

TECHNICAL DDT

Specification WHO/SIT/1.R7
Approved 25 September 1989

Preliminary remark

The present method used in the DDT specifications is based on a GLC method which determines specifically the p,p'-isomer content. However, as the dose rates of this insecticide are still expressed on the basis of technical products, it is necessary to relate the p,p'-DDT content to the technical product and vice versa.

The following conversion tables are based on a technical product containing an average of 720 g/kg p,p'-DDT.

DDT TECHNICAL CONTENT	p,p'-DDT CONTENT	p,p'-DDT CONTENT	DDT TECHNICAL CONTENT
200 g/kg	144 g/kg	150 g/kg	208 g/kg
250 g/kg	180 g/kg	175 g/kg	243 g/kg
400 g/kg	288 g/kg	250 g/kg	347 g/kg
500 g/kg	360 g/kg	400 g/kg	556 g/kg
600 g/kg	432 g/kg	500 g/kg	694 g/kg
750 g/kg	540 g/kg	540 g/kg	750 g/kg

This table has to be adapted for other p,p'-DDT contents in technical products.

1. Specification

1.1 Material

The material shall consist of DDT together with related manufacturing compounds and shall be in the form of white or cream-colored granules, flakes, or powder, 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 *Setting point*

The setting point¹ determined by the method described in section 2.1 shall not be less than 89°C.

1.2.2 *p,p'-DDT content (g/kg basis)*

The p,p'-DDT content shall be declared (not less than 720 g/kg) and, when determined by the method described in section 2.2, the content obtained shall not differ from that declared by more than ± 20 g/kg.

1.2.3 *Chloral hydrate content*

The chloral hydrate content determined by the method described in section 2.3 shall not be higher than 0.25 g/kg.

1.2.4 *Acidity*

The acidity of the material determined by the method described in WHO/M/3 shall not be higher than 3.0 g/kg calculated as H₂SO₄.

1.2.5 *Material insoluble in acetone*

The material insoluble in acetone as determined by the method described in WHO/M/21.R1, shall not be higher than 10 g/kg.

1.2.6 *Water content*

The water content, determined by the method described in section 2.4, shall not be higher than 10 g/kg.

1.3 **Packing and marking of packages**

The technical DDT 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 DDT to specification WHO/SIT/1.R7
p,p'-Isomer content, ... g/kg
Batch or reference number, and date of test
Net weight of contents

¹ It is recommended that technical DDT with a higher setting point be used for formulating 540 g/kg p,p'-DDT water-dispersible powders in order to attain the quality specified under WHO/SIF/1.R7 and WHO/SIF/26.R4 especially in regard to suspensibility, sieving after heat stability treatment, and storage life.

and the following minimum cautionary notice:

Keep well away from foodstuffs and animal feed and their containers.

2. Methods of determining chemical and physical properties

2.1 Setting-point

2.1.1 Outline of method

The sample is melted and then allowed to cool. The setting-point is the temperature at which solidification occurs.

2.1.2 Procedure

Place in a 20 cm boiling-tube of 2.5 cm internal diameter, with a wall thickness of 2 ± 0.1 mm, a sufficient amount of the sample to give, when melted, a depth of liquid of approximately 7.5 cm. Melt carefully by immersing the tube to a depth of 10 cm in an oil-bath at a maximum temperature of 100°C . If hydrogen chloride is evolved during the melting, another portion of the sample must be taken and melted in a bath at a lower temperature.

Fit the boiling-tube with a cork collar and insert it to within 1.3 cm of the bottom of a 15 cm boiling-tube of approximately 4 cm diameter; then immerse the two tubes to a depth of 10 cm in a water-bath maintained at a temperature $5\text{-}8^{\circ}\text{C}$ below the anticipated setting-point. Place in the inner boiling tube, a stirrer, consisting of a stainless steel rod 1.5 mm in diameter bent in the form of a helix with six turns of 1.8 cm outside diameter. A thermometer graduated in one-tenths of a degree is then clamped in a central position with its bulb 2.5 cm from the bottom of the tube. Stir at the rate of about two strokes per second, by moving the stirrer up and down, until the material begins to thicken at which point stir vigorously to work into the melt any material that has solidified on the walls of the tube. Stop stirring when the temperature ceases dropping and remains constant for some time². Record this temperature as the setting-point after making any corrections necessary for thermometer calibration and emergent stem.

Emergent-stem correction. The correction for the emergent stem in mercury-filled thermometers, to be added to the temperature reading, is calculated from the following formula:

$$TC = N \times 0.00015 \times (T - t)$$

- Tc = correction to be applied to the observed temperature of the setting point.
N = number of degrees on the scale of the thermometer between the top of the inner boiling-tube and the level of the mercury.
T = temperature reading on the thermometer in the melt.
t = temperature of the stem at the midpoint of the exposed mercury thread.

² In some instances, there may be a slight rise in the temperature of the material; if this occurs, record the highest steady temperature as the setting-point.

2.2 p,p'-DDT content

2.2.1 Outline of method

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

2.1.2 Special apparatus

1. **Gas-liquid chromatograph.** The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame-ionization detector, an electrometer having a sensitivity of at least 10^{-11} amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1 mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.
2. **Chromatographic column.** The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. **Column-packing material.** Chromosorb W-HP (100-120 mesh) treated with 5% OV-210.
4. **Glass wool, silane-treated.**

2.2.3 Special reagents

p,p'-DDT standard. Analytical grade of known purity.

Internal standard. 2,2'-Dinitrobiphenyl.

2.2.4 Preparation of standard solutions

Internal standard solution. Prepare a 28 g/l solution of the internal standard in chloroform. This solution is stable for four weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

p,p'-DDT calibration solutions. Weigh (to the nearest 0.1 mg) about 150, 200, and 300 mg quantities of the *p,p'*-DDT standard directly into separate 50 ml stoppered conical flasks equipped with teflon-lined screw caps.

To each flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap each flask tightly and gently swirl the contents of each flask for 1 minute using a rotational motion of the wrist. Allow each flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow each flask to stand for 30 minutes. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.2.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity specifications described in section 2.2.7 can be met.

2.2.5 *Preparation and conditioning of column*

See method WHO/M/20.

2.2.6 *Operating conditions for gas liquid chromatography*

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

Temperatures

Oven	170 ⁰ C
Injection port	250 ⁰ C
Detector	275 ⁰ C

Gas flow rates

Hydrogen	30 ml/min
Air	300 ml/min
Carrier gas (helium or nitrogen)	30 ml/min

Retention times

<i>p,p'</i> -DDT peak	12.4 min
Internal standard peak	19.5 min

2.2.7 *Linearity check*

The gas-liquid chromatograph should be checked for linearity at least once a week, and the same check should be carried out whenever new calibration solutions are prepared and whenever a column, new or used, is installed in the instrument. Using digital integration for peak area measurements, determine the appropriate attenuation setting and the quantity (between 2 and 4 μl) of calibration solution B that must be injected to yield an area count of at least 100 000 (optimum electrometer output with acceptable noise level). The attenuation setting quantity so determined should be used for all samples and calibration solutions in the set.

Inject triplicate aliquots of appropriate volume (as determined above) of calibration solutions A, B and C into the gas-liquid chromatograph, determine the response ratio for each injection, and average the resulting ratios for each solution. Divide the average response ratio for each solution by the corresponding p,p'-DDT content (in mg) and compare the resulting response factors. These factors should agree to within 2%.

Failure to meet this requirement indicates either a weighing error in the preparation of one of the calibration solutions or instrumental difficulties, which must be corrected before proceeding with the analysis of samples.

2.2.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of active ingredient (p,p'-DDT) into a 50 ml stoppered conical flask equipped with teflon-lined screw cap (the amount of sample to be taken should be 278 mg for a 720 g/kg p,p'-DDT content in the technical material). To the flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap the flask tightly, and gently swirl the contents for 1 minute using a rotational motion of the wrist. Allow the flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow the flask to stand for 30 minutes.

Inject duplicate aliquots of appropriate volume (as determined in section 2.2.7) of calibration solution B. The response ratios should agree to within 2%. If this precision limit is not met, inject two more aliquots of the solution. Failure to meet the precision requirement with the second pair of injections indicates instrumental difficulties, which must be resolved before proceeding with the analyses.

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

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

Use this average to calculate the p,p-DDT content of the sample.

2.2.9 Calculation

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

$$r = \frac{\text{area of } p, p' - \text{DDT peak}}{\text{area of internal standard peak}}$$

$$p, p' - \text{DDT content (g / kg)} = \frac{r_2 \times m_1 \times P}{r_1 \times m_2}$$

- r_1 = average response ratio for calibration solution B
 r_2 = average response ratio for sample solution
 m_1 = mass (g) of p,p'-DDT standard in the calibration solution B
 m_2 = mass (g) of sample taken
 P = purity of p,p'-DDT standard (g/kg)

2.3 Chloral hydrate content

2.3.1 Outline of method

The sample is mixed with water and the chloral hydrate distilled off. The distillate is treated with sodium hydroxide and pyridine, and the resulting color is compared with that from a standard chloral hydrate solution.

2.3.2 Special reagent

Standard chloral hydrate solution. Dissolve 5 mg of chloral hydrate in 100 ml of distilled water.

2.3.3 Special apparatus

Distillation apparatus consisting of:

- a 500 ml round-bottomed flask, fitted with two necks
- a mechanical stirrer, mercury sealed, to fit flask
- a distillation set to fit flask, the condenser should be a double surface pattern, e.g., Davies type.

2.3.4 Procedure

Place 20 g of the sample and 200 ml of carbon dioxide-free distilled water in a 500 ml round-bottomed flask equipped with a mercury-sealed mechanical stirrer. Heat in an oil-bath at a temperature between 140⁰C and 160⁰C, with rapid stirring to prevent superheating, and distil the mixture through a well cooled condenser, at such a rate that

100 ml of distillate are obtained in not less than 3 minutes and not more than 1 hour. Collect exactly 100 ml of distillate in a centrifuge tube and centrifuge to effect complete separation of the water-insoluble material.

Place 2 ml of a 400 g/l sodium hydroxide solution in a test-tube and add 1 ml of colorless pyridine and 4 ml of the distillate. In another test-tube, place 2 ml of the same 400 g/l sodium hydroxide solution and add 1 ml of colorless pyridine and 4 ml of standard chloral hydrate solution. Shake the two tubes and heat in a bath of boiling water for 1 minute. The red color that develops in the pyridine layer shall not be darker in the sample solution than in the standard solution.

2.4 Water content

Determine the water content by the Karl Fischer electrometric titration method (see WHO/M/7.R1) or by the Dean and Stark distillation method (see WHO/M/8.R1). The latter may not always be practicable owing to its unreliability at very low water contents. In the event of a dispute, the Karl Fischer method shall be the referee method.

DDT WATER-DISPERSIBLE POWDER*

Specification WHO/SIF/1.R7
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical DDT together with filler(s) and other necessary formulants, and shall be in the form of a fine, free-flowing, white to cream-colored powder that wets out readily on stirring into water and does not produce undue foaming under normal conditions of use. The technical DDT used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/1.R7.

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 *p,p'*-DDT content (g/kg basis)

The content of *p,p'*-DDT, 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 360 g/kg ¹	± 6% of the nominal content
Above 360 g/kg ¹	± 20 g/kg

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

1.2.2 *Acidity or alkalinity*

The acidity or alkalinity of the powder, determined by the method described in WHO/M/3, shall not be higher than 2 g/kg calculated as H₂SO₄ or 2 g/kg calculated as NaOH

* If DDT water-dispersible powder for overseas shipment is required, specification WHO/SIF/26.R4 should be used.

¹ This 360 g/kg nominal content of *p,p'*-DDT corresponds to 500 g/kg technical DDT of which the *pp'*-DDT content is 720 g/kg (see "Preliminary remarks" in technical DDT specification WHO/SIT/1.R7)

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 60% of the p,p'-DDT (10.8 g/l) shall be in suspension 30 minutes after agitating a suspension containing 18 g/l of p,p'-DDT; prepared in standard hard water from the powder subjected to the heat stability treatment described in section 2.3.

1.2.5 *Heat stability*

The powder 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 DDT water-dispersible powder shall be packed in suitable, clean drums, as specified in the order. The drums shall have a minimum capacity of 2 litres for every kilogram of DDT powder and 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
DDT water-dispersible powder to specification WHO/SIF/1.R7
p,p'-DDT, ... g/kg, corresponding to ... g/kg technical DDT
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Keep well away from foodstuffs and animal feed and their containers.

2. Methods of determining chemical and physical properties

2.1 p,p'-DDT content

2.1.1 *Outline of method*

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

2.1.2 *Special apparatus*

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame-ionization detector, and electrometer having a sensitivity of at least 10^{-11} amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1 mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.
2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 5% OV-210.
4. *Glass wool, silane-treated*

2.1.3 *Special reagents*

p,p'-DDT standard. Analytical grade, of known purity.
Internal standard. 2,2'-Dinitrobiphenyl.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Prepare a 28 g/l solution of the internal standard in chloroform. This solution is stable for four weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

p,p'-DDT calibration solutions. Weigh (to the nearest 0.1 mg) about 150, 200, and 300 mg quantities of the p,p'-DDT standard directly into separate 50 ml stoppered conical flasks equipped with teflon-lined screw caps. To each flask add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap each flask tightly and gently swirl the contents of each flask for 1 minute using a rotational motion of the wrist. Allow each flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow each flask to stand for 30 minutes. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.1.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity specifications described in section 2.1.7 can be met.

2.1.5 *Preparation and conditioning of column*

See method WHO/M/20.

2.1.6 *Operating conditions for gas liquid chromatography*

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

Temperatures

Oven	170°C
Injection port	250°C
Flame-ionization detector	275°C

Gas flow rates

Hydrogen	30 ml/min.
Air	300 ml/min.
Carrier gas (helium or nitrogen)	30 ml/min.

Retention times

p,p'-DDT peak	12.4 min.
Internal standard peak	19.5 min.

2.1.7 *Linearity check*

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

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

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

2.1.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of active ingredient (p,p'-DDT) into a 50 ml stoppered conical flask equipped with teflon-lined screw cap (to estimate the amount of sample which must be taken, consider the nominal percentage of technical DDT for the formulation and assume 720 g/kg p,p'-DDT in the technical material). To the flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap the flask tightly and gently swirl the contents for 1 minute using a rotational motion of the wrist. Allow the flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow the flask to stand for 30 minutes. Take a 10 ml aliquot centrifuge or filter before injection.

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

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

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

Use this average to calculate the p,p'-DDT content of the sample.

2.1.9 Calculation

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

$$r = \frac{\text{area of } p, p' - \text{DDT peak}}{\text{area of internal standard peak}}$$

$$p, p' - \text{DDT content (g / kg)} = \frac{r_2 \times m_1 \times P}{r_1 \times m_2}$$

- r_1 = average response ratio for calibration solution B
 r_2 = average response ratio for sample solution
 m_1 = mass (g) of p,p'-DDT standard in the calibration solution B
 m_2 = mass (g) of sample taken
 P = purity of p,p'-DDT standard (g/kg)

2.2 Suspending ability after heat stability treatment

2.2.1 Outline of method

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

2.2.2 Special apparatus

1. A 250 ml graduated cylinder with a ground-glass stopper and a distance of 20-21.5 cm between the bottom and the 250 ml calibration mark.
2. A glass tube, about 40 cm long and about 5 mm in internal diameter, pointed at one end to an opening of 2-3 mm, the other end being connected to a suitable source of suction.

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 the method WHO/M/26 and correct if appropriate.

2.2.4 *Procedure*

Weigh (to the nearest 10 mg) into a 100 ml beaker an amount of the sample to form 250 ml of a suspension containing 18 g/l of p,p'-DDT. Add a volume of water² at $30 \pm 1^\circ\text{C}$ 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 4 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 $30 \pm 1^\circ\text{C}$ for rinsing and again avoid mechanical disintegration of lumps. Immediately add sufficient water at $30 \pm 1^\circ\text{C}$ 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 $30 \pm 1^\circ\text{C}$, 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.

Determine the mass of p,p'-DDT in the retained bottom one-tenth of the suspension, including the sediment, by transferring it quantitatively with water into a tared large evaporating dish (w'g). Evaporate the water by heating on a boiling water-bath. Remove the dish as soon as the last traces of water have evaporated. Dry in an oven at 100°C for 15 minutes. Cool and reweigh (w g).

Alternatively, evaporate the water by heating in a forced-draught oven at 100°C . Remove the dish from the oven as soon as the last traces of water have evaporated in order to avoid overheating the sample. Cool and reweigh (w g).

² Whenever water is mentioned in this section, use standard hard water.

$$m = \text{mass of residue (in g)} = \underline{w} - \underline{w}'$$

\underline{w} = mass of the evaporating dish containing the residue (in g)

\underline{w}' = mass of the evaporating dish (in g).

Homogenize carefully the residue. Transfer a quantity of sample containing about 200 mg of p,p'-DDT³ weighed to the nearest 0.1 mg to a 50 ml stoppered conical flask equipped with teflon-lined screw cap. Add by pipette 5 ml of internal standard solution and 20 ml of chloroform and continue as described in section 2.1.8.

Calculate the p,p'-DDT content (\underline{p} g/kg) according to section 2.1.9. The total mass of p,p'-DDT (\underline{m}_1) in the retained bottom one-tenth of the suspension is:

$$\underline{m}_1 = \frac{\underline{p} \times \underline{m}}{1000}$$

where: \underline{m} = mass of residue (g) determined here above.

2.2.5 Calculation

From the value obtained in section 2.1 for the content of p,p'-DDT (g/kg), calculate the mass of p,p'-DDT (\underline{m}_2) in the initial sample taken for the suspensibility test.

$$\text{Suspensibility (\%)} = \frac{(\underline{m}_2 - \underline{m}_1) \times 111.1}{\underline{m}_2}$$

where: \underline{m}_1 = total mass of p,p'-DDT in the retained bottom one-tenth of the suspension (g).

\underline{m}_2 = mass of p,p'-DDT in the initial sample (g).

³ The sample weight should be 555 mg assuming that the nominal content of the formulation is 500 g/kg of technical DDT with p,p'-DDT content of 720 g/kg. But if segregation between p,p'-DDT and formulants occurs during the sedimentation, the sample weight has to be adapted accordingly.

2.3 Heat stability treatment

Fill a 50 ml⁴ wide-mouthed glass bottle to within 1 cm of the top with the sample. Seal the bottle with a phenolic plastic cap having a soft liner. Turn the cap firmly to ensure a tight seal and place the bottle in a forced-draught oven maintained at $54 \pm 2^\circ\text{C}$ for 3 days. At the end of the heating period, remove the bottle from the oven and allow it to come to room temperature before removing the cap.

After completion of the heat stability treatment, the sample should be exposed to heat, bright sunshine, or high atmospheric humidity.

⁴ 100 ml if a larger quantity of the sample is required.

DDT WATER-DISPERSIBLE POWDER FOR OVERSEAS SHIPMENT

Specification WHO/SIF/26.R4
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical DDT together with filler(s) and any other necessary formulants. It shall be in the form of a fine, free-flowing, white to cream-colored powder that wets out readily on stirring into water and does not produce undue foaming under normal conditions of use. The technical DDT used in the manufacture of the water-dispersible powder shall comply with the requirements of specification WHO/SIT/1.R7.

1.2 Chemical and physical requirements

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

1.2.1 *p,p'*-DDT content (g/kg basis)

The content of *p,p'*-DDT, determined by the method described in section, 2.1, shall not differ from the nominal content by more than the following amounts:

Nominal content
360 g/kg and above²

Tolerance permitted
± 20 g/kg

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

1.2.2 *Acidity or alkalinity*

The acidity or alkalinity of the powder, determined by the method described in WHO/M/3, shall not be greater than 2 g/kg, calculated as H₂SO₄ or 2 g/kg calculated as NaOH.

¹ Samples for the storage life test (section 1.2.5) should be taken at the same time as samples for tests for the other chemical and physical requirements.

² This 360 g/kg content of *p,p'*-DDT corresponds to 500 g/kg technical DDT of which the *p,p'*-DDT content is 720 g/kg (see "Preliminary remarks" in technical DDT specification WHO/SIT/1.R7).

1.2.3 *Sieving after heat stability treatment*

Not less than 98% of the powder after heat stability treatment, as described in section 2.4, shall pass through a 75 mm sieve when tested by the method described in section 2.3.

1.2.4 *Suspensibility*

In standard soft water without pretreatment. When tested by the method described in section 2.2, a minimum of 70% of the p,p'-DDT (12.6 g/l) shall be in suspension 30 minutes after agitating a suspension containing 18 g/l of p,p'-DDT, prepared in standard soft water from the powder as received.

In standard hard water after heat stability treatment. When tested by the method described in section 2.2, a minimum of 65% of the p,p'-DDT (11.7 g/l) shall be in suspension 30 minutes after agitating a suspension containing 18 g/l of p,p'-DDT, prepared in standard hard water from the powder subjected to the heat stability treatment described in section 2.4.

1.2.5 *Storage life*³

Any sample⁴ of powder taken from a consignment at the time of offer by the manufacturer and stored in moisture-proof containers at an ambient temperature between 21⁰C and 38⁰C for a period of up to 12 months shall have a minimum of 50% (9 g/l) of the p,p'-DDT in suspension 30 minutes after agitating a suspension containing 18 g/l of p,p'-DDT prepared in standard hard water when tested by the method described in section 2.2.

³ This requirement may be waived in certain circumstances, but it should be maintained for all purchases of DDT powders intended for use in malaria control programs, in order to ensure that powders meeting all other requirements will maintain minimum suspensibility for a period of at least 12 months. It is recommended that before the end of the 12-month storage period, the purchaser or his agent should examine at his discretion a part or all of the batch samples held in storage for compliance with this requirement. The following procedure is suggested. (1) After at least 9 months in storage, examine 20% of the batch samples in a given consignment by the visual suspensibility test (WHO/M/2.R2) conducted in standard hard water without pretreatment. If any of the samples exhibit questionable suspensibility, retest them by the procedure described in section 2.2, using standard hard water. (2) If any of the samples so examined fail to pass the test, all the batch samples in the consignment should be examined as described in (1).

⁴ A sampling procedure is described in method WHO/M/1. However, this does not preclude the purchaser from sampling in any way he considers desirable. Samples should be taken at the same time as those for the tests described in sections 1.2.1, 1.2.2, 1.2.3, and 1.2.4.

1.3 Packing and marking of packages

The DDT water-dispersible powder shall be packed in suitable clean drums or boxes⁵ as specified in the order. The containers shall have a minimum capacity of 2 litres for every kilogram of powder and 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
DDT water-dispersible powder to specification WHO/SIF/26.R4
p,p'-DDT, ... g/kg, corresponding to ... g/kg technical DDT
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Keep well away from foodstuffs and animal feed and their containers.

2. Methods of determining chemical and physical properties

2.1 p,p'-DDT content

2.1.1 *Outline of method*

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

2.1.2 *Special apparatus*

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

⁵ Specifications for boxes of adequate strength to withstand overseas shipment may be obtained, on request, from the World Health Organization, 1211 Geneva 27, Switzerland

should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.

2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 5% OV-210.
4. *Glass wool, silane-treated.*

2.2.3 *Special reagents*

p,p'-DDT standard. Analytical grade of known purity.

Internal standard. 2,2'-Dinitrobiphenyl.

2.2.4 *Preparation of standard solutions*

Internal standard solution. Prepare a 28 g/l solution of the internal standard in chloroform. This solution is stable for four weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

p,p'-DDT calibration solutions. Weigh (to the nearest 0.1 mg) about 150, 200, and 300 mg quantities of the p,p'-DDT standard directly into separate 50 ml stoppered conical flasks equipped with teflon-lined screw caps. To each flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap each flask tightly and gently swirl the contents of each flask for 1 minute using a rotational motion of the wrist. Allow each flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow each flask to stand for 30 minutes. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.2.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity specifications described in section 2.2.7 can be met.

2.2.5 *Preparation and conditioning of column*

See method WHO/M/20.

2.2.6 *Operating conditions for gas liquid chromatography*

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

Temperatures

Oven	170 ⁰ C
Injection port	250 ⁰ C
Detector	275 ⁰ C

Gas flow rates

Hydrogen	30 ml/min
Air	300 ml/min
Carrier gas (helium or nitrogen)	30 ml/min

Retention times

p,p'-DDT peak	12.4 min
Internal standard peak	19.5 min

2.2.7 *Linearity check*

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

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

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

2.2.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of active ingredient (p,p'-DDT) into a 50 ml stoppered conical flask equipped with teflon-lined screw cap (to estimate the amount of sample which must be taken, consider the nominal percentage of technical DDT for the formulation and assume 720 g/kg p,p'-DDT in the technical material).

To the flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap the flask tightly, and gently swirl the contents for 1 minute using a rotational motion of the wrist. Allow the flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow the flask to stand for 30 minutes. Take a 10 ml aliquot and centrifuge or filter before injection. Inject duplicate aliquots of appropriate volume (as determined in section 2.1.7) of calibration solution B. The response ratios should agree to within 2%. If this precision limit is not met, inject two more aliquots of the solution. Failure to meet the precision requirement with the second pair of injections indicates instrumental difficulties, which must be resolved before proceeding with the analyses.

Inject duplicate aliquots of the sample solution, using the same volume as that used in the preceding step. The precision considerations discussed in the preceding step apply here also. Average the response ratios for each sample solution. In a series of analyses, after every two sample solution injections, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solution injections.

Use this average to calculate the p,p-DDT content of the sample.

2.1.9 *Calculation*

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

$$p, p' - \text{DDT content (g / kg)} = \frac{r_2 \times m_1 \times P}{r_1 \times m_2}$$

$$r = \frac{\text{area of } p, p' - \text{DDT peak}}{\text{area of internal standard peak}}$$

where r_1 = average response ratio for calibration solution B
 r_2 = average response ratio for sample solution
 m_1 = mass (g) of p,p'-DDT standard in the calibration solution B
 m_2 = mass (g) of sample taken
 P = purity of p,p'-DDT standard (g/kg)

2.2 Suspensibility

2.2.1 Outline of method

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

2.2.2 Special apparatus

1. A 100 ml glass-stoppered, graduated cylinder having the 100 ml mark situated 18.0 ± 1.5 cm from the bottom and 5.5 ± 0.5 cm from the top excluding the neck.
2. A 25 ml pipette fitted with a device (e.g. a rubber stopper with a vent in the side) to permit its insertion into the 100 ml cylinder so that it is held with the tip exactly at the 50 ml mark.
3. A constant-temperature water-bath into which the 100 ml graduated cylinder can be immersed to the 100 ml mark and which 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.

2.2.3 Special reagents

Standard hard water. Dissolve 0.304 g of anhydrous calcium chloride and 0.139 g of magnesium chloride hexahydrate in distilled water and make up to 1 litre. This provides water with a hardness of 342 mg/l calculated as calcium carbonate. Check the hardness by the method WHO/M/26 and correct if appropriate.

2.2.4 Procedure

Weigh (to the nearest 10 mg) into a 100 ml beaker an amount of the sample to form 100 ml of a suspension containing 18 g/l of p,p'-DDT. Add 50 ml of water⁶ at $30 \pm 1^{\circ}\text{C}$, and allow to stand for 30 seconds. Stir the mixture with a glass rod by hand for 30 seconds and then transfer to the 100 ml graduated 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

⁶ Whenever water is mentioned in this section use standard soft water for testing the powder without pretreatment (section 1.2.4) and standard hard water for testing the powder after heat stability treatment (section 1.2.4) and after storage at $21-28^{\circ}\text{C}$ for the storage life test (section 1.2.5).

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.

At the end of the 30 minute 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, see section 2.4, the remaining 75 ml of the suspension should be retained for the sieving test as described in section 2.3). Transfer the 25 ml aliquot to a 50 ml stoppered conical flask equipped with a teflon-lined screw cap and evaporate the water in a forced-draught oven at 100°C . Remove the flask from the oven as soon as the last traces of water have evaporated in order to avoid overheating the sample. Add to the residue exactly 5 ml of internal standard solution (section 2.1.4) and 20 ml of chloroform. Cap and treat as indicated in section 2.1.8. Determine p,p'-DDT content according to section 2.1, except that solution C is used as the working calibration solution.

2.2.5 Calculation

$$\text{Suspensibility (\%)} = m \times 222.2$$

where: m = mass (g) of p,p'-DDT found in the 25 ml aliquot.

2.3 Sieving test after heat stability treatment

Pour the 75 ml of suspension retained from the suspensibility test (see section 2.2.4) on to a 75 mm sieve and proceed with the sieving test as described in method WHO/M/4R1. 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.

2.4 Heat stability treatment

Weigh 5 g of the powder into a 25 x 200 mm test-tube. If the height of the powder in the tube exceeds 6.0 cm, gently tap the tube until the level is reduced to 6.0 cm. Immerse the tube to a depth of at least 9.0 cm in an oil-bath maintained at $70 \pm 0.1^{\circ}\text{C}$. The bath should be equipped with an electric stirrer and the tube must not be stoppered. Allow the sample to remain in the bath for 2 hours, then remove and cool to room temperature. After completion of the heat stability treatment, the sample should not be exposed to heat, bright sunshine, or high atmospheric humidity.

DDT EMULSIFIABLE CONCENTRATE

Specification WHO/SIF/4.R7
approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of technical DDT 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 DDT used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/1.R7.

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 *p,p'*-DDT content (g/kg basis)¹

The content of *p,p'*-DDT, 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 360 g/kg ²	±6% of the nominal content

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

1.2.2 *Water content*

The water content determined by the method described in WHO/M/7.R1 shall not be greater than 5 g/kg.

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 0.5 g/kg, calculated as H₂SO₄ or 0.5 g/kg calculated as NaOH.

¹ Certain batches of emulsion concentrate with *p,p'*-DDT content greater than 210 g/kg have given rise to spraying difficulties. Intending purchasers of such concentrates should consult Operational Research/ Division of Control of Tropical Diseases, World Health Organization, 1211 Geneva 27, Switzerland.

² This 360 g/kg nominal content of *p,p'*-DDT corresponds to 500 g/kg technical DDT of which the *p,p'*-DDT content is 720 g/kg (see "preliminary remarks" in technical DDT specification WHO/SIT/1.R7).

1.2.4 *Cold test*

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

1.2.5 *Flash point*

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

1.2.6 *Stability of the emulsion*

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

1.2.7 *Heat stability*

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

1.2.8 *Staining and odour*

The staining of surfaces produced by the diluted emulsion, prepared as described in WHO/M/13.R3 and applied at the recommended rate, shall not be greater than that produced by equal volumes of (1) standard hard water and (2) xylene when these liquids are sprayed separately at the same rate of application. The DDT deposit left after the application shall be ignored in assessing the staining, but the deposit must be free from objectionable odour after standing for 24 hours in a still atmosphere at room temperature.

1.3 Packing and marking of packages

The DDT 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
DDT emulsifiable concentrate to specification WHO/SIF/4.R7
p,p'-DDT, ... g/kg, corresponding to ... g/kg technical DDT
Batch or reference number, and date of test
Net weight of contents
Instruction for dilution
Date of formulation

and the following minimum cautionary notice:

Keep well away from foodstuffs and animal feed and their containers.

2. Methods of determining chemical and physical properties

2.1 p,p'-DDT content

2.1.1 *Outline of method*

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

2.1.2 *Special apparatus*

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

3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 5% OV-210.
4. *Glass wool, silane-treated.*

2.1.3 *Special reagents*

p,p'-DDT standard. Analytical grade of known purity.

Internal standard. 2,2'-Dinitrobiphenyl.

2.1.4 *Preparation of standard solutions*

Internal standard solution. Prepare a 28 g/l solution of the internal standard in chloroform. This solution is stable for four weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

p,p'-DDT calibration solutions. Weigh (to the nearest 0.1 mg) about 150, 200, and 300 mg quantities of the *p,p'*-DDT standard directly into separate 50 ml stoppered conical flasks equipped with teflon-lined screw caps. To each flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap each flask tightly and gently swirl the contents of each flask for 1 minute using a rotational motion of the wrist. Allow each flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow each flask to stand for 30 minutes. Label the three calibration solutions "A", "B", and "C", respectively. Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.1.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration. Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity specifications described in section 2.1.7 can be met.

2.1.5 *Preparation and conditioning of column*

See method WHO/M/20.

2.1.6 *Operating conditions for gas liquid chromatography*

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

<i>Temperatures</i>	
Oven	170°C
Injection port	250°C
Flame-ionization detector	275°C
<i>Gas flow rates</i>	
Hydrogen	30 ml/min
Air	300 ml/min
Carrier gas (helium or nitrogen)	30 ml/min
<i>Retention times</i>	
p,p'-DDT peak	12.4 min
Internal standard peak	19.5 min

2.1.7 *Linearity check*

The gas-liquid chromatograph should be checked for linearity at least once a week, and the same check should be carried out whenever new calibration solutions are prepared and whenever a column, new or used, is installed in the instrument. Using digital integration for peak area measurements, determine the appropriate attenuation setting and the quantity (between 2 and 4 µl) of calibration solution B that must be injected to yield an area count of at least 100 000 (optimum electrometer output with acceptable noise level). The attenuation setting quantity so determined should be used for all samples and calibration solutions in the set.

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

2.1.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of active ingredient (p,p'-DDT) into a 50 ml stoppered conical flask equipped with teflon-lined screw cap (to estimate the amount of sample which must be taken, consider the nominal percentage of technical DDT for the formulation and assume 720 g/kg p,p'-DDT in the technical material). To the flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap the flask tightly, and gently swirl the contents for 1 minute using a rotational motion of the wrist. Allow the flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow the flask to stand for 30 minutes.

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

Inject duplicate aliquots of the sample solution, using the same volume as that used in the preceding step. The precision considerations discussed in the preceding step apply here also. Average the response ratios for each sample solution. In a series of analyses, after every two sample solution injections, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solution injections. Use this average to calculate the p,p'-DDT content of the sample.

2.1.9 Calculation

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

$$r = \frac{\text{area of } p, p' - \text{DDT peak}}{\text{area of internal standard peak}}$$

$$p, p' - \text{DDT content (g/kg)} = \frac{r_2 \times \underline{m}_1 \times p}{r_1 \times \underline{m}_2}$$

- where r_1 = average response ratio for calibration solution B
 r_2 = average response ratio for sample solution
 \underline{m}_1 = mass (g) of p,p'-DDT standard in the calibration solution B
 \underline{m}_2 = mass (g) of sample taken
 p = purity of p,p'-DDT standard (g/kg)

2.2 Heat stability

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

DDT DUSTABLE POWDER

Specification WHO/SIF/16.R6
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical DDT together with carriers and any other necessary formulants. It shall be a fine, free-flowing, white, cream, or grey powder free from hard lumps. The technical DDT used in the manufacture of the powder shall comply with the requirements of specification WHO/SIT/1.R7.

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 *p,p'*-DDT content (g/kg basis)

The content of *p,p'*-DDT, determined by the method described in section 2.1, shall not differ from the nominal *p,p'*-DDT content by more than $\pm 10\%$.

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

1.2.2 *Acidity or alkalinity*

The acidity or alkalinity of the powder, determined by the method described in WHO/M/3, shall not be higher than 1 g/kg, calculated as H_2SO_4 or 2 g/kg calculated as NaOH.

1.2.3 *Sieving after heat stability treatment*

Not less than 98% of the powder after heat stability treatment, as described in section 2.2, shall pass through a 150 mm sieve when tested by the method described in WHO/M/4.R1. For powders intended for personal use, the residue remaining on the sieve shall be free from grittiness.

1.2.4 *Dustability after heat stability treatment*

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

1.2.5 *Heat stability*

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

1.3 **Packing and marking of packages**

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

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

Manufacturer's name
DDT dustable powder to specification WHO/SIF/16.R6
p,p'-DDT, ... g/kg, corresponding to ... g/kg technical DDT
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

Keep well away from foodstuffs and animal feed and their containers.

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

2.1 **p,p'-DDT content**

2.1.1 *Outline of method*

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

¹ Equipment for vector control, 3rd edition, World Health Organization, Geneva, 1990, p. 128.

2.1.2 *Special apparatus*

1. *Gas-liquid chromatograph.* The instrument should be one that is designed for use with glass columns and that is equipped with an on-column injection system, a high-sensitivity flame-ionization detector, an electrometer having a sensitivity of at least 10^{-11} amperes and a drift of less than 1% per hour, and a strip-chart recorder with a range of 1 mV. It is also recommended that the instrument be equipped with a solid-state amplifier with a field-effect transistor input and an electronic digital integrator or a computer for area measurement. The integrator should have independent controls for the selection of slope sensitivities, so that start and stop integration points can be selected. An automated sample injection system also contributes significantly to the accuracy of the assay.
2. *Chromatographic column.* The column should be a borosilicate glass tube 183 cm long, 2 mm in internal diameter, and 6 mm in external diameter, bent to fit the chromatograph.
3. *Column-packing material.* Chromosorb W-HP (100-120 mesh) treated with 5% OV 210.
4. *Glass wool, silane-treated.*

2.2.3 *Special reagents*

p,p'-DDT standard. Analytical grade of known purity.

Internal standard. 2,2'-Dinitrobiphenyl.

2.2.4 *Preparation of standard solutions*

Internal standard solution. Prepare a 28 g/l solution of the internal standard in chloroform. This solution is stable for four weeks if kept tightly sealed and under refrigeration. Allow the solution to warm to room temperature before use.

p,p'-DDT calibration solutions. Weigh (to the nearest 0.1 mg) about 150, 200, and 300 mg quantities of the *p,p'*-DDT standard directly into separate 50 ml stoppered conical flasks equipped with teflon-lined screw caps. To each flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap each flask tightly and gently swirl the contents of each flask for 1 minute using a rotational motion of the wrist. Allow each flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow each flask to stand for 30 minutes. Label the three calibration solutions "A", "B", and "C", respectively.

Solution B is the working calibration solution for gas chromatography; solutions A and C are used to check the linearity of the gas chromatograph (section 2.2.7) and to guard against weighing error in the preparation of the working calibration solution. These solutions are stable for 4 weeks if kept tightly sealed and under refrigeration.

Allow the solutions to warm to room temperature before use. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity specifications described in section 2.2.7 can be met.

2.2.5 *Preparation and conditioning of column*
See method WHO/M/20.

2.2.6 *Operating conditions for gas liquid chromatography*

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

Temperatures

Oven	170 ⁰ C
Injection port	250 ⁰ C
Detector	275 ⁰ C

Gas flow rates

Hydrogen	30 ml/min.
Air	300 ml/min.
Carrier gas (helium or nitrogen)	30 ml/min.

Retention times

p,p'-DDT peak	12.4 min.
Internal standard peak	19.5 min.

2.2.7 *Linearity check*

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

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

Inject triplicate aliquots of appropriate volume (as determined above) of calibration solutions A, B and C into the gas-liquid chromatograph, determine the response ratio for each injection, and average the resulting ratios for each solution. Divide the average response ratio for each solution by the corresponding p,p'-DDT content (in mg) and compare the resulting response factors.

These factors should agree to within 2%. Failure to meet this requirement indicates either a weighing error in the preparation of one of the calibration solutions or instrumental difficulties, which must be corrected before proceeding with the analysis of samples.

2.2.8 *Sample preparation and analysis*

Weigh (to the nearest 0.1 mg) a quantity of the sample containing about 200 mg of active ingredient (p,p'-DDT) into a 50 ml stoppered conical flask equipped with teflon-lined screw cap (to estimate the amount of sample which must be taken, consider the nominal percentage of technical DDT for the formulation and assume 720 g/kg p,p'-DDT in the technical material). To the flask, add by pipette 5 ml of internal standard solution and 20 ml of chloroform. Cap the flask tightly, and gently swirl the contents for 1 minute using a rotational motion of the wrist. Allow the flask to stand at least 5 minutes and then shake for 1 minute, either by hand or using a reciprocal shaker. Ensure that no leaking occurs around the cap. Allow the flask to stand for 30 minutes. Take a 10 ml aliquot and centrifuge or filter before injection.

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

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

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

Use this average to calculate the p,p'-DDT content of the sample.

2.2.9 Calculation

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

$$r = \frac{\text{area of } p, p' \text{ - DDT peak}}{\text{area of internal standard peak}}$$

$$p, p' \text{ - DDT content (g / kg)} = \frac{r_2 \times m_1 \times P}{r_1 \times m_2}$$

- r_1 = average response ratio for calibration solution B
- r_2 = average response ratio for sample solution
- m_1 = mass (g) of p, p' -DDT standard in the calibration solution B
- m_2 = mass (g) of sample taken
- P = purity of p, p' -DDT standard (g/kg)

2.2 Heat stability

For the sieve test, section 1.2.3, place 20 g of the sample in a 100 ml wide-mouthed bottle with a vinyl-plastic-lined screw-cap. For the dustability test, section 1.2.4, place 250 g of the sample in a 1 litre wide-mouthed bottle fitted with an vinyl-plastic-lined screw-cap. For the other tests to be repeated, section 1.2.5, place 25 g of the sample in a 100 ml wide-mouthed bottle with a vinyl-plastic buried screw-cap.

Place the bottles in an oven maintained at $54 + 2^{\circ}\text{C}$ for 3 days. Remove the samples from the oven and allow them to cool to room temperature before removing the caps.

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