Sesame seeds, rapeseeds, sunflower seeds, safflower seeds, cotton seeds and other oil seeds	Seeds only
Almond, ginkgo nut, chestnut, walnut, pecan and other nuts	With the husks removed
Cacao beans and coffee beans	Beans without the pods
Tea	Tea leaves
Hop	Dried flowers
Other spices and other herbs	Edible portions

(3) 2, 4, 5-T analytical method

1. Equipment

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 to be 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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for

compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance that interferes with the 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.

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. When 5 μl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Reagent for butyl esterification: Ten grams of boron trifluoride ether complex is dissolved in 25 ml of *n*-butanol.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. When 5 μl of the concentrated sample is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Water: Distilled water is used. In cases where a substance that 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 that 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.

Methanol: Three hundred ml of methanol is concentrated using a rotary vacuum evaporator. After removing the methanol, the residue is dissolved in 5 ml of *n*-hexane. When 5 μl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

3. Reference material

2,4,5-T: This product should consist of 98% or more 2,4,5-T.

Melting point: 156°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 to the obtained sample and left to stand for two hours.

Then, acetone (100 ml) and 4 mol/l hydrochloric acid (5 ml) are added. After homogenizing for three minutes, the 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 ethyl acetate 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 ethyl acetate layer is transferred to a 300-ml conical flask. Fifty ml of ethyl acetate is added to the aqueous layer, and, after repeating the above procedure, the ethyl acetate 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 ethyl acetate 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 concentrated to approximately 1 ml at 40°C or lower, and then the solvent is evaporated to near

dryness in a nitrogen stream at room temperature.

Thirty ml of *n*-hexane is added to the dried residue and the mixture is transferred to a 100-ml separating funnel, to which 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 a 200-ml separating funnel. After adding *n*-hexane-saturated acetonitrile (30 ml) to the *n*-hexane layer, the above procedure is repeated twice and the acetonitrile layer is added to the separating funnel above. Fifty ml of *n*-hexane saturated with acetonitrile is also added into the separating funnel, which is lightly shaken and left to stand. The acetonitrile layer is then transferred into a rotary vacuum evaporator and concentrated to approximately 1 ml at 40°C or lower, and then the solvent is evaporated to near dryness in a nitrogen stream at room temperature.

ii. Fruit, vegetables, matcha and hops

Fruit and vegetables are weighed accurately to prepare a sample of about one kilogram. An appropriate amount of water is measured and 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.

Hops are first crushed into pieces and weighed to prepare a 5.00-gram sample, to which 20 ml of water is added and left to stand for two hours.

Acetone (100 ml) and 4 mol/l hydrochloric acid (5 ml) are added to the obtained sample. The mixture, after homogenizing for three minutes, 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 ethyl acetate to obtain the washings, which are combined in the separating funnel above. The mixture is shaken vigorously for five minutes using a shaker before being left to stand and the ethyl acetate layer is transferred to a 300-ml conical flask. Fifty ml of ethyl acetate is added to the aqueous layer and, after repeating the above procedure, the ethyl acetate layer is combined 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 ethyl acetate 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 concentrated to approximately 1 ml at 40°C or lower, and then the solvent is evaporated to near dryness in a nitrogen stream at room temperature.

iii. Teas 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 18 g of sodium chloride and 4 mol/l hydrochloric acid are added to adjust the pH to 1 or lower. This solution is transferred to a 1,000-ml separating funnel already containing 100 ml of ethyl acetate before shaking vigorously for five minutes using a shaker. The shaken mixture is left to stand and the ethyl acetate layer is transferred to a 300-ml conical flask. One hundred ml of ethyl acetate is added to the aqueous layer and, after repeating the above procedure, the ethyl acetate layer is combined 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 ethyl acetate 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 concentrated to approximately 1 ml at 40°C or lower, and then the solvent is evaporated to near dryness in a

nitrogen stream at room temperature.

iv. Foods other than those listed in i to iii above

Extracts are obtained by the methods described in i or ii.

b. Hydrolysis

The residue obtained by the extraction method described in “a. Extraction methods” is dissolved in 20 ml of methanol and transferred to a 100-ml eggplant-shaped flask, to which 10 ml of a 1.5 mol/l sodium hydroxide solution is added. A reflux condenser is attached to the flask, which is then heated for 30 minutes in a water bath at 80°C and allowed to cool. The solution is transferred to a rotary vacuum evaporator and most of the methanol is removed at 40°C or lower. The residue is then filtered by suction through a glass filter (pore size G3). The filtrate is transferred to a 300-ml separating funnel (I). The residue on the glass filter is washed with a small amount of acetone and water and the washings are added to the separating funnel above, to which 50 ml of ether and 100 ml of 10% sodium chloride solution are also added. The mixture is shaken vigorously for five minutes using a shaker before being left to stand, and then the aqueous layer is transferred to a 300-ml separating funnel (II), to which 4 mol/l hydrochloric acid is added to adjust the pH to 1 or lower. Fifty ml of ethyl acetate is added to the adjusted solution before being vigorously shaken for five minutes using a shaker and left to stand. The ethyl acetate layer is then transferred to a 300-ml conical flask. Fifty ml of ethyl acetate is added to the aqueous layer and, after repeating the above procedure, the ethyl acetate layer is combined in the conical flask described above. 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 ethyl acetate to obtain the washings, with which the residue on the surface of the filter paper is washed. The washings are added to the rotary vacuum evaporator and concentrated to approximately 1 ml at 40°C or lower.

c. Butyl esterification

The solution obtained by the hydrolysis described in “b. Hydrolysis” is transferred to a 20-ml eggplant-shaped flask, and then the solvent

is evaporated to near dryness in a nitrogen stream at room temperature. After the desiccation, one ml of a butyl esterificated agent is added. A reflux condenser is attached to the eggplant-shaped flask described above, which is then heated for 30 minutes in a water bath at 90°C and allowed to cool. The cooled mixture is transferred to a 200-ml separating funnel already containing 50 ml of 10% sodium chloride solution and 50 ml of *n*-hexane before shaking vigorously for five minutes using a shaker. The shaken mixture is left to stand and the *n*-hexane layer is transferred to a 200-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 combined in the conical flask described above. 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 10 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed. The washings are added to the rotary vacuum evaporator and concentrated to approximately 2 ml at 40°C or lower.

d. Clean-up

Five grams of florisil for column chromatography suspended in *n*-hexane is added to 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 of the column until only a small amount remains on the packing of the column, into which the solution obtained by the butyl esterification described in “c. Butyl esterification” is poured. Then, 50 ml of a mixture of ether and *n*-hexane (1:19) is also poured into the column and the effluent is discarded. In addition, 150 ml of a mixture of ether and *n*-hexane (3:17) is also poured into the column and the eluate is collected in a rotary vacuum evaporator and concentrated to approximately 1 ml at 40°C or lower, and then the solvent is evaporated to near dryness in a nitrogen stream at room temperature. The residue obtained is dissolved in *n*-hexane to make exactly 10 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 must be the same as the results obtained in the reference material under the procedure described in “c. Butyl esterification” in “4. Preparation of test solutions.”

Testing conditions

Column: A silicate glass capillary column (inner diameter: 0.25 mm and length: 30 m) coated with 5% phenyl 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 125°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: 260°C

Detector: Should be operated at 300°C

Gas flow rate: Nitrogen or helium is used as the carrier gas. The flow rate should be adjusted so that *n*-butyl (2,4,5-trichlorophenoxy) acetate flows out in approximately 15 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 same conditions as those described in “a. Qualitative tests.” Test results obtained in the reference material must be the same as the results obtained under the procedure described in “c. Butyl esterification” in “4. Preparation of test solutions.” The quantity may be determined by either the peak height or peak area method, if required.

(4) Analytical method for azocyclotin and cyhexatin

1. Equipment

A gas chromatograph with a flame photometric detector (interference filter for tin determination, wavelength: 610nm) 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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

3 mol/l ethylmagnesium bromide-ethereal solution: 3 mol/l ethylmagnesium bromide-ethereal solution.

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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance that 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.

Cyhexatin standard solution: A mixture of acetic acid and ethyl acetate (1:99) is added to 10.0 mg of cyhexatin to make a 100-ml solution. Of the 100-ml solution, 10 ml is taken out and *n*-hexane is added to make 100 ml of mix.

Sodium dodecyl sulfate: A reagent with a purity of 85% or higher is

used.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. When 5 µl of the concentrated sample is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Water: Distilled water is used. In cases where a substance that 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 that 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

Cyhexatin: This product should consist of 99% or more cyhexatin.

Melting point: 195-198°C

4. Preparation of test solutions

a. Extraction methods

i. Legumes/pulses and seeds

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 to the obtained sample and left to stand for two hours.

Then, 100 ml of a mixture of acetone and acetic acid (99:1) is added. The mixture, after homogenizing for three minutes, 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 50 ml of a mixture of acetone and acetic acid (99:1) 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 500-ml separating funnel already containing 200 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 *n*-hexane is removed at 40°C or lower.

Twenty ml of *n*-hexane is added to the residue and the mixture is transferred to a 100-ml separating funnel, to which 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. 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 *n*-hexane to make exactly 5 ml of solution.

ii. Cereal grains, fruit and vegetables

Cereal grains 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 to the obtained sample and then left to stand for two hours.

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

One hundred ml of a mixture of acetone and acetic acid (99:1) is added to the 20-gram sample before being finely crushed for three minutes, and 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 50 ml of a mixture of acetone and acetic acid (99:1) 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 500-ml separating funnel already containing 200 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. Matcha and hops

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.

Hops are first crushed into pieces and weighed to prepare a 5.00-gram sample, to which 20 ml of water is added and left to stand for two hours.

One hundred ml of a mixture of acetone and acetic acid (99:1) is added to this sample. The mixture, after homogenizing for three minutes, 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 50 ml of a mixture of acetone and acetic acid (99:1) 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 500-ml separating funnel already containing 200 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 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 added to the rotary vacuum evaporator and concentrated to approximately 5 ml at 40°C or lower, to which *n*-hexane is added to make exactly 10 ml of solution.

iv. Teas 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 separating funnel.

To this conical flask, 30 g of sodium chloride, 2 ml of 2% sodium dodecyl sulfate solution and 100 ml of *n*-hexane are also added. The mixture is shaken vigorously for five minutes using a shaker 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 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 *n*-hexane is removed at 40°C or lower. The residue is dissolved in *n*-hexane to make exactly 6 ml of solution.

v. Foods other than those listed in i to iv above

Extracts are obtained by the methods described in i, ii, or iii.

b. Ethylation

One ml (2 ml for cereal grains, teas and hops) of the solution obtained by the method described in “a. Extraction methods” is transferred to a 50-ml test tube with a glass stopper, to which 1 ml (2 ml for cereal grains, teas and hops) of a 3 mol/l ethylmagnesium bromide-ethereal solution is added. The mixture is left to stand for 20 minutes at room temperature.

Then, 10 ml of 0.5 ml/l sulfuric acid is gradually added, followed by the addition of 10 ml of water to be mixed in. Ten ml of *n*-hexane is added to the mixture before being vigorously shaken for one minute. After being left to stand, the *n*-hexane layer is transferred to a 50 ml conical flask. Five ml of *n*-hexane is added to the aqueous layer and, after repeating the above procedure twice, 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 5 ml of *n*-hexane to obtain the washings, with which the residue on the surface of the filter paper is washed twice. The washings from the repeated washing are then added to the rotary vacuum evaporator and concentrated to 2 ml at 40°C or lower.

c. Clean-up

Five grams of florisil for column chromatography suspended in *n*-hexane is added to 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 the solution obtained by the ethylation described in “b. Ethylation” is poured. Then, the eggplant-shaped flask of the rotary vacuum evaporator is washed with 15 ml of *n*-hexane to obtain the washings, which are transferred to a column. The eluate is collected in the

rotary vacuum evaporator. Then, 50 ml of a mixture of ether and *n*-hexane (1:99) is poured in and the eluate is collected in the rotary vacuum evaporator, and the ether and *n*-hexane are removed at 40°C or lower. The residue obtained 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 must be the same as the results obtained in the cyhexatin reference material under the procedure described in “b. Ethylation” in “4. Preparation of test solutions.” Azocyclotin is modified by ethylation into the same substance as cyhexatin.

Testing conditions

Column: A silicate glass capillary column (inner diameter: 0.32 mm-0.53 mm and length: 30 m) coated with 5% phenyl methyl silicone for gas chromatography to a thickness of 1.5 μm is used.

Column temperature: The column temperature is held at 120°C for two minutes, followed by an increase of 10°C every minute until reaching 200°C, after which the temperature is increased by 20°C every minute until reaching 300°C, where it is held for five minutes.

Inlet temperature: 280°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 cyhexatin flows out in approximately 13-15 minutes. The flow rates of air and hydrogen are adjusted to optimal conditions.

b. Quantitative tests

The quantity is determined from the test results obtained under the same 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 same conditions described in “a. Qualitative tests.” Test results obtained must be the same as the results obtained in the reference material under the procedure described in “b. Ethylation” in “4. Preparation of test solutions.” The quantity may be determined by either the peak height or peak area method, if required.

(5) Amitrole analytical method

1. Equipment

A high-performance liquid chromatograph with a fluorescence detector and liquid 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.

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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Ethanol: Three hundred ml of ethanol is concentrated using a rotary vacuum evaporator. After removing the ethanol, the residue is dissolved in 5 ml of *n*-hexane. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Diatomaceous earth: Diatomaceous earth for chemical analysis is used.

Acetic acid buffer solution: 0.05 mol/l sodium acetate solution is added to 800 ml of 0.05 mol/l acetic acid solution to make a 1,000-ml solution.

Weak acid cation exchange resin: Weak acid cation exchange resin produced for column choromatography is first washed with 1 mol/l hydrochloric acid, secondly with a 2.8% aqueous ammonia, and thirdly with 1 mol/l hydrochloric acid again. Thereafter the washings are further washed with water until they become neutral.

Fluorescamin: A reagent with a purity of 98% or higher is used.

Water: Distilled water is used. In cases where a substance that 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.

Phosphate buffer solution: 10% phosphoric acid solution is added to a 0.05 ml/l monosodium phosphate solution to adjust the pH to 3.0.

3. Reference material

Amitrole: This product should consist of 98% or more amitrole.

Melting point: 157-159°C

4. Preparation of test solutions

a. Extraction methods

i. Cereal grains, legumes/pulses, seeds, fruit, vegetables, matcha and hops

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 30.0-gram sample.

Fruit and vegetables are weighed accurately to prepare a sample of about one kilogram. An appropriate amount of water is measured and added to the sample, if required. After homogenizing, a sample equivalent to 30.0 g is measured out.

Matcha is weighed to prepare a 30.0-gram sample.

Hops are crushed into pieces and weighed to prepare a 30.0-gram sample.

Eighty ml of ethanol is added to the obtained sample before being crushed finely 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 and the filtrate is transferred to a 200-ml graduated cylinder. The residue on the surface of the filter paper is collected and 40 ml of 60% ethanol is added before homogenizing for three minutes. After repeating the above procedure, the filtrate is added to the graduated cylinder and the amount of filtrate is measured.

Ten ml of the filtrate is transferred to a 200-ml round bottom flask, to which 1 ml of hydrogen peroxide solution is added. A reflux condenser is attached to the flask, which is then heated for 30 minutes in a water bath at 75°C and allowed to cool.

ii. Teas except matcha

A 10.00-gram sample soaked in 600 ml of water at 100°C is left to stand at room temperature for five minutes before being filtered. From the cooled filtrate, 12 ml is transferred into a 200-ml round bottom flask, to which 1 ml of hydrogen peroxide solution is added. A reflux condenser is attached to the flask, which is then heated for 30 minutes in a water bath at 75°C and allowed to cool.

iii. Foods except those listed in i and ii above

Extracts are obtained by the methods described in i.

b. Clean-up

One ml of strong acid cation exchange resin (particle size: 0.063-0.156 μm) in a water suspension is poured into a chromatograph tube (inner diameter: 10 mm and length: 300 mm). The water is then spilt out until only a small amount remains on the packing of the column, into which 5 ml of water is poured and the effluent is discarded. The solution obtained by the method described in “a. Extraction methods” is then poured into the column. The round bottom flask described above is then washed with 10 ml of water and the washings are also poured into the column and the effluent is discarded. Subsequently, 12 ml of 2.8% aqueous ammonia is added to the column. The eluate is collected in a rotary vacuum evaporator and 30 ml of *n*-propanol is added before removing the water and *n*-propanol at 45°C or lower. Five ml of water is added to the residue to dissolve it.

In a chromatograph tube (inner diameter: 10 mm and length: 300 mm), 5 ml of weak acid cation exchange resin (particle size: 0.33-0.50 μm) in a water suspension is poured. The water is then spilt out until only a small amount remains on the packing of the column, into which 10 ml of water is poured and the effluent is discarded. The above solution is poured in and the effluent is discarded. Fifty ml of water is also poured in and the effluent is discarded. Subsequently, 35 ml of 2.8% aqueous ammonia is added to the column. The eluate is collected in a rotary vacuum evaporator and 100 ml *n*-propanol is added before removing the aqueous ammonia and *n*-propanol at 45°C or lower.

c. Derivatization

Two ml of acetic acid buffer solution is added to the residue to dissolve it well. To 1 ml of this solution, 100 μl of 0.25% fluorescamin-acetone solution is added, which is then shaken well before being allowed to stand for one hour. Then, 0.5 ml of 0.05 mol/l sodium borate solution is also added to prepare the test solution.

5. Determination

a. Qualitative tests

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

reference material under the procedure described in “c. Derivatization” in “4. Preparation of test solutions.”

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 5 μm) is used.

Column: A stainless tube (inner diameter: 4.6 mm and length: 150 mm) is used.

Column temperature: 40°C

Detector: Should be operated with an excitation wavelength of 380 nm and a fluorescent wavelength of 484 nm.

Mobile phase: A mixture of acetonitrile and phosphate buffer solution (3:7) is used. The flow rate should be adjusted so that the amitrole flows out in approximately 15 minutes.

b. Quantitative tests

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

c. Confirmation tests

Liquid chromatography/mass spectrometry is performed under the same conditions described in “a. Qualitative tests.” Test results obtained in the reference material must be the same as the results obtained under the procedure described in “c. Derivatization” in “4. Preparation of test solutions.” The quantity may be determined by either the peak height or peak area method, if required.

(6) Captafol analytical method

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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance that 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.

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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. When 5 µl of the concentrated sample is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Water: Distilled water is used. In cases where a substance that 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 that 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

Captafol: This product should consist of 98% or more captafol.

Melting point: 159-161°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 3% phosphoric acid solution is added to the obtained sample, which is then left to stand for two hours.

Acetone (100 ml) is then added. The mixture, after homogenizing for three minutes, 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 then 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.

Thirty ml of *n*-hexane is added to the residue and the mixture is then transferred to a 100-ml separating funnel, to which 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 *n*-hexane.

ii. Fruit, vegetables, matcha and hops

Fruit and vegetables are weighed accurately to prepare a sample of about one kilogram, to which 500 ml of 10% phosphoric acid solution is added before homogenizing. Then, 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 3% phosphoric acid solution is added and left to stand for two hours.

Hops are first crushed into pieces and weighed to prepare a 5.00-gram sample, to which 20 ml of 3% phosphoric acid solution is added and left to stand for two hours.

One hundred ml of acetone is then added to the mixture 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. The residue is dissolved in 5 ml of *n*-hexane.

iii. Teas except 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.

Thirty ml of phosphoric acid, 100 ml of acetone and 2 ml of saturated lead acetate solution are also added to this conical flask. The mixture 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, to which 30 g of sodium chloride and 100 ml of *n*-hexane are also added. 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 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 *n*-hexane is removed at 40°C or lower.

The residue is dissolved in 5 ml of *n*-hexane.

iv. Foods except those listed in i to iii above

Extracts are obtained by the methods described in i or ii.

b. Clean-up

In a chromatograph tube (inner diameter: 15 mm and length: 300 mm), 5 g of florisil for column chromatography suspended in *n*-hexane is added, 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 the solution obtained by the extraction method described in “a. Extraction methods” is poured. Then, 100 ml of *n*-hexane is also poured in and the effluent is discarded. Subsequently, 150 ml of a mixture of ethyl acetate and *n*-hexane (1:9) is added and the eluate is collected in a rotary vacuum evaporator, and the ethyl acetate and *n*-hexane are removed at 40°C or lower. The residue is dissolved in *n*-hexane 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: 0.25 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 to the optimal condition.

Testing conditions 2

Column: A silicate glass capillary column (inner diameter: 0.25 mm and length: 10-30 m) coated with 5% phenyl 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 125°C, after which the temperature is increased by 10°C every minute until reaching 300°C, where it is held for three 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 to the optimal condition.

b. Quantitative tests

The quantity is determined from the test results obtained under the same 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 same 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) Carbadox analytical method

Quinoxaline-2-carboxylic acid is analyzed.

1. Equipment

A high-performance liquid chromatograph with an ultraviolet spectrophotometric detector and a liquid 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.

Octadecylsilane-bonded silica gel minicolumn (500 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 500 mg of octadecylsilane-bonded silica gel or one with the same separation characteristics is used.

Strong basic anion exchanger minicolumn (360 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 360 mg of

trimethylamino acrylamide copolymer silane-bonded silica gel or one with the same separation characteristics is used.

Water: Water produced for liquid chromatography is used.

Sodium sulfate (anhydrous): Sodium sulfate (anhydrous) (special grade). In cases where a substance that 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.

Phosphate buffer solution (pH 2.5): Monopotassium dihydrogen monophosphate (1.36 g) is dissolved in water to make a 800-ml solution, to which phosphoric acid is added to adjust the pH to 2.5. Water is added to the adjusted solution to make a 1,000-ml solution.

3. Reference material

Quinoxaline-2-carboxylic acid: This product should consist of 99% or more quinoxaline-2-carboxylic acid.

Melting point: 208°C

4. Preparation of test solutions

a. Extraction methods

After homogenizing, a sample of 5.00 g is measured out. For muscle, the fat layer should be removed as much as possible before chopping. One hundred ml of a mixture of methanol and 0.3% metaphosphoric acid solution (3:7) is added to the measured-out sample. The mixture, after homogenizing, is filtered by suction into a rotary vacuum evaporator using filter paper covered with a two-millimeter-thick layer of diatomaceous earth. The residue on the surface of the filter paper is then washed with 10 ml of a mixture of methanol and 0.3% metaphosphoric acid solution (3:7) to collect the washings, which are then filtered by suction and added into the rotary vacuum evaporator. The mixture is concentrated to 30 ml at 45°C or lower, and 0.1 ml of phosphoric acid is added.

b. Clean-up

i. Octadecylsilane-bonded silica gel column chromatography

Five ml of methanol is added to an octadecylsilane-bonded silica gel minicolumn (500 mg), followed by 10 ml of water. The effluent is discarded. The solution obtained by the extraction method described in “a. Extraction methods” is poured into this column and subsequently 20 ml of phosphate buffer solution (pH 2.5) is also added. The effluent is discarded. Ten ml of methanol is poured into

the column. The eluate is collected in a rotary vacuum evaporator, and the methanol is removed at 40°C or lower. The residue is dissolved in 5 ml of water.

ii. Strong basic anion exchanger column chromatography

Five ml of water is poured into a strong basic anion exchanger minicolumn (360 mg) and the effluent is discarded. The solution obtained in “i. Octadecylsilane-bonded silica gel column chromatography” is poured into this column. Two ml of water, 10 ml of ethanol and then 5 ml of water are also added in that order. The effluent is discarded. Five ml of 0.1 mol/l hydrochloric acid is poured into this column and the eluate is collected in a 50-ml test tube. Three ml of 3 mol/l hydrochloric acid and 15 ml of ethyl acetate are also added before shaking vigorously using a shaker for five minutes. After leaving to stand, the ethyl acetate layer is transferred into a 100-ml conical flask. Fifteen ml of ethyl acetate is added to the aqueous layer, and after repeating the above procedure, the ethyl acetate 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, and the ethyl acetate is removed at 40°C or lower. The residue is dissolved in 1.0 ml of a mixture of acetonitrile and phosphate buffer solution (pH 2.5) (1:4), which is used as the test solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 5 µm) is used.

Column: A stainless tube (inner diameter: 4.0-6.0 mm and length: 150 mm) is used.

Column temperature: 40°C

Detector: Should be operated at an absorption wavelength of 245 nm.

Mobile phase: A mixture of acetonitrile and phosphate buffer solution

(pH 2.5) (1:4) is used. The flow rate should be adjusted so that quinoxaline-2-carboxylic acid flows out in approximately 10 minutes.

b. Quantitative tests

The quantity is determined from the test results obtained under the same 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 same conditions described in “a. Qualitative tests,” but the mobile phase should be a mixture of acetonitril and water (1:4). 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.

(8) Coumaphos analytical method

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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for

compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance that 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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. When 5 µl of the concentrated sample is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Water: Distilled water is used. In cases where a substance that 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 that 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

Coumaphos: This product should consist of 98% or more coumaphos.
Melting point: 95°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 to the obtained sample and it is left to stand for two hours.

Acetone (100 ml) is then added. The mixture, after homogenizing for three minutes, 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 then 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 then 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 then 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 and the mixture is then transferred to a 100-ml separating funnel, to which 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, vegetables, teas and hops

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

The tea is weighed to prepare a sample of 5.00 g, to which 20 ml of water is added and left to stand for two hours.

Hops are first crushed into pieces and 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 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 30ml at 40℃ 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 then added to the separating funnel above. The mixture is shaken vigorously for five minutes using a shaker before being left to stand, and the layers of ethyl acetate and *n*-hexane are then 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. The residue is dissolved in 5 ml of a mixture of acetone and *n*-hexane (1:1).

iii. Foods except those listed in i and ii above

Extracts are obtained by the methods described in i or ii.

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. Then, the mixture of acetone and *n*-hexane (1:1) is spilt out until only a small amount remains in the tip 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 same 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 same 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.

(9) Chloramphenicol analytical method

1. Equipment

Liquid chromatograph mass spectrometer is 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: Acetonitrile produced for liquid chromatography is used.

Octadecylsilane-bonded silica gel minicolumn (500 mg): A polyethylene column with an inner diameter of 8-9 mm packed with

500 mg of octadecylsilane-bonded silica gel or one with the same separation characteristics is used.

Strong basic anion exchanger minicolumn (360 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 360 mg of trimethylamino acrylamide copolymer silane-bonded silica gel or one with the same separation characteristics is used.

Water: Water produced for liquid chromatography is used.

Sodium sulfate (anhydrous): Sodium sulfate (anhydrous) (special grade). In cases where a substance that interferes with analysis of an ingredient of the agricultural chemical product concerned is contained, it should be rinsed out with ethyl acetate.

Phosphate buffer solution (pH 2.5): Monopotassium dihydrogen monophosphate (1.36 g) is dissolved in water to make a 800-ml solution, to which phosphoric acid is added to adjust the pH to 2.5. Water is added to the adjusted solution to make a 1,000-ml solution.

3. Reference material

Chloramphenicol: This product should consist of 99% or more chloramphenicol.

Decomposition point: 208°C

4. Preparation of test solutions

a. Extraction methods

After homogenizing, a sample of 5.00 g is measured out. For muscle, the fat layer should be removed as much as possible before chopping. One hundred ml of a mixture of methanol and 0.3% metaphosphoric acid solution (3:7) is added to the measured out sample. After finely crushing for three minutes, the mixture is filtered by suction into a rotary vacuum evaporator using filter paper covered with a two-millimeter-thick layer of diatomaceous earth. The residue on the surface of the filter paper is then washed with 10 ml of a mixture of methanol and 0.3% metaphosphoric acid solution (3:7) to collect the washings, which are then filtered by suction into the rotary vacuum evaporator. The mixture is concentrated to 30 ml at 45°C or lower, to which 0.1 ml of phosphoric acid is added.

b. Clean-up

i. Octadecylsilane-bonded silica gel column chromatography

Five ml of methanol is added to the octadecylsilane-bonded silica gel minicolumn (500 mg), followed by an additional 10 ml of water.

The effluent is discarded. The solution obtained by the extraction method described in “a. Extraction methods” is poured into this column, and subsequently 20 ml of phosphate buffer solution (pH 2.5) is also added. The effluent is discarded. Ten ml of methanol is poured into the column. The eluate is collected in a rotary vacuum evaporator, and the methanol is removed at 40°C or lower. The residue is dissolved in 5 ml of water.

ii. Strong basic anion exchanger column chromatography

Five ml of water is poured into a strong basic anion exchanger minicolumn (360 mg) and the effluent is discarded. The solution obtained in “i. Octadecylsilane-bonded silica gel column chromatography” is poured into the column. Two ml of water, 10 ml of ethanol and then 5 ml of water are also added in that order. The effluent is discarded. Five ml of 0.1 mol/l hydrochloric acid is poured into the column and the eluate is collected in a 50-ml test tube. Three ml of 3 mol/l hydrochloric acid and 15 ml of ethyl acetate are also added before shaking vigorously using a shaker for five minutes. After leaving to stand, the ethyl acetate layer is transferred into a 100-ml conical flask. Fifteen ml of ethyl acetate is added to the aqueous layer, and after repeating the above procedure, the ethyl acetate 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, and the ethyl acetate is removed at 40°C or lower. The residue is dissolved in 1.0 ml of a mixture of acetonitrile and water (3:7), which is used as the test solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 2-5 μm) is used.

Column: A stainless tube (inner diameter: 2.0-6.0 mm and length: 100-250 mm) is used.

Column temperature: 40°C

Mobile phase: A mixture of acetonitrile and 0.5% acetic acid (3:7) is used. The flow rate should be adjusted so that chloramphenicol flows out in approximately 15 minutes.

b. Quantitative tests

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

(10) Chlorpromazine analytical method

1. Equipment

Liquid chromatograph tandem mass spectrometer is 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.

Acetonitrile: Acetonitrile produced for liquid chromatography is used.

Strong acid cation exchanger minicolumn (500 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 500 mg of benzenesulfonyl propyl silane-bonded silica gel or one with the same separation characteristics is used.

Water: Water produced for liquid chromatography is used.

Methanol: Methanol produced for liquid chromatography is used.

3. Reference material

Chlorpromazine hydrochloride: This product should consist of 98% or more chlorpromazine hydrochloride.

Melting point: 194-196°C

4. Preparation of test solutions

a. Extraction methods

After homogenizing, a sample of 5.00 g is measured out, to which 25 ml of ethyl acetate and 1 ml of 4 mol/l potassium carbonate solution are added. The mixture is finely crushed before being centrifuged at 3,000 rpm for five minutes. Then, the ethyl acetate layer is transferred to a rotary vacuum evaporator. Twenty-five ml of ethyl acetate is added to the residue and crushed and centrifuged in the same manner as above. The ethyl acetate layer is then added to the rotary vacuum evaporator, and the ethyl acetate is removed at 40°C or lower. Thirty ml each of acetonitrile and acetonitrile-saturated

n-hexane are added to the residue before shaking vigorously for five minutes using a shaker. The shaken mixture is left to stand, and then the acetonitrile layer is transferred to a 100-ml separating funnel, to which 30 ml of acetonitrile-saturated *n*-hexane is added. The above procedure is repeated and the acetonitrile layer is collected in the rotary vacuum evaporator, and the acetonitrile is removed at 40°C or lower. The residue is dissolved in 10 ml of a mixture of methanol and 1.2% metaphosphoric acid solution (2:3), which is filtered through a cotton plug.

b. Clean-up

Three ml of methanol followed by 3 ml of water are poured into a strong acid cation exchanger minicolumn (500 mg) and the effluent is discarded. The solution obtained by the method described in “a. Extraction methods” is poured into this column. Water (5 ml) is also added and the effluent is discarded. Fifteen ml of a mixture of methanol and 0.1 mol/l dipotassium hydrogen orthophosphate solution (9:1) are added to the column, and the eluate is collected in a rotary vacuum evaporator,and the water and methanol are removed at 40°C or lower. The residue is dissolved in 1.0 ml of methanol, which is used as the sample solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 2-5 μm) is used.

Column: A stainless tube (inner diameter: 2.0-6.0 mm and length: 100-250 mm) is used.

Column temperature: 40°C

Mobile phase: A mixture of acetonitrile, formic acid and water (500:1:500) is used. The flow rate should be adjusted so that chlorpromazine flows out in approximately 15 minutes.

b. Quantitative tests

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

peak height or peak area method.

(11) Diethylstilbestrol analytical method

1. Equipment

Liquid chromatograph tandem mass spectrometer is 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: Acetonitrile produced for liquid chromatography is used.

Glucuronidase solution: Should contain 100,000 units/ml of beta-D-glucuronidase extracted from *Helix pomatia* and refined.

Dichloromethane: Dichloromethane (special grade).

Weak basic anion exchange resin minicolumn (500 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 500 mg of diethylaminopropyl weak basic anion exchange resin produced for column chromatography or one with the same separation characteristics is used.

Water: Water produced for liquid chromatography is used.

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

3. Reference material

Diethylstilbestrol: This product should consist of 99% or more diethylstilbestrol.

Decomposition point: 208°C

4. Preparation of test solutions

a. Extraction methods

After homogenizing, a sample of 5.00 g is measured out, to which 11 ml of 0.04 mol/l sodium acetate solution is added. The mixture is finely crushed before adding acetic acid to adjust the pH to 4.25-4.75. One hundred µl of glucuronidase solution is added to the solution and left to stand for 14 hours at 37°C. Then, 16 ml of acetonitrile is added.

The mixture is shaken vigorously for five minutes using a shaker before being centrifuged at 3,000 rpm for five minutes. The supernatant liquid is collected in a rotary vacuum evaporator. Sixteen ml of acetonitrile is added to the residue and the above procedure is repeated twice. The supernatant liquid is again collected in the rotary vacuum evaporator and concentrated to approximately 3 ml at 50°C or lower. Fifty ml of dichloromethane and 200 ml of 5% sodium chloride solution are added to the concentrated sample, which is then vigorously shaken for five minutes using a shaker. The dichloromethane layer is then collected and dehydrated with sodium sulfate (anhydrous) and filtered. Fifty ml of dichloromethane is added to the aqueous layer and the above procedure is repeated twice. The dichloromethane layer is then mixed. An adequate amount of sodium sulfate (anhydrous) is added to the mixture, which is then left to stand for 15 minutes and shaken from time to time. Then, the content is filtered into a rotary vacuum evaporator, and the dichloromethane is removed at 40°C or lower. The residue is dissolved in 4 ml of a mixture of *n*-hexane and benzene (3:1).

b. Clean-up

Ten ml of a mixture of *n*-hexane and benzene (3:1) is poured into a weak basic anion exchange resin minicolumn (500 mg) and the effluent is discarded. The solution obtained by the method described in “a. Extraction methods” is poured into this column. Two ml of a mixture of *n*-hexane and benzene (3:1) and then 4 ml of dichloromethane are also added in that order. The effluent is discarded. Eight ml of a mixture of dichloromethane and methanol (9:1) is poured into the column and the eluate is collected in a rotary vacuum evaporator, and the dichloromethane and methanol are removed at 40°C or lower. The residue is dissolved in 0.5 ml of a mixture of acetonitrile and water (1:1), which is used as the sample solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 2-5 μm) is used.

Column: A stainless tube (inner diameter: 2.0-6.0 mm and length: 100-250 mm) is used.

Column temperature: 40°C

Mobile phase: A mixture of acetonitrile and 0.002 mol/l ammonium acetate (1:1) is used. The flow rate should be adjusted so that diethylstilbestrol flows out in approximately seven minutes.

b. Quantitative tests

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

(12) Analytical method for dimetridazole, metronidazole and ronidazole

1. Equipment

Liquid chromatograph tandem mass spectrometer is 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.

Acetonitrile: Acetonitrile produced for liquid chromatography is used.

Methanol: Methanol produced for liquid chromatography is used.

Water: Water produced for liquid chromatography is used.

3. Reference material

Dimetridazole: This product should consist of 99% or more dimetridazole.

Melting point: 138-139°C

Metronidazole: This product should consist of 99% or more metronidazole.

Melting point: 158-160°C

Ronidazole: This product should consist of 99% or more ronidazole.

Melting point: 167-169°C

4. Preparation of test solutions

After homogenizing, a sample of 5.00 g is measured out, to which 20 ml of acetonitrile is added. The mixture is finely crushed again before being centrifuged at 3,000 rpm for five minutes. Then, the acetonitrile layer is transferred to a 100-ml separating funnel, to which 20 ml of *n*-hexane is also added and shaken vigorously. The shaken mixture is

left to stand, and then the acetonitrile layer is transferred to a rotary vacuum evaporator, to which 5 ml of *n*-propanol is added to remove the acetonitrile and *n*-propanol at 40°C or lower. The residue is dissolved in 1.0 ml of methanol and filtered through a membrane filter with a pore size of 0.2 μm. This filtered solution is used as the test solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 2-5 μm) is used.

Column: A stainless tube (inner diameter: 2.0-6.0 mm and length: 100-250 mm) is used.

Column temperature: 40°C

Mobile phase: A mixture of acetonitril and water (1:9) is used. The flow rate should be adjusted so that dimetridazole, metronidazole and ronidazole flow out in 4-10 minutes.

b. Quantitative tests

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

(13) Daminozide analytical method

1. Equipment

A gas chromatograph with an alkali flame ionization detector or highly-sensitive nitrogen phosphorus detector, a gas chromatograph-mass spectrometer, and steam distillation apparatus are used. The steam distillation apparatus should be made of glass and roughly as shown in the following figure:

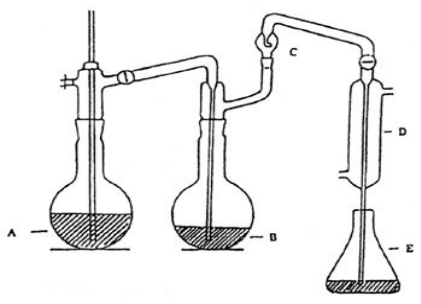
A: 1,000-ml round bottom flask (for steam generation)

B: 1,000-ml round bottom flask (for distillation)

C: Distillation trap

D: Cooling tube

E: 100-ml conical flask



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.

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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Basic alumina minicolumn (1,710 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 1,710 mg of basic alumina or one with the same separation characteristics is used.

Silicone for defoaming: Silicone produced for defoaming is used.

o-Nitrobenzaldehyde: *o*-Nitrobenzaldehyde (special grade).

1% *o*-Nitrobenzaldehyde-methanol solution: *o*-Nitrobenzaldehyde (100 mg) is dissolved in 10 ml of methanol. Should be prepared immediately before use.

Phenolphthalein reagent: Phenolphthalein (1 g) is dissolved in 100 ml of ethanol.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. When 5 µl of the concentrated sample is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatograph must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Water: Distilled water is used. In cases where a substance that 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.

Methanol: Three hundred ml of methanol is concentrated using a rotary vacuum evaporator. After removing the methanol, the residue is dissolved in 5 ml of *n*-hexane. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Phosphate buffer solution (pH 5): Monopotassium dihydrogen monophosphate (13.15 g) and dipotassium hydrogen orthophosphate (0.59 g) are dissolved in water to make a 100-ml solution.

3. Reference material

Dimethylhydrazine: This product should consist of 97% or more 1,1-dimethylhydrazine.

Boiling point: 62-64°C

4. Preparation of test solutions

a. Extraction methods

i. Cereal grains, legumes/pulses, seeds, fruit, vegetables, matcha and hops

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 5.0-gram sample.

Fruit and vegetables are weighed accurately to prepare a sample of about one kilogram. An appropriate amount of water is measured and added to the sample, if required. After homogenizing, a sample equivalent to 10.0 g is measured out.

Matcha is weighed to prepare a 5.0-gram sample.

Hops are crushed into pieces and weighed to prepare a 5.0-gram sample.

Eighty ml of water is added to the obtained sample before shaking vigorously for 30 minutes. The shaken mixture is filtered by suction using a glass fiber filter. The residue on the filter is collected and 40 ml of water is added before shaking for five minutes. After repeating the above procedure, the filtrate is transferred to a 1,000-ml round bottom flask (for distillation).

ii. Teas except matcha

A 6.0-gram sample soaked in 360 ml of water at 100°C is left to stand at room temperature for five minutes before being filtered. From the cooled filtrate, 120 ml is transferred into a 1,000-ml round bottom flask (for distillation).

iii. Foods except those listed in i and ii above

Extracts are obtained by the methods described in i.

b. Distillation

Sixty grams of sodium hydroxide (65 g for vegetables and fruit) is added bit by bit to the round bottom flask described above so that it

dissolves as the water cools. Immediately after adding one to two drops of defoaming silicone to the dissolved solution, the flask is attached to a distillation apparatus. Separately, a 100-ml conical flask containing 5 ml of phosphate buffer solution (pH 5) and one drop of phenolphthalein reagent is attached to a steam distiller and a 1,000-ml round bottom flask (for steam generation) is heated. The solution is steam distilled until the distillate comes to 45 ml to confirm that the distillate remains colorless. The heat should be adjusted so as to complete the distillation in about 15 minutes.

c. Derivatization

One ml of 1% *o*-nitrobenzaldehyde-methanol solution is added to the above distillate. The mixture is shaken and left to stand for two hours at 30°C. Then, 50 ml of *n*-hexane is added before shaking again for five minutes. The shaken mixture is left to stand and the *n*-hexane layer is collected to be filtered in a 200-ml eggplant-shaped flask using a liquid phase separation filter paper. Fifty ml of *n*-hexane is added to the aqueous layer, and after repeating the above procedure, the *n*-hexane layer is combined in the eggplant-shaped flask. The residue on the filter is washed with 10 ml of *n*-hexane to obtain the washings, which are combined in the eggplant-shaped flask to remove the *n*-hexane at 40°C or lower. The residue is then dissolved in 5 ml of a mixture of acetone and *n*-hexane (1:19).

d. Clean-up

Ten ml of a mixture of acetone and *n*-hexane (1:19) is poured into a basic alumina minicolumn (1,710 mg) and the effluent is discarded. The solution obtained in “c. Derivatization” is poured into this column, followed by the injection of 10 ml of a mixture of acetone and *n*-hexane (1:19). 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 test solution.

5. Determination

a. Qualitative tests

Qualitative tests are performed under the following conditions. Test results obtained must be the same as the results obtained in the reference material under the same procedure described in “c. Derivatization” in “4. Preparation of test solutions.”

Testing conditions

Column: A silicate glass capillary column (inner diameter: 0.25 mm and length: 10-30 m) coated with 5% phenyl methyl silicone for gas chromatography to a thickness of 0.25 μm is used.

Column temperature: The column temperature is held at 60°C for two minutes, followed by an increase of 10°C every minute until reaching 280°C, where it is held for five minutes.

Inlet temperature: 280°C

Detector: Should be operated at 280°C.

Gas flow rate: Helium is used as the carrier gas. The flow rate should be adjusted so that dimethylhydrazine derivatives flow out in approximately 13 minutes. The flows of air and hydrogen should also be adjusted to the optimal conditions.

b. Quantitative tests

The content of dimethylhydrazine is determined from the test results obtained under the same conditions described in “a. Qualitative tests,” using either the peak height or peak area method. The content of daminozide is also determined using the following formula:

Daminozide content (ppm) = 2.67 x dimethylhydrazine content (ppm)

c. Confirmation tests

Gas chromatography/mass spectrometry is performed under the same conditions described in “a. Qualitative tests.” Test results obtained in the reference material must be the same as the results obtained under the procedure described in “c. Derivatization” in “4. Preparation of test solutions.” The quantity may be determined by either the peak height or peak area method, if required.

d. Calibration curve

A solution of dimethylhydrazine (1 ml) combined with 5 ml of phosphate buffer solution (pH 5) and 40 ml of water is assayed with the procedure described in “c. Derivatization” in “4. Preparation of test solutions.”

(14) Nitrofurans analytical method

3-amino-2-oxazolidinone, 1-aminohydantoin, 3-amino-5-morpholinomethyl -2-oxazolidinone and semicarbazide are analysed.

1. Equipment

Liquid chromatograph tandem mass spectrometer is 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: Acetonitrile produced for liquid chromatography is used.

Porous diatomaceous earth column (to hold 20 ml of solution): A polyethylene column with an inner diameter of 20-30 mm packed with granular porous diatomaceous earth produced for column chromatography that can hold 20 ml of solution, or one with the same separation characteristics, is used.

o-Nitrobenzaldehyde: *o*-Nitrobenzaldehyde (special grade).

Water: Water produced for liquid chromatography is used.

3. Reference material

3-amino-2-oxazolidinone: This product should consist of 99% or more 3-amino-2-oxazolidinone.

Decomposition point: 65-67°C

1-aminohydantoin hydrochloride: This product should consist of 90% or more 1-aminohydantoin hydrochloride.

Decomposition point: 201-205°C

3-amino-5-morpholinomethyl-2-oxazolidinone: This product should consist of 99% or more 3-amino-5-morpholinomethyl-2- oxazolidinone.

Decomposition point: 115-120°C

Semicarbazide hydrochloride: This product should consist of 99% or more semicarbazide hydrochloride.

Decomposition point: 175-177°C

4. Preparation of test solutions

a. Extraction methods and derivatization

After homogenizing, a sample of 5.00 g is measured out, to which 10 ml of 0.1 mol/l hydrochloric acid is added. The mixture is finely crushed before adding 0.4 ml of 0.05 mol/l *o*-nitrobenzaldehyde -dimethyl sulfoxide solution. The mixture is then left to stand for 16 hours at 37°C. Then, 5 ml of 0.1 mol/l dipotassium hydrogen orthophosphate solution is added. Approximately 0.8 ml of a 1 mol/l sodium hydroxide solution is also added to adjust the pH to 7-8. This

solution is centrifuged at 2,500 rpm for five minutes and the supernatant is collected.

b. Clean-up

The supernatant obtained by the procedure stated in “a. Extraction methods and derivatization” is injected into a porous diatomaceous earth column (to hold 20 ml of solution). The column is left to stand for five minutes before adding 100 ml of ethyl acetate. The eluate is collected in a rotary vacuum evaporator, and the ethyl acetate is removed at 40°C or lower. The residue is dissolved in 1.0 ml of a mixture of acetonitrile and water (1:1), which is used as the test solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilane-bonded silica gel (particle size: 2-5 μm) is used.

Column: A stainless tube (inner diameter: 2.0-6.0 mm and length: 100-250 mm) is used.

Column temperature: 40°C

Mobile phase: A concentration gradient of a mixture of acetonitrile and 0.1% acetic acid solution from 1:4 to 4:1 should be created in 15 minutes. The flow rate should be adjusted so that 3-amino-2-oxazolidinone flows out in approximately 12 minutes.

b. Quantitative tests

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

(15) Propham analytical method

1. Equipment

Gas chromatograph mass spectrometer is 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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$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. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Sodium chloride: Sodium chloride (special grade). In cases where a substance that 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.

Octadecylsilane-bonded silica gel minicolumn (1,000 mg): A polyethylene column with an inner diameter of 12-13 mm packed with 1,000 mg of octadecylsilane-bonded silica gel or one with the same separation characteristics is used.

Graphite carbon and aminopropylsilane-bonded silica gel laminated minicolumn (500 mg/ 500 mg): A polyethylene column with an inner diameter of 12-13 mm packed with 500 mg each of graphite carbon and aminopropylsilane-bonded silica gel, or one with the same separation characteristics, is used.

Diatomaceous earth: Diatomaceous earth for chemical analysis is used.

Toluene: Three hundred ml of toluene is concentrated in a rotary vacuum evaporator. After removing the toluene, the residue is dissolved in 5 ml of *n*-hexane. When 5 µl of the dissolved residue is injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

n-Hexane: Three hundred ml of *n*-hexane is concentrated to 5 ml using a rotary vacuum evaporator. When 5 µl of the concentrated sample is

injected into the GC-ECD for analysis, the heights of peaks for compounds other than *n*-hexane on the gas chromatogram must be the same as or lower than the peak for gamma-BHC at $2x10^{-11}$g.

Water: Distilled water is used. In cases where a substance that 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 that 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.

0.5 mol/l phosphate buffer solution (pH 7.0): 136.85 g of secondary sodium phosphate (dodecahydrate) and 17.92 g of sodium phosphate monobasic (dihydrate) are measured out to be dissolved in water to make a 1,000-ml solution.

3. Reference material

Propham: This product should consist of 99% or more propham.

Melting point: 87°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 to the obtained sample and it is then left to stand for 15 minutes.

Acetonitrile (50 ml) is then added. After homogenizing for three minutes, the mixture is filtered by suction 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 20 ml of acetonitrile is added. The mixture is crushed finely for three minutes. The above procedure is repeated and the filtrate is combined. Acetonitrile is then added to make a 100-ml solution.

Twenty ml of the above solution is transferred to a 100-ml separating funnel, to which 10 g of sodium chloride and 20 ml of 0.5 mol/l phosphate buffer solution (pH 7.0) are added. The mixture is shaken vigorously for 10 minutes using a shaker and left to stand. The aqueous layer is then discarded and the acetonitrile layer is collected.

Ten ml of acetonitrile is poured in an octadecylsilane-bonded silica gel minicolumn (1,000 mg) and the effluent is discarded. The above acetonitrile layer is poured into this column. After the eluate is collected in a 50-ml conical flask, 2 ml of acetonitrile is also poured into the column. The eluate is combined 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 washed with 10 ml of acetonitrile 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 acetonitrile is removed at 40°C or lower. The residue is dissolved in 2 ml of a mixture of toluene and acetonitril (1:3).

ii. Fruit, vegetables, teas and hops

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

Teas and hops are first crushed into pieces and weighed to prepare a 5.00-gram sample, to which 20 ml of water is added and left to stand for 15 minutes.

Then, 50 ml of acetonitrile is added before finely crushing for three minutes. The crushed sample is filtered by suction 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 acetonitrile (20 ml) is added before homogenizing for three minutes. The above procedure is repeated and the filtrate is combined, and then acetonitrile is added to make a 100-ml solution.

Twenty ml of this solution is transferred to a 100-ml separating funnel, to which 10 g of sodium chloride and 20 ml of 0.5 mol/l phosphate buffer solution (pH 7.0) are added. The mixture is shaken vigorously for 10 minutes using a shaker and left to stand. The aqueous layer is then discarded. The acetonitrile layer is collected in a 50-ml conical flask. An adequate amount of sodium sulfate (anhydrous) is also added to the flask, which is then 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 washed with 10 ml of acetonitrile 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 acetonitrile is removed at 40°C or lower. The residue is dissolved in 2 ml of a mixture of toluene and acetonitril (1:3).

iii. Foods except those listed in i and ii above

Extracts are obtained by the methods described in i or ii.

b. Clean-up

Ten ml of a mixture of toluene and acetonitrile (1:3) is poured into a graphite carbon and aminopropylsilane-bonded silica gel laminated minicolumn (500 mg/ 500 mg) and the effluent is discarded. The solution obtained by the extraction method described in “a. Extraction methods” is poured into this column and subsequently 20 ml of a mixture of toluene and acetonitrile (1:3) is also added. The eluate is transferred into a rotary vacuum evaporator, and the toluene and acetonitrile are removed at 40°C or lower. The residue is dissolved in a mixture of acetone and *n*-hexane (1:1) to make exactly 2 ml (1 ml for cereal grains, legumes/pulses, seeds, teas and hops) of solution, which is used as the test solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column: A silicate glass capillary column (inner diameter: 0.25 mm and length: 30 m) coated with 5% phenyl 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 125°C, after which the temperature is increased by 10°C every minute until reaching 300°C, where it is held for 6.5 minutes.

Inlet temperature: 250°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 optimal conditions.

Injection method: Splitless injection method

Measured mass number (m/z): 93, 137 and 179

b. Quantitative tests

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

6. Regardless of the provisions in 5, 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. In association with this regulation, foods listed in the “foods” column in the table in (2) below 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) below, 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 (10) below.

(1) The maximum residue limits of substances used as ingredients of agricultural chemicals in foods (***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
Other legumes/pulses	Seeds without the pods