

# TECHNICAL MALATHION

Specification WHO/SIT/10.R6  
Approved 25 September 1989

## 1. Specification

### 1.1 Material

The material shall consist of malathion together with related manufacturing compounds and shall be in the form of a clear, colourless to light amber liquid free from extraneous impurities or added modifying agents.

### 1.2 Chemical and physical requirements

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

#### 1.2.1 *Malathion content (g/kg basis)*

The malathion content shall be declared (not less than 940 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  $\pm 20$  g/kg.

#### 1.2.2 *Acidity*

The acidity of the material, determined by the method described in WHO/M/3, shall not be higher than 5 g/kg, calculated as H<sub>2</sub>SO<sub>4</sub>.

#### 1.2.3 *Material insoluble in acetone*

The material, insoluble in acetone determined by the method described in WHO/M/21.R1, shall not be higher than 5 g/kg.

#### 1.2.4 *Water content*

The water content, determined by the method described in section 2.2, shall not be higher than 1 g/kg.

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<sup>1</sup> If the material is for use inside buildings, the requirement that it should be odourless should also be specified.

### 1.3 Packing and marking of packages

The technical malathion 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 malathion to specification WHO/SIT/10.R6  
Batch or reference number, and date of test  
Net weight of contents  
Date of manufacture

and the following minimum cautionary notice:

Malathion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. 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 and pralidoxime are specific antidotes and artificial respiration may be needed. Store in a cool place and use as soon as possible after manufacture to avoid decomposition, which may occur under conditions of prolonged tropical storage.

## 2. Methods of determining chemical and physical properties

### 2.1 Malathion content

#### 2.1.1 *Outline of method*

The sample is dissolved in chloroform to which an internal standard is added. An aliquot is introduced into a gas-liquid chromatograph and the ratio of the response of the malathion to that of the internal standard is determined. This is compared with the response of a standard of known purity to give the malathion content in the sample.

#### 2.1.2 *Special apparatus*

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame-ionization detector, an electrometer having a sensitivity of at least  $10^{-11}$  amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital

integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample

injection system also contributes significantly to the accuracy of the assay.

2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210.
4. *Glass wool, silane-treated.*

### 2.1.3 *Special reagents*

*Malathion standard.* Analytical grade, of known purity.

*Internal standard.* 1,3-Diphenoxybenzene.

### 2.1.4 *Preparation of standard solutions*

*Internal standard solution.* Prepare a 30 g/l solution of the internal standard in chloroform. This solution is stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

*Malathion calibration solutions.* Weigh (to the nearest 0.1 mg) about 425, 500, and 575 mg quantities of the malathion standard, directly into separate, weighed 50 ml volumetric flasks. Add by pipette exactly 5 ml of the internal standard solution to each flask. Dilute to the mark with chloroform. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.1.7) and to guard against weighing error in the preparation of the working calibration solution.

These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C provided the linearity specifications described in section 2.1.7 can be met.

### 2.1.5 *Preparation and conditioning of column*

See method WHO/M/20.

### 2.1.6 *Operating conditions for gas-liquid chromatography*

The temperatures, gas flow rates, and retention times given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

*Temperatures*

Oven	180°C
Injection port	190°C
Flame-ionization detector	250°C

*Gas flow rates*

Hydrogen	30 ml/min.
Air	350 ml/min.
Carrier gas (helium or nitrogen)	30 ml/min.

*Retention times*

Malathion peak	10 min.
Internal standard peak	7 min.

*2.1.7 Linearity check*

The gas-liquid chromatograph should be checked for linearity at least once a week, and the same check should be carried out whenever new calibration solutions are prepared and whenever a column, new or used, is installed in the instrument.

Using digital integration for peak area measurements, determine the appropriate attenuation setting and the quantity (between 2 and 4 ml) of calibration solution B that must be injected to yield an area count of at least 100 000 (optimum electrometer output with acceptable noise level). The attenuation setting quantity so determined should be used for all samples and calibration solutions in the set.

Inject triplicate aliquots of appropriate volume (as determined above) of calibration solutions A, B, and C into the gas-liquid chromatograph, determine the response ratio for each injection, and average the resulting ratios for each solution. Divide the average response ratio for each solution by the corresponding malathion content (in mg) and compare the resulting response factors. These factors should agree to within 2%. Failure to meet this requirement indicates either a weighing error in the preparation of one of the calibration solutions or instrumental difficulties, which must be corrected before proceeding with the analysis of samples.

*2.1.8 Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 500 mg of malathion directly into a weighed 50 ml volumetric flask. Add by pipette exactly 5 ml of the internal standard solution to the volumetric flask, dilute to the mark with chloroform, and mix well. Inject duplicate aliquots of appropriate volume (as determined in section 2.1.7) of calibration solution B. The response ratios should agree to within 2%. If this accuracy limit is not met, inject two more aliquots of the solution. Failure to meet the accuracy requirement with the second pair of injections indicates instrumental difficulties, which must be resolved before proceeding with the analyses.

Inject duplicate aliquots of the sample solution, using the same volume as that used in the preceding step. The accuracy considerations discussed in the preceding step apply here also. Average the response ratios for each sample solution.

In a series of analyses, after every two sample solution injections, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solution injections.

Use this average to calculate the malathion content of the sample.

### 2.1.9 Calculation

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

$$r = \frac{\text{area of malathion peak}}{\text{area of internal standard peak}}$$

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

where  $r_1$  = average response ratio for calibration solution B  
 $r_2$  = average response ration for sample solution  
 $m_1$  = mass of malathion standard in the calibration solution B (mg)  
 $m_2$  = mass of sample taken (mg)  
 $P$  = purity of malathion standard (g/kg)

## 2.2 Water content

Determine the water content by the Karl Fischer electrometric titration method (see WHO/M/7.R1) 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.

# MALATHION WATER-DISPERSIBLE POWDER

Specification WHO/SIF/10.R6  
Approved 25 September 1989

## 1. Specification

### 1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical malathion together with filler(s) and other necessary formulants and shall be in the form of a fine free-flowing, yellow to light-brown powder that wets out readily on stirring into water. The technical malathion used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/10.R6.

### 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.<sup>1</sup>

#### 1.2.1 Malathion content (g/kg basis)

The content of malathion 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 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 Isomalathion content (g/kg) after heat stability treatment at 54°C

The content of isomalathion determined by the method described in section 2.2 on a sample subjected to the heat stability treatment at 54°C described in section 2.5.1 shall not be higher than 1.8% of the nominal malathion content.

#### 1.2.3 Acidity

The acidity of the powder, determined by the method described in WHO/M/3, shall not be higher than 5 g/kg calculated as H<sub>2</sub>SO<sub>4</sub>.

<sup>1</sup> If the material is for use inside buildings, the requirement that it should be odourless should also be specified.

1.2.4 *Sieving after heat stability treatment at 90<sup>0</sup>C*

Not less than 98% of the powder after 90<sup>0</sup>C heat stability treatment (section 2.5.2) shall pass through a 75 mm sieve when tested by the method described in section 2.4.

1.2.5 *Suspensibility*

*In standard soft water without pretreatment.* When tested by the method described in section 2.3, a minimum of 65% of the malathion (16.25 g/l) shall be in suspension 30 minutes after agitating a suspension containing 25 g/l of malathion, prepared in standard soft water from the powder as received.

*In standard hard water after 90<sup>0</sup>C heat stability treatment.* When tested by the method described in section 2.3, a minimum of 50% of the malathion (12.5 g/l) shall be in suspension 30 minutes after agitating a suspension containing 25 g/l of malathion, prepared in standard hard water from powder subjected to the 90<sup>0</sup>C heat stability treatment described in section 2.5.2.

1.2.6 *Heat stability*

The powder after treatment as described in section 2.5.1 shall comply with the requirements of sections 1.2.1 and 1.2.3 of this specification.

**1.3 Packing and marking of packages**

The malathion water-dispersible powder shall be packed in clean drums or boxes,<sup>2 2</sup> as specified in the order. The containers shall contain an inner liner or bag of polyethylene or equivalent with a nominal thickness of 0.1 mm. The lining or bag shall be hermetically sealed after filling. All packages shall bear, durably and legibly marked on the container, the following:

Manufacturer's name  
Malathion water-dispersible powder to specification WHO/SIF/10.R6  
Malathion ... g/kg  
Batch or reference number, and date of test  
Net weight of contents  
Date of manufacture

and the following minimum cautionary notice:

Malathion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. Keep the material out of the reach of children and well away from foodstuffs and animal feed and their containers.

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<sup>2</sup> Specifications for boxes of adequate strength to withstand overseas shipment may be obtained, on request, from the Division of Control of Tropical Diseases, Schistosomiasis Control Unit, World Health Organization, 1211 Geneva 27, Switzerland

If poisoning occurs, call a physician. Atropine and pralidoxime are specific antidotes and artificial respiration may be needed.

Store in a cool place and use as soon as possible after manufacture to avoid decomposition, which may occur under conditions of prolonged tropical storage.

## 2. Methods of determining chemical and physical properties

### 2.1 Malathion content

#### 2.1.1 *Outline of method*

Malathion is extracted from the sample with chloroform, an internal standard is added, an aliquot is introduced into a gas-liquid chromatograph, and the ratio of the response of the malathion to that of the internal standard is determined. This is compared with the response of a standard of known purity to give the malathion content in the sample.

#### 2.1.2 *Special apparatus*

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame ionization detector, an electrometer having a sensitivity of at least  $10^{-11}$  amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.
2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210.
4. *Glass wool, silane-treated.*

### 2.1.3 *Special reagents*

*Malathion standard.* Analytical grade, of known purity.

*Internal standard.* 1,3-Diphenoxybenzene.

### 2.1.4 *Preparation of standard solutions*

*Internal standard solution.* Prepare a 30 g/l solution of the internal standard in chloroform. This solution is stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

*Malathion calibration solutions.* Weigh (to the nearest 0.1 mg) about 425, 500, and 575 mg quantities of the malathion standard, directly into separate, weighed 50 ml volumetric flasks. Add by pipette exactly 5 ml of the internal standard solution to each flask. Dilute to the mark with chloroform. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.1.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C provided the linearity specifications described in section 2.1.7 can be met.

### 2.1.5 *Preparation and conditioning of column*

See method WHO/M/20.

### 2.1.6 *Operating conditions for gas liquid chromatography*

The temperatures, gas flow rates, and retention times given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

#### *Temperatures*

Oven	180 <sup>0</sup> C
Injection port	190 <sup>0</sup> C
Flame-ionization detector	250 <sup>0</sup> C

#### *Gas flow rates*

Hydrogen	30 ml/min.
Air	350 ml/min.
Carrier gas (helium or nitrogen)	30 ml/min.

#### *Retention times*

Malathion peak	10 min.
Internal standard peak	7 min.

### 2.1.7 *Linearity check*

The gas-liquid chromatograph should be checked for linearity at least once a week, and the same check should be carried out whenever new calibration solutions are prepared and whenever a column, new or used, is installed in the instrument.

Using digital integration for peak area measurements, determine the appropriate attenuation setting and the quantity (between 2 and 4 ml) of calibration solution B that must be injected to yield an area count of at least 100 000 (optimum electrometer output with acceptable noise level). The attenuation setting quantity so determined should be used for all samples and calibration solutions in the set.

Inject triplicate aliquots of appropriate volume (as determined above) of calibration solutions A, B, and C into the gas-liquid chromatograph, determine the response ratio for each injection, and average the resulting ratios for each solution. Divide the average response ratio for each solution by the corresponding malathion content (in mg) and compare the resulting response factors. These factors should agree to within 2%. Failure to meet this requirement indicates either a weighing error in the preparation of one of the calibration solutions or instrumental difficulties, which must be corrected before proceeding with the analysis of samples.

### 2.1.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 500 mg of malathion, transfer it to a 200 ml screw-capped bottle, and add from a pipette, 5 ml of the internal standard solution. Add 50 ml of chloroform and shake for approximately 30 seconds. Filter a few ml of the supernatant solution and hold for gas-liquid chromatography analysis. The filtration step is best accomplished by drawing 4-5 ml of the supernatant solution into a 10-ml Varipet syringe,<sup>3</sup> fitting the syringe with a 13 mm Swinnex filter holder (Millipore SX00'01300) with a glass-fibre filter (Gelman Type A-E, 13 mm, or equivalent) and forcing the solution through the filter into a small screw-capped vial.

Inject duplicate aliquots of appropriate volume (as determined in section 2.1.7) of calibration solution B. The response ratios should agree to within 2%. If this accuracy limit is not met, inject two more aliquots of the solution. Failure to meet the accuracy requirement with the second pair of injections indicates instrumental difficulties, which must be resolved before proceeding with the analyses. Inject duplicate aliquots of the sample solution, using the same volume as that used in the preceding step. The accuracy considerations discussed in the preceding step apply here also. Average the response ratios for each sample solution.

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<sup>3</sup> Available from Manostat Co., 519 8th Avenue, New York, NY 10018, USA

In a series of analyses, after every two sample solution injections, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solution injections.

Use this average to calculate the malathion content of the sample.

### 2.1.9 Calculation

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

$$r = \frac{\text{area of malathion peak}}{\text{area of internal standard peak}}$$

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

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

## 2.2 Determination of isomalathion content

### 2.2.1 Outline of method

Chloroform is added to a sample of malathion water-dispersible powder to dissolve the active ingredient as well as any isomalathion present. An internal standard is added to the solution and an aliquot is injected into a gas-liquid chromatograph. The ratio of the response of the isomalathion to that of the internal standard is determined. This is compared with the response of a standard containing a known quantity of isomalathion to give the percentage of isomalathion in the sample.

### 2.2.2 Special apparatus

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame-ionization detector, an electrometer having a sensitivity of at least  $10^{-11}$  amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1mV.

It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.

2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210.
4. *Glass wool, silane-treated.*

### 2.2.3 *Special reagents*

*Isomalathion standard.* A solution of a known quantity of isomalathion (approximately 20 mg/g) dissolved in purified malathion with tetraethyl thiodisuccinate added.

*Internal standard.* 1,3-Diphenoxybenzene.

### 2.2.4 *Preparation of standard solutions*

*Internal standard solution.* Prepare a 4 g/l solution of the internal standard in reagent-grade chloroform. This solution is stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

*Isomalathion calibration solution.* Weigh (to the nearest 0.1 mg) about 1.5 g of the isomalathion standard in a 25 ml volumetric flask. Add 2.0 ml of internal standard solution to the flask and make up to volume with chloroform. Multiply the weight (in g) of the isomalathion standard by the concentration of isomalathion in the standard (in mg/g) to determine the weight (in mg) of isomalathion in the calibration solution.

### 2.2.5 *Preparation and conditioning of column*

See method WHO/M/20.

### 2.2.6 *Operating conditions for gas-liquid chromatography*

The temperatures, gas flow rates, and retention times given below are typical values and may have to be adjusted to obtain optimum results from a specific apparatus.

*Temperatures*

Oven	180 <sup>0</sup> C <sup>4</sup>
Injection port	190 <sup>0</sup> C
Flame-ionization detector	250 <sup>0</sup> C

*Gas flow rates*

Hydrogen	30 ml/min.
Air	350 ml/min.
Carrier gas (helium or nitrogen)	30 ml/min.

*Retention times*

Isomalathion peak	26 min.
Internal standard peak	7 min.

2.2.7 *Resolution check*

The efficiency of the gas chromatographic column must be sufficient to effect partial resolution of the diastereoisomers of tetraethyl thiodisuccinate. The resolution may be considered satisfactory when the distance measured from the top of the tetraethyl thiodisuccinate peaks to the valley between the peaks is at least 10% of the height of the peaks. This measurement should be made on a chromatogram of the isomalathion calibration solution.

2.2.8 *Preparation and analysis of sample*

Weigh (to the nearest 0.1 mg) a quantity of the sample equivalent to about 1.5 g of malathion in a 30 ml screw-capped bottle. Add 2.0 ml of the internal standard solution and about 25 ml of chloroform. Shake to dissolve the malathion, allow the phases to separate, filter a portion of the supernatant solution, and hold it for gas chromatographic analysis.

Inject duplicate aliquots of 3 ml of the isomalathion calibration solution. Calculate the response ratios by dividing the area of the isomalathion peak by the area of the internal standard peak. Response ratios should agree to within 2%. If the accuracy limit is not met, inject two more aliquots of the solution. Failure to meet the accuracy requirement with the second pair of injections indicates that the apparatus is not functioning properly, and this problem must be resolved before proceeding with the analyses. Average the duplicate response ratios obtained with the isomalathion calibration solution.

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<sup>4</sup> When a sample is run, the temperature of the oven must be programmed to rise to 240<sup>0</sup>C following the emergence of tetraethyl thiodisuccinate in order to remove another minor component such as tetraethyl dithiodisuccinate. After several minutes at 240<sup>0</sup>C, the oven may be returned to 180<sup>0</sup>C to begin the analysis of the next sample.

Inject duplicate aliquots (3 ml each) of the sample solution. Calculate the response ratios, which, as for the calibration solution, should agree to within 2%. Average the duplicate response ratios obtained with the sample solution.

### 2.2.9 Calculation

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

$$\text{Isomalathion content (g / kg of sample)} = \frac{r_2 \times m_1}{r_1 \times m_2}$$

where  $r_1$  = average response ratio for calibration isomalathion solution  
 $r_2$  = average response ratio for sample solution  
 $m_1$  = mass (mg) of isomalathion in the calibration solution  
 $m_2$  = mass (g) of sample taken

## 2.3 Suspending ability

### 2.3.1 Outline of method

A suspension of known concentration of malathion in standard waters is prepared, poured into a 100 ml graduated cylinder, maintained at a constant temperature and allowed to remain undisturbed for 30 minutes. A 25 ml aliquot is drawn off at mid-height of the suspension and its malathion content is determined, so allowing to evaluate the active ingredient mass still in suspension after 30 minutes.

### 2.3.2 Special apparatus

1. A 100 ml glass-stoppered graduated cylinder having the 100 ml mark situated  $18.0 \text{ cm} \pm 1.5 \text{ cm}$  from the bottom.
2. A 25 ml pipette fitted with a device (e.g. a rubber stopper with a vent in the side) to permit its insertion into the 100 ml cylinder so that it is held with the tip exactly at the 50 ml mark.
3. A constant-temperature water-bath into which the 100 ml graduated cylinder can be immersed to the 100 ml mark and that can be maintained at  $30 \pm 1^\circ\text{C}$ . The bath must be free from any vibration caused by stirring motors or other equipment.

### 2.3.3 *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.

*Standard soft water.* Take 100 ml of standard hard water and add 900 ml of distilled water. This provides water with a hardness of 34.2 mg/l, calculated as calcium carbonate.

### 2.3.4 *Procedure*

Weigh (to the nearest 1 mg) into a 100 ml beaker an amount of the sample to form 100 ml of a suspension containing 25 g/l of malathion. Add 50 ml of water<sup>5</sup> 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, and then immediately transfer the sample quantitatively to the

100 ml cylinder using additional water for the transfer. Add sufficient water at  $30 \pm 1^{\circ}\text{C}$  to make 100 ml of suspension. Stopper the cylinder and mix by inverting and righting it 30 times at the rate of approximately one cycle every 2 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 100 ml mark in the water-bath maintained at  $30 \pm 1^{\circ}\text{C}$ . The preparation of the suspension, from the first addition of water to the placing of the cylinder in the constant-temperature bath, should be a continuous operation and should be completed within 3 minutes. Allow the cylinder to stand for 30 minutes in the water-bath at  $30 \pm 1^{\circ}\text{C}$ . During this period care should be taken that the bath and cylinder are free from vibrations.

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

At the end of the 30 minutes settling period, remove the cylinder from the water-bath, insert the specially fitted pipette so that the tip is exactly at the 50 ml mark and remove a 25 ml aliquot. (If this test is being performed on a sample after heat stability treatment, the remaining 75 ml of the suspension should be retained for the sieving test as described in section 2.4). Transfer the 25 ml aliquot to a 100 ml beaker and add 2 g of potassium bromide. Allow to stand for about 5 minutes to permit the powder to coagulate. Place a 5.5 cm glass-fibre filter paper<sup>6</sup> in a 5.5 cm Buchner funnel, insert the funnel in a 250 ml suction flask, and wet the filter paper with water. Transfer the

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<sup>5</sup> Whenever water is mentioned in this section use standard soft water for testing the powder without pretreatment and standard hard water for testing the powder after heat stability at  $90^{\circ}\text{C}$  (section 2.5.2)

<sup>6</sup> Reeve Angel No. 934AH or Whatman GF/A or equivalent.

coagulated 25 ml aliquot to the filter paper and wash with four 20 ml portions of water. Transfer the funnel to a dry 250 ml suction flask and extract the malathion with six 20 ml portions of acetone. Evaporate the acetone in the flask in a steam-bath to a volume of approximately 10 ml.

Transfer the residue quantitatively to a tared 50 ml beaker using additional acetone. Evaporate the acetone at 60°C in a stream of dry air. Add two 5 ml portions of propan-2-ol during the evaporation to remove the traces of water. Dry the sample in an oven at 55°C for 20 minutes and weigh as malathion.

### 2.3.5 Calculation

Suspensibility (%) =  $m \times 160$

where  $m$  = mass of malathion (in g) found in the 25 ml aliquot (section 2.3.4).

## 2.4 Sieving test after 90°C heat stability treatment

Pour the 75 ml of suspension retained from the suspensibility test (section 2.3.4) on to a 75 mm sieve and proceed with the sieving test described in method WHO/M/4.R1. In this procedure it is assumed that all the particles in the 25 ml aliquot taken from the centre of the suspension would have passed through the 75 mm sieve.

## 2.5 Heat stability treatment

### 2.5.1 54°C heat stability treatment

Fill a 50 ml<sup>7</sup> 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 6 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.

### 2.5.2 90°C heat stability treatment

Fill a 50 ml<sup>7</sup> 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  $90 \pm 2^\circ\text{C}$  for 20 hours. 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 heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

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<sup>7</sup> If a larger quantity of the sample is required for the tests, use a 100 ml bottle.

# MALATHION EMULSIFIABLE CONCENTRATE

Specification WHO/SIF/14.R6  
Approved 25 September 1989

## 1. Specification

### 1.1 Description and ingredients

The material shall consist of technical malathion dissolved in suitable solvents, with other necessary formulants added. It shall be in the form of a stable liquid, free from suspended matter and sediment. The technical malathion used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/10.R6.

### 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.<sup>1</sup>

#### 1.2.1 *Malathion content (g/kg basis)*

The content of malathion 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 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 *Water content*

The water content determined by the method described in WHO/M/7.R1 shall not be higher than 2 g/kg.

#### 1.2.3 *Acidity*

The acidity of the concentrate, determined by method WHO/M/3 shall not be higher than 5 g/kg calculated as H<sub>2</sub>SO<sub>4</sub>.

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<sup>1</sup> If the material is for use inside buildings, the requirement that it should be odourless should also be specified.

#### 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 determined by the method WHO/M/10.R1, 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 100 ml of emulsion prepared in standard soft water with 5 ml of concentrate shall not exceed 2 ml when tested as described in WHO/M/13.R3.

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

#### 1.2.7 *Heat stability*

The concentrate, after treatment as described in section 2.2 shall comply with the requirements of sections 1.2.1, 1.2.3 and 1.2.6 or this specification.

### 1.3 **Packing and marking of packages**

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

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

Manufacturer's name  
Malathion emulsifiable concentrate to specification WHO/SIF/14.R6  
Malathion ... g/kg  
Batch or reference number, and date of test  
Net weight of contents  
Instructions for dilution  
Date of manufacture

and the following minimum cautionary notice:

Malathion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. 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 and pralidoxime are specific antidotes and artificial respiration may be needed. Store in a cool place and use as soon as possible after manufacture to avoid decomposition, which may occur under conditions of prolonged tropical storage.

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

### **2.1 Malathion content**

#### *2.1.1 Outline of method*

The sample is dissolved in chloroform to which an internal standard is added. An aliquot is introduced into a gas-liquid chromatograph and the ratio of the response of the malathion to that of the internal standard is determined. This is compared with the response of the standard of known purity to give the malathion content in the sample.

#### *2.1.2 Special apparatus*

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame-ionization detector, an electrometer having a sensitivity of at least  $10^{-11}$  amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.
2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210.
4. *Glass wool, silane-treated.*

### 2.1.3 *Special reagents*

*Malathion standard.* Analytical grade, of known purity.

*Internal standard.* 1,3-Diphenoxybenzene.

### 2.1.4 *Preparation of standard solutions*

*Internal standard solution.* Prepare a 30 g/l solution of the internal standard in chloroform. This solution is stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

*Malathion calibration solutions.* Weigh (to the nearest 0.1 mg) about 425, 500, and 575 mg quantities of the malathion standard, directly into separate, weighed 50 ml volumetric flasks. Add by pipette exactly 5 ml of the internal standard solution to each flask. Dilute to the mark with chloroform. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.1.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C provided the linearity specifications described in section 2.1.7 can be met.

### 2.1.5 *Preparation and conditioning of column*

See method WHO/M/20.

### 2.1.6 *Operating conditions for gas-liquid chromatography*

The temperatures, gas flow rates, and retention times given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

#### *Temperatures*

Oven	180°C
Injection port	190°C
Flame-ionization detector	250°C

#### *Gas flow rates*

Hydrogen	30 ml/min.
Air	350 ml/min.
Carrier gas (helium or nitrogen)	30 ml/min.

#### *Retention times*

Malathion peak	10 min.
Internal standard peak	7 min.

### 2.1.7 *Linearity check*

The gas-liquid chromatograph should be checked for linearity at least once a week, and the same check should be carried out whenever new calibration solutions are prepared and whenever a column, new or used, is installed in the instrument.

Using digital integration for peak area measurements, determine the appropriate attenuation setting and the aliquot (between 2 and 4 ml) of calibration solution B that must be injected to yield an area count of at least 100 000 (optimum electrometer output with acceptable noise level). The attenuation setting quantity so determined should be used for all samples and calibration solutions in the set.

Inject triplicate aliquots of appropriate volume (as determined above) of calibration solutions A, B, and C into the gas-liquid chromatograph, determine the response ratio for each injection, and average the resulting ratios for each solution. Divide the average response ratio for each solution by the corresponding malathion content (in mg) and compare the resulting response factors. These factors should agree to within 2%. Failure to meet this requirement indicates either a weighing error in the preparation of one of the calibration solutions or instrumental difficulties, which must be corrected before proceeding with the analysis of samples.

### 2.1.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 500 mg of malathion directly into a weighed 50 ml volumetric flask. Add by pipette exactly 5 ml of the internal standard solution to the volumetric flask, dilute to the mark with chloroform, and mix well.

Inject duplicate aliquots of appropriate volume (as determined in section 2.1.7) of calibration solution B. The response ratios should agree to within 2%. If this accuracy limit is not met, inject two more aliquots of the solution. Failure to meet the accuracy requirement with the second pair of injections indicates instrumental difficulties, which must be resolved before proceeding with the analyses.

Inject duplicate aliquots of the sample solution, using the same volume as that used in the preceding step. The accuracy considerations discussed in the preceding step apply here also. Average the response ratios for each sample solution.

In a series of analyses, after every two sample solution injections, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solution injections.

Use this average to calculate the malathion content of the sample.

### 2.1.9 Calculation

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

$$r = \frac{\text{area of malathion peak}}{\text{area of internal standard peak}}$$

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

where  $r_1$  = average response ratio for calibration solution B

$r_2$  = average response ration for sample solution

$m_1$  = mass of malathion standard in the calibration solution B (mg)

$m_2$  = mass of sample taken (mg)

$P$  = purity of malathion standard (g/kg)

## 2.2 Heat stability

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

# MALATHION DUSTABLE POWDER

Specification WHO/SIF/22.R5  
Approved 25 September 1989

## 1. Specification

### 1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical malathion together with carriers and any other necessary formulants. It shall be a fine free-flowing powder free from visible extraneous matter and hard lumps. The technical malathion used in the manufacture of the powder shall comply with the requirements of specification WHO/SIT/10.R6.

### 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.<sup>1</sup>

#### 1.2.1 Malathion content (g/kg basis)

The content of malathion determined by the method described in section 2.1 shall not differ from the nominal content by more than -10% to + 25%. The average content of all samples taken shall not be lower than the nominal content.

#### 1.2.2 Acidity<sup>2</sup>

The acidity or alkalinity of the powder, determined by the method described in WHO/M/3, shall not be higher than 1 g/kg calculated as H<sub>2</sub>SO<sub>4</sub>.

#### 1.2.3 Sieving after heat stability treatment at 90<sup>o</sup>C

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 the method described in WHO/M/4.R1. For powder intended for personal use, the residue remaining on the sieve shall be free from grittiness.

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<sup>1</sup> If the material is for use inside buildings, the requirement that it should be odourless should also be specified.

<sup>2</sup> Alkaline carriers should not be used.

#### 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.R2.<sup>3</sup>

#### 1.2.5 *Heat stability*

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

### 1.3 **Packing and marking of packages**

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

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

Manufacturer's name  
Malathion dustable powder to specification WHO/SIF/22.R5  
Malathion ... g/kg  
Batch or reference number, and date of test  
Net weight of contents  
Date of manufacture

and the following minimum cautionary notices:

**(a) Malathion content up to 10 g/kg:**

The powder may be hazardous if swallowed. Keep away from foodstuffs and animal feed and their containers. If poisoning occurs, call a physician. Atropine and pralidoxime are specific antidotes and artificial respiration may be needed.

Store in a cool place and use as soon as possible after manufacture to avoid decomposition, which may occur under prolonged storage in warm conditions.

**(b) Malathion content above 10 g/kg:**

Not for application to skin, clothing or bedding. Malathion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. 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 and pralidoxime are specific antidotes and artificial respiration may be needed. Store in a cool place and use as soon as possible after manufacture to avoid decomposition, which may occur under prolonged storage in warm conditions.

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<sup>3</sup> Equipment for vector control. 3rd ed. Geneva, World Health Organization, 1990, P. 128.

## 2. Methods of determining chemical and physical properties

### 2.1 Malathion content

#### 2.1.1 *Outline of method*

Malathion is extracted from the sample with chloroform, an internal standard is added. An aliquot is introduced into a gas-liquid chromatograph, and the ratio of the response of the malathion to that of the internal standard is determined. This is compared with the response of a standard of known purity to give the malathion content in the sample.

#### 2.1.2 *Special apparatus*

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame ionization detector, an electrometer having a sensitivity of at least  $10^{-11}$  amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.
2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210.
4. *Glass wool, silane-treated.*

#### 2.1.3 *Special reagents*

*Malathion standard.* Analytical grade, of known purity.

*Internal standard.* 1,3-Diphenoxybenzene.

2.1.4 *Preparation of standard solutions*

*Internal standard solution.* Prepare a 30 g/l solution of the internal standard in chloroform. This solution is stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

*Malathion calibration solutions.* Weigh (to the nearest 0.1 mg) about 425, 500, and 575 mg quantities of the malathion standard, directly into separate, weighed 50 ml volumetric flasks. Add by pipette exactly 5 ml of the internal standard solution to each flask. Dilute to the mark with chloroform. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.1.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C provided the linearity specifications described in section 2.1.7 can be met.

2.1.5 *Preparation and conditioning of column*  
See method WHO/M/20.2.1.6 *Operating conditions for gas liquid chromatography*

The temperatures, gas flow rates, and retention times given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

*Temperatures*

Oven	180°C
Injection port	190°C
Flame-ionization detector	250°C

*Gas flow rates*

Hydrogen	30 ml/min
Air	350 ml/min
Carrier gas (helium or nitrogen)	30 ml/min

*Retention times*

Malathion peak	10 min
Internal standard peak	7 min

### 2.1.7 *Linearity check*

The gas-liquid chromatograph should be checked for linearity at least once a week, and the same check should be carried out whenever new calibration solutions are prepared and whenever a column, new or used, is installed in the instrument.

Using digital integration for peak area measurements, determine the appropriate attenuation setting and the quantity (between 2 and 4 ml) of calibration solution B that must be injected to yield an area count of at least 100 000 (optimum electrometer output with acceptable noise level). The attenuation setting and aliquot so determined should be used for all samples and calibration solutions in the set.

Inject triplicate aliquots of appropriate volume (as determined above) of calibration solutions A, B, and C into the gas-liquid chromatograph, determine the response ratio for each injection, and average the resulting ratios for each solution. Divide the average response ratio for each solution by the corresponding malathion content (in mg) and compare the resulting response factors. These factors should agree to within 2%. Failure to meet this requirement indicates either a weighing error in the preparation of one of the calibration solutions or instrumental difficulties, which must be corrected before proceeding with the analysis of samples.

### 2.1.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 500 mg of malathion, transfer it to a 200 ml screw-capped bottle, and add from a pipette, 5 ml of the internal standard solution. Add 50 ml of chloroform and shake for approximately 30 seconds. Filter a few ml of the supernatant solution and hold for gas-liquid chromatography analysis. The filtration step is best accomplished by drawing 4-5 ml of the supernatant solution into a 10-ml Varipet syringe,<sup>4</sup> fitting the syringe with a 13 mm Swinnex filter holder (Millipore SX00'01300) with a glass-fibre filter (Gelman Type A-E, 13 mm, or equivalent) and forcing the solution through the filter into a small screw-capped vial.

Inject duplicate aliquots of appropriate volume (as determined in section 2.1.7) of calibration solution B. The response ratios should agree to within 2%. If this accuracy limit is not met, inject two more aliquots of the solution. Failure to meet the accuracy requirement with the second pair of injections indicates instrumental difficulties, which must be resolved before proceeding with the analyses. Inject duplicate aliquots of the sample solution, using the same volume as that used in the preceding step. The accuracy considerations discussed in the preceding step apply here also. Average the response ratios for each sample solution.

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<sup>4</sup> Available from Manostat Co., 519 8th Avenue, New York, NY 10018, USA.

In a series of analyses, after every two sample solution injections, inject duplicate aliquots of calibration solution B. Average the response ratios for the calibration solution injections immediately before and after the sample solution injections.

Use this average to calculate the malathion content of the sample.

### 2.1.9 Calculation

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

$$r = \frac{\text{area of malathion peak}}{\text{area of internal standard peak}}$$

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

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

## 2.2 Heat stability treatment

For the sieve test, section 1.2.3 place 20 g of the sample in a 100 ml wide-mouthed bottle fitted with a vinyl-plastic-lined screw cap. 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. For the other tests to be repeated, section 1.2.5, place 25 g of the sample in a 100 ml wide-mouthed bottle with a vinyl-plastic-lined screw-cap.

Place the bottles in a forced-draught oven maintained at  $70 \pm 2^\circ\text{C}$  for 2 hours. Remove 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.