

TECHNICAL BENDIOCARB

Specification WHO/SIT/23.R1
Approved 25 September 1989

1. Specification

1.1 Material

The material shall consist of bendiocarb together with related manufacturing compounds and shall be in the form of a white or off-white solid, practically odourless, and 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 Bendiocarb content (g/kg basis)

The bendiocarb content shall be declared (not less than 960 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 Water content

The water content, determined by the method described in section 2.2, shall not be higher than 5 g/kg.

1.2.3 Melting point

The melting point of the material determined by the method described in WHO/M/5.R1 shall not be lower than 125°C and shall not be depressed on admixture with an equal quantity of pure bendiocarb.

1.3 Packing and marking of packages

The technical bendiocarb 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 bendiocarb to specification WHO/SIT/23.R1
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Bendiocarb is a carbamate compound that inhibits cholinesterase. It is poisonous if swallowed or inhaled. Keep the material out of the reach of children and well away from foodstuffs and animal feed and their containers. If poisoning occurs call a physician. Atropine is a specific antidote and artificial respiration may be needed.

2. Methods of determining chemical and physical properties

2.1 Bendiocarb content

2.1.1 *Outline of method*

The sample is dissolved in acetonitrile with propiophenone added as internal standard. The bendiocarb content is determined by high-performance liquid chromatography (HPLC), using a reverse-phase column and a mixture of acetonitrile and water as mobile phase.

2.1.2 *Special apparatus*

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate a pressure of 17 Mpa and a UV spectrophotometer detector able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 25 cm long and 4.6 mm in internal diameter, packed with $\leq 10 \mu\text{m}$ C-18 bonded silica gel (Partisil 10 ODS-2 or equivalent).

2.1.3 *Special reagents*

Bendiocarb standard. Analytical grade, of known purity.

Internal standard. Propiophenone.

Acetonitrile. HPLC grade.

Water. HPLC grade.

Mobile phase. A volume of 400 ml acetonitrile diluted to 1 l with water. The mixture is degassed by applying reduced pressure until the solvent just boils. Maintain this pressure for 10 minutes.

2.1.4 Preparation of standard solutions

Internal standard solution. Prepare a 1 ml/l solution of propiophenone in acetonitrile.

Bendiocarb calibration solution. Weigh (to the nearest 0.1 mg) about 0.5 g of bendiocarb standard into 100 ml glass-stoppered conical flask. Add by pipette 25.0 ml of internal standard solution and mix to dissolve.

2.1.5 Operating conditions for high-performance liquid chromatography

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

Column temperature	Ambient.
Flow rate	2 ml/min (adjust to give the bendiocarb peak at 3 to 5 minutes).
Wavelength	254 nm.
Injection volume	5 µl.
Retention times:	
bendiocarb	3-5 min.
internal standard	4.5-7.5 min.

2.1.6 Sample preparation and analysis

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 0.5 g of bendiocarb into a 100 ml glass-stoppered conical flask. Add, using the same pipette as that used for the calibration solution, 25.0 ml of internal standard solution and dissolve.

Pump 50 ml of acetonitrile through the column, followed by 50 ml of a mixture of 750 ml/l acetonitrile in water. Change to the mobile phase mixture and pump until the system is equilibrated (flat baseline). Inject 5 µl of the calibration solution and adjust the flow rate to give the bendiocarb peak at 3-5 min (the internal standard peak will follow at 1.5 times the retention time of bendiocarb).

Make repetitive injections of calibration solution until the response is stable and the ratios of bendiocarb peak area (or height) to the internal standard peak area (or height) for successive injections agree to within 1%.

Inject the sample solution. The peak area (or height) ratio for the sample solution must not differ by more than 10% from the peak area (or height) ratio for the calibration solution. If it differs, reweigh samples to match the calibration solution. If not, reinject the sample solution. Peak area (or height) ratios for 2 sample solution injections must agree to within 1%. Average the peak area (or height) ratios for the 2 calibration solution injections immediately preceding and following the sample solution injections. These must agree to within 1%. If not, repeat the analysis.

2.1.7 Calculation

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

$$r = \frac{\text{area (or height) of bendiocarb peak}}{\text{area (or height) of internal standard peak}}$$

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

where

- r_1 = average response ratio for calibration solution.
- r_2 = average response ratio for sample solution.
- m_1 = mass of bendiocarb standard in the calibration solution (g).
- m_2 = mass of sample taken (g).
- P = purity of bendiocarb standard (g/kg).

2.2 Water content

Determine the water content by the Karl Fischer electrometric titration method (see WHO/M/7.R.1) or by the Dean and Stark distillation method (see 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 method.

BENDIOCARB WATER-DISPERSIBLE POWDER

Specification WHO/SIT/41.R1
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical bendiocarb together with filler(s) and other necessary formulants and shall be in the form of a fine, free-flowing powder that wets out readily on stirring into water. The technical bendiocarb used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/23.R1.

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 *Bendiocarb content (g/kg basis)*

The content of bendiocarb shall not differ from the nominal content by more than the following amounts:

<i>Nominal content</i>	<i>Tolerance permitted</i>
Up to 500 g/kg	± 5% of the nominal content
Above 500 g/kg	± 25 g/kg

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

1.2.2 *Sieving after heat stability treatment*

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

1.2.3 *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 bendiocarb (5.0 g/l) shall be in suspension 30 minutes after agitating a suspension containing 10 g/l of bendiocarb

prepared in standard hard water from powder subjected to the heat stability treatment described in section 2.3.

1.2.4 *Heat stability*

The powder after treatment as described in section 2.3 shall comply with the requirement of sections 1.2.1 of this specification.

1.3 **Packing and marking of packages**

The bendiocarb water-dispersible powder with nominal content up to and including 200 g/kg shall be packed in suitable clean drums, kegs or boxes, as specified in the order. The packages shall contain a lining or bag of polyethylene or equivalent, with a nominal thickness of 0.1 mm. The lining or bag shall be hermetically sealed after filling.

The bendiocarb water-dispersible powder with nominal content higher than 200 g/kg shall be prepacked in individual, sealed, laminated foil/plastic sachets in the amount corresponding to one pump-charge.

The design of the sachet should be such that it can be opened and easily emptied without spillage. The sachets shall be packed in suitable clean drums, kegs, or boxes, as specified in the order. The packs shall contain a lining or bag of polyethylene or equivalent, with a nominal thickness of 0.1 mm.

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

Manufacturer's name
Bendiocarb water-dispersible powder to specification WHO/SIF/41.R1
Bendiocarb g/kg
Batch or reference number, and date of test
Net weight of contents
Date of formulation

and the following minimum cautionary notice:

Bendiocarb is a carbamate compound that inhibits cholinesterase. It is poisonous if swallowed or inhaled. Keep the material out of the reach of children and well away from foodstuffs and animal feed and their containers. If poisoning occurs call a physician. Atropine is a specific antidote and artificial respiration may be needed.

2. Methods of determining chemical and physical properties

2.1 Bendiocarb content

2.1.1 *Outline of method*

Bendiocarb is extracted from the sample with acetonitrile to which propiophenone is added as internal standard. The bendiocarb content is determined by high-performance liquid chromatography (HPLC), using a reverse-phase column and a mixture of acetonitrile and water as mobile phase.

2.1.2 *Special apparatus*

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate a pressure of 17 Mpa and a UV spectrophotometer detector able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 25 cm long and 4.6 mm in internal diameter, packed with $\leq 10 \mu\text{m}$ C-18 bonded silica gel (Partisil 10 ODS-2, or equivalent).

2.1.3 *Special reagents*

Bendiocarb standard. Analytical grade, of known purity.

Internal standard. Propiophenone.

Acetonitrile. HPLC grade

Water HPLC grade

Mobile phase. Volume of 400 ml acetonitrile diluted to 1l with water. The mixture is degassed by applying reduced pressure until the solvent just boils. Maintain this pressure for 10 minutes.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Prepare a 1 ml/l solution of propiophenone in acetonitrile.

Bendiocarb calibration solution. Weigh (to the nearest 0.1 mg) about 0.5 g of bendiocarb standard into a 100 ml glass-stoppered conical flask. Add by pipette 25.0 ml of internal standard solution and mix to dissolve.

2.1.5 *Operating conditions for high-performance liquid chromatography*

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

Column temperature	Ambient.
Flow rate	2 ml/min (adjust to give the bendiocarb peak at 3 to 5 minutes).
Wavelength	254 nm.
Injection volume	5 µl.
Retention times:	
bendiocarb	3-5 min.
internal standard	4.5-7.5 min.

2.1.6 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of sample containing about 0.5 g of bendiocarb into a 100 ml glass-stoppered conical flask. Add, using the same pipette as that used for the calibration solution, 25.0 ml of internal standard solution. Swirl to dissolve the bendiocarb. Filter through a suitable filter to remove the insoluble particles and use the filtrate for the HPLC determination.

Pump 50 ml of acetonitrile through the column, followed by 50 ml of a mixture of 75% acetonitrile and 25% water. Change to the mobile phase mixture and pump until the system is equilibrated (flat baseline).

Inject 5 µl of the calibration solution and adjust the flow rate to give the bendiocarb peak at 3-5 min (the internal standard peak) will follow at 1.5 times the retention time of bendiocarb).

Make repetitive injections of calibration solution until the response is stable and the ratios of bendiocarb peak area (or height) to the internal standard peak area (or height) for successive injections agree to within 1%.

Inject the sample solution. The peak area (or height) ratio for the sample solution must not differ by more than 10% from the peak area (or height) ratio for the calibration solution. If it differs, reweigh samples to match the calibration solution.

If not, reinject the sample solution. Peak area (or height) ratios for 2 sample solution injections must agree to within 1%. Average the peak area (or height) ratios for the 2 calibration solution injections immediately preceding and following the sample solution injections. These must agree to within 1%. If not repeat the analysis.

2.1.7 Calculation

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

$$r = \frac{\text{area (or height) of bendiocarb peak}}{\text{area (or height) of internal standard peak}}$$

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

where

r_1	=	average response ratio for calibration solution.
r_2	=	average response ratio for sample solution.
m_1	=	mass of bendiocarb standard in the calibration solution (g).
m_2	=	mass of sample taken (g).
P	=	purity of bendiocarb standard (g/kg).

2.2 Suspensibility after heat stability treatment

2.2.1 Outline of method

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

2.2.2 Special apparatus

1. *A 250 ml graduated cylinder.* With a ground-glass stopper and a distance of 20-21.5 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 end to an opening of 2-3 mm, the other end being connected to a suitable source of suction.
3. *Suitable HPLC apparatus* as described in section 2.1.2.

2.2.3 *Special reagents*

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 1 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.

Reagents. Same as those used in the determination of bendiocarb content (section 2.1.3), plus *dichloromethane*, reagent grade.

2.2.4 *Procedure*

Weigh sufficient sample to contain 2.5 g of active ingredient into a 100 ml beaker. Add 50 ml of water¹ at $30 \pm 1^\circ\text{C}$. Stir the mixture with a glass rod by hand for 30 seconds, making no deliberate attempt to break up any lumps. Then immediately transfer the sample quantitatively to the 250 ml cylinder using additional water for the transfer. Add sufficient water at $30 \pm 1^\circ\text{C}$ to make 250 ml of suspension. Stopper the cylinder and mix by inverting and righting it 30 times at a rate of approximately one complete cycle every two seconds. This operation should be carried out as smoothly as possible, keeping the axis of rotation fixed. The cylinder must be thermally insulated from the hands to maintain the prescribed temperature of the suspension. Immerse the cylinder up to the 250 ml mark in a water bath maintained at $30 \pm 1^\circ\text{C}$. Allow the graduated cylinder to stand for 30 minutes in the water-bath, 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 during 10-15 seconds by means of the suction tube nine-tenths of the suspension, i.e., 225 ml. This is achieved by maintaining the tip of the glass tube just below the sinking surface of the suspension. Discard the suspension withdrawn.

Pipette 200 ml of dichloromethane into the remaining 25 ml of suspension, stopper, and shake vigorously for 2 minutes. Transfer the suspension to a separating funnel and allow to separate so that at least 75 ml of solvent can be withdrawn. Filter the solvent through anhydrous sodium sulphate (2-3 g). Discard the first 3-5 ml of the filtrate and collect the remainder in a dry flask.

¹ Whenever water is mentioned in this section, use standard hard water.

Pipette 50 ml into another flask and evaporate almost to dryness. Add 10 ml of the internal standard solution (section 2.1.4) to the flask containing the dried residue and dissolve. Hold this sample solution for HPLC analysis and continue as described in section 2.1.

2.2.5 Calculation

Mass (m_1) in g of bendiocarb² in the retained one-tenth of the suspension:

$$m_1 = \frac{r_1 \times m_o \times 1.6}{r_o}$$

where r_o = average response ratio for calibration solution
 r_1 = average response ratio for sample solution
 m_o = mass (g) of bendiocarb standard in the calibration solution

From the value obtained in section 2.1.7 for the content of bendiocarb, calculate the weight of bendiocarb (m_2) in the initial sample taken for the suspensibility test.

$$\text{Suspensibility (\%)} = \frac{(m_2 - m_1) \times 111.1}{m_2}$$

m_1 = mass (g) of bendiocarb found in the retained one-tenth of the suspension
 m_2 = mass (g) of bendiocarb in the initial sample

2.3 Heat stability treatment

Fill a 50 ml³ 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 a forced-draught oven maintained at $54 \pm 2^\circ\text{C}$ 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 completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

² If the volume of the internal standard solution added for dissolving the residue is not 10 ml but V ml, the following equation must be applied:

$$m_1 = \frac{r_1 \times m_o \times 0.16V}{r_o}$$

³ If a larger quantity of the sample is required for the tests, use a 100 ml bottle.

BENDIOCARB DUSTABLE POWDER

Specification WHO/SIF/54
Approved September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical bendiocarb together with carriers and any other necessary formulants. It shall be a fine, free-flowing powder, free from hard lumps. The technical bendiocarb used in the manufacture of the powder shall comply with the requirements of specification WHO/SIT/23.R1

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 *Bendiocarb content (g/kg basis)*

The content of bendiocarb, determined by the method described in section 2.1 shall not differ from the nominal content by more than -10% to +35%. The average content of all the samples taken shall not be lower than the nominal content.

1.2.2 *pH value*

The pH value shall be in the range 6.8 - 7.2 when determined by the method described in WHO/M/25.

1.2.3 *Sieving after heat stability treatment*

Not less than 98% of the powder after heat stability treatment, as described in section 2.2, shall pass through a 150 µm sieve when tested by method described in WHO/M/4.R1.

1.2.4 *Dustability after heat stability treatment*

After heat stability treatment, as described in section 2.2, the powder shall issue freely without clogging or bridging, when tested in a hand-dusting apparatus conforming to specification WHO/EQP/4.R2¹

¹ Equipment for vector control, 3rd ed. Geneva. World Health Organisation, 1990, p.128.

1.2.5 *Heat stability*

The powder after treatment as described in section 2.2 shall comply with the requirement of sections 1.2.1 and 1.2.2 of this specification.

1.3. **Packing and marking of packages**

The bendiocarb dustable powder shall be packed in suitable clean and airtight drums, as specified in the order.

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

Manufacturer's name
Bendiocarb water-dispersible powder to specification WHO/SIF/54
Bendiocarb g/kg
Batch or reference number, and date of test
Net weight of contents
Date of formulation

and the following minimum cautionary notice:

Bendiocarb is a carbamate compound that inhibits cholinesterase. It is poisonous if swallowed or inhaled. Keep the material out of the reach of children and well away from foodstuffs and animal feed and their containers. If poisoning occurs call a physician. Atropine is a specific antidote and artificial respiration may be needed.

2. **Methods of determining chemical and physical properties**

2.1 **Bendiocarb content**

2.1.1 *Outline of method*

Bendiocarb is extracted from the sample with acetonitrile to which propiophenone is added as internal standard. The bendiocarb content is determined by high-performance liquid chromatography (HPLC), using a reverse-phase column and a mixture of acetonitrile and water as mobile phase.

2.1.2 *Special apparatus*

- 1 *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate a pressure of 17 MPa and a UV spectrophotometer detector able to measure UV absorbance at 254 nm.

2. *Liquid chromatographic column.* the column should be a stainless steel tube 25 cm long and 4.6 mm in internal diameter, packed with $\leq 10 \mu\text{m}$ C-18 bonded silica gel (Partisil 10 ODS-2, or equivalent).

2.1.3 *Special reagents*

Bendiocarb standard. Analytical grade, of known purity.

Internal standard. Propiophenone.

Acetonitrile. HPLC grade

Water. HPLC grade

Mobile phase. A volume of 400 ml acetonitrile diluted to 1 l with water. The mixture is degassed by applying reduced pressure until the solvent just boils. Maintain this pressure for 10 minutes.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Prepare a 1 ml/l solution of propiophenone in acetonitrile.

Bendiocarb calibration solution. Weigh (to the nearest 0.1 mg) about 0.5 g of bendiocarb standard into a 100 ml glass-stoppered conical flask. Add by pipette 25.0 ml of internal standard solution and mix to dissolve.

2.1.5 *Operating conditions for high-performance liquid chromatography*

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

Column temperature	Ambient.
Flow rate	2 ml/min (adjust to give the bendiocarb peak at 3 to 5 minutes).
Wavelength	254 nm.
Injection volume	5 ml.
Retention times:	
Bendiocarb	3-5 min.
Internal standard	4.5-7.5 min.

2.1.6 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of sample containing about 0.5 g of bendiocarb into a 250 ml beaker. Add 100 ml of dichloromethane and a few anti-bumping granules. Warm, with precaution, on a steam bath stirring continuously for 2 minutes. Cool and filter the slurry under vacuum through a suitable filter, rinsing the beaker with 20 ml dichloromethane then washing the filter cake with 2 x 30 ml portions of dichloromethane.

Add a few anti-bumping granules to the filtrate and evaporate just to dryness on a steam bath. Cool, then add, using the same pipette as that used for the calibration solution 25.0 ml of internal standard solution. Swirl to dissolve the bendiocarb. Use this solution for the HPLC determination.

Pump 50 ml of acetonitrile through the column followed by 50 ml of a mixture of 75% acetonitrile and 25% water. Change to the mobile phase mixture and pump until the system is equilibrated (flat baseline).

Inject 5 µl of the calibration solution and adjust the flow rate to give the bendiocarb peak at 3-5 min (the internal standard peak will follow at 1.5 times the retention time of bendiocarb).

Make repetitive injections of calibration solution until the response is stable and the ratios of bendiocarb peak area (or height) to the internal standard peak area (or height) for successive injections agree to within 1%.

Inject the sample solution. The peak area (or height) ratio for the sample solution must not differ by more than 10% from the peak area (or height) ratio for the calibration solution. If it differs, reweigh samples to match the calibration solution. If not, reinject the sample solution. Peak area (or height) ratios for 2 sample solution injections must agree to within 1%. Average the peak area (or height) ratios for the 2 calibration solution injections immediately preceding and following the sample solution injections. These must agree to within 1%. If not repeat the analysis.

2.1.7 Calculation

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

$$r = \frac{\text{area (or height) of bendiocarb peak}}{\text{area (or height) of internal standard peak}}$$

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

where

r_1	=	average response ratio for calibration solution.
r_2	=	average response ratio for sample solution.
m_1	=	mass of bendiocarb standard in the calibration solution (g).
m_2	=	mass of sample taken (g).
P	=	purity of bendiocarb standard (g/kg).

2.2 Heat stability treatment

Fill a 100 ml wide-mouthed bottle fitted with a vinyl-plastic-lined screw-cap to within 1 cm from the top with the sample. Moreover for the dustability test, section 1.2.4, place 250 g of the sample in a 1 litre wide-mouthed bottle fitted with a vinyl-plastic-lined screw-cap. Place the bottles in an oven maintained at $54 \pm 2^{\circ}\text{C}$ for 3 days. Take the samples from the oven and allow them to cool to room temperature before removing the caps.

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

BENDIOCARB ULTRA-LOW VOLUME LIQUID

Specification WHO/SIF/58
Approved September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of bendiocarb dissolved in suitable solvents with any other necessary formulants. It shall be in the form of a stable liquid free from suspended matter and sediment. The technical bendiocarb used in the manufacture of the ULV liquid shall comply with the requirements of specification WHO/SIT/23.R1.

1.2 Chemical and physical requirements

The material sampled from any part of the consignment¹ (see method WHO/M/1), shall comply with the requirements of section 1.1 and with the following requirements.

1.2.1 *Bendiocarb content (g/kg basis)*

The content of bendiocarb, 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>
Up to 200 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 *Cold test*

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

1.2.3 *Water content*

The water content of the product, determined by the method described in WHO/M/7.R1 shall not be higher than 10 g/kg.

¹ A sampling procedure is described in method WHO/M/1². However this does not preclude the purchases from sampling in any considered desirable.

² Specification for Pesticides used in Public Health, Geneva 1985, (6th Edition).

1.2.4 *pH range*

The pH of an aqueous dispersion of the product shall be in the range of 3.0 to 3.5, when tested by the method described in WHO/M/25.

1.2.5 *Flash point*

The flash point determined by the method WHO/M/10.R1 shall not be lower than 22.8⁰C and shall comply with all national and international regulations on handling and transport of flammable materials.

1.2.6 *Kinematic viscosity*

The kinematic viscosity of the product determined by the method WHO/M/22 but at 22⁰C, shall not be greater than 36 mm².s⁻¹.

1.2.7 *Volatility*

The volatility (evaporation rate) of the product determined by the method described in WHO/M/24¹ shall not exceed 130 g/kg.

1.2.8 *Heat stability*

The material after treatment as described in section 2.2 shall comply with the requirements of sections 1.2.1, 1.2.4 and 1.2.6.

1.3 **Packing and marking of packages**

The bendiocarb ultra-low volume liquid 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
Bendiocarb ultra-low volume liquid to specification WHO/SIF/
Bendiocarb, ... g/kg
Batch or reference number, and date of test
Net weight of contents
Date of manufacture
Type of equipment to be used for application if required

and the following minimum cautionary notice

Bendiocarb is a cholinesterase inhibitor; do not use if under medical advice not to work with such compounds. Product is poisonous if swallowed or inhaled. Avoid skin, mouth and eye contact; do not breathe spray mist. Toxic to fish - do not contaminate waterways with product or container. Store in a safe place out of the reach of children and away from foodstuffs and animal feed/containers. If poisoning occurs, call a physician immediately; atropine sulphate is the recommended antidote.

2. Methods of determining chemical and physical properties

2.1 Bendiocarb content

2.1.1 Summary of method

The bendiocarb content is determined by high performance liquid chromatography (HPLC) using a reverse-phase column and a mixture of acetonitrile and water as mobile phase with propiophenone as internal standard.

2.1.2 Special apparatus

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate a pressure of 17 x MPa and a UV spectrophotometer detector able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 25 cm long and 4.6 mm in internal diameter, packed with $\leq 10 \mu\text{m}$ C-18 bonded silica gel (Partisil 10 ODS-2 or equivalent).

2.1.3 Special reagents

Bendiocarb standard. Analytical grade of known purity.

Internal standard. Propiophenone.

Acetonitrile. HPLC grade.

Water. HPLC grade.

Mobile phase. A volume of 400 ml acetonitrile diluted to 1 litre with water. The mixture is degassed by applying reduced pressure until the solvent just boils. Maintain this pressure for 10 min.

2.1.4 Preparation of standard solutions

Internal standard solution. Prepare a 1 ml/l solution of propiophenone in acetonitrile.

Bendiocarb calibration solution. Weigh (to the nearest 0.1 mg) about 0.5 g of bendiocarb standard into a 100 ml glass-stoppered conical flask. Add by pipette 25.0 ml of internal standard solution and mix to dissolve.

2.1.5 Operating conditions for high performance liquid chromatography

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

Column temperature	Ambient.
Flow rate	2 ml/min (adjust to give the bendiocarb peak at 3 to 5 minutes).
Wavelength	254 nm.
Injection volume	5 µl.
Retention times	
bendiocarb	3-5 min.
internal standard	4.5 - 7.5 min.

2.1.6 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of sample containing about 0.5 g of bendiocarb into a 100 ml glass-stoppered conical flask. Then add, using the same pipette as that used for the calibration solution, 25.0 ml of internal standard solution. Swirl to mix the solution. Use this solution for the HPLC determination.

Pump 50 ml of acetonitrile through the column, followed by 50 ml of a mixture of 750 ml/l acetonitrile in water. Change to the mobile phase mixture and pump until the system is equilibrated (flat baseline). Inject 5 µl of the calibration solution and adjust the flow rate to give the bendiocarb peak at 3-5 minutes (the internal standard peak will follow at 1.5 times the retention time of bendiocarb).

Make repetitive injections of calibration solution until the response is stable and the ratios of bendiocarb peak area (or height) to the internal standard peak area (or height) for successive injections agree to within 1%. Inject the sample solution. The peak area (or height) ratio for the sample solution must not differ by more than 10% from the peak area (or height) ratio for the calibration solution. If it differs, reweigh samples to match the calibration solution. If not, re-inject the sample solution. Peak area (or height) ratios for 2 sample solution injections must agree to within 1%.

Average the peak area (or height) ratios for the two calibration solution injections immediately preceding and following the sample solution injections. These must agree to within 1%. If not, repeat the analysis.

2.1.7 Calculation

For each injection the response ratio (r) is given by the equation

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

$$r = \frac{\text{area (or height) of bendiocarb peak}}{\text{area (or height) of internal standard peak}}$$

where

r_1	=	average response ratio for calibration solution
r_2	=	average response ratio for sample solution
m_1	=	mass of bendiocarb standard in the calibration solution (g)
m_2	=	mass of sample taken (g)
P	=	purity of bendiocarb standard (g/kg)

2.2 Heat stability

Keep 50 ml of the sample for 6 days at a temperature of $50 \pm 1^{\circ}\text{C}$ in a glass container sealed to avoid loss of volatile solvent, and then cool to room temperature.