Analytical Method for Parathion
(Targeted to Agricultural Products)

The target compound to be determined is parathion.

1. Instrument

A gas chromatograph with an alkali flame ionization detector (GC-FID), a flame photometric detector (GC-FPD, interference filter for phosphorus determination, wavelength: 526 nm), or a highly-sensitive nitrogen phosphorus detector (GC-NPD), and a gas chromatograph/mass spectrometer (GC/MS or GC/MS/MS)

2. Reagents and test solutions

In addition to the reagents and test solutions listed below, use those listed in Section C *Reagents/Test Solutions*, Etc., Part II *Food additives*. Reagents designated as "special grade" in this section must meet the requirements for "special grade" specified in the Japan Industrial Standards for the reagents.

Acetonitrile: Use a rotary vacuum evaporator on 300 mL of acetonitrile to concentrate to the point of dryness. Dissolve the residue in 5 mL of n-hexane. For analysis, inject 5 μL of the solution into a GC-ECD. Peaks other than that of n-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10- 11g.

Acetone: Use a rotary vacuum evaporator on 300 mL of acetone to concentrate to the point of dryness. Dissolve the residue in 5 mL of n-hexane. For analysis, inject 5 μL of the solution into a GC-ECD. Peaks other than that of n-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10- 11g.

Sodium chloride: Sodium chloride (special grade). If the reagent is found to include any substance that may interfere with the analysis of substances to be analyzed from agricultural chemicals, wash with a solvent such as n-hexane before use.

Silica gel for column chromatography (63-200 μm in particle size): Heat silica gel made for column chromatography (63-200 μm in particle size) at 130°C for 12 hours or longer. Cool down to room temperature in a desiccator.

Diatomaceous earth: Diatomaceous earth for chemical analysis.

Ethyl acetate: Use a rotary vacuum evaporator on 300 mL of ethyl acetate to concentrate to the point of dryness. Dissolve the residue in 5 mL of n-hexane. For

analysis, inject 5 μL of the solution into a GC-ECD. Peaks other than that of n-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10- 11g.

n-Hexane: Use a rotary vacuum evaporator on 300 mL of n-hexane to evaporate until 5 mL is left. For analysis, inject 5 μL of the solution into a GC-ECD. Peaks other than that of n-hexane in the resulting chromatogram should be as high as or lower than the peaks of γ-BHC at 2 x 10- 11g.

Water: Distilled water. If the distilled water is found to include any substance that may interfere with analysis of the target compositional substances from agricultural chemicals, wash with a solvent such as n-hexane before use.

Sodium sulfate (anhydrous): Sodium sulfate (anhydrous)(special grade). If the reagent is found to include any substance that may interfere with analysis of the target compositional substances from agricultural chemicals, wash with a solvent such as n-hexane before use.

3. Reference standard

Parathion: This product contains not less than 97% of parathion, and its boiling point is 375°C.

4. Procedure

a. Extraction

i. Cereal grains and legumes/pulses

Weigh 10.0 g of test sample, previously ground so as to pass through a standard mesh sieve (420 μm). Add 20 ml of water to the obtained sample and leave it to stand for two hours. Then, add 100 ml of acetone and homogenize the mixture for three minutes. Filter the homogenized sample by suction into a rotary vacuum evaporator through a filter paper covered with a 1-cm thick layer of diatomaceous earth. Collect the residue on the surface of the filter paper and add 50 ml of acetone, and then homogenize the mixture for three minutes. Repeat the above procedure and combine the filtrate into the rotary vacuum evaporator, and then concentrate the mixtue to approximately 30ml at 40℃ or lower. Transfer the concentrated solution to a 300-ml separating funnel already containing 100 ml of saturated sodium chloride solution. Wash the eggplant-shaped flask of the above rotary vacuum evaporator with 100 ml of ethyl acetate/n-hexane (1:4) and add the washings to the separating funnel above. Shake the mixture vigorously for five minutes using a shaker and leave it to stand, and then transfer the layers of ethyl acetate and n-hexane to a 300-ml conical flask. Add 50 ml of ethyl acetate/n-hexane

(1:4) to the aqueous layer and repeat the above procedure, and then add the layers of ethyl acetate and n-hexane to the conical flask above. Add an adequate amount of sodium sulfate (anhydrous) to the flask and leave it to stand for 15 minutes with occasional shaking. Filter the content of the flask into a rotary vacuum evaporator. Wash the flask with 20 ml of n-hexane and wash twice the residue on the surface of the filter paper with the washings. Add the washing into the rotary vacuum evaporator and remove the ethyl acetate and n-hexane at 40°C or lower. Add 30 ml of n-hexane the residue and transfer the mixture to a 100-ml separating funnel, and then add 30 ml of n-hexane-saturated acetonitrile. Shake the mixture vigorously for five minutes using a shaker and leave it to stand, and then transfer the acetonitrile layer into the rotary vacuum evaporator. Add 30 ml of n-hexane-saturated acetonitrile to the n-hexane layer and repeat the above procedure twice, and then combine the acetonitrile layer into the rotary vacuum evaporator above. Remove the acetonitrile at 40°C or lower and dissolve the residue in 5 ml of acetone/n-hexane (1:1).

ii. Fruit and vegetables

Weigh accurately 1 kg of the test sample and add an appropriate amount of water to the sample, if required. Homogenize the mixture and measure out a sample equivalent to 20.0 g. Then, add 100 ml of acetone and homogenize the mixture for three minutes. Filter the homogenized sample by suction into a rotary vacuum evaporator using filter paper covered with a 1-cm thick layer of diatomaceous earth. Collect the residue on the surface of the filter paper and add 50 ml of acetone, and then homogenize the mixture for three minutes. Repeat the above procedure and combine the filtrate into the rotary vacuum evaporator, and then concentrate the mixture to approximately 30 ml at 40°C or lower. Transfer the concentrated solution to a 300-ml separating funnel already containing 100 ml of saturated sodium chloride solution. Wash the eggplant-shaped flask of the above rotary vacuum evaporator with 100 ml of ethyl acetate/n-hexane (1:4) and add the washing to the separating funnel above. Shake the mixture vigorously for five minutes using a shaker and leave it to stand, and then transfer the layers of ethyl acetate and n-hexane to a 300-ml conical flask. Add 50 ml of ethyl acetate/n-hexane (1:4) to the aqueous layer and repeat the above procedure twice, and then combine the ethyl acetate and n-hexane layer to the conical flask above. Add an adequate amount of sodium sulfate (anhydrous) to the flask and leave it to stand for 15 minutes with occasional shaking. Filter the content of the flask into a rotary vacuum evaporator. Wash the flask with 20 ml of n-hexane and wash twice the residue on the surface of the filter paper. Add the washings into the rotary vacuum already containing

100 ml of saturated sodium chloride solution. Wash the eggplant-shaped flask of the above rotary vacuum evaporator with 100 ml of ethyl acetate/n-hexane (1:4) and add the washing to the separating funnel above. Shake the mixture vigorously for five minutes using a shaker and leave it to stand, and then transfer the layers of ethyl acetate and n-hexane to a 300-ml conical flask. Add 50 ml of ethyl acetate/n-hexane (1:4) to the aqueous layer and repeat the above procedure twice, and then combine the ethyl acetate and n-hexane layer to the conical flask above. Add an adequate amount of sodium sulfate (anhydrous) to the flask and leave it to stand for 15 minutes with occasional shaking. Filter the content of the flask into a rotary vacuum evaporator. Wash the flask with 20 ml of n-hexane and wash twice the residue on the surface of the filter paper. Add the washings into the rotary vacuum.

5. Determination

a. Qualitative tests

Perform qualitative tests under the following conditions. Test results obtained must be the same as those obtained for the reference standard.

Testing conditions 1

Column: A silicate glass capillary column (0.53 mm in inner diameter, 10-30 m in length) coated with methyl silicone for gas chromatography to a thickness of 1.5 μm.

Column temperature: Hold the column temperature at 80°C for one minute, followed by an increase of 8°C every minute until reaching 250°C, and hold for five minutes.

Inlet temperature: 230°C

Detector: Operate at 280°C

Gas flow rate: Use helium as the carrier gas. Adjust the flow rate to the optimal condition. Adjust the flows of air and hydrogen to the optimal conditions.

Testing conditions 2

Column: A silicate glass capillary column (0.32 mm in inner diameter, 10-30 m in length) coated with 50% trifluoro propyl methyl silicone for gas chromatography to a thickness of 0.25 μm.

Column temperature: Hold the column temperature at 70°C for one minute, followed by an increase of 25°C every minute until reaching 125°C, after which increase the temperature by 10°C every minute until reaching 235°C, and hold

for 12 minutes.
Inlet temperature: 230°C
Detector: Operate at 280°C
Gas flow rate: Use helium as the carrier gas. Adjust the flow rate to the optimal condition. Adjust the flows of air and hydrogen to the optimal conditions.

b. Quantitative tests

Determine the quantity from the test results obtained under the conditions described in 5-a using either the peak height or peak area method.

c. Confirmation tests

Perform gas chromatography/mass spectrometry under the conditions described in 5-a. Test results obtained must be the same as those obtained for the reference standard. Determine the quantity usingeither the peak height or peak area method, if required.

6. Limit of Quantification