TECHNICAL DIAZINON

Specification WHO/SIT/9.R6
Approved 25 September 1989

1. Specification

1.1 Material

The material shall consist of diazinon 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 other than the stabilizer[1].

1.2 Chemical and physical requirements

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

1.2.1 *Diazinon content (g/kg basis)*

The diazinon content shall be declared[1] (g/kg) and when determined by the method described in section 2.1, the content obtained shall not differ from that declared by more than ±25 g.

1.2.2 *Impurities*

1.2.2.1 *O,O,O',O'-tetraethyl thiopyrophosphate (O,S-TEPP)*

The O,S-TEPP content determined by the method described in section 2.2 shall not be higher than 0.2 g/kg.

1.2.2.2 O,O,O',O'-tetraethyl dithiopyrophosphate (S,S-TEPP)

The S,S-TEPP content determined by the method described in section 2.2 shall not be higher than 2.5 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 0.3 g/kg calculated as H_2SO_4.

[1] A stabilizer may be present (maximum: 100 g/kg). Then the diazinon content of the remainder of the material (i.e., excluding the stabilizer) shall be not less than 950 g/kg.

1.2.4 *Material insoluble in acetone*

The material insoluble in acetone, determined by the method described in WHO/M/21.R1, shall not be higher than 1.5 g/kg.

1.2.5 *Water content*

The water content, determined by the method described in WHO/M/7.R1, shall not be higher than 0.6 g/kg.

1.3 Packing and marking of packages

The technical diazinon 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 diazinon to specification WHO/SIT/9.R6
Batch or reference number, and date of test
Net weight of content
Date of manufacture

and the following minimum cautionary notice:

Diazinon is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. 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 Diazinon content

2.1.1 *Outline of method*

The sample is dissolved in acetone to which aldrin is added as internal standard. An aliquot of this mixture is introduced into a gas-liquid chromatograph and the ratio of the response of the diazinon to that of the internal standard is determined. This is compared with the response of a standard of known purity to give the diazinon 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 and a high-sensitivity flame-ionization detector.

2. *Chromatographic column.* The column should be a borosilicate glass tube 180 cm long, 4 mm in internal diameter and 6 mm in external diameter, bent to fit the chromatograph.

3. *Column-packing material.* Gas-Chrom Q (80-100 mesh) treated with 10% silicone DC 200.

2.1.3 *Special reagents*

Diazinon standard. Analytical grade, of known purity.
Internal standard. Aldrin should be at least 900 g/kg HHDN purity and should contain no impurities that elute at the same retention time as diazinon.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weigh (to the nearest 0.1 g) about 4 g of technical aldrin into a 600 ml beaker. Mix with 400 ml of acetone to dissolve, filter through paper into a 1000 ml volumetric flask, washing with several 100 ml portions of acetone. Dilute to volume and mix well.

Diazinon calibration solution. Weigh (to the nearest 0.1 mg) about 125 mg of diazinon standard into a 125 ml round bottle fitted with an aluminium-lined screw cap. Pipette in 50 ml of internal standard solution and shake mechanically for 30 minutes.

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	190 ± 10°C.
Injection port	240°C.
Flame-ionization detector	240°C.

Gas flow rates

Hydrogen	As recommended for the detector
Air	by the manufacturer
Carrier gas (nitrogen or helium)	80-100 ml/min.

Attenuation

Adjust until the peak heights of the diazinon and internal standard are 60 to 80% full scale.

Retention times

Diazinon peak	5-6 min.
Internal standard peak	10-12 min.

2.1.7 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of sample containing about 125 mg of diazinon directly into a 125 ml round bottle fitted with an aluminium-lined screw cap. Pipette in 50 ml of internal standard as used for the preparation of the diazinon calibration solution and shake mechanically for 30 minutes. Let insoluble materials, if any, to settle or centrifuge a portion to obtain a clear solution.

Inject 3 µl aliquots of calibration solution until peak area (or height) ratios of diazinon to internal standard agree to within 1%. Then make duplicate injections of sample solution followed by duplicate injections of calibration solution. Peak area (or height) ratios of calibration solution injections must agree to within ±1% of first accepted calibration solution values. If not, repeat series of injections. Repeat for additional samples.

2.1.8 *Calculation*

Calculate the peak area (or height) ratios for both duplicate calibration solution injections preceding and following the sample solution injections. Average the four values.

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

Calculate and average the peak area (or height) ratios of the two sample solution injections. For each injection the response ratio (r) is given by the equation:

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

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

2.2 Impurities (O,S-TEPP and S,S-TEPP) content

2.2.1 *Outline of method*

The sample is dissolved in methanol containing diethyl phthalate as internal standard. This solution is passed through a strong cation exchange resin column to remove diazinon. The eluate is reconcentrated, dissolved in 1,1,1-trichloroethane and O,S-TEPP and S,S-TEPP are determined by capillary gas-liquid chromatography with flame-ionization detection.

2.2.2 *Special apparatus*

1. *Gas-liquid chromatograph.* Carlo Erba HRGC 5160 or equivalent, equipped with cold on-column injection system and flame-ionization detector.

2. *Gas-liquid chromatographic column.* The column should be a fused silica 15 m x 0.32 mm id coated with OV 1701, film thickness 1 mm (supplier: J & W Scientific Inc). Precolumn: fused silica 20 cm x 0.53 mm id, uncoated, deactivated, connected to column by pressfit connector.

3. *Electrometer. Sensitivity 10^{-9} A/V.*

4. *Chromatographic columns.* 250 x 10 (id) mm.

5. *Rotary evaporator.*

2.2.3 *Special reagents*

1. *Dowex 50 W X 2.* Strongly acidic cation exchange resin, 50-100 mesh; H^+-form (Fluka 44455 or Aldrich 21744-1).

2. *Reference standard O,S-TEPP* of known purity.

3. *Reference standard S,S-TEPP* of known purity.

4. *Internal standard.* Diethyl phthalate (Merck 822323) GLC grade.

2.2.4 *Preparation of standard solutions*

O,S-TEPP/internal standard stock solution. Accurately weigh ca. 10 mg O,S-TEPP reference standard and 50 mg diethyl phthalate internal standard into a 50 ml volumetric flask, dilute to volume with 1,1,1-trichloroethane and mix.

Internal standard solution. Accurately weigh ca. 20 mg internal standard into a 100 ml volumetric flask, dilute to volume with methanol and mix.

Calibration solution. Accurately weigh ca. 12.5 mg S,S-TEPP reference standard into a 100 ml volumetric flask. Add 5.0 ml O,S-TEPP/internal standard stock solution by pipette, dilute with 1,1,1-trichloroethane to volume and mix. (Approximate weights in final dilution (100 ml): 1 mg O,S-TEPP, 12.5 mg S,S-TEPP and 5 mg internal standard.)

2.2.5 *Preparation of ion exchange column*

Mix the ion exchange resin (ca. 7 g for one column) with methanol. Allow the slurry to stand for at least 1 hour. Pour the slurry into chromatographic column to give bed height of 8 to 10 cm after settling. Wash resin with methanol until effluent is clear, colourless and neutral. Do not let liquid level drain below the top of the resin bed.

2.2.6 *Operating conditions for gas-liquid chromatography*

The temperature, 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	programme: 1 min at 60°C; increase from
Injection port	60 to 250°C at the rate of 5°C/min - Maintain 250°C for 12 min.
Flame-ionization detector	270°C.
Electrometer sensitivity	10^{-9} A/V
Injection volume	0.5 µl.
Gas flow rates	
Carrier gas	H, linear velocity 40 cm/sec measured with dichloromethane at 60°C.
Make up gas	N, 50 ml/min.
Detector gases	H, 25 ml/min; air, 350 ml/min.
Retention times	
Internal standard peak	ca. 23.93 min.
O,S-TEPP peak	25.70 min.
S,S-TEPP	26.15 min.

2.2.7 *Preparation of sample and analysis*

Accurately weigh sufficient sample to contain ca. 1 g diazinon (based on nominal value) into a 10 ml volumetric flask, add 5.0 ml internal standard solution by pipette, dilute to volume with methanol and mix. Transfer 1.0 ml of this solution to the ion exchange column and allow the liquid level to reach the top of resin bed. Elute with 20 ml methanol. Collect the eluate in a 20 ml round-bottomed flask. Evaporate to dryness at 40°C and reduced pressure using a rotary evaporator. Dissolve the residue with 2.0 ml 1,1,1-trichloroethane. (Approximate weights in final dilution (2 ml): one-tenth of sample weight and 0.1 mg internal standard.)

Inject 0.5 µl aliquots of the calibration solution until the peak height ratio of O,S-TEPP/internal standard and S,S-TEPP/internal standard response varies <5% for successive injections. Then make duplicate injections of the sample followed by one injection of the calibration solution.

2.2.8 *Calculation*

Calculate the calibration factor f from chromatograms of the calibration solution.

$$f = \frac{W_C \times A_{CI}}{A_C \times W_{CI}}$$

where A_C = peak area of O,S-TEPP (or S,S-TEPP) in the calibration solution.
A_{CI} = peak area of the internal standard in the calibration solution.
W_C = mass of O,S-TEPP (or S,S-TEPP) in the final dilution of the calibration solution (mg).
W_{CI} = mass of internal standard in the final dilution of the calibration solution (mg).

Calculate the content of O,S-TEPP or S,S-TEPP from the chromatograms of the sample solution, as follows:

$$\textit{Content of O,S-TEPP (or S,S-TEPP) (g/kg)} = \frac{W_{SI} \times A_S \times f \times P}{A_{SI} \times W_S}$$

where A_S = peak area of O,S-TEPP (or S,S-TEPP) in the sample solution.
A_{SI} = peak area of the internal standard in the sample solution.
W_S = mass of O,S-TEPP or S,S-TEPP in the final dilution of the sample solution (mg).
W_{SI} = mass of internal standard in the final dilution of the sample solution (mg).
P = purity of O,S-TEPP or S,S-TEPP reference standards (g/kg).

The value of f to be used in the above equation is the average calibration factor of duplicate calibration solution injections preceding and following the sample solution injections.

DIAZINON WATER-DISPERSIBLE POWDER

Specification WHO/SIF/9.R6
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical diazinon 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 diazinon used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/9.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.

1.2.1 *Diazinon content (g/kg basis)*

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

Nominal content	*Tolerance permitted*
Up to 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 *pH of the aqueous dispersion*

The pH of the aqueous dispersion, determined by the method described in WHO/M/25, shall be lower than 7.0 and higher than 10.5.

1.2.3 *Sieving after heat stability treatment*

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

1.2.4 *Suspensibility*

In standard hard water after heat stability treatment. When tested by the method described in section 2.2, a minimum of 50% of the diazinon (5 g/l) shall be in suspension 30 minutes after agitating a suspension containing 10 g/l of diazinon, prepared in standard hard water from powder subjected to the heat stability treatment described in section 2.3.

1.2.5 *Heat stability*

The material after treatment as described in section 2.3 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 diazinon water-dispersible powder shall be packed in suitable clean drums, as specified in the order. The drums shall contain an inner liner or bag of polyethylene or equivalent, with a nominal thickness of 0.1 mm. The inner liner or bag shall be hermetically sealed after filling.

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

Manufacturer's name
Diazinon water-dispersible powder to specification WHO/SIF/9.R6
Diazinon .... g/kg
Batch or reference number, and date of test
Net weight of contents
Date of formulation

and the following minimum cautionary notice:

Diazinon is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. 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 Diazinon content

2.1.1 *Outline of method*

Diazinon is extracted from the sample with acetone to which aldrin is added as internal standard. An aliquot of the extract is introduced into a gas-liquid chromatograph and the ratio of the response of the diazinon to that of the internal standard is determined.

This is compared with the response of a standard of known purity to give the diazinon 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 and a high-sensitivity flame-ionization detector.

2. *Chromatographic column.* The column should be a borosilicate glass tube 180 cm long, 4 mm in internal diameter and 6 mm in external diameter, bent to fit the chromatograph.

3. *Column-packing material.* Gas-Chrom Q (80-100 mesh) treated with 10% silicone DC 200.

2.1.3 *Special reagents*

Diazinon standard. Analytical grade, of known purity.
Internal standard. Aldrin should be at least 900 g/kg HHDN purity and should contain no impurities that elute at the same retention time as diazinon.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weigh (to the nearest 0.1 g) about 4 g of technical aldrin into a 600 ml beaker. Mix with 400 ml of acetone to dissolve, filter through paper into a 1 litre volumetric flask, washing with several 100 ml portions of acetone. Dilute to volume and mix well.

Diazinon calibration solution. Weigh (to the nearest 0.1 mg) about 125 mg of diazinon standard into a 125 ml round bottle fitted with an aluminium-lined screw cap. Pipette in 50 ml of internal standard solution and shake mechanically for 30 minutes.

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	190 ± 10°C.
Injection port	240°C.
Flame-ionization detector	240°C.
Gas flow rates	
Hydrogen	As recommended for the detector
Air	by the manufacturer
Carrier gas (nitrogen or helium)	80-100 ml/min.
Attenuation	
Adjust until the peak heights of the diazinon and internal standard are 60 to 80% full scale.	
Retention times	
Diazinon peak	5-6 min.
Internal standard peak	10-12 min.

2.1.7 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of sample containing about 125 mg of diazinon directly into a 125 ml round bottle fitted with an aluminium-lined screw cap. Pipette in 50 ml of internal standard as used for the preparation of the diazinon calibration solution and shake mechanically for 30 minutes. Let insoluble materials settle or centrifuge a portion of the extract to obtain a clear solution.

Inject 3 µl aliquots of calibration solution until peak area (or height) ratios of diazinon to internal standard agree to within 1%. Then make duplicate injections of sample solution followed by duplicate injections of calibration solution. Peak area (or height) ratios of calibration solution injections must agree to within ±1% of first accepted calibration solution values. If not, repeat series of injections. Repeat for additional samples.

2.1.8 *Calculation*

Calculate the peak area (or height) ratios for both duplicate calibration solution injections preceding and following the sample solution injections. Average the four values.

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

Calculate and average the peak area (or height) ratios of the two sample solution injections. For each injection the response ratio (r) is given by the equation:

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

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

2.2 Suspensibility after heat stability treatment

2.2.1 *Outline of method*

A suspension of known concentration of diazinon in standard hard water is prepared, poured into a 250 ml graduated cylinder, maintained at a constant temperature, and allowed to remain undisturbed for 30 minutes. The top nine-tenths are drawn off and the content of diazinon in the bottom one-tenth is determined, so allowing to evaluate the active ingredient mass still in suspension after 30 minutes.

2.2.2 *Special apparatus*

1. *A 250 ml graduated cylinder* with a ground-glass stopper and a distance of 20-21.5 cm between the bottom and the 250 ml graduation.

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

2.2.3 *Special reagent*

Standard hard water. Dissolve 0.304 g of anhydrous calcium chloride and 0.139 g of magnesium chloride hexahydrate in distilled water and make up to 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.

2.2.4 *Procedure*

Weigh (to the nearest 1 mg) into a 100 ml beaker an amount of the sample to form 250 ml of a suspension containing 10 g/l of diazinon. Add a volume of water[1] at 30 ± 1°C equal to at least twice the mass of the sample taken. Allow to stand for 30 seconds and then stir by hand for 30 seconds with a glass rod, 4-6 mm in diameter, at not more than four revolutions per second, making no deliberate attempt to break up any lumps. Then immediately transfer the mixture quantitatively to the 250 ml graduated cylinder, using water at 30 ± 1°C for rinsing, and again avoiding mechanical disintegration of lumps. Immediately add sufficient water at 30 ± 1°C to bring the volume to the 250 ml mark. Stopper the cylinder and mix by inverting and righting it 30 times at a rate of one complete 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. Allow the graduated cylinder to stand for 30 minutes in a water-bath at 30 ± 1°C, taking care that the bath is free from vibrations.

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

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

Shake the retained one-tenth of the suspension for a short time and transfer to a 500 ml separating funnel using 100-150 ml of light petroleum (boiling range: 40-60°C) and 20 ml ethanol. Add 10 ml of a saturated aqueous solution of aluminium sulfate and shake the mixture. Let the layers separate with occasional slight horizontal swirling of the funnel. Drain the aqueous layer into a second separating funnel containing 100 ml light petroleum. If a stable emulsion is formed between the two phases, separate off the aqueous layer as far as the emulsion, then add 10 ml of a saturated aqueous solution of sodium chloride, shake, and run off the aqueous layer completely.

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

Shake the second separating funnel and then allow the layers to settle. Run off the aqueous layer into a third separating funnel and repeat the extraction with 100 ml light petroleum as above.

All the material soluble in light petroleum should have been extracted and the aqueous layer can be discarded. The three light petroleum extracts are then individually washed with a mixture of 50 ml distilled water and 10 ml of a saturated aqueous solution of sodium chloride using it successively for all three extracts. Separate each extract, carefully, from the last water droplets and then filter, successively, the first, second, and third extract, through a plug of cotton wool, into and Erlenmeyer flask. Rinse the plug of cotton wool with light petroleum. Distil off the solvent to about 100-150 ml volume and transfer to a 250 ml volumetric flask. Make up to volume with light petroleum and homogenize. Pipette 25 ml[2] of this solution into another flask and evaporate the solvent. Add to the residue exactly 50 ml of internal standard solution (section 2.1.4). Hold this sample solution for gas-liquid chromatographic analysis and continue as described in section 2.1.

Calculation

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

$$m_1 = \frac{r_1 \times m_o \times 10}{r_o} \quad 2.2.5$$

where r_0 = average response ratio for calibration solution
r_1 = average response ratio for sample solution
m_0 = mass (g) of diazinon standard in the calibration solution

From the value obtained in section 2.1.8 for the content of diazinon, calculate the mass of the active ingredient in the initial sample taken for the suspensibility test. Then

$$\textit{suspensibility (\%)} = \frac{(m_2 - m_1) \times 111.1}{m_2}$$

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

[2] If the volume taken to obtain the residue for analysis is not 25 ml out of 250 ml, correct the equation accordingly.

2.3 Heat stability treatment

Fill a 50 ml[3] 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 ± 1°C for 2 hours. After completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

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

DIAZINON EMULSIFIABLE CONCENTRATE

Specification WHO/SIF/13.R6
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of technical diazinon 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 diazinon used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/9.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.

1.2.1 *Diazinon content (g/kg basis)*

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

Nominal content	*Tolerance permitted*
Up to 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 *Impurities*

1.2.2.1 *O,O,O',O'-tetraethyl thiopyrophosphate (O,S-TEPP)*

The O,S-TEPP content determined by the method described in section 2.2 shall not be higher than 0.22 x x mg/kg, where x is the diazinon content in g/kg as found by the determination of the active ingredient in the formulation.

1.2.2.2 *O,O,O',O'-tetraethyl dithiopyrophosphate (S,S-TEPP)*

The S,S-TEPP content determined by the method described in section 2.2 shall not be higher than 2.8 x x mg/kg, where x is the diazinon content in g/kg as found by the determination of the active ingredient in the formulation.

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 the method described in WHO/M/3, shall not be higher than 0.5 g/kg calculated as H_2SO_4.

1.2.5 *Cold test*

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

1.2.6 *Flash point*

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

1.2.7 *Stability of the emulsion*

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

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

1.2.8 *Heat stability*

The emulsifiable 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 diazinon 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
Diazinon emulsifiable concentrate to specification WHO/SIF/13.R6
Diazinon .... g/kg
Batch or reference number, and date of test
Net weight of contents
Instructions for dilution
Date of formulation

and the following minimum cautionary notice:

Diazinon is an organophosphorus compound that inhibits cholinesterase. It is poisonous if swallowed. 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 Diazinon content

2.1.1 *Outline of method*

The sample is dissolved in acetone to which aldrin is added as internal standard. An aliquot of this mixture is introduced into a gas-liquid chromatograph and the ratio of the response of the diazinon to that of the internal standard is determined. This is compared with the response of a standard of known purity to give the diazinon 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 and a high-sensitivity flame-ionization detector.

2. *Chromatographic column.* The column should be a borosilicate glass tube 180 cm long, 4 mm in internal diameter and 6 mm in external diameter, bent to fit the chromatograph.

3. *Column-packing material.* Gas-Chrom Q (80-100 mesh) treated with 10% silicone DC 200.

2.1.3 *Special reagents*

Diazinon standard. Analytical grade, of known purity.
Internal standard. Aldrin should be at least 900 g/kg HHDN purity and should contain no impurities that elute at the same retention time as diazinon.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Weigh (to the nearest 100 mg) about 4 g of technical aldrin into a 600 ml beaker. Mix with 400 ml of acetone to dissolve, filter through paper into a 1000 ml volumetric flask, washing with several 100 ml portions of acetone. Dilute to volume and mix well.

Diazinon calibration solution. Weigh (to the nearest 0.1 mg) about 125 mg of diazinon standard into a 125 ml round bottle fitted with an aluminium-lined screw cap. Pipette in 50 ml of internal standard solution and shake mechanically for 30 minutes.

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	190 ± 10°C.
Injection port	240°C.
Flame-ionization detector	240°C.
Gas flow rates	
Hydrogen	As recommended for the detector
Air	by the manufacturer
Carrier gas (nitrogen or helium)	80-100 ml/min.

Attenuation
Adjust until the peak heights of the diazinon and internal standard are 60 to 80% full scale.

Retention times	
Diazinon peak	5-6 min.
Internal standard peak	10-12 min.

2.1.7 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 125 mg of diazinon directly into a 125 ml round bottle fitted with an aluminium-lined screw cap. Pipette in 50 ml of internal standard as used for the preparation of the diazinon calibration solution and shake mechanically for 30 minutes. Let insoluble materials, if any, to settle or centrifuge a portion to obtain a clear solution.

Inject 3 µl aliquots of calibration solution until peak area (or height) ratios of diazinon to internal standard agree to within 1%. Then make duplicate injections of sample solution followed by duplicate injections of calibration solution. Peak area (or height) ratios of calibration solution injections must agree to within ±1% of the first accepted calibration solution values. If not, repeat the series of injections. Repeat for additional samples.

2.1.8 *Calculation*

Calculate the peak area (or height) ratios for both duplicate calibration solution injections preceding and following the sample solution injections. Average the four values.

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

Calculate and average the peak area (or height) ratios of the two sample solution injections. For each injection the response ratio (r) is given by the equation:

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

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

2.2 Impurities (O,S-TEPP and S,S-TEPP) content

2.2.1 *Outline of method*

The sample is dissolved in methanol containing diethyl phthalate as internal standard. This solution is passed through a strong cation exchange resin column to remove diazinon. The eluate is reconcentrated, dissolved in 1,1,1-trichloroethane and O,S-TEPP and S,S-TEPP are determined by capillary gas-liquid chromatography with flame-ionization detection.

2.2.2 *Special apparatus*

1. *Gas-liquid chromatograph.* Carlo Erba HRGC 5160 or equivalent, equipped with cold on-column injection system and flame-ionization detector.

2. *Gas-liquid chromatographic column.* The column should be a fused silica 15 m x 0.32 mm id coated with OV 1701, film thickness 1 mm (supplier: J & W Scientific Inc). Precolumn: fused silica 20 cm x 0.53 mm id, uncoated, deactivated, connected to column by pressfit connector.

3. *Electrometer.* Sensitivity 10^{-9} A/V.

4. *Chromatographic columns.* 250 x 10 (id) mm.

5. *Rotary evaporator.*

2.2.3 *Special reagents*

1. *Dowex 50 W X 2.* strongly acidic cation exchange resin, 50-100 mesh; H^+-form (Fluka 44455 or Aldrich 21744-1).

2. *Reference standard O,S-TEPP* of known purity.

3. *Reference standard S,S-TEPP* of known purity.

4. *Internal standard.* Diethyl phthalate (Merck 822323) GLC grade.

2.2.4 *Preparation of standard solutions*

O,S-TEPP/internal standard stock solution. Accurately weigh ca. 10 mg O,S-TEPP reference standard and 50 mg diethyl phthalate internal standard into a 50 ml volumetric flask, dilute to volume with 1,1,1-trichloroethane and mix.

Internal standard solution. Accurately weigh ca. 20 mg internal standard into a 100 ml volumetric flask, dilute to volume with methanol and mix.

Calibration solution. Accurately weigh ca. 12.5 mg S,S-TEPP reference standard into a 100 ml volumetric flask. Add 5.0 ml O,S-TEPP/internal standard stock solution by pipette, dilute with 1,1,1-trichloroethane to volume and mix. (Approximate weights in final dilution (100 ml): 1 mg O,S-TEPP, 12.5 mg S,S-TEPP and 5 mg internal standard.)

2.2.5 *Preparation of ion exchange column*

Mix the ion exchange resin (ca. 7 g for one column) with methanol. Allow the slurry to stand for at least 1 hour. Pour the slurry into chromatographic column to give bed height of 8 to 10 cm after settling. Wash resin with methanol until effluent is clear, colourless and neutral. Do not let liquid level drain below the top of the resin bed.

2.2.6 *Operating conditions for gas-liquid chromatography*

The temperature, 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	programme: 1 min at 60°C; increase from
Injection port	60 to 250°C at the rate of 5°C/min - Maintain 250°C for 12 min.
Flame-ionization detector	270°C.
Electrometer sensitivity	0^{-9} A/V
Injection volume	0.5 µl.
Gas flow rates	
Carrier gas	H, linear velocity 40 cm/sec measured with dichloromethane at 60°C.
Make up gas	N, 50 ml/min.
Detector gases	H, 25 ml/min; air, 350 ml/min.
Retention times	
Internal standard peak	ca. 23.95 min.
O,S-TEPP peak	25.70 min.
S,S-TEPP	26.15 min.

2.2.7 *Preparation of sample and analysis*

Accurately weigh sufficient sample to contain ca. 1 g diazinon (based on nominal value) into a 10 ml volumetric flask, add 5.0 ml internal standard solution by pipette, dilute to volume with methanol and mix. Transfer 1.0 ml of this solution to the ion exchange column and allow the liquid level to reach the top of resin bed. Elute with 20 ml methanol. Collect the eluate in a 20 ml round-bottomed flask. Evaporate to dryness at 40°C and reduced pressure using a rotary evaporator. Dissolve the residue with 2.0 ml 1,1,1-trichloroethane. (Approximate weights in final dilution (2 ml): one-tenth of sample weight and 0.1 mg internal standard.)

Inject 0.5 µl aliquots of the calibration solution until the peak height ratio of O,S-TEPP/internal standard and S,S-TEPP/internal standard response varies <5% for successive injections. Then make duplicate injections of the sample followed by one injection of the calibration solution.

2.2.8 *Calculation*

Calculate the calibration factor f from chromatograms of the calibration solution.

$$f = \frac{W_C \times A_{CI}}{A_C \times W_{CI}}$$

where A_C = peak area of O,S-TEPP (or S,S-TEPP) in the calibration solution
A_{CI} = peak area of the internal standard in the calibration solution
W_C = mass of O,S-TEPP (or S,S-TEPP) in the final dilution of the calibration solution (mg)
W_{CI} = mass of internal standard in the final dilution of the calibration solution (mg)

Calculate the content of O,S-TEPP or S,S-TEPP from the chromatograms of the sample solution, as follows:

$$\text{Content of O,S-TEPP (or S,S-TEPP) (g/kg)} = \frac{W_{SI} \times A_S \times f \times P}{A_{SI} \times W_S}$$

where A_S = peak area of O,S-TEPP (or S,S-TEPP) in the sample solution
A_{SI} = peak area of the internal standard in the sample solution
W_S = mass of O,S-TEPP or S,S-TEPP in the final dilution of the sample solution (mg)
W_{SI} = mass of internal standard in the final dilution of the sample solution (mg)
P = purity of O,S-TEPP or S,S-TEPP reference standards (g/kg)

The value of f to be used in the above equation is the average calibration factor of duplicate calibration solution injections preceding and following the sample solution injections.

Note

In emulsifiable concentrate formulations components of solvents or other auxiliaries may co-elute in the chromatogram with the internal standard or O,S-TEPP or S,S-TEPP. To confirm the specificity of the method a mass selective detector can be used. In this case adjust the concentrations of calibration and sample solutions by dilution if necessary. Typical m/z-values: O,S-TEPP: 97, 129, 194, 306; S,S-TEPP: 93, 97, 121, 322; diethyl phthalate: 149, 177, 222.

2.3 Heat stability

Keep 100 ml of the sample for three days at a temperature of 54 ± 2°C in a glass container sealed to avoid loss of volatile solvent, and then cool to room temperature.