

TECHNICAL TEMEPHOS

Specification WHO/SIT/19.R3
Approved 25 September 1989

1. Specification

1.1 Material

The material shall consist of temephos together with related manufacturing compounds and shall be in the form of an oily liquid, free from extraneous impurities or added modifying agents.

1.2 Chemical and physical requirements

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

1.2.1 *Temephos content (g/kg basis)*

The temephos content shall be declared (not less than 900 g/kg) and, when determined by the method described in section 2.1, the content obtained shall not differ from that declared by more than ± 20 g.

1.2.2 *Acidity*

The acidity of the material, determined by the method described in WHO/M/3, shall not be higher than 3 g/kg, calculated as H_2SO_4 .

1.2.3 *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 5 g/kg.

1.2.4 *Water content*

The water content, determined by the method described in section 2.2, shall not be higher than 2 g/kg.

1.3 Packing and marking of packages

The technical temephos 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 temephos to specification WHO/SIT/19.R3
Batch or reference number, and date of test
Net weight of contents
Date of manufacture

and the following minimum cautionary notice:

Temephos is an organophosphorous compound that inhibits cholinesterase.

Keep the material out of the reach of children and well away from foodstuffs and animal feed and their containers.

2. Methods of determining chemical and physical properties

2.1 Temephos content

2.1.1 *Outline of method*

The sample is dissolved in ethyl acetate and either 4-nitrophenyl 4-nitrobenzoate or dimethyl 4-nitrophthalate is added as internal standard. The temephos content is determined by high-performance liquid chromatography (HPLC), using a silica gel column and a mixture of n-hexane and ethyl acetate as the mobile phase.

2.1.2 *Special apparatus*

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate more than 14 MPa pressure and a UV detector spectrophotometer able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 3.9 mm in internal diameter, packed with 10 µm silica gel (µm Porasil, or equivalent).

2.1.3 *Special reagents*

Temephos standard. Analytical grade of known purity.

Internal standard. Pure 4-nitrophenyl 4-nitrobenzoate.

Dimethyl 4-nitrophthalate may be used as an alternative internal standard.

Ethyl acetate. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 µm Millipore filter or equivalent.

n-Hexane. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 µm Millipore filter or equivalent.

Mobile phase. A degassed mixture of 10 ml of ethyl acetate and 90 ml of n-hexane.

2.1.4 Preparation of standard solutions

Internal standard solution. Weigh 1.5 g of 4-nitrophenyl 4-nitrobenzoate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate. Store under refrigeration. Warm to room temperature before using. Alternatively, weigh 2.75 g of dimethyl 4-nitrophthalate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate.

Temephos calibration solutions. Weigh (to the nearest 0.1 mg) approximately 50-, 60-, and 70 mg quantities of temephos standard into separate 50 ml volumetric flasks. Add by pipette 5.0 ml of internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution of the standard and dilute to volume with n-hexane.

Label these solutions as "A", "B", and "C". Solution B is the working calibration solution for liquid chromatography. Solutions A and C are used to check the linearity of the liquid chromatograph (see section 2.1.7) and to guard against weighing error in the preparation of the calibration solution. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity requirements described under section 2.1.7 can be met.

2.1.5 Equilibration of the liquid chromatographic column

To render the column essentially dry, pump 50 ml of anhydrous methanol through the column followed by 100 ml of dry ethyl acetate. After this treatment pump sufficient mobile phase through the column to equilibrate the system. When the column has come to equilibrium at the conditions described below, inject 5 (ml) aliquots of calibration solution B until a constant response is obtained. To meet this requirement, the response ratios (area of temephos peak/area of internal standard peak) of three consecutive injections must agree to within 2%.

2.1.6 Operating conditions for high-performance liquid chromatography

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Column temperature	ambient
Flow rate	1.0 ml/min (at about 3.2 MPa)
Wavelength	254 nm
Retention times:	
internal standard	9.6 min
temephos	11.5 min
alternative internal standard	18 min

2.1.7 *Linearity check*

The 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. When digital integration is used for peak area measurements, 5 ml injections of calibration solution B should yield an area count of at least 100 000. If less than 100 000 counts are observed, the injection volume may be increased to satisfy this requirement. Alternatively, peak height may be used as a measure of chromatographic response. In this case determine the appropriate attenuation and injection volume (3-6 ml) of calibration solution B for a peak height for the internal standard between 50 and 60% of full-scale recorder deflection. The conditions 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 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 temephos 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) in duplicate a quantity of the sample containing about 60 mg of temephos directly into two 50 ml volumetric flasks. The sample should be warmed and thoroughly mixed before weighing. Add by pipette exactly 5.0 ml of internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution and dilute to volume with n-hexane.

Inject duplicate aliquots of appropriate volume (as determined under section 2.1.7) of calibration solution B. Calculate the response ratios by dividing the area (or height) of the temephos peak by that of the internal standard peak. Response ratios should agree to within 2%. Average the duplicate response ratios obtained with the calibration solution B. Inject duplicate aliquots (same volume as that used in the preceding step) of each of the sample solutions. The precision considerations discussed in the preceding step apply here also. Average the duplicate response ratios for each sample solution¹.

¹ After the first injection of any sample, let the instrument run for at least 30 min after the emergence of the temephos peak in order to detect late-eluting peaks due to impurities. Subsequent injections should be timed so that late-eluting peaks from sample injections do not interfere with the internal standard or temephos peaks of subsequent samples.

After duplicate injections of two sample solutions, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solutions. Use the average to calculate the temephos content of the two sample solutions. Each determination of response ratio should give a yield equal to within 2% of the previously determined ratio.

2.1.9 Calculation

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

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

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

where

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

2.2 Water content

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

TEMEPHOS EMULSIFIABLE CONCENTRATE*

Specification WHO/SIF/31.R3
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of technical temephos dissolved in suitable solvents, with other necessary formulants added. It shall be in the form of a stable liquid, free from extraneous impurities. The technical temephos used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/19.R3.

1.2 Chemical and physical requirements

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

1.2.1 *Temephos content (g/kg basis)*

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

<i>Nominal content</i>	<i>Tolerance permitted</i>
Up to 500 g/kg	± 5% of the nominal content
Above 500 g/kg	± 25 g/kg

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

1.2.2 *Water content*

The water content, determined by the method described in section 2.2, shall not be higher than 2 g/kg.

1.2.3 *Acidity*

The acidity of the concentrate, determined by method WHO/M/3 shall not be higher than 5 g/kg calculated as H₂SO₄.

* If the emulsifiable concentrate is required for Simulium control, then the specification WHO/SIF/34.R2 should be used.

1.2.4 *Cold test*

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

1.2.5 *Flash point*

The flash point of the product 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 the 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 the method WHO/M/13.R3.

1.2.7 *Heat stability*

The concentrate, after treatment as described in section 2.3, shall comply with the requirements of sections 1.2.1, 1.2.3 and 1.2.6 of this specification.

1.3 **Packing and marking of packages**

The temephos 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
Temephos emulsifiable concentrate to specification WHO/SIF/31.R3
Temephos ... g/kg
Batch or reference number, and date of test
Net weight of contents
Instructions for dilution
Date of formulation

and the following minimum cautionary notice:

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

2. Methods of determining chemical and physical properties

2.1 Temephos content

2.1.1 Outline of method

The sample is dissolved in ethyl acetate and either 4-nitrophenyl 4-nitrobenzoate or dimethyl 4-nitrophthalate is added as internal standard. The temephos content is determined by high-performance liquid chromatography (HPLC) using a silica gel column and a mixture of n-hexane and ethyl acetate as the mobile phase.

2.1.2 Special apparatus

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate more than 14 MPa pressure and a UV detector spectrophotometer able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 3.9 mm in internal diameter packed with 10 μ m silica gel (m Porasil or equivalent).

2.1.3 Special reagents

Temephos standard. Analytical grade of known purity.

Internal standard. Pure 4-nitrophenyl 4-nitrobenzoate.

Dimethyl 4-nitrophthalate may also be used as an alternative internal standard.

Ethyl acetate. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 μ m Millipore filter or equivalent.

n-Hexane. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 μ m Millipore filter or equivalent.

Mobile phase. A degassed mixture of 10 ml of ethyl acetate and 90 ml of n-hexane.

2.1.4 Preparation of standard solutions

Internal standard solution. Weigh 1.5 g of 4-nitrophenyl 4-nitrobenzoate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate. Store under refrigeration. Warm to room temperature before using.

Alternatively, weigh 2.75 g of dimethyl 4-nitrophthalate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate.

Temephos calibration solutions. Weigh (to the nearest 0.1 mg) approximately 50-, 60-, and 70 mg quantities of temephos standard into separate 50 ml volumetric flasks. Add by pipette 5.0 ml of internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution of the standard and dilute to volume with n-hexane.

Label these solutions as "A", "B", and "C". Solution B is the working calibration solution for liquid chromatography. Solutions A and C are used to check the linearity of the liquid chromatograph (see section 2.1.7) and to guard against weighing error in the preparation of the calibration solution. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity requirement described under section 2.1.7 can be met.

2.1.5 *Equilibration of the liquid chromatographic column*

To render the column essentially dry, pump 50 ml of anhydrous methanol through the column followed by 100 ml of dry ethyl acetate. After this treatment pump sufficient mobile phase through the column to equilibrate the system. When the column has come to equilibrium at the conditions described below, inject 5 ml aliquots of calibration solution B until a constant response is obtained. To meet this requirement, the response ratios (area of temephos peak/area of internal standard peak) of three consecutive injections must agree to within 2%.

2.1.6 *Operating conditions for high-performance liquid chromatography*

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Column temperature	ambient
Flow rate	1.0 ml/min (at about 3.2 MPa)
Wavelength	254 nm
Retention times:	
Internal standard	9.6 min
temephos	11.5 min
alternative internal standard	18 min

2.1.7 *Linearity check*

The 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. When digital integration is used for peak area measurements, 5 ml injections of calibration solution B should yield an area count of at least 100 000. If less than 100 000 counts are observed, the injection volume may be increased to satisfy this requirement. Alternatively, peak height may be used as a measure of chromatographic response.

In this case, determine the appropriate attenuation and injection volume (3-6 ml) of calibration solution B for a peak height for the internal standard between 50 and 60% of full-scale recorder deflection. The conditions 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 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 temephos 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) in duplicate a quantity of the sample containing about 60 mg of temephos directly into two 50 ml volumetric flasks. Add by pipette exactly 5.0 ml of the internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution and dilute to volume with n-hexane.

Inject duplicate aliquots of appropriate volume (as determined under section 2.1.7) of calibration solution B. Calculate the response ratios by dividing the area (or height) of the temephos peak by that of the internal standard peak. Response ratios should agree to within 2%. Average the duplicate response ratios obtained with the calibration solution B. Inject duplicate aliquots (same volume as that used in the preceding step) of each of the sample solutions. The precision considerations discussed in the preceding step apply here also.

Average the duplicate response ratios for each sample solution². After duplicate injections of two sample solutions, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solutions. Use the average to calculate the temephos content of the two sample solutions. Each determination of response ratio should give a value equal to within 2% of the previously determined ratio.

² After the first injection of any sample, let the instrument run for at least 30 min after the emergence of the temephos peak in order to detect late-eluting peaks due to impurities. Subsequent injections should be timed so that late-eluting peaks from sample injections do not interfere with the internal standard or temephos peaks of subsequent samples.

2.1.9 Calculation

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

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

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

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

2.2 Water content

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

2.3 Heat stability

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

TEMEPHOS EMULSIFIABLE CONCENTRATE FOR SIMULIUM CONTROL

Specification WHO/SIF/34.R2
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of technical temephos dissolved in suitable solvents, with other necessary formulants added. It shall be in the form of a stable liquid, free from extraneous impurities. At the rates of application ordinarily used, it must not be toxic to fish, domestic animals, man, or plant life. The technical temephos used in the manufacture of the concentrate shall comply with the requirements of specification WHO/SIT/19.R3.

1.2 Chemical and physical requirements

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

1.2.1 *Temephos content (g/kg basis)*

The content of temephos shall be 200 g/kg with a tolerance of ± 10 g/kg when determined by the method given in section 2.1.

The average content of all samples taken shall not be less than the 200 g/kg nominal content.

1.2.2 *Density*

The density as determined at 28⁰C by the method described in section 2.2, shall not be less than 0.950 g/ml and not higher than 0.980 g/ml.

1.2.3 *Emulsion type*

The concentrate, when tested in standard soft water as described in section 2.3, shall have a minimum absorbance of 2.0 at 436 nm.

1.2.4 *Emulsion stability*

The emulsion stability in standard soft water shall be at least 90.0% when tested by the method given in section 2.4.

1.2.5 *Acidity*

The acidity of the concentrate, determined by the method described in WHO/M/3 shall not be higher than 5 g/kg calculated as H₂SO₄.

1.2.6 *Cold test*

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

1.2.7 *Flash point*

The flash point of the concentrate shall not be lower than 38⁰C when determined by the method described in section 2.5.

1.2.8 *Heat stability*

The concentrate, after treatment as described in section 2.6, shall comply with the requirements of sections 1.2.1, 1.2.3, 1.2.4 and 1.2.5 of this specification.

1.3 **Packing and marking of packages**

The temephos 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
- Temephos emulsifiable concentrate for Simulium control to specification WHO/SIF/34.R2
- Temephos ... g/kg
- Batch or reference number, and date of test
- Net weight of contents
- Instructions for dilution
- Date of formulation

and the following minimum cautionary notice:

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

2. Methods of determining chemical and physical properties

2.1 Temephos content

2.1.1 Outline of method

The sample is dissolved in ethyl acetate and either 4-nitrophenyl 4-nitrobenzoate or dimethyl 4-nitrophthalate is added as internal standard. The temephos content is determined by high-performance liquid chromatography (HPLC) using a silica gel column and a mixture of n-hexane and ethyl acetate as the mobile phase.

2.1.2 Special apparatus

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate more than 14 MPa pressure and a UV detector spectrophotometer able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 3.9 mm in internal diameter packed with 10 μ m silica gel (m Porasil or equivalent).

2.1.3 Special reagents

Temephos standard. Analytical grade of known purity.

Internal standard. Pure 4-nitrophenyl 4-nitrobenzoate.

Dimethyl 4-nitrophthalate may also be used as an alternative internal standard.

Ethyl acetate. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 μ m Millipore filter or equivalent.

n-Hexane. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 μ m Millipore filter or equivalent.

Mobile phase. A degassed mixture of 10 ml of ethyl acetate and 90 ml of n-hexane.

2.1.4 Preparation of standard solutions

Internal standard solution. Weigh 1.5 g of 4-nitrophenyl 4-nitrobenzoate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate. Store under refrigeration. Warm to room temperature before using.

Alternatively, weigh 2.75 g of dimethyl 4-nitrophthalate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate.

Temephos calibration solutions. Weigh (to the nearest 0.1 mg) approximately 50-, 60-, and 70 mg quantities of temephos standard into separate 50 ml volumetric flasks. Add by pipette 5.0 ml of internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution of the standard and dilute to volume with n-hexane.

Label these solutions as "A", "B", and "C". Solution B is the working calibration solution for liquid chromatography. Solutions A and C are used to check the linearity of the liquid chromatograph (see section 2.1.7) and to guard against weighing error in the preparation of the calibration solution. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity requirement described under section 2.1.7 can be met.

2.1.5 *Equilibration of the liquid chromatographic column*

To render the column essentially dry, pump 50 ml of anhydrous methanol through the column followed by 100 ml of dry ethyl acetate. After this treatment pump sufficient mobile phase through the column to equilibrate the system. When the column has come to equilibrium at the conditions described below, inject 5 ml aliquots of calibration solution B until a constant response is obtained. To meet this requirement, the response ratios (area of temephos peak/area of internal standard peak) of three consecutive injections must agree to within 2%.

2.1.6 *Operating conditions for high-performance liquid chromatography*

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Column temperature	ambient
Flow rate	1.0 ml/min (at about 3.2 MPa)
Wavelength	254 nm
Retention times:	
internal standard	9.6 min
temephos	11.5 min
alternative internal standard	18 min

2.1.7 *Linearity check*

The 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. When digital integration is used for peak area measurements, 5 ml injections of calibration solution B should yield an area count of at least 100 000. If less than 100 000 counts are observed, the injection volume may be increased to satisfy this requirement.

Alternatively, peak height may be used as a measure of chromatographic response. In this case, determine the appropriate attenuation and injection volume (3-6 ml) of calibration solution B for a peak height for the internal standard between 50 and 60% of full-scale recorder deflection. The conditions 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 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 temephos 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) in duplicate a quantity of the sample containing about 60 mg of temephos directly into two 50 ml volumetric flasks. Add by pipette exactly 5.0 ml of the internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution and dilute to volume with n-hexane.

Inject duplicate aliquots of appropriate volume (as determined under section 2.1.7) of calibration solution B. Calculate the response ratios by dividing the area (or height) of the temephos peak by that of the internal standard peak. Response ratios should agree to within 2%. Average the duplicate response ratios obtained with the calibration solution B. Inject duplicate aliquots (same volume as that used in the preceding step) of each of the sample solutions. The precision considerations discussed in the preceding step apply here also. Average the duplicate response ratios for each sample solution¹.

After duplicate injections of two sample solutions, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solutions. Use the average to calculate the temephos content of the two sample solutions. Each determination of response ratio should give a value equal to within 2% of the previously determined ratio.

¹ After the first injection of any sample, let the instrument run for at least 30 min after the emergence of the temephos peak in order to detect late-eluting peaks due to impurities. Subsequent injections should be timed so that late-eluting peaks from sample injections do not interfere with the internal standard or temephos peaks of subsequent samples.

2.1.9 Calculation

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

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

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

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

2.2 Density

Determine the density at 28°C by means of a pycnometer.

2.3 Emulsion type

Add 0.1 ml of the sample to 100 ml of standard soft water² in a 100 ml glass stoppered graduated cylinder. The concentrate may be added by pipette or syringe with a tip against the wall of the cylinder and about 5 mm above the surface of the water. Stopper the cylinder, invert five times, and pour the emulsion into a 1 cm cell. Measure the absorbance against water in a spectrophotometer at 436 nm.

2.4 Emulsion stability

Dissolve 20 mg of "Oil Red O" in 10 ml of the emulsion sample. Add 1 ml of this dyed sample, by means of a syringe, to each of two 100 ml glass stoppered graduated cylinders A and B containing 99 ml of standard soft water.

Stopper cylinder A, invert five times, allow to stand, and after 15 minutes pipette a 25 ml aliquot from the centre of the emulsion into a clean 100 ml cylinder. Shake cylinder B well and immediately pipette a 25 ml aliquot into another clean 100 ml cylinder. Add sufficient isopropanol to each cylinder to make up to 60 ml. Measure the absorbance of each of these red solutions in 1 cm cells in a spectrophotometer at 525 nm.

² Standard soft water is prepared by diluting 1 part of standard hard water with 9 parts of distilled water; this provides water with a hardness of 34.2 mg/l, calculated as calcium carbonate. Standard hard water is prepared as follows: 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. Check the hardness by method WHO/M/26 and correct if appropriate.

$$\textit{Emulsion stability (\%)} = \frac{\textit{absorbance of sample A}}{\textit{absorbance of sample B}} \times 100$$

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).

2.6 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.

TEMEPHOS 10 g/kg SAND GRANULES

Specification WHO/SIF/40.R1
Approved 25 September 1989

1. Specification

1.1 Description and ingredients

The material shall consist of a homogeneous mixture of technical temephos, silica sand and any necessary formulants and shall be in the form of dry free-flowing and essentially non-dusting granules. The technical temephos used in the manufacture of the sand granules shall comply with the requirements of specification WHO/SIT/19.R3.

1.2 Chemical and physical requirements

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

1.2.1 *Temephos content (g/kg basis)*

The content of temephos shall be 10 g/kg with a tolerance of - 1 g/kg to + 2 g/kg, when determined by the method given in section 2.1.

The average content of all samples taken shall not be less than the 10 g/kg nominal content.

1.2.2 *Sieving*

Not less than 98% of the granules shall pass through a 1.25 mm sieve and not more than 2% shall pass through a 250 mm sieve when tested by the method described in section 2.2.

1.2.3 *Apparent density*

The apparent density shall be not less than 1.30 g/ml and not more than 1.60 g/ml when tested by the method described in section 2.3.

1.3 Packing and marking of packages

The temephos 10 g/kg sand granules shall be packed in suitable, clean, airtight containers, as specified in the order.

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

Manufacturer's name
Temephos sand granules to specification WHO/SIF/40.R1
Temephos 10 g/kg
Batch or reference number, and date of test
Net weight of contents
Date of formulation

and the following minimum cautionary notice:

Temephos is an organophosphorus compound that inhibits cholinesterase. Keep the material out of the reach of children and well away from foodstuffs, animal feed, and their containers.

2. Methods of determining chemical and physical properties

2.1 Temephos content

2.1.1 *Outline of method*

Temephos is extracted from the sample with ethyl acetate and either 4-nitrophenyl 4-nitrobenzoate or dimethyl 4-nitrophthalate is added as internal standard.

The temephos content is determined by high-performance liquid chromatography (HPLC) using a silica gel column and a mixture of n-hexane and ethyl acetate as the mobile phase.

2.1.2 *Special apparatus*

1. *Liquid chromatograph.* The instrument should be one that is designed for use with stainless steel columns and that is equipped with a pumping system able to generate more than 14 MPa pressure and a UV detector spectrophotometer able to measure UV absorbance at 254 nm.
2. *Liquid chromatographic column.* The column should be a stainless steel tube 30 cm long and 3.9 mm in internal diameter packed with 10 mm silica gel (m Porasil or equivalent).

2.1.3 *Special reagents*

Temephos standard. Analytical grade of known purity.

Internal standard. Pure 4-nitrophenyl 4-nitrobenzoate.

Dimethyl 4-nitrophthalate may also be used as an alternative internal standard.

Ethyl acetate. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 μ m Millipore filter or equivalent.

n-Hexane. HPLC grade. Dry over a molecular sieve (0.5 nm, 8-12 mesh beads) and filter through a 0.45 µm Millipore filter or equivalent.

Mobile phase. A degassed mixture of 10 ml of ethyl acetate and 90 ml of n-hexane.

2.1.4 Preparation of standard solutions

Internal standard solution. Weigh 1.5 g of 4-nitrophenyl 4-nitrobenzoate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate. Store under refrigeration. Warm to room temperature before using.

Alternatively, weigh 2.75 g of dimethyl 4-nitrophthalate into a 250 ml volumetric flask and dilute to volume with dry ethyl acetate.

Temephos calibration solutions. Weigh (to the nearest 0.1 mg) approximately 50-, 60- and 70 mg quantities of temephos standard into separate 50 ml volumetric flasks. Add by pipette 5.0 ml of internal standard solution and 25.0 ml of dry ethyl acetate to each flask. Shake the flasks to ensure dissolution of the standard and dilute to volume with n-hexane.

Label these solutions as "A", "B", and "C". Solution B is the working calibration solution for liquid chromatography. Solutions A and C are used to check the linearity of the liquid chromatograph (see section 2.1.7) and to guard against weighing error in the preparation of the calibration solution. The supply of solution B can be replenished from time to time without preparing new supplies of solutions A and C, provided the linearity requirement described under section 2.1.7 can be met.

2.1.5 Equilibration of the liquid chromatographic column

To render the column essentially dry, pump 50 ml of anhydrous methanol through the column followed by 100 ml of dry ethyl acetate. After this treatment pump sufficient mobile phase through the column to equilibrate the system. When the column has come to equilibrium at the conditions described below, inject 5 ml aliquots of calibration solution B until a constant response is obtained. To meet this requirement, the response ratios (area of temephos peak/area of internal standard peak) of three consecutive injections must agree to within 2%.

2.1.6 Operating conditions for high-performance liquid chromatography

The conditions given below are typical values and may have to be adjusted to obtain optimum results from a given apparatus.

Column temperature	ambient
Flow rate	1.0 ml/min (at about 3.2 MPa)
Wavelength	254 nm
Retention times:	
internal standard	9.6 min
temephos	11.5 min
alternative internal standard	18 min

2.1.7 *Linearity check*

The 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. When digital integration is used for peak area measurements, 5 ml injections of calibration solution B should yield an area count of at least 100 000. If less than 100 000 counts are observed, the injection volume may be increased to satisfy this requirement. Alternatively, peak height may be used as a measure of chromatographic response. In this case, determine the appropriate attenuation and injection volume (3-6 ml) of calibration solution B for a peak height for the internal standard between 50 and 60% of full-scale recorder deflection. The conditions 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 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 temephos 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) in duplicate a quantity of the sample containing about 60 mg of temephos directly into two 50 ml bottles fitted with plastic screw-caps. Add by pipette 5.0 ml of the internal standard solution and 25.0 ml of dry ethyl acetate and shake for 1 minute. Add 20 ml of n-hexane, mix thoroughly and allow the particles to settle. Filter a portion of the solution and hold for HPLC analysis. (In some cases centrifugation may be sufficient to remove insoluble particles before HPLC analysis).

Inject duplicate aliquots of appropriate volume (as determined under section 2.1.7) of calibration solution B. Calculate the response ratios by dividing the area (or height) of the temephos peak by that of the internal standard peak. Response ratios should agree to within 2%. Average the duplicate response ratios obtained with the calibration solution B.

Inject duplicate aliquots (same volume as that used in the preceding step) of each of the sample solutions. The precision considerations discussed in the preceding step apply here also. Average the duplicate response ratios for each sample solution¹.

After duplicate injections of two sample solutions, inject duplicate aliquots of calibration solution B. Average the response ratios of the calibration solution injections immediately before and after the sample solutions. Use the average to calculate the temephos content of the two sample solutions. Each determination of response ratio should give a value equal to within 2% of the previously determined ratio.

2.1.9 Calculation

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

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

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

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

2.2 Sieving test

Place 100 g of the sample (weighed to the nearest 0.1 g) on a clean, dry, 1.25 mm sieve, nested over a 250 mm sieve and a receiver. Screen in an appropriate sieving machine² for five minutes. Alternatively, shake the sieves by hand in a rotary horizontal direction and vertically by tapping on a hard surface for five minutes. Disconnect the sieves and brush away any material on the underside of each sieve on to the sieve or receiver below it. Using an analytical balance, weigh the contents of the 1.25 mm sieve and of the

¹ After the first injection of any sample, let the instrument run for at least 30 min after the emergence of the temephos peak in order to detect late-eluting peaks due to impurities. Subsequent injections should be timed so that late-eluting peaks from sample injections do not interfere with the internal standard or temephos peaks of subsequent samples.

² Different sieving apparatuses are commercially available. Choose, preferably, the one which operates with a horizontal, circular motion and a tapping action.

receiver, obtained by inverting each of them individually over pieces of weighed glassine paper and brushing the retained material on the paper.

Calculate the portion collected and express the values in g/kg of the amount of sample taken as follows:

Material passing through the 1.25 mm sieve:

$$100 - m_1 (\% \text{ m/m})$$

Material passing through the 250 μ m sieve:

$$m_2 (\% \text{ m/m})$$

where: m_1 = mass (g) of the residue on the 1.25 mm sieve

m_2 = mass (g) of the product on the receiver

2.3 Apparent density

Place approximately 75 ml of sample in a 250 ml beaker. Pour the material into a second beaker, holding the spout of the first approximately 2 cm above the rim of the second. Transfer the material in a similar manner twice more. Transfer approximately 50 ml of this sample to a weighed 100 ml graduated cylinder, tilting the cylinder and pouring the material slowly down its side. Drop the cylinder three times through a distance of 2.5 cm on to a hardwood surface. Note the volume of the sample and weigh the cylinder and sample to the nearest 0.1 g. Subtract the weight of the cylinder to determine the weight of the sample. The apparent density is calculated by dividing the weight (g) of sample taken by the volume (ml) of sample in the cylinder.