Analytical Method for aldrin, endrin and dieldrin
(Targeted to Agricultural, Animal and Fishery Products)

The target compound to be determined is aldrin, endrin and dieldrin.

1. Instrument

A gas chromatograph with an electron capture detector (GC-ECD) and a gas chromatograph/mass spectrometer (GC/MS or GC/MS/MS)

2. Reagents

Use the reagents listed in Section C *Reagent/Test Solution, Etc*., Part II *Food Additives*, except the following.

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.

Ether: Use a rotary vacuum evaporator on 300 mL of diethyl ether 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.

Synthetic magnesium silicate (Florisil) for column chromatography: Heat florisil (150-250 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.

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

Aldrin: This product contains not less than 97% of aldrin, and its melting point is 102-104°C.

Endrin: This product contains not less than 98% of endrin, and its decomposition point is 200°C.

Dieldrin: This product contains not less than 98% of dieldrin, and its melting point is 177-179°C.

4. Procedure

a. Extraction

i. Cereal grains, legumes/pulses and seeds

Weigh 10.0 g of test samples, previously ground so as to passthrough a standard mesh sieve (420 μm). Add 20 mL of water to theobtained 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 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 10% sodium chloride solution. Wash the eggplant-shaped flask of the above rotary vacuum evaporator with 100 mL of *n*-hexane 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 *n*-hexane layer to a 300-mL conical flask. Add 50 mL of *n*-hexane to the aqueous layer and repeat the above procedure, and then add the *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 with the washings. Add the washing into the rotary vacuum evaporator and remove the *n*-hexane at 40°C or lower. Add 20 mL of *n*-hexane to the residue and transfer the mixture to a 100-mL separating funnel, and then add 40 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 40 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. Remove the acetonitrile at 40°C or lower and dissolve the residue in *n*-hexane to make exactly 5 mL of solution.

ii. Fruit, vegetables, matcha

In case of 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. In case of matcha, weigh 5.00 g of the test sample and add 20 mL of water, and then 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 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 10% sodium chloride solution. Wash the eggplant-shaped flask of the above rotary vacuum evaporator with 100 mL of *n*-hexane 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 *n*-hexane layer to a 300-mL conical flask. Add 50 mL of *n*-hexane to the aqueous layer and repeat the above procedure twice, and then combine the *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 evaporator and remove the *n*-hexane at 40°C or lower. Dissolve the residue in *n*-hexane to make exactly 10 mL of

solution.

iii. Teas (limited to unfermented tea) other than matcha

Weigh 9.00 g of the test sample and soak it in 540 mL of water at 100°C, and the leave it to stand at room temperature for five minutes. Transfer 360 mL of the cooled filtrate into a 500-mL conical flask. Add 100 mL of acetone and 2 mL of saturated lead acetate solution to the above flask and leave it to stand for one hour at room temperature. Filter the mixtue by suction into a 1,000-mL separating funnel through a filter paper covered with a 1-cm thick layer of diatomaceous earth. Wash the above conical flask with 50 mL of acetone and wash the residue on the surface of the filter paper with the washings. Add the washings into the above separating funnel and also add 30 g of sodium chloride and 100 mL of *n*-hexane. Shake the mixture vigorously for five minutes and leave it to stand, and then transfer the *n*-hexane layer to a 300-mL conical flask. Add 100 mL of *n*-hexane to the aqueous layer and repeat the above procedure, and then add the *n*-hexane layer to the conical flask above. Add an adequate amount of asodium 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 20 mL of *n*-hexane and wash twice the residue on the surface of the filter paper with the washings. Add the washings into the rotary vacuum evaporator and remove the *n*-hexane at 40°C or lower. Dissolve the residue in *n*-hexane to make exactly 5 mL of solution.

b. Clean-up

Add 10 g of florisil for column chromatography suspended in *n*-hexane into a chromatograph tube (15 mm in inner diameter, 300 mm in length) and add over approximately 5 g of sodium sulfate (anhydrous) into the column. Spill out the *n*-hexane until only a small amount remains on the packing of the column and pour 2 mL of the solution obtained by the extraction described in 4-a into the column. Pour 200 mL of ether/*n*-hexane (3:17) into the column and collect the eluate into a rotary vacuum evaporator, and then remove the ether and *n*-hexane at 40°C or lower. Dissolve the residue in *n*-hexane to make exactly 2 mL of solution, which is used as the sample solution.

5. Determination

a. Qualitative tests

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

Testing conditions 1

Column: A silicate glass capillary column (0.25 mm in inner diameter,10-30 m in length) coated with methyl silicone for gaschromatography to a thickness of 0.25 μm.

Column temperature: Hold the column temperature at 50°C for one minute, followed by an increase of 25°C every minute until reaching 175°C, after which increase the temperature by 10°C every minute until reaching 300°C, and hold for five minutes.

Inlet temperature: 230°C

Detector: Operate at 300°C

Gas flow rate: Use helium as the carrier gas. Adjust the flow rate so that aldrin flows out in approximately 10 minutes.

Testing conditions 2

Column: A silicate glass capillary column (0.25 mm in inner diameter, 10-30 m in length) coated with 14% cyanopropylphenyl-methyl silicone for gas chromatography to a thickness of 0.25 μm.

Column temperature: Hold the column temperature at 80°C for two minutes, followed by an increase of 30°C every minute until reaching 190°C, after which increase the temperature by 3.6°C every minute until reaching 250°C, and hold for eight minutes.

Inlet temperature: 230°C

Detector: Operate at 300°C

Gas flow rate: Use helium as the carrier gas. Adjust the flow rate so that aldrin flows out in approximately 10 minutes.

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 using either the peak height or peak area method, if required.

6. Limit of quantification

0.005 mg/kg