

# TECHNICAL FENITROTHION

Specification WHO/SIT/17.R3  
Approved 25 September 1989

## 1. Specification

### 1.1 Material

The material shall consist of fenitrothion together with related manufacturing compounds and shall be in the form of a yellow to brown liquid free from extraneous impurities or added modifying agents.

### 1.2 Chemical and physical requirements

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

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

The fenitrothion content shall be declared (not less than 930 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/kg.

#### 1.2.2 *S-methyl fenitrothion*

The S-methyl fenitrothion content determined by the method described in section 2.2, shall not be higher than 20 g/kg.

#### 1.2.3 *Acidity*

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

#### 1.2.4 *Water content*

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

### 1.3 Packing and marking of packages

The technical fenitrothion 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 fenitrothion to specification WHO/SIT/17.R3  
Batch or reference number, and date of test  
Net weight of contents  
Date of manufacture

and the following minimum cautionary notice:

Fenitrothion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed or inhaled. It may be absorbed through the skin. Avoid skin contact; wear protective gloves, clean protective clothing, and a respirator when handling the material. Wash thoroughly with soap and water after using. 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.

## 2. Methods of determining chemical and physical properties

### 2.1 Fenitrothion content

#### 2.1.1 *Outline of method*

The sample is dissolved in chloroform and an internal standard is added. The fenitrothion content is determined by gas-liquid chromatography using a flame-ionization detector.

#### 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 and a high-sensitivity flame-ionization detector.
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, acid washed and dry, bent to fit the chromatograph.
3. *Column-packing material*<sup>1</sup>. Chromosorb W-HP (100-120 mesh) treated with 30 ml/l polyphenyl ether with polymerization degree 6 (PPE-6R).

---

<sup>1</sup> Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210 can also be used as an alternative packing material. However, note that if this packing material is used, the column should be 150 cm long and 3 mm in internal diameter.

### 2.1.3 *Special reagents*

*Fenitrothion standard.* Analytical grade, of known purity, better than 995 g/kg

*Internal standard.* Fluoranthene, pure.

If the alternative column-packing (OV-210) is used, the internal standard should be dibutyl sebacate.

### 2.1.4 *Preparation of standard solutions*

*Fluoranthene internal standard solution.* Weigh about 1.5 g of fluoranthene into a 100 ml volumetric flask, dissolve in chloroform, dilute to volume, and mix.

*Dibutyl sebacate internal standard solution.* Weigh about 3 g of dibutyl sebacate into a 100 ml volumetric flask, dissolve in chloroform, dilute to volume, and mix.

*Fenitrothion calibration solution.* Weigh (to the nearest 0.1 mg) about 200 mg of fenitrothion standard into a 50 ml screw-capped bottle. Add by pipette 5.0 ml of internal standard solution and 20.0 ml of chloroform and shake for 30 seconds.

### 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 specific apparatus.

Temperatures	PPE.6R	OV-210
Column	195 <sup>0</sup> C	165 <sup>0</sup> C
Injection port	200 <sup>0</sup> C	190 <sup>0</sup> C
lame-ionization detector	250 <sup>0</sup> C	250 <sup>0</sup> C
Gas flow rates		
Hydrogen and air	As recommended for the detector by the manufacturer.	
Carrier gas (nitrogen)	30 ml/min.	30 ml/min.
Approximate retention times		
Fenitrothion peak	16 min.	26 min.
Internal standard peak	26 min.	30 min.

### 2.1.7 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of fenitrothion into a 50 ml screw-capped bottle. Add by pipette 5.0 ml of the internal standard solution and 20.0 ml of chloroform and shake for 30 seconds.

Inject 2 µl portions of the calibration solution of fenitrothion until the response ratios (area or peak height) for fenitrothion to internal standard agree to within 2%. Make duplicate injections of the calibration solution followed by duplicate injections of the sample solution<sup>2</sup>.

Recalibrate after not more than 4 injections of sample solutions.

### 2.1.8 Calculation

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

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

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

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

## 2.2 S-methyl fenitrothion content<sup>3</sup>

### 2.2.1 Outline of method

The sample is dissolved in chloroform. The S-methyl fenitrothion content is determined by normal phase liquid chromatography with UV detection.

### 2.2.2 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 UV detector, a loop injector and an electronic integrator.

---

2 In order to avoid interference from a late-emerging impurity (retention time, approximately 45 min.), injection of subsequent samples must be made not earlier than 7 min. after the elution of the internal standard. Thus, the total run time for each sample is about 35 min.

3 A GLC alternative method is available on request from WHO Schistosomiasis Control Unit, Control of Tropical Diseases, CH 1211 Geneva 27, Switzerland.

2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 4 mm in internal diameter. It shall be packed with 5 $\mu$  Bondapak CN (Waters Assoc.) or equivalent. The column should be cleaned with a mixture of dichloromethane - methanol (1+1) at a flow rate of 3 ml/min. for 30 minutes before or after analysis of each day.

### 2.2.3 *Special reagents*

*S-methyl fenitrothion* of known purity

*Chloroform*, HPLC grade

*Hexane*, HPLC grade

*Dichloromethane*, HPLC grade

*Methanol*, HPLC grade

*Mobile phase.* A mixture of 500 ml hexane, 20 ml dichloromethane and 2 ml methanol. Filter and degas before use.

### 2.2.4 *Preparation of standard solution*

*S-methyl fenitrothion calibration solutions.* Weigh (to the nearest 0.1 mg) about 25 mg of S-methyl fenitrothion into a 50 ml volumetric flask. Dissolve, make up to volume with chloroform and mix thoroughly. Transfer 2,3 and 4 ml of this solution into separate 20 ml volumetric flasks, make up to volume with chloroform and mix thoroughly. Label the three calibration solutions "A", "B", and "C" respectively

### 2.2.5 *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	1.5 ml/min.
Wavelength	254 nm
Injection volume	5 $\mu$ l
Retention times	
S-methyl fenitrothion	10 min.
(fenitrothion)	5 min.

### 2.2.6 *Linearity check*

The liquid chromatograph should be checked for linearity at least twice a month, 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.

Inject 5 ml aliquots of calibration solutions A, B and C into the liquid chromatograph. Determine the response factor for each injection and compare the response factors.

$$\text{Response factor} = \frac{W_{smf}}{A_{smf}}$$

These factors should agree to within 3%.

where:  $W_{smf}$  = weight of S-methyl fenitrothion in each calibration solution (mg/20 ml)  
 $A_{smf}$  = peak area of S-methyl fenitrothion.

### 2.2.7 Sample preparation and analysis

Weigh (to the nearest 0.1 mg) a quantity<sup>4</sup> of sample containing about 1.5 mg S-methyl fenitrothion into a 30 ml screw-capped bottle, add by pipette 20 ml chloroform and shake for approximately 30 seconds. Filter if needed.

Inject 5 ml aliquots of the calibration solution B into the liquid chromatograph to stabilize the instrument. Make repetitive injections until the response factors for successive injections agree to within 2%.

Inject two 5 µl aliquots of the calibration solution B, two 5 ml aliquots of the sample solution and two 5 ml aliquots of the calibration solution B.

$$F = \frac{\text{weight of S - methyl fenitrothion in solution B (mg)}}{\text{peak area of S - methyl fenitrothion}}$$

Average the response factors of the calibration solution B

---

4 The quantity of sample depends on the S-methyl fenitrothion content in the material. As a first approximation, weigh a quantity based on the following formula:

$$\frac{200\ 000}{D \times S} \text{ mg}$$

D = declared content of fenitrothion in the sample (g/kg).

S = specified percentage of S-methyl fenitrothion versus fenitrothion in the sample

### 2.2.8 Calculation

Calculate for each of the sample injection the:

$$S\text{-methyl fenitrothion content (g / kg)} = \frac{A_s \times F \times P}{W_s}$$

where  $A_s$  = peak area of S-methyl fenitrothion for the sample solution

$W_s$  = weight of the sample (mg)

$P$  = purity of the standard S-methyl fenitrothion (g/kg)

$F$  = response factor

Average the two results.

# FENITROTHION EMULSIFIABLE CONCENTRATE

Specification WHO/SIF/37.R2  
Approved 25 September 1989

## 1. Specification

### 1.1 Description and ingredients

The material shall consist of technical fenitrothion dissolved in suitable solvents, with other necessary formulants added. It shall be in the form of a stable liquid, free from extraneous impurities. The technical fenitrothion used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/17.R3.

### 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 *Fenitrothion content (g/kg basis)*

The content of fenitrothion, 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 *S-methyl fenitrothion*

The S-methyl fenitrothion content determined by the method described in section 2.2 shall not be higher than 2.5% of the fenitrothion content found in section 1.2.1.

#### 1.2.3 *Water content*

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

#### 1.2.4 *Acidity*

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

#### 1.2.5 *Cold test*

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

#### 1.2.6 *Flash point*

The flash point of the product determined by method WHO/M/10.R1, shall comply with all national and/or international transport regulations.

#### 1.2.7 *Stability of emulsion*

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

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

#### 1.2.8 *Heat stability*

The concentrate, after treatment as described in section 2.3, shall comply with the requirements of sections 1.2.1, 1.2.2, 1.2.4 and 1.2.7 of this specification.

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

The fenitrothion 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  
Fenitrothion emulsifiable concentrate to specification WHO/SIF/37.R2  
Fenitrothion, ... 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:

Fenitrothion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed or inhaled. It may be absorbed through the skin. Avoid skin contact; wear protective gloves, clean protective clothing, and a respirator when handling the material. Wash thoroughly with soap and water after using.

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.

## 2. Methods of determining chemical and physical properties

### 2.1 Fenitrothion content

#### 2.1.1 *Outline of method*

The sample is dissolved in chloroform and an internal standard is added. The fenitrothion content is determined by gas-liquid chromatography using a flame-ionization detector.

#### 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 and a high-sensitivity flame-ionization detector.
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, acid washed and dry, bent to fit the chromatograph.
3. *Column-packing material*<sup>1</sup>. Chromosorb W-HP (100-120 mesh) treated with 30 ml/l polyphenyl ether with polymerization degree 6 (PPE-6R).

#### 2.1.3 *Special reagents*

*Fenitrothion standard.* Analytical grade, of known purity, better than 995 g/kg.

*Internal standard.* Fluoranthene, pure. If the alternative column-packing (OV-210) is used, the internal standard should be dibutyl sebacate.

---

<sup>1</sup> Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210 can also be used as an alternative packing material. However, note that if this packing material is used, the column should be 150 cm long and 3 mm in internal diameter.

#### 2.1.4 Preparation of standard solutions

*Fluoranthene internal standard solution.* Weigh about 1.5 g of fluoranthene into a 100 ml volumetric flask, dissolve in chloroform, dilute to volume, and mix.

*Dibutyl sebacate internal standard solution.* Weigh about 3 g of dibutyl sebacate into a 100 ml volumetric flask, dissolve in chloroform, dilute to volume, and mix.

*Fenitrothion calibration solution.* Weigh (to the nearest 0.1 mg) about 200 mg of fenitrothion standard into a 50 ml screw-capped bottle. Add the pipette 5.0 ml of internal standard solution and 20.0 ml of chloroform and shake for 30 seconds.

#### 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 specific apparatus.

<i>Temperatures</i>	PPE.6R	OV-210
Column	195 <sup>0</sup> C	165 <sup>0</sup> C
Injection port	200 <sup>0</sup> C	190 <sup>0</sup> C
Flame-ionization detector	250 <sup>0</sup> C	250 <sup>0</sup> C
<i>Gas flow rates</i>		
Hydrogen and air	As recommended for the detector by the manufacturer.	
Carrier gas (nitrogen)	30 ml/min.	30 ml/min.
<i>Approximate retention times</i>		
Fenitrothion peak	16 min.	26 min.
Internal standard peak	26 min.	30 min.

#### 2.1.7 Sample preparation and analysis

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of fenitrothion into a 50 ml screw-capped bottle. Add by pipette 5.0 ml of the internal standard solution and 20.0 ml of chloroform and shake for 30 seconds. Inject 2- $\mu$ l portions of the calibration solution of fenitrothion until the response ratios (area or peak height) for fenitrothion to internal standard agree to within 2%. Make duplicate injections of the calibration solution followed by duplicate injections of the sample solution<sup>2</sup>. Recalibrate after not more than 4 injections of sample solutions.

---

2 In order to avoid interference from a late-emerging impurity (retention time, approximately 45 min), injection of subsequent samples must be made not earlier than 7 min after the elution of the internal standard. Thus, the total run time for each sample is about 35 min.

2.1.8 *Calculation*

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

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

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

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

2.2 **S-methyl fenitrothion content**<sup>3</sup>2.2.1 *Outline of method*

The sample is dissolved in chloroform. The S-methyl fenitrothion content is determined by normal phase liquid chromatography with UV detection.

2.2.2 *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 UV detector, a loop injector and an electronic integrator.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 4 mm in internal diameter. It shall be packed with 5 $\mu$ m/Bondapak CN (Waters Assoc.) or equivalent.

The column should be cleaned with a mixture of dichloromethane - methanol (1+1) at a flow rate of 3 ml/min. for 30 minutes before or after analysis of each day.

---

3 A GLC alternative method is available on request from WHO Schistosomiasis Control Unit, Control of Tropical Diseases, CH 1211 Geneva 27, Switzerland.

### 2.2.3 *Special reagents*

*S*-methyl fenitrothion of known purity

Chloroform, HPLC grade

Hexane, HPLC grade

Dichloromethane, HPLC grade

Methanol, HPLC grade

*Mobile phase.* A mixture of 500 ml hexane, 20 ml dichloromethane and 2 ml methanol. Filter and degas before use.

### 2.2.4 *Preparation of standard solution*

*S*-methyl fenitrothion calibration solutions. Weigh (to the nearest 0.1 mg) about 25 mg of *S*-methyl fenitrothion into a 50 ml volumetric flask. Dissolve, make up to volume with chloroform and mix thoroughly. Transfer 2,3 and 4 ml of this solution into separate 20 ml volumetric flasks, make up to volume with chloroform and mix thoroughly. Label the three calibration solutions "A", "B", and "C" respectively.

### 2.2.5 *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	1.5 ml/min.
Wavelength	254 nm
Injection volume	5 µl
Retention times	
<i>S</i> -methyl fenitrothion	10 min.
(fenitrothion)	5 min.

### 2.2.6 *Linearity check*

The liquid chromatograph should be checked for linearity at least twice a month, 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. Inject 5 µl aliquots of calibration solutions A, B and C into the liquid chromatograph. Determine the response factor for each injection and compare the response factors.

These factors should agree to within 3%. where:

$$\text{Response factor} = \frac{W_{smf}}{A_{smf}}$$

$W_{smf}$  = weight of *S*-methyl fenitrothion in each calibration solution (mg/20 ml)

$A_{smf}$  = peak area of *S*-methyl fenitrothion.

### 2.2.7 Sample preparation and analysis

Weigh (to the nearest 0.1 mg) a quantity<sup>4</sup> of sample containing about 1.5 mg S-methyl fenitrothion into a 30 ml screw-capped bottle, add by pipette 20 ml chloroform and shake for approximately 30 seconds. Filter if needed. Inject 5 µl aliquots of the calibration solution B into the liquid chromatograph to stabilize the instrument. Make repetitive injections until the response factors for successive injections agree to within 2%. Inject two 5 µl aliquots of the calibration solution B, two 5 µl aliquots of the sample solution and two 5 ml aliquots of the calibration solution B.

$$F = \frac{\text{weight of S - methyl fenitrothion in solution B (mg)}}{\text{peak area of S - methyl fenitrothion}}$$

Average the response factors of the calibration solution B

### 2.2.8 Calculation

Calculate for each of the sample injection the:

$$\text{S - methyl fenitrothion content (g / kg)} = \frac{As \times F \times P}{Ws}$$

As = peak area of S-methyl fenitrothion for the sample solution

Ws = weight of the sample (mg)

P = purity of the standard S-methyl fenitrothion (g/kg)

F = response factor

Average the two results and calculate the ratio (%) of S-methyl fenitrothion versus fenitrothion as follows:

$$\text{S - methyl fenitrothion (\%)} = \frac{Cs \times 100}{Cf}$$

where:

Cs = content of S-methyl fenitrothion in the sample

Cf = content of fenitrothion in the sample determined in section 2.1.

## 2.3 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.

4 The quantity of sample depends on the S-methyl fenitrothion content in the material. As a first approximation, weigh a quantity based on the following formula:

$$\frac{200\,000}{D \times S} \text{ mg}$$

where: D =

declared content of fenitrothion in the sample (g/kg).

S =

specified percentage of S-methyl fenitrothion versus fenitrothion in the sample

# FENITROTHION WATER-DISPERSIBLE POWDER

Specification WHO/SIF/29.R3  
Approved 25 September 1989

## 1. Specification

### 1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical fenitrothion 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 fenitrothion used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/17.R3.

### 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 *Fenitrothion content (g/kg basis)*

The content of fenitrothion, 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 *S-methyl fenitrothion*

The S-methyl fenitrothion content determined by the method described in section 2.2 shall not be higher than 2.5% of the fenitrothion content found in section 1.2.1.

#### 1.2.3 *Acidity or alkalinity*

The acidity or alkalinity 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> or 2 g/kg calculated as NaOH.

#### 1.2.4 *Sieving after heat stability treatment*

Not less than 98% of the powder after heat stability treatment (section 2.5) shall pass through a 75 µm sieve when tested by the method described in section 2.4.

#### 1.2.5 *Suspensibility*

*In standard hard water after heat stability treatment.* When tested by the method described in section 2.3, a minimum of 50% of the fenitrothion (12.5 g/l) shall be in suspension 30 minutes after agitating a suspension containing 25 g/l fenitrothion, prepared in standard hard water from the powder subjected to the heat stability treatment described in section 2.5.

#### 1.2.6 *Heat stability*

The material after treatment as described in section 2.5, shall comply with the requirements of section 1.2.1, 1.2.2 and 1.2.3.

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

The fenitrothion 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 0.1 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  
Fenitrothion water-dispersible powder to specification WHO/SIF/29.R3  
Fenitrothion, ... g/kg  
Batch or reference number, and date of test  
Net weight of contents  
Date of manufacture

and the following minimum cautionary notice:

Fenitrothion is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed or inhaled. It may be absorbed through the skin. Avoid skin contact; wear protective gloves, clean protective clothing, and a respirator when handling the material. Wash thoroughly with soap and water after using.

Keep 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.

## 2. Methods of determining chemical and physical properties

### 2.1 Fenitrothion content

#### 2.1.1 Outline of method

Fenitrothion is extracted from the sample with chloroform and an internal standard is added. The fenitrothion content is determined by gas-liquid chromatography using a flame-ionization detector.

#### 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 and a high-sensitivity flame-ionization detector.
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, acid washed and dry, bent to fit the chromatograph.
3. *Column-packing material*<sup>1</sup>. Chromosorb W-HP (100-120 mesh) treated with 30 ml/l polyphenyl ether with polymerization degree 6 (PPE-6 R).

#### 2.1.3 Special reagents

*Fenitrothion standard.* Analytical grade, of known purity, better than 995 g/kg.

*Internal standard.* Fluoranthene, pure. If the alternative column-packing (OV-210) is used, the internal standard should be dibutyl sebacate.

#### 2.1.4 Preparation of standard solutions

*Fluoranthene internal standard solution.* Weigh about 1.5 g of fluoranthene into a 100 ml volumetric flask, dissolve in chloroform, dilute to volume, and mix.

*Dibutyl sebacate internal standard solution.* Weigh about 3 g of dibutyl sebacate into a 100 ml volumetric flask, dissolve in chloroform, dilute to volume, and mix.

*Fenitrothion calibration solution.* Weigh (to the nearest 0.1 mg) about 200 mg of fenitrothion standard into a 50 ml screw-capped bottle. Add by pipette 5.0 ml of internal standard solution and 20.0 ml of chloroform and shake for 30 seconds.

---

<sup>1</sup> Chromosorb W-HP (100-120 mesh) treated with 7.5% OV-210 can also be used as an alternative packing material. However, note that if this packing material is used, the column should be 150 cm long and 3 mm in internal diameter.

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 specific apparatus.

<i>Temperatures</i>	PPE.6R	OV-210
Column	195 <sup>0</sup> C	165 <sup>0</sup> C
Injection port	200 <sup>0</sup> C	190 <sup>0</sup> C
Flame-ionization detector	250 <sup>0</sup> C	250 <sup>0</sup> C

*Gas flow rates*

Hydrogen and air	As recommended for the detector by the manufacturer.	
Carrier gas (nitrogen)	30 ml/min.	30 ml/min.

*Approximate retention times*

Fenitrothion peak	16 min.	26 min.
Internal standard peak	26 min.	30 min.

2.1.7 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of sample containing about 200 mg of fenitrothion into a 50 ml screw-capped bottle. Add by pipette 5.0 ml of the internal standard solution and 20.0 ml of chloroform. Shake for 30 seconds. Filter or centrifuge to remove the insoluble particles.

Inject 2 µl portions of the calibration solution of fenitrothion until the response ratios (area or peak height) for fenitrothion to internal standard agree to within 2%. Make duplicate injections of the calibration solution followed by duplicate injections of the sample solution<sup>2</sup>.

Recalibrate after not more than 4 injections of sample solutions.

---

2 In order to avoid interference from a late-emerging impurity (retention time, approximately 45 min), injection of subsequent samples must be made not earlier than 7 min after the elution of the internal standard. Thus, the total run time for each sample is about 35 min.

### 2.1.8 Calculation

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

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

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

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

## 2.2 S-methyl fenitrothion content<sup>3</sup>

### 2.2.1 Outline of method

S-methyl fenitrothion is extracted from the sample with chloroform and its content is determined by normal phase liquid chromatography with UV detection

### 2.2.2 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 UV detector, a loop injector and an electronic integrator.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 4 mm in internal diameter. It shall be packed with 5m Bondapak CN (Waters Assoc.) or equivalent.

The column should be cleaned with a mixture of dichloromethane-methanol (1+1) at a flow rate of 3 ml/min for 30 minutes, before or after analysis of each day.

---

3 A GLC alternative method is available on request from WHO Schistosomiasis Control Unit, Control of Tropical Diseases, CH 1211 Geneva 27, Switzerland

### 2.2.3 *Special reagents*

*S-methyl fenitrothion* of known purity

*Hexane*, HPLC grade

*Chloroform*, HPLC grade

*Dichloromethane*, HPLC grade

*Methanol*, HPLC grade

*Mobile phase.* A mixture of 500 ml hexane, 20 ml dichloromethane and 2 ml methanol. Filter and degas before use.

### 2.2.4 *Preparation of standard solutions*

*S-methyl fenitrothion calibration solutions.* Weigh (to the nearest 0.1 mg) about 25 mg of S-methyl fenitrothion into a 50 ml volumetric flask. Dissolve, make up to volume with chloroform and mix thoroughly.

Transfer 2, 3 and 4 ml of this solution into separate 20 ml volumetric flasks, make up to volume with chloroform and mix thoroughly. Label the three calibration solutions "A", "B", and "C" respectively.

### 2.2.5 *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	1.5 ml/min.
Wavelength	254 nm
Injection volume	5 ml
Retention times	
S-methyl fenitrothion	10 min.
(fenitrothion)	5 min.

### 2.2.6 *Linearity check*

The liquid chromatograph should be checked for linearity at least twice a month, 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.

Inject 5 ml aliquots of calibration solutions A, B and C into the liquid chromatograph.

Determine the response factor for each injection and compare the response factors. These factors should agree to within 3%.

$$\text{Response factor} = \frac{W_{smf}}{A_{smf}}$$

$W_{smf}$  = weight of S-methyl fenitrothion in each calibration solution (mg/20 ml).  
 $A_{smf}$  = peak area of S-methyl fenitrothion.

### 2.2.7 Sample preparation and analysis

Weigh (to the nearest 0.1 mg) a quantity<sup>4</sup> of sample containing about 1.5 mg S-methyl fenitrothion into a 30 ml screw-capped bottle, add by pipette 20 ml chloroform and shake for approximately 30 seconds. Filter or centrifuge the solution.

Inject 5  $\mu$ l aliquots of the calibration solution B into the liquid chromatograph to stabilize the instrument. Make repetitive injections until the response factors for successive injections agree to within 2%.

Inject two 5  $\mu$ l aliquots of the calibration solution B, two 5  $\mu$ l aliquots of the sample solution and two 5  $\mu$ l aliquots of the calibration solution B.

Average the response factors of the calibration solution B

$$F = \frac{\text{weight of S - methyl fenitrothion in solution B (mg)}}{\text{peak area of S - methyl fenitrothion}}$$

---

4 The quantity of sample depends on the S-methyl fenitrothion content in the material. As a first approximation, weigh a quantity based on the following formula:

$$\frac{200\ 000}{D \times S} \text{ mg}$$

D = declared content of fenitrothion in the sample (g/kg).  
S = specified percentage of S-methyl fenitrothion versus fenitrothion in the sample.

### 2.2.8 Calculation

Calculate for each of the sample injection the:

$$S\text{-methyl fenitrothion content (g / kg)} = \frac{A_s \times F \times P}{W_s}$$

$A_s$  = peak area of S-methyl fenitrothion for the sample solution

$W_s$  = weight of the sample (mg)

$P$  = purity of the standard S-methyl fenitrothion (g/kg)

$F$  = response factor

Average the two results and calculate the ratio (%) of S-methyl fenitrothion versus fenitrothion as follows:

$$S\text{-methyl fenitrothion (\%)} = \frac{C_s \times 100}{C_f}$$

$C_s$  = content of S-methyl fenitrothion in the sample

$C_f$  = content of fenitrothion in the sample determined in section 2.1.

## 2.3 Suspensibility after heat stability treatment

### 2.3.1 Outline of method

A suspension of known concentration of fenitrothion in standard hard water 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 fenitrothion 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 a 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.

### 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 fenitrothion. 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 paper with water.

---

5 Whenever water is mentioned in this section use standard hard water.

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

Transfer the coagulated 25 ml aliquot to the paper and wash with four 20 ml portions of water. Transfer the funnel to a dry 250 ml suction flask and extract the fenitrothion 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 traces of water. Dry the sample in an oven at 55°C for 20 minutes and weigh as fenitrothion.

### 2.3.5 Calculation

Suspensibility (%) =  $m \times 160$

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

## 2.4 Sieving test after 70°C heat stability treatment

Pour the 75 ml of suspension retained from the suspensibility test (section 2.3.3) 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

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  $70 \pm 2^\circ\text{C}$  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 completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

---

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