

(5) Analytical method for aldrin, endrin and dieldrin

1. Equipment

A gas chromatograph with an electron capture detector (GC-ECD) and a gas chromatograph-mass spectrometer are used.

2. Reagents/Test solutions

In addition to the reagents and test solutions listed below, those listed in Section C *Reagents/Test Solutions, Etc.*, Part II *Food additives* are used.

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: Three hundred ml of acetonitrile is concentrated using a rotary vacuum evaporator. After removing the acetonitrile, the residue is dissolved in 5 ml of *n*-hexane. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Acetone: Three hundred ml of acetone is concentrated using a rotary vacuum evaporator. After removing the acetone, the residue is dissolved in 5 ml of *n*-hexane. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Ether: Three hundred ml of diethyl ether is concentrated using a rotary vacuum evaporator. After removing the diethyl ether, the residue is dissolved in 5 ml of *n*-hexane. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance which interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with a solvent such as *n*-hexane.

Synthetic magnesium silicate (Florisil) for column chromatography: Florisil (particle size: 150-250 µm) produced for column chromatography is heated at 130°C for more than 12 hours before

being allowed to cool in a desiccator.

Diatomaceous earth: Diatomaceous earth for chemical analysis is used.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Water: Distilled water is used. In cases where a substance which interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with a solvent such as *n*-hexane.

Sodium sulfate (anhydrous): Sodium sulfate (anhydrous) (special grade). In cases where a substance which interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with a solvent such as *n*-hexane.

3. Reference material

Aldrin: This product should consist of 97% or more aldrin.

Melting point: 102-104°C

Endrin: This product should consist of 98% or more endrin.

Decomposition point: 200°C

Dieldrin: This product should consist of 98% or more dieldrin.

Melting point: 177-179°C

4. Preparation of test solutions

a. Extraction methods

i. Cereal grains, legumes/pulses and seeds

Cereal grains, legumes/pulses and seeds are crushed so as to pass through a standard mesh sieve (420 µm) before being weighed to prepare a 10.0-gram sample. Twenty ml of water is added and left to stand for two hours. Acetone (100 ml) is added and finely crushed for three minutes. The crushed mixture is filtered by suction into a rotary vacuum evaporator using filter paper covered with a one-centimeter-thick layer of diatomaceous earth. The residue on the surface of the filter paper is collected and acetone (50 ml) is added before homogenizing for three minutes. The above procedure is repeated and the filtrate is added to the rotary vacuum evaporator and concentrated to approximately 30 ml at 40°C or lower.

The concentrated solution is transferred to a 300-ml separating

funnel already containing 100 ml of 10% sodium chloride solution. The eggplant-shaped flask of the above rotary vacuum evaporator is washed with 100 ml of *n*-hexane to obtain the washings, which are added to the separating funnel above. The mixture is shaken vigorously for five minutes using a shaker before being left to stand, and then the *n*-hexane layer is transferred to a 300-ml conical flask. Fifty ml of *n*-hexane is added to the aqueous layer and, after repeating the above procedure, the *n*-hexane layer is added to the conical flask. An adequate amount of sodium sulfate (anhydrous) is also added to the flask, which is left to stand for 15 minutes and shaken from time to time. The content of the flask is then filtered into a rotary vacuum evaporator. The conical flask is then washed with 20 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed twice. The washings obtained from the repeated washing are then added to the rotary vacuum evaporator, and the *n*-hexane is removed at 40°C or lower.

Twenty ml of *n*-hexane is added to the residue, which is transferred to a 100-ml separating funnel, where 40 ml of *n*-hexane-saturated acetonitrile is added. After shaking the mixture vigorously for five minutes using a shaker, the funnel is left to stand and the acetonitrile layer is transferred to the rotary vacuum evaporator. After adding *n*-hexane-saturated acetonitrile (40 ml) to the *n*-hexane layer, the above procedure is repeated twice and the acetonitrile layer is also added to the rotary vacuum evaporator and the acetonitrile is removed at 40°C or lower. The residue is dissolved in *n*-hexane to make exactly 5 ml of solution.

ii. Fruit, vegetables, matcha

Fruit and vegetables are weighed accurately to prepare a sample of about one kilogram. An appropriate amount of measured water is added to the sample, if required. After homogenizing, a sample equivalent to 20.0 g is measured out.

Matcha is weighed to prepare a 5.00-gram sample, to which 20 ml of water is added and left to stand for two hours. Then, 100 ml of acetone is added and the mixture is finely crushed for three minutes. The crushed mixture is filtered by suction into a rotary vacuum evaporator using filter paper covered with a one-centimeter-thick

layer of diatomaceous earth. The residue removed from the surface of the filter paper is collected and 50 ml of acetone is added before homogenizing for three minutes. The above procedure is repeated and the filtrate is added to the rotary vacuum evaporator and concentrated to approximately 30 ml at 40°C or lower.

The concentrated solution is transferred to a 300-ml separating funnel already containing 100 ml of 10% sodium chloride solution. The eggplant-shaped flask of the above rotary vacuum evaporator is washed with 100 ml of *n*-hexane to obtain the washings, which are added to the separating funnel above. The mixture is shaken vigorously for five minutes using a shaker before being left to stand, and then the *n*-hexane layer is transferred to a 300-ml conical flask. Fifty ml of *n*-hexane is added to the aqueous layer and, after repeating the above procedure, the *n*-hexane layer is added to the conical flask. An adequate amount of sodium sulfate (anhydrous) is also added to the flask, which is left to stand for 15 minutes and shaken from time to time. The content of the flask is then filtered into a rotary vacuum evaporator. The conical flask is then washed with 20 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed twice. The washings obtained from the repeated washing are then added to the rotary vacuum evaporator, and the *n*-hexane is removed at 40°C or lower. The residue is dissolved in *n*-hexane to make exactly 10 ml of solution.

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

A 9.00-gram sample soaked in 540 ml of water at 100°C is left to stand at room temperature for five minutes before being filtered. From the cooled filtrate, 360 ml is transferred into a 500-ml conical flask, to which 100 ml of acetone and 2 ml of saturated lead acetate solution are added. This solution is left to stand for one hour at room temperature before being filtered by suction using filter paper covered with a one-centimeter-thick layer of diatomaceous earth. The filtrate is transferred to a 1,000-ml separating funnel. The conical flask is then washed with 50 ml of acetone to obtain the washings, with which the residue on the surface of the filter paper is washed. The washings are added to the separating funnel above. Thirty grams of sodium chloride and 100 ml of *n*-hexane are also

added to the separating funnel. The mixture is shaken vigorously for five minutes and left to stand. The *n*-hexane layer is then transferred to a 300-ml conical flask. One hundred ml of *n*-hexane is added to the aqueous layer and, after repeating the above procedure, the *n*-hexane layer is added in the conical flask. An adequate amount of sodium sulfate (anhydrous) is also added to the flask, which is left to stand for 15 minutes and shaken from time to time. The content of the flask is then filtered into a rotary vacuum evaporator. The conical flask is then washed with 20 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed twice. The washings obtained from the repeated washing are then added to the rotary vacuum evaporator, and the *n*-hexane is removed at 40°C or lower. The residue is dissolved in *n*-hexane to make exactly 5 ml of solution.

b. Clean-up

Ten grams of florisil for column chromatography suspended in *n*-hexane is added into a chromatograph tube (inner diameter: 15 mm and length: 300 mm), over which approximately 5 g of sodium sulfate (anhydrous) is further added. The *n*-hexane is then spilt out until only a small amount remains on the packing of the column, into which 2 ml of the solution obtained by the method described in “a. Extraction methods” is poured. Subsequently, 200 ml of a mixture of ether and *n*-hexane (3:17) is also poured into the column. The eluate is transferred to a rotary vacuum evaporator, and the ether and *n*-hexane are removed at 40°C or lower. The residue is dissolved in *n*-hexane to make exactly 2 ml of solution, which is used as the sample solution.

5. Determination

a. Qualitative tests

Qualitative tests are performed under the following conditions. Test results obtained under any of the conditions must be the same as the results obtained in the reference material.

Testing conditions 1

Column: A silicate glass capillary column (inner diameter: 0.25 mm and length: 10-30 m) coated with methyl silicone for gas chromatography to a thickness of 0.25 μm is used.

Column temperature: The column temperature is held at 50°C for one minute, followed by an increase of 25°C every minute until reaching

175°C, after which the temperature is increased by 10°C every minute until reaching 300°C, where it is held for five minutes.

Inlet temperature: 230°C

Detector: Should be operated at 300°C

Gas flow rate: Helium is used as the carrier gas. The flow rate should be adjusted so that aldrin flows out in approximately 10 minutes.

Testing conditions 2

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

Column temperature: The column temperature is held at 80°C for two minutes, followed by an increase of 30°C every minute until reaching 190°C, after which the temperature is increased by 3.6°C every minute until reaching 250°C, where it is held for eight minutes.

Inlet temperature: 230°C

Detector: Should be operated at 300°C

Gas flow rate: Helium is used as the carrier gas. The flow rate should be adjusted so that aldrin flows out in approximately 10 minutes.

b. Quantitative tests

The quantity is determined from the test results obtained under the conditions described in “a. Qualitative tests,” using either the peak height or peak area method.

c. Confirmation tests

Gas chromatography/mass spectrometry is performed under the conditions described in “a. Qualitative tests.” Test results obtained must be the same as the results obtained in the reference material. The quantity may be determined by either the peak height or peak area method, if required.

(6) Captafol analytical method

Should be performed according to 5 (6).

(7) Quinoxaline-2-carboxylic acid analytical method

Should be performed according to 5 (7).

(8) Cyhexatin analytical method

Should be performed according to 5 (4).

(9) Daminozide analytical method

Should be performed according to 5 (13).

(10) Analytical method for triazophos and parathion

1. Equipment

A gas chromatograph with an alkali flame ionization detector, a flame photometric detector (interference filter for phosphorus determination, wavelength: 526 nm), or a highly-sensitive nitrogen phosphorus detector, and a gas chromatograph-mass spectrometer are used.

2. Reagents/Test solutions

In addition to the reagents and test solutions listed below, those listed in Section C *Reagents/Test Solutions, Etc.*, Part II *Food additives* are used.

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: Three hundred ml of acetonitrile is concentrated using a rotary vacuum evaporator. After removing the acetonitrile, the residue is dissolved in 5 ml of *n*-hexane. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Acetone: Three hundred ml of acetone is concentrated using a rotary vacuum evaporator. After removing the acetone, the residue is dissolved in 5 ml of *n*-hexane. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance which interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with a solvent such as *n*-hexane.

Silica gel for column chromatography (particle size: 63-200 µm): Silica gel (particle size: 63-200 µm) produced for column chromatography is heated at 130°C for more than 12 hours before being allowed to cool in a desiccator.

Diatomaceous earth: Diatomaceous earth for chemical analysis is used.

Ethyl acetate: Three hundred ml of ethyl acetate is concentrated using a rotary vacuum evaporator. After removing the ethyl acetate, the residue is dissolved in 5 ml of *n*-hexane. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. Then, 5 µl of the dissolved residue is injected into the GC-ECD for analysis, when the heights of peaks for compounds other than *n*-hexane on the gas chromatogram should be the same as or lower than the peak for gamma-BHC at 2×10^{-11} g.

Water: Distilled water is used. In cases where a substance which interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with a solvent such as *n*-hexane.

Sodium sulfate (anhydrous): Sodium sulfate (anhydrous) (special grade). In cases where a substance which interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with a solvent such as *n*-hexane.

3. Reference material

Triazophos: This product should consist of 98% or more triazophos.

Melting point: 0-5°C

Parathion: This product should consist of 97% or more parathion.

Boiling point: 375°C

4. Preparation of test solutions

a. Extraction methods

i. Cereal grains and legumes/pulses

Cereal grains and legumes/pulses are crushed so as to pass through a standard mesh sieve (420 µm) before being weighed to prepare a 10.0-gram sample. Twenty ml of water is added and left to stand for two hours. Acetone (100 ml) is added and finely crushed for three minutes. The crushed mixture is filtered by suction into a rotary vacuum evaporator using filter paper covered with a one-centimeter-thick layer of diatomaceous earth. The residue on the surface of the filter paper is collected and acetone (50 ml) is added before homogenizing for three minutes. The above procedure

is repeated, and the filtrate is added to the rotary vacuum evaporator and concentrated to approximately 30ml at 40°C or lower.

The concentrated solution is transferred to a 300-ml separating funnel already containing 100 ml of saturated sodium chloride solution. The eggplant-shaped flask of the above rotary vacuum evaporator is washed with 100 ml of a mixture of ethyl acetate and *n*-hexane (1:4) to obtain the washings, which are added to the separating funnel above. The mixture is shaken vigorously for five minutes using a shaker before being left to stand, and then the layers of ethyl acetate and *n*-hexane are transferred to a 300-ml conical flask. Fifty ml of a mixture of ethyl acetate and *n*-hexane (1:4) is added to the aqueous layer, and after repeating the above procedure, the layers of ethyl acetate and *n*-hexane are added to the conical flask. An adequate amount of sodium sulfate (anhydrous) is also added to the flask, which is left to stand for 15 minutes and shaken from time to time. The content of the flask is then filtered into a rotary vacuum evaporator. The conical flask is then washed with 20 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed twice. The washings obtained from the repeated washing are then added to the rotary vacuum evaporator, and the ethyl acetate and *n*-hexane are removed at 40°C or lower.

Thirty ml of *n*-hexane is added to the residue, which is then transferred to a 100-ml separating funnel, where 30 ml of *n*-hexane-saturated acetonitrile is added. After shaking the mixture vigorously for five minutes using a shaker, the funnel is left to stand and the acetonitrile layer is transferred to the rotary vacuum evaporator. After adding *n*-hexane-saturated acetonitrile (30 ml) to the *n*-hexane layer, the above procedure is repeated twice. The acetonitrile layer is then added to the rotary vacuum evaporator, and the acetonitrile is removed at 40°C or lower. The residue is dissolved in 5 ml of a mixture of acetone and *n*-hexane (1:1).

ii. Fruit and vegetables

Fruit and vegetables are weighed accurately to prepare a sample of about one kilogram. An appropriate amount of measured water is added to the sample, if required. After chopping into pieces of equal size, a sample equivalent to 20.0 g is taken.

Then 100 ml of acetone is added before finely crushing for three minutes. The crushed sample is filtered by suction into a rotary vacuum evaporator using filter paper covered with a one-centimeter-thick layer of diatomaceous earth. The residue on the surface of the filter paper is collected and acetone (50 ml) is added before homogenizing for three minutes. The above procedure is repeated and the filtrate is added to the rotary vacuum evaporator and concentrated to approximately 30 ml at 40°C or lower.

The concentrated solution is transferred to a 300-ml separating funnel already containing 100 ml of saturated sodium chloride. The eggplant-shaped flask of the above rotary vacuum evaporator is washed with 100 ml of a mixture of ethyl acetate and *n*-hexane (1:4) to obtain the washings, which are added to the separating funnel above. The mixture is shaken vigorously for five minutes using a shaker before being left to stand, and then the layers of ethyl acetate and *n*-hexane are transferred to a 300-ml conical flask. Fifty ml of a mixture of ethyl acetate and *n*-hexane (1:4) is added to the aqueous layer, and after repeating the above procedure, the layers of ethyl acetate and *n*-hexane are added to the conical flask. An adequate amount of sodium sulfate (anhydrous) is also added to the flask, which is left to stand for 15 minutes and shaken from time to time. The content of the flask is then filtered into a rotary vacuum evaporator. The conical flask is then washed with 20 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed twice. The washings obtained from the repeated washing are then added to the rotary vacuum evaporator, and the ethyl acetate and *n*-hexane at 40°C or lower. The residue is dissolved in 5 ml of a mixture of acetone and *n*-hexane (1:1).

b. Clean-up

Five grams of silica gel for column chromatography (particle size: 63-200 µm) suspended in a mixture of acetone and *n*-hexane (1:1) is added into a chromatograph tube (inner diameter: 15 mm and length: 300 mm), over which about 5 g of sodium sulfate (anhydrous) is also poured in. Then, the mixture of acetone and *n*-hexane (1:1) is spilt out until only a small amount remains on the packing of the column. The solution obtained by the method described in “a. Extraction methods” is poured into this column. Then 100 ml of a mixture of

acetone and *n*-hexane (1:1) is also added. The eluate is collected in a rotary vacuum evaporator, and the acetone and *n*-hexane are removed at 40°C or lower. The residue is dissolved in acetone to make exactly 5 ml of solution, which is used as the sample solution.

5. Determination

a. Qualitative tests

Qualitative tests are performed under the following conditions. Test results obtained under any of the conditions must be the same as the results obtained in the reference material.

Testing conditions 1

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

Column temperature: The column temperature is held at 80°C for one minute, followed by an increase of 8°C every minute until reaching 250°C, where it is held for five minutes.

Inlet temperature: 230°C

Detector: Should be operated at 280°C

Gas flow rate: Helium is used as the carrier gas. The flow rate should be adjusted to the optimal condition. The flows of air and hydrogen should also be adjusted to the optimal conditions.

Testing conditions 2

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

Column temperature: The column temperature is held at 70°C for one minute, followed by an increase of 25°C every minute until reaching 125°C, after which the temperature is increased by 10°C every minute until reaching 235°C, where it is held for 12 minutes.

Inlet temperature: 230°C

Detector: Should be operated at 280°C

Gas flow rate: Helium is used as the carrier gas. The flow rate should be adjusted to the optimal condition. The flows of air and hydrogen should also be adjusted to the optimal conditions.

b. Quantitative tests

The quantity is determined from the test results obtained under the conditions described in “a. Qualitative tests,” using either the peak

height or peak area method.

c. Confirmation tests

Gas chromatography/mass spectrometry is performed under the conditions described in “a. Qualitative tests.” Test results obtained must be the same as the results obtained in the reference material. The quantity may be determined by either the peak height or peak area method, if required.

7. In addition to the substances stipulated under 6, ingredients of agricultural chemicals and other chemical substances listed in the first column of the table in (1) must not be contained in foods at levels exceeding the limits stipulated in the third column of the same table according to the food categories shown in the second column of the same table. Concerning this, foods listed in the “foods” column in the table in (2) shall be tested using the part listed in the “samples” column in the table as a sample. In addition, in the substances used as ingredients of agricultural chemicals and other chemical substances listed in the first column of the table in (1), which are stipulated to be “Not detected” in the third column of the same table in the foods listed in the second column of the same table, no ingredient of agricultural chemicals or other chemical substances shall be detected when these foods are tested using the test methods stipulated in (3) to (8).”

(1) The maximum residue limits of substances used as ingredients of agricultural chemicals in foods (*Provisional MRLs List*)

(2) Samples

Food	Sample material
Barley and buckwheat	Threshed seeds
Wheat and rye	Husked seeds
Rice (brown rice)	Husked seeds
Corn (maize)	Seeds with the husk, the silk and the cores removed
Other cereal grains	Threshed seeds
Peas, beans (dry)*, broad beans and soybeans (dry)	Seeds without the pods
Peanuts, dry	With the shells removed