 µl of one of the etofenprox 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 1.0 µl 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 1.0 µl of the etofenprox 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 x 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 etofenprox 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 = Area of etofenprox peak from the etofenprox calibration solution.
I_r = Area of di-cyclohexyl phthalate peak in the etofenprox calibration solution.
M = Mass of etofenprox analytical standard in the etofenprox calibration solution (g)
P = Purity of the etofenprox analytical standard (g/kg).

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

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

2.1.9 *Calculation*

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

$$\text{Etofenprox content (g/kg)} = \frac{H_w}{f \times I_q \times \underline{w}}$$

where

f = mean relative response factor
H_w = area of the etofenprox peak in the sample solution
I_q = area of the di-cyclohexyl phthalate peak in the sample solution
$\underline{w}$ = mass of sample (g).

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

Calculate the etofenprox content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Etofenprox	
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 0.5 % of their respective mean values (U and V). U and V should agree to within 1% of their mean values. Take the mean of the two values U and V as the total etofenprox content.

ETOFENPROX WATER-DISPERSIBLE POWDER

Specification WHO/IS/97.24.2

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical etofenprox together with filler(s) and other necessary formulants. It shall be in the form of a fine, free-flowing, whitish powder free from visible extraneous matter and hard lumps that wets out readily on stirring into water. The technical etofenprox used in the manufacture of the wettable powder shall comply with the requirements of specification WHO/IS/97.24.1.

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 *Etofenprox content (g/kg basis)*

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

Nominal content	*Tolerance permitted*
Up to 100 g/kg	10% of the nominal content
Above 100 up to 250 g/kg	6% of the nominal content
Above 250 up to 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 *Water content*

The water content, determined by the method described in WHO/M/7.R1 shall not be higher than 30 g/kg.

1.2.3 *pH of aqueous dispersion*

The pH of an aqueous dispersion, determined by the method described in WHO/M/25, shall lie in the range 5.5 to 9.0.

1.2.4 *Sieving after heat stability treatment*

Not less than 98% of the powder after the heat stability treatment (section 2.3) shall pass through a 75 µm sieve when tested by the method described in WHO/M/4.

1.2.5 *Suspensibility*

In standard hard water after heat stability treatment. When tested by the method described in section 2.2, a minimum of 60% of the etofenprox (3 g/l) shall be in suspension 30 minutes after agitating a suspension containing 5 g/l of etofenprox prepared in standard hard water from the powder subjected to the heat stability treatment described in section 2.3.

1.2.6 *Persistent foam*

The persistent foam at the top of 100 ml of suspension prepared in standard hard water with 5 g of powder, shall not exceed 10 ml when tested by the method described in CIPAC MT 47.

1.2.7 *Wetting of the product*

The product shall be completely wetted in 2 minutes without swirling when tested by the method described in CIPAC MT 53.3.1.

1.2.8 *Heat stability*

The powder after treatment as described in section 2.3 shall comply with the requirements of section 1.2.1 and 1.2.2 of this specification.

1.3 Packing and marking of packages

The etofenprox water dispersible powder 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
Etofenprox water dispersible powder to specification WHO/IS/97.24.2
Etofenprox .... g/kg
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice.

Etofenprox is an insecticide with an action similar to the pyrethroids that act predominantly on the central nervous system. It may be hazardous if swallowed. Do not inhale spray mist. Avoid skin contact; wear protective gloves, clean protective clothing, and a face mask (surgical type) when handling the product. Wash hands and exposed skin thoroughly after using.

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

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

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

WHO has classified etofenprox as unlikely to present acute hazard in normal use.

2. Methods of determining chemical and physical properties

2.1 Etofenprox content

2.1.1 *Outline of method*

The sample is dissolved in cyclohexane containing di-cyclohexyl phthalate as internal standard. Separation is carried out by gas-liquid chromatography with a flame ionization detector on a column of Chromosorb W-HP coated with silicone AN-600. The etofenprox is determined by comparison with calibration solutions.

2.1.2 *Special apparatus*

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

Chromatographic column. Glass column 2 m long, 3 mm internal diameter packed with 5% silicone AN-600 on Chromosorb W-HP (60-80 mesh) or equivalent.

Injector volume. 1.0 µl.

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

Before use condition a freshly prepared column by purging with nitrogen overnight at 290 . 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*

Cyclohexane, p.a.

Internal standard. Di-cyclohexyl phthalate. Select for use a batch which, when chromatographed under the conditions given below for the determination of etofenprox, gives no peak with a similar retention time to etofenprox.

Etofenprox working standard. Analytical standard of known etofenprox content (minimum 999 g/kg).

2.1.4 *Preparation of standard solutions*

Internal standard solution

Dissolve 1 g di-cyclohexyl phthalate in 200 ml cyclohexane. 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. Keep the solution in thermostated bath if room temperature varies by more than 1.

Etofenprox calibration solution

Weigh in duplicate (to the nearest 0.1 mg) about 0.12 g of etofenprox standard (M_A and M_B, g) into 100 ml stoppered volumetric flasks. Add 20.0 ml of di-cyclohexyl phthalate internal standard solution, shake to dissolve the etofenprox and dilute to 100 ml with cyclohexane. (Solutions C_A and C_B). Keep the solution in thermostated bath if room temperature varies by more than 1.

Prepare a solution without internal standard by dissolving about 0.12 g of standard in 100 ml of cyclohexane. (Solution C_O).

2.1.5 *Operating conditions*

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

Temperatures

Column oven	230
Injector	270
Detector	270

Adjust the column oven temperature if required to obtain retention time windows for etofenprox (approximately 19 min) and di-cyclohexyl phthalate (approximately 9.5 min), but not exceeding 300 .

Gas flow rates

Nitrogen	50 ml min^{-1}
Hydrogen	40 ml min^{-1}
Air	500 ml min^{-1}

2.1.6 *Sample preparation*

Sampling. Homogenize the bulk material before taking at least 100 g 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 about 0.12 g of etofenprox into 100 ml stoppered volumetric flasks. Add to each flask 20 ml of di-cyclohexyl phthalate internal standard solution by pipette, shake the flasks thoroughly to dissolve the etofenprox and dilute to 100 ml with cyclohexane. (Solutions S_A and S_B).

Prepare a solution without internal standard by dissolving about 0.12 g of etofenprox in 100 ml of cyclohexane. (Solution S_O).

2.1.7 *Equilibration of the system*

Inject at least 3 x 1.0 µl of the etofenprox 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 1.0 µl 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 injection of 1.0 µl of the etofenprox 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 x 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 etofenprox 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 = Area of etofenprox peak from the etofenprox calibration solution.
I_r = Area of di-cyclohexyl phthalate peak in the etofenprox calibration solution.
M = Mass of etofenprox analytical standard in the etofenprox calibration solution (g).
P = Purity of the etofenprox analytical standard (g/kg)

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

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

2.1.9 *Calculation*

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

$$\text{Etofenprox content (g/kg)} = \frac{H_w}{f \times I_q \times \underline{w}}$$

where:

f = mean relative response factor
H_w = area of etofenprox peak in the sample solution
I_q = area of the di-cyclohexyl phthalate peak in the sample solution
$\underline{w}$ = mass of sample (g).

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

Calculate the etofenprox content of the sample as the mean of the four determinations as follows:

Sample injection	Use relative response factor from	Etofenprox	
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 0.5 % of their mean values (U and V). U and V should agree to within 1% of their mean values. Take the mean of the two values U and V as the total etofenprox content.

2.2 Suspensibility after heat stability treatment

2.2.1 *Outline of method*

A suspension of known concentration of etofenprox in standard hard water is prepared, placed in a 250 ml graduated cylinder at a constant temperature, and allowed to remain undisturbed for 30 minutes. The top 9/10ths are drawn off and the content of etofenprox in the bottom 1/10th determined, so allowing to evaluate the active ingredient still in suspension after 30 minutes.

2.2.2 *Special apparatus*

1. A 250 ml graduated cylinder with ground-glass stopper and a distance of 20.0 - 21.5 cm between the bottom and the 250 ml calibration mark.

2. A glass tube, about 40 cm long and about 5 mm in internal diameter, pointed at one and to an opening of 2-2 mm, the other and being connected to a suitable source of suction.

2.2.3 *Special reagent*

Standard hard water. Dissolve 0.304 g of anhydrous calcium chloride and 0.139 g of magnesium chloride hexahydrate in distilled water and make up to one litre. This provides water with a hardness of 342 mg/l, calculated as calcium carbonate. Check the hardness by method WHO/M/26 and correct if appropriate.

2.2.4 *Procedure*

Weigh (to the nearest 1 mg) into 100 ml beaker an amount of the sample to form 250 ml of a suspension containing 5 g/l of etofenprox. Add a volume of water at 30 1 equal to at least twice the mass of the sample taken. Allow to stand for 30 seconds and then stir by hand for 30 seconds with a glass rod 4 - 6 mm in diameter, at not more than four revolutions per second, making no deliberate attempt to break up any lumps. Then immediately transfer the mixture quantitatively to the 250 ml graduated cylinder, using water at 30 1 for rinsing, and again avoiding mechanical disintegration of lumps.

Immediately add sufficient water at 30 1 to bring the volume up to the 250 ml mark. Insert the stopper and invert the cylinder end over end 30 times at the rate of one complete cycle every 2 seconds. During agitation the cylinder must be thermally insulated from the hands to maintain the prescribed temperature of the suspension. This operation should be carried out as smoothly as possible, keeping the axis of rotation fixed. Allow the graduated cylinder to stand for 30 minutes in a water-bath at 30 1, taking care that the bath is free from vibrations.

Should excessive flocculation occur during the test, the material is unsatisfactory.

At the end of the 30 minute settling period, insert the glass tube into the cylinder and, with a minimum of disturbance, withdraw nine-tenths of the suspension (i.e. 225 ml) by means of the suction tube in a period of 10 - 15 seconds. This is achieved by maintaining the tip of the glass tube just below the sinking surface of the suspension. Discard the suspension withdrawn.

2.2.5 *Determination of etofenprox in the retained one-tenth of the suspension*

Transfer the bottom one-tenth of suspension from the suspensibility test quantitatively to a 250 ml glass-stoppered separating funnel. Use a maximum volume of 25 ml of distilled water to rinse the 250 ml graduated cylinder and combine the suspension and washings.

Add 25 ml of cyclohexane to the separating funnel, stopper, and shake for one minute. Formation of an emulsion at this stage may be overcome by adding 1 g of sodium chloride crystals to the aqueous layer and reshaking the contents of the funnel.

Run the separating cyclohexane layer through phase-separating paper into a clean, dry 250 ml round-bottom flask. Repeat the extraction with a further three 25 ml aliquots of cyclohexane, combining all four extracts.

Remove the cyclohexane under reduced pressure at 60 using a rotary evaporator and dissolve the residue in the 250 ml flask in 2.0 ml of di-cyclohexyl phthalate internal standard solution, and make up to 25 ml with cyclohexane.

Determine the etofenprox content of the solution by gas chromatography as described in section 2.1.8 injecting duplicate 1 microlitre aliquots of sample and standard solutions.

2.2.6 *Calculation*

Weight of etofenprox (g) in the bottom one-tenth of suspension

$$a = \frac{r_2 \times P \times m_1}{r_1 \times 10^4}$$

where r_1 = average response ration for the standard solution
r_2 = average response ratio for the sample solution
m_1 = mass (g) of etofenprox standard taken
p = purity (g/kg) of etofenprox standard

Then percentage suspensibility of sample $= \frac{10\,(b-a)}{9} \times \frac{100}{b} = \frac{111(b-a)}{b}$

where a = mass (g) of etofenprox in bottom one-tenth of suspension.
b = mass (g) of etofenprox in the sample used in the suspensibility test (calculated from the mass of sample and its percentage etofenprox content).

2.3 Heat stability treatment

Fill a 50 ml wide-mouthed glass bottle to within 1 cm of the top with the sample. Seal the bottle with a phenolic plastic cap having a soft liner. Turn the cap firmly to ensure a tight seal and place the bottle in an oven maintained at 54 ± 2°C for 14 days. At the end of the heating period, remove the bottle from the oven and allow it to come to room temperature before removing the cap.

After completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

TECHNICAL BRODIFACOUM

Interim specification: WHO/IS/1.RO1.1.rev1

1. Specification

1.1 Material

The material shall consist of brodifacoum together with related manufacturing impurities and shall be in the form of a white to pale buff powder, free from odour and extraneous matter and 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 *Brodifacoum content (g/kg basis)*

The brodifacoum content shall be declared (not less than 900 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 20 g.

1.2.2 *Brodifacoum cis-trans isomer ratio*

The cis-trans isomer ratio shall be declared and shall be in the range of 50: 50 to 80: 20 when determined by the method described in section 2.2.

1.2.3 *Loss on drying at 100°C*

The maximum loss on drying at 100°C when determined by the method described in section 2.3 shall be 5 g/kg.

1.3 Packing and Marking of Packages

The technical brodifacoum 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 brodifacoum to specification WHO/IS/1.RO1.1.rev1
Actual 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.

Brodifacoum is an indirect anticoagulant and is hazardous if swallowed. Avoid skin contact; wear protective gloves, clean protective clothing, and suitable respiratory protection when handling the material. Open and handle only in a cabinet with adequate exhaust ventilation. Wash hands and exposed skin thoroughly after using and destroy contaminated clothing.

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

Brodifacoum 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 Brodifacoum

2.1.1 *Summary of method*

A weighed sample of technical brodifacoum is dissolved in triphenylbenzene internal standard solution and determined by reverse phase HPLC with UV detection

2.1.2 *Special Apparatus*

Liquid chromatograph, equipped with 10 µl loop injector. UV detector capable of operating at 254 nm and a suitable electronic integrator or laboratory data system.

Chromatographic column, stainless steel, 250 x 4.6 mm (i.d.) with Zorbax ODS 5 µm, reverse phase column (DuPont Instruments Inc), or equivalent.

2.1.3 *Special reagents*

1,3,5 Triphenylbenzene, internal standard, purity greater than 97%.
Acetic acid, glacial, HPLC grade
Dichloromethane, HPLC grade
Methanol, HLPC grade
Water, HLPC grade
Brodifacoum of known purity

Diluting solvent: dichloromethane-methanol (2+3)
Eluting solvent: methanol-water-acetic acid (94.2 + 5.0 + 0.8)

Mix 942 ml methanol, 50 ml water and 8 ml acetic acid, filter and degas.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weight about 100 mg of 1,3,5 triphenylbenzene into a 500 ml volumetric flask, dissolve in dichloromethane (200 ml) and dilute to volume with methanol.

Mix thoroughly. Check for interfering components by injecting 10 µl into the liquid chromatograph. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

Brodifacoum calibration solution. Weigh (to the nearest 0.1 mg) about 100 mg brodifacoum (s g) into a 100 ml volumetric flask, dissolve in dichloromethane (40 ml) and make up to volume with methanol. Mix thoroughly. Transfer by pipette 10.0 ml into a 50 ml volumetric flask, add by pipette internal standard solution (10.0 ml) and make up to volume with the diluting solvent. Mix thoroughly. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

2.1.5 *Operating conditions*

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

Eluting solvent flow rate	1 ml min^{-1}
Temperature	ambient
Injection volume	10 µl
Wavelength	254 nm
Retention times	Brodifacoum: 6.2 min, internal standard: 11.7 min

2.1.6 *Sample preparation*

Weigh (to the nearest 0.1 mg) into a 100 ml volumetric flask enough sample (w g) to contain about 100 mg brodifacoum. Dissolve in dichloromethane (40 ml) and dilute to volume with methanol. Mix thoroughly. Transfer 10.0 ml into a 50 ml volumetric flask, add by pipette internal standard solution (10.0 ml) and make up to volume with the diluting solvent. Mix thoroughly.

2.1.7 *Equilibration of the system*

Inject two or more 10 µl aliquots of the calibration solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument. Continue with injections until the peak area ratios of the brodifacoum peaks to the internal standard peaks for successive injections agree within 2%.

2.1.8 *Analysis of sample*

Carry out duplicate injections of calibration and sample solutions (in the order calibration, sample, sample, and calibration). Average the peak area ratios of the calibration injection (R′) and of the sample injections. (R).

$$(R) = \frac{\text{area brodifacoum peak}}{\text{area internal standard peak}}$$

2.1.9 *Calculation*

$$\text{Brodifacoum content} = \frac{R \times \underline{s} \times P}{R' \times \underline{w}} \quad \text{g/kg}$$

R	=	average peak area ratio of brodifacoum and internal standard for the sample
R′	=	average peak area ratio of brodifacoum and internal standard for the calibration solution
$\underline{w}$	=	mass of sample (g)
$\underline{s}$	=	mass of brodifacoum in the calibration solution (g)
P	=	purity of brodifacoum (g/kg).

2.2 Determination of cis/trans isomer ratio

2.2.1 *Summary of method*

A weighed sample of technical brodifacoum is dissolved in dichloromethane and the isomer ratio determined by HPLC with UV detection

2.2.2 *Special apparatus*

Liquid chromatograph, equipped with 10 µl loop injection, UV detector capable of operating at 254 nm and a suitable recorder, or electronic integrator, or laboratory data system.

Chromatographic column, stainless steel, 250x4.6 mm (i.d.) with Spherisorb S5W or Lichrosorb SI60.

2.2.3 *Special reagents*

Acetic acid, glacial, HLPC grade
Dichloromethane, HLPC grade
Hexane, HLPC grade
Brodifacoum of known purity
Eluting solvent: Hexane-dichloromethane-acetic acid (74.8 + 25 + 0.2)

Mix 748 ml hexane, 250 ml dichloromethane and 2 ml acetic acid, filter and degas.

2.2.4 *Operating conditions*

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

Eluting solvent flow rate	2 ml min^{-1}
Temperature	ambient
Injection volume	10 µl
Wavelength	254 nm
Retention times	Trans isomer 14 minutes
	Cis isomer 15.7 minutes

2.2.5 *Sample preparation*

Weigh (to the nearest 0.1 mg) into a 50 ml volumetric flask, enough sample to contain 20 mg brodifacoum. Dissolve in dichloromethane (20 ml) and dilute to volume with dichloromethane. Mix thoroughly.

2.2.6 *Equilibration of the system*

Inject two or more 10 µl aliquots of sample solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument.

2.2.7 *Analysis of sample*

Inject two 10 µl aliquots of sample solution in succession and record the integrated areas for each peak. Average the integrated areas for each of the peaks.

2.2.8 *Calculation*

$$\text{Cis isomer content of sample} = \frac{C}{C + T} \times 100\%$$

$$\text{Trans isomer content of sample} = \frac{T}{C + T} \times 100\%$$

C = mean area of cis isomer peaks
T = mean area of trans isomer peaks

2.3 Loss on drying at 100°C

Outline of Method

The material is heated at 100°C for 4h, and the loss of water and volatile materials calculated.

Apparatus
Squat type weighing bottle - 50 mm diameter, 30 mm high
Oven at 100 ± 2°C
Desiccator

Procedure

Heat the weighing bottle at 100 ± 2°C for 1h, cool in a desiccator and weigh. Place about 2.5 g of the sample in a thin layer in the tared weighing bottle (x g) and weigh (y g). Heat (without lid) to 100 ± 2°C for 4h, replace lid, cool in the desiccator, and reweigh (z g).

$$\text{Loss on drying} = \frac{100\,(y-z)}{(y-x)}\ \%\ w/w$$

BRODIFACOUM
5 and 25 g/kg CONCENTRATES

Interim specification: WHO/IS/11.RO1.1.rev1

1. Specification

1.1 Material

The material shall consist of a solution of technical brodifacoum as its triethanolamine salt, together with any necessary formulants and a red or blue dye. It shall be free from visible suspended matter and sediment. The technical brodifacoum used in the manufacture of the concentrate shall comply with the requirements of specification WHO/IS/1.RO1.1.rev1

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 sections 1.1 and with the following requirements.

1.2.1 *Brodifacoum content (g/kg basis)*

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

Nominal content	Tolerance permitted
Up to 25g/kg	± 15% of the declared content

The average content of all samples taken shall not be lower than the nominal content.

1.3 Packing and marking of packages

The brodifacoum 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
Brodifacoum concentrate to specification WHO/IS/11.RO1.1.rev1
Brodifacoum .....mg/kg
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Brodifacoum is an indirect anticoagulant and is hazardous if swallowed. Avoid skin contact; wear protective gloves and clean protective clothing when handling the material. Wash hands and exposed skin thoroughly after using.

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

Brodifacoum 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 Brodifacoum content

2.1.1 *Summary of method*

A weighed sample of brodifacoum concentrate is dissolved in triphenylbenzene internal standard solution and determined by reverse phase HLPC with UV detection.

2.1.2 *Special apparatus*

Liquid chromatograph, equipped with 10 µl loop injector. UV detector capable of operating at 254 nm and a suitable electronic integrator, or laboratory data system.
Chromatographic column, stainless steel, 250x4.6 mm (i.d.) with Zorbax ODS 5 µm, reverse phase column (DuPont Instruments Inc), or equivalent.

2.1.3 *Special reagents*

1,3,5-Triphenylbenzene internal standard purity greater than 97%.
Acetic acid, glacial, HPLC grade
Dichloromethane, HLPC grade
Methanol, HLPC grade
Water, HLPC grade
Brodifacoum of known purity
Diluting solvent: dichloromethane-methanol (2+3)
Eluting solvent: methanol-water-acetic acid (94.2 + 5.0 + 0.8)

Mix 942 methanol, 50 ml water and 8 ml acetic acid, filter and degas

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weigh about 100 mg 1,3,5-triphenylbenzene into a 500 ml volumetric flask, dissolve in dichloromethane (200 ml) and dilute to volume with methanol. Mix thoroughly. Check for interfering components by injecting 10 µl into the liquid chromatograph. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

Brodifacoum calibration solution. Weigh (to the nearest 0.1 mg) about 100 mg brodifacoum (s g) into a 100 ml volumetric flask, dissolve in dichloromethane (40 ml) and make up to volume with methanol. Mix thoroughly. Transfer by pipette 10.0 ml into a 50 ml volumetric flask, add by pipette internal standard solution (10.0 ml) and make up to volume with the diluting solvent. Mix thoroughly. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

2.1.5 *Operating conditions*

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

Eluting solvent flow rate	1 ml min^{-1}
Temperature	ambient
Injection volume	10 µl
Wavelength	254nm
Retention times	Brodifacoum: 6.2 min, internal standard: 11.7 min

2.1.6 Sample preparation

Weigh (to the nearest 0.1 mg) into a 100 ml volumetric flask enough sample (w g) to contain about 100mg of brodifacoum. Dissolve in dichloromethane (40 ml) and dilute to volume with methane. Mix thoroughly. Transfer by pipette 10.0 ml into a 50 ml volumetric flask, add by pipette internal standard solution (10.0 ml) and make up to the mark with diluting solvent. Mix thoroughly and filter through a 0.45 µm filter.

2.1.7 *Equilibration of the system*

Inject two or more 10 µl aliquots of the calibration solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument. Continue with injections until the peak area ratios of the brodifacoum peaks to the internal standard peaks for successive injections agree within 2%.

2.1.8 *Analysis of sample*

Carry out duplicate injections of calibration and sample solutions (in the order calibration, sample, sample and calibration). Average the peak area ratios of the calibration injections (R′) and of the two sample injections (R).

$$(R) = \frac{\text{area brodifacoum peak}}{\text{area internal standard peak}}$$

2.1.9 *Calculation*

$$\text{Brodifacoum content} = \frac{R \times \underline{s} \times P}{R' \times \underline{w}} \text{ g/kg}$$

Where:

R	=	average peak area ratio of brodifacoum and internal standard for the sample.
R′	=	average peak area ratio of brodifacoum and internal standard for the calibration solution
$\underline{w}$	=	mass of sample (g)
$\underline{s}$	=	mass of brodifacoum in the calibration solution (g)
P	=	purity of brodifacoum (g/kg)

BRODIFACOUM
10 & 50 mg/kg BAITS

Interim specification: WHO/IS/7.RO1.1.rev1

1. Specification

1.1 Material

The material shall consist of pellets containing ground whole wheat, palatability agents, pigments and china clay filler, with brodifacoum added as concentrate.

The technical brodifacoum used in the manufacture of the baits shall comply with the requirements of specification WHO/IS/1.RO1.1.rev1.

The brodifacoum concentrate used in the manufacture of the baits shall comply with the requirements of specification WHO/IS/11.RO1.1.rev1.

1.2 Chemical and physical requirements

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

1.2.1 *Brodifacoum content (mg/kg basis)*

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

Nominal content	Tolerance permitted
Up to 25 mg/kg	- 25% to + 30% the nominal content
Above 25 mg/kg	- 20% to + 30% of the nominal content

The average content of all samples taken shall not be lower than the nominal content.

1.2.2 *Water content*

The water content determined by the method WHO/M/7, shall not be greater than 100g/kg.

1.3 Packing and marking of packages

The brodifacoum bait shall be packed in suitable clean containers, as specified in the order, and complying with standard UN specification packaging.

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

Manufacturer's name
Brodifacoum baits to specification WHO/IS/7.RO1.1.rev1
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Brodifacoum is an indirect anticoagulant and is hazardous if swallowed. Avoid skin contact; wear protective gloves when handling the material. Wash hands and exposed skin thoroughly after using.

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

Brodifacoum 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 Brodifacoum content

2.1.1 *Summary of method*

A weighed sample of brodifacoum is dissolved in triphenylbenzene internal standard solution and determined by reverse phase HPLC with UV detection.

2.1.2 *Special apparatus*

Liquid chromatograph, equipped with 10 µl loop injector. UV detector capable of operating at 254 nm and a suitable electronic integrator, or laboratory data system.
Chromatograph column, stainless steel, 250x4.6 mm (i.d.) with Zorbax ODS 5 µm, reverse phase column (DuPont Instruments Inc) or equivalent.
Rotary evaporator
Macerator, with 400 ml stainless steel cup, impeller assembly, e.g. Sorvall Omnimixer
Centrifuge, equipped with 15 ml capped tubes
Analytical Mill
Hartley type funnel (125 mm)
Whatman GF/C fibreglass filters (125 mm)
Whatman 1 - PS phase separating filters (125 mm)

2.1.3 *Special reagents*

1,3,5-Triphenylbenzene internal standard purity greater than 97%.
Acetic acid, glacial, HLPC grade
Dichloromethane, HPLC grade
Water, HLPC grade
Brodifacoum of known purity
Diluting solvent: dichloromethane-methanol (2+3)
Eluting solvent: ethanol-water-acetic acid (94.2 + 5.0 + 0.8)
Mix 942 methanol, 50 ml water and 8 ml acetic acid, filter and degas
Formic acid, purity greater than 90%
Extracting solution, dichloromethane-formic acid. Saturate 1 litre of dichloromethane with 55 ml of formic acid, mixing in a separating funnel. Allow the two phases to separate. Draw off the cloudy lower layer and add 80 ml of dichloromethane. Mix before use.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weigh about 100 mg 1,3,5-triphenylbenzene into a 500 ml volumetric flask, dissolve in dichloromethane (200 ml) and dilute to volume with methanol. Mix thoroughly. Check for interfering components by injecting 10 µl into the liquid chromatograph. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

Brodifacoum calibration solution. For 50mg/kg bait, weigh (to the nearest 0.1 mg) about 100 mg brodifacoum (s g) into a 100 ml volumetric flask, dissolve in dichloromethane (40 ml) and make up to volume with methanol. Mix thoroughly. Transfer 10.0 ml into a 50 ml volumetric flask, add by pipette internal standard solution (10.0 ml) and make up to volume with the diluting solvent. Mix thoroughly. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

For 10mg/kg bait, weigh (to the nearest 0.1 mg) about 100 mg brodifacoum (s g) into a 100 ml volumetric flask, dissolve in dichloromethane (40 ml) and make up to volume with methanol. Mix thoroughly. Transfer 10.0 ml into a 50 ml volumetric flask, add by pipette internal standard solution (10.0 ml) and make up to volume with the diluting solvent. Mix thoroughly. Store in a tightly capped dark glass bottle to avoid evaporation and decomposition.

2.1.5 *Operating conditions*

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

Eluting solvent flow rate	1 ml min^{-1}
Temperature	ambient
Injection volume	10 µl
Wavelength	254 nm
Retention times	Brodifacoum: 6.2 min, internal standard: 11.7 min

2.1.6 *Sample preparation*

Grind sufficient sample (e.g. 50 g) in an analytical sample mill.

For 50mg/kg bait, weigh (to the nearest 0.1g) into a macerator cup enough of this sample (w g) to contain about 2 mg brodifacoum. Add extracting solution (250 ml) and homogenise for 10 minutes. Wash off the macerator head with extracting solution (25 ml), collecting in the macerator cup. Pour the mixture in the cup through a Hartley type filter, fitted with two 125 mm GF/C fibreglass filters and a 125 mm phase separating filter, collecting in a 500 ml rotary evaporator flask. Wash out the cup with extracting solution (50 ml) and pour this through the funnel. Wash the filter with extracting solution (50 ml) by pouring through the funnel. Evaporate the filtrate using the rotary evaporator at 60 ± 2°C under vacuum. Add 48 ml of diluting solvent and internal standard solution (2.0ml), using a pipette. Mix thoroughly. Centrifuge the solution to remove remaining solids.

For 10mg/kg bait, weigh (to the nearest 0.1g) into a macerator cup enough of this sample (w g) to contain about 0.4 mg brodifacoum. Add extracting solution (250 ml) and homogenise for 10 minutes. Wash off the macerator head with extracting solution (25 ml), collecting in the macerator cup. Pour the mixture in the cup through a Hartley type filter, fitted with two 125 mm GF/C fibreglass filters and a 125 mm phase separating filter, collecting in a 500 ml rotary evaporator flask. Wash out the cup with extracting solution (50 ml) and pour this through the funnel. Wash the filter with extracting solution (50 ml) by pouring through the funnel. Evaporate the filtrate using the rotary evaporator at 60 ± 2°C under vacuum. Add 24ml of diluting solvent and internal standard solution (0.40ml) using a 2ml burette. Mix thoroughly. Centrifuge to remove remaining solids.

2.1.7 *Equilibration of the system*

Inject two or more 10 µl aliquots of the calibration solution into the liquid chromatograph to set the integration parameters and to stabilise the instrument. Continue with injections until the peak area ratios of the brodifacoum peaks to the internal standard peaks for successive injections agree within 2%.

2.1.8 *Analysis of sample*

Carry out duplicate injections of calibration and sample solutions (in the order calibration, sample, sample, calibration).

Average the peak area ratios of the calibration solutions (R′) and of the two sample solutions (R).

$$(R) = \frac{\text{area brodifacoum peak}}{\text{area internal standard peak}}$$

2.1.9 *Calculation*

$$\text{Brodifacoum content} = \frac{R \times \underline{s} \times P \times 1000}{R' \times \underline{w} \times K} \text{ mg/kg}$$

Where:

R	=	average peak area ratio of brodifacoum and internal standard for the sample.
R′	=	average peak area ratio of brodifacoum and internal standard for the calibration solution.
w	=	mass of sample (g)
s	=	mass of brodifacoum in the calibration solution (g)
P	=	purity of brodifacoum (g/kg)
K	=	dilution factor (K = 50 for 50mg/kg bait and K = 250 for 10mg/kg bait)