TECHNICAL DIAZINON

Full specification WHO/SIT/9.R7
Revised 10 December 1999

1. Specification

1.1 Description

The material shall consist of diazinon together with related manufacturing impurities and shall be in the form of a yellow to brown liquid, free from visible extraneous matter 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.R1), shall comply with the requirements of section 1.2 and with the following requirements.

1.2.1 *Identity test*

Where the identity of the active ingredient is in doubt, then it shall be comply with at least one additional test.

1.2.2 *Diazinon content (g/kg basis)*

The diazinon content of the remainder of the material (i.e. excluding the stabilizer) shall be declared (not less than 950 g/kg) and when determined by the method described in section 2.1, the mean measured content shall not be lower than the declared content.

1.2.3 *Impurities*

1.2.3.1 *$\underline{0},\underline{0},\underline{0}',\underline{0}'$-tetraethyl thiopyrophosphate ($\underline{O},\underline{S}$-TEPP)*

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

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[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.

1.2.3.2 $\underline{0},\underline{0},\underline{0}',\underline{0}'$-tetraethyl dithiopyrophosphate ($\underline{S},\underline{S}$-TEPP)

The $\underline{S},\underline{S}$-TEPP content determined by the method described in section 2.2 shall not be higher than 2.5 g/kg.

1.2.4   *Acidity*

The acidity of the material, determined by the CIPAC method MT 31 (CIPAC Handbook F, p.96), shall not be higher than 0.3 g/kg calculated as H_2SO_4.

1.2.5   *Material insoluble in acetone*

The material insoluble in acetone, determined by the CIPAC method MT 27 (CIPAC Handbook F, p.88), shall not be higher than 1.5 g/kg.

1.2.6   *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   g/kg
> 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 *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, or equivalent.

2.1.3 *Reagents*

Diazinon standard. Analytical grade, of known purity.
Internal standard. Aldrin (technical grade) 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 *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°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.

2.1.6   *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.

2.1.7   *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.
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:

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

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

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   *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 μm (supplier: J & W Scientific Inc, or equivalent). Precolumn: fused silica 20 cm x 0.53 mm id, uncoated, deactivated, connected to column by pressfit connector, or equivalent.

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

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

5.   *Rotary evaporator.*

2.2.3   *Reagents*

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

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, or equivalent) 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 60 to 250°C at the rate of 5°C/min - Maintain 250°C for 12 min.
Injection port	250°C
Flame-ionization detector	270°C.
Electrometer sensitivity	10^{-9} A/V
Injection volume	0.5 μL.
Gas flow rates	
Carrier gas: hydrogen	linear velocity 40 cm/sec measured with dichloromethane at 60°C.
Make up gas: nitrogen	50 mL/min.
Detector gases: hydrogen	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 about 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.

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

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:

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

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 WETTABLE POWDER

Full specification WHO/SIF/9.R7
Revised 10 December 1999

1.   Specification

1.1   Description

The material shall consist of a homogeneous mixture of technical diazinon, complying with the requirements of WHO specification WHO/SIT/9.R7, in the form of a fine, free-flowing powder that wets out readily on stirring into water, together with filler(s) and other necessary formulants. It shall be free from visible extraneous matter and hard lump.

1.2   Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1.R1), 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 declared content by more than the following amounts:

Declared content	*Tolerance permitted*
Above 250 up to 500 g/kg	±5% of the declared content
Above 500 g/kg	±25 g/kg

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

1.2.2   *pH of the aqueous dispersion*

The pH of the aqueous dispersion, determined by the CIPAC method MT 75 (CIPAC Handbook F, p.205), shall be not lower than 7.0 and not higher than 10.5.

1.2.3   *Wet 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 CIPAC method MT 59.3 (CIPAC Handbook F, p.179).

1.2.4   *Suspensibility after heat stability treatment*

In WHO standard hard water. When tested by the method described in section 2.2 (equivalent to CIPAC method MT 15.1, CIPAC Handbook F, p.45), a minimum of 50% of the diazinon (5.0 g/l) shall be in suspension 30 minutes after agitating a suspension containing 10 g/L of diazinon, prepared in WHO standard hard water from powder subjected to the heat stability treatment described in section 2.3.

1.2.5   *Persistent foam*

In WHO standard soft water (WHO/M/29). When tested by the method CIPAC MT 47.2 (CIPAC Handbook F, p.152) a maximum of 60 mL of foam shall be observed after 1 minute.

1.2.6   *Wettability*

In WHO standard hard water (WHO/M/29) after heat stability treatment. When tested by the method CIPAC MT 53.3 (CIPAC Handbook F, p.164) the formulation shall be completely wetted in 2 min. without swirling

1.2.7   *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 wettable 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 wettable powder
> Diazinon .... g/kg
> Batch or reference number, and date of test
> Net weight of contents
> Date of formulation
> Instruction for use

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 *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, or equivalent.

2.1.3 *Reagents*

Diazinon standard. Analytical grade, of known purity.
Internal standard. Aldrin (technical grade) 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   *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°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.

2.1.6   *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. 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:

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

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

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

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   *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   *Reagent*

WHO standard hard water. See method WHO/M/29

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 $30 \pm 2°C$ equal to at least twice the mass of the sample taken. Allow to stand for 30 seconds and then stir by hand for 30 seconds with a glass rod, 4-6 mm in diameter, at

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

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 $30 \pm 2°C$ for rinsing, and again avoiding mechanical disintegration of lumps. Immediately add sufficient water at $30 \pm 2°C$ 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. Allow the graduated cylinder to stand for 30 minutes in a water-bath at $30 \pm 2°C$, 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.

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

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

sample solution for gas-liquid chromatographic analysis and continue as described in section 2.1.

2.2.5   *Calculation*

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

$$m_1 \ = \ \frac{r_1 \ x \ m_o \ x \ 10}{r_o}$$

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

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

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

2.3   **Heat stability treatment**

54°C ± 2°C for 14 days (CIPAC method MT 46.1, CIPAC Handbook F, p.149), unless other temperatures and times are requested (FAO Manual on the development and use of FAO specifications for plant protection products, n° 149, p.33).

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

If required the test should be conducted in commercial type pack.

DIAZINON EMULSIFIABLE CONCENTRATE

Full specification WHO/SIF/13.R7
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1.   Specification

1.1   Description

The material shall consist of technical diazinon, complying with the WHO specification WHO/SIT/9.R7, dissolved in suitable solvents, together with any necessary formulants. It shall be in the form of a stable homogeneous liquid, free from visible suspended matter and sediment to be applied as an emulsion after dilution in water.

1.2   Chemical and physical requirements

The material, sampled from any part of the consignment (see method WHO/M/1.R1), 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 declared content by more than the following amounts:

Declared content	*Tolerance permitted*
Above 250 up to 500 g/kg	$\pm$ 5% of the declared content
Above 500 g/kg	$\pm$ 25 g/kg

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

1.2.2   *Impurities*

1.2.2.1 $\underline{O},\underline{O},\underline{O}',\underline{O}$*-tetraethyl thiopyrophosphate (*$\underline{O}$,$\underline{S}$*-TEPP)*

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

1.2.2.2   *0,0,0',0'-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 $\underline{x}$ mg/kg, where $\underline{x}$ 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 CIPAC method MT 31.2 (CIPAC Handbook F, p.99), 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 by the CIPAC method MT 39 (CIPAC Handbook F,p.128).

1.2.6   *Flash point*

The flash point of the product, determined by the CIPAC method MT 12 (CIPAC Handbook F, p.75), shall comply with all national and/or international transport regulations.

1.2.7   *Stability of the emulsion*

In WHO 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.R4.

In WHO 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.R4.

1.2.8   *Persistent foam*

In WHO standard soft water: When tested by the CIPAC method MT 47.2 (CIPAC Handbook F, p.152) a maximum of 60 mL of foam shall be observed after 1 minute.

1.2.9   *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
> Diazinon .... g/kg
> Batch or reference number, and date of test
> Net weight of contents
> Instructions for use
> 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   *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, or equivalent.

2.1.3   *Reagents*

Diazinon standard.   Analytical grade, of known purity.
Internal standard.   Aldrin (technical grade) 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   *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°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.

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

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:.

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

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   *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 μm (supplier: J & W Scientific Inc, or equivalent). Precolumn: fused silica 20 cm x 0.53 mm id, uncoated, deactivated, connected to column by pressfit connector, or equivalent.

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

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

5. *Rotary evaporator.*

2.2.3   *Reagents*

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

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, or equivalent) 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 60 to 250°C at the rate of 5°C/min - Maintain 250°C for 12 min.

Injection port:	270°C
Flame-ionization detector	270°C.
Electrometer sensitivity	10^{-9} A/V
Injection volume	0.5 μL.

Gas flow rates

Carrier gas: hydrogen	linear velocity 40 cm/sec measured with dichloromethane at 60°C.
Make up gas: nitrogen	50 mL/min.
Detector gases: hydrogen	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 about 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.

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

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:

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

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 treatment

54°C ± 2°C for 14 days (CIPAC method MT 46.1, CIPAC Handbook F, p.149), unless other temperatures and times are requested (FAO Manual on the development and use of FAO specifications for plant protection products, n° 149, p.33).

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

If required the test should be conducted in commercial type pack.