Provisional Translation
from Japanese Original

Specifications and Standards for Food, Food Additives, Etc.

Ministry of Health and Welfare Notification No. 370, 1959

Based on Notification No. 499, 2005, the Minister of Health, Labour and Welfare has revised the specifications and standards (Section A ***General Compositional Standards for Food***, Part I ***Food***, ***Specifications and Standards for Food, Food Additives, Etc.***, Notification No. 370, 1959), which were established based on Article 11 Paragraph 1 of the Food Sanitation Law, as given below. (Effective on May 29, 2006)

Part I Food

Section A General Compositional Standards for Food

1. Foods shall not contain any antibiotics or chemically synthesized antibacterial substances (substances obtained by instigating chemical reactions to elements and/or compounds through chemical methods, except for decomposition; this applies hereinafter in this paragraph), except for the following cases:

(1) When the substance concerned is identical to the food additive determined by the Minister of Health, Labour and Welfare as having no potential to cause damage to human health under Article 10 of the Food Sanitation Law (Law No. 233, 1947, hereinafter “the Law.”)

(2) When compositional standards are set forth in 5, 6, 7, 8 or 9 below for the substance concerned.

(3) When the food product concerned has been manufactured or processed using a food ingredient that meets the compositional standards given in 5, 6, 7, 8 or 9 below (except for foods containing antibiotics or chemically synthesized antibacterial substances for which compositional standards are not set forth in 5, 6, 7, 8 or 9 below.)

2.~4. (Omitted)

5. Foods shall not contain substances (including substances produced by chemical transformation: this applies hereinafter in this paragraph) used as ingredients of agricultural chemicals and other chemical substances listed in the table in (1) below. The agricultural chemicals and other

chemical substances stated above, here and also later in this paragraph, refer to substances used for purposes designated by the Agriculture, Forestry and Fisheries Ministerial Ordinance according to the provision of Paragraph 3 of Article 2 of the Law Concerning Safety Assurance and Quality Improvement of Agricultural Chemicals and Feeds (Law No. 35, 1953), which is stipulated under Paragraph 1 of Article 1-2 of the Agricultural Chemicals Regulation Law (Law No. 82, 1948), with such aims as adding to, mixing with, or infiltrating into feeds (the feeds stipulated under Paragraph 2 of Article 2 of Law No. 35), or medical products to be used for animals, which are stipulated in Paragraph 1 of Article 2 of the Pharmaceutical Affairs Law (Law No. 145, 1960). In association with this regulation, a sample of the 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 by the testing methods described in (3) to (15) below. No ingredients of agricultural chemicals or other chemical substances shall be detected in these tests.

(1) Substances used as ingredients of agricultural chemicals and other chemical substances that are stipulated to be "Not detected" in foods

1. 2, 4, 5-T
2. Azocyclotin and cyhexatin
3. Amitrol
4. Captafol
5. Carbadox
6. Coumaphos
7. Chloramphenicol
8. Chlorpromazine
9. Diethylstilbestrol
10. Dimetridazole
11. Daminozide
12. Nitrofurans
13. Propham
14. Metronidazole
15. Ronidazole

(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 husks, the silk and the cores removed
Other cereal grains	Threshed seeds
Peas, beans (dry)(including butter beans, cowbeans (red beans), lentil, lima beans, pegia, sultani, sultapya and white beans)*, broad beans and soybeans (dry))	Seeds without the pods
Peanuts	With the shells removed
Other legumes/pulses	Seeds without the pods
Apricot, mume plum, cherry, Japanese plum (including prune) and nectarine	With the peduncle and the seeds removed
Peach	With the skins and the seeds removed
Orange (including navel orange), grapefruit, citrus *natsudaidai* (whole), lime and lemon	Whole fruit
Citrus *natsudaidai* (pulp) and *unshu* orange (pulp)	With the peels removed
Citrus *natsudaidai*, peels	With the calyxes removed
Other citrus fruits	Whole fruit
Pear, Japanese pear, quince and apple	With the blossom scars, the cores and the peduncles removed
Loquat	With the peduncles, the skins and the seeds removed
Avocado and mango	With the seeds removed
Kiwifruit	With the skins removed
Guava	With the calyxes removed
Date	With the calyxes and the seeds removed

Pineapple	With the tops removed
Passion fruit and papaya	Whole fruit
Banana	With the pedicels removed
Strawberry, cranberry, huckleberry, blackberry and blueberry	With the calyxes removed
Raspberry	Whole fruit
Other berries	With the calyxes removed
Japanese persimmon	With the calyxes and the seeds removed
Watermelon, *makuwauri* melon and melons	With the rinds removed
Grape	With the peduncles removed
Other fruits	Edible portions
Turnip (roots) and Japanese radish (roots, including radish)	With the dirt lightly rinsed off with water
Turnip (leaves), watercress, kale, Japanese radish (leaves, including radish), and brussels sprouts	With the decayed leaves removed
Cauliflower and broccoli	With the leaves removed
Cabbage and Chinese cabbage	A sample consisting of one portion from each of four heads, with each head equally cut into four portions, without the decayed outer leaves and the cores
Kyona and *komatsuna* (Japanese mustard spinach)	With the roots and the decayed leaves removed
Horseradish	Roots with the dirt lightly rinsed off with water
Qing-geng-cai and other cruciferous vegetables	Edible portions
Sweet potato, konjac, taro, potato, yam and other potatoes	With the dirt lightly rinsed off with water
Pumpkin (including squash), cucumber (including gherkin) and oriental pickling melon (vegetable)	With the vines removed
Other cucurbitaceous vegetables	Edible portions
Artichoke, endive and chicory	With the decayed leaves removed

Burdock and salsify	A sample thinly sliced then ground with a meat grinder, the leaves having been removed and the dirt having been lightly rinsed off with water
Shungiku	With the roots and the decayed leaves removed
Lettuce (including cos lettuce and leaf lettuce)	With the decayed outer leaves and the cores removed
Other composite vegetables	Edible portions
Shiitake mushroom, button mushroom and other mushrooms	Edible portions
Celery, parsley and *mitsuba*	With the roots and the decayed leaves removed
Carrot and parsnip	With the dirt lightly rinsed off with water
Other umbelliferous vegetables	Edible portions
Tomato, egg plant and pimiento (sweet pepper)	With the calyxes removed
Other solanceous vegetables	Edible portions
Asparagus	Stems
Onion, garlic, welsh (including leek) and multiplying onion	With the outer skins and the root hair removed
Nira and other liliaceous vegetables	Edible portions
Green soybeans, kidney beans (immature, with pods) and peas (immature, with pods)	With the pedicels removed
Okra	With the calyxes removed
Sugarcane	With the husks removed
Ginger	With the leaves removed, and with the dirt lightly rinsed off with water
Sugar beet	With the dirt lightly rinsed off with water
Spinach	With red roots left on, and with the root hair and the decayed leaves removed
Bamboo shoots and other vegetables	Edible portions

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. Apparatus

Liquid chromatograph-mass spectrometer (LC/MS or LC/MS/MS)

2. Reagents and test solutions

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

Acetonitrile: Acetonitrile produced for liquid chromatography.

Divinylbenzene-*N*-vinylpyrrolidine Copolymer Cartridge Column (60 mg): Use a polyethylene column of 12–13 mm in inner diameter packed with 60 mg of divinylbenzen -*N*-vinylpyrrolidone copolymer, or a column equivalent to the specified one in separation capability.

Divinylbenzene-*N*-vinylpyrrolidine Copolymer Cartridge Column (200

mg): Use a polyethylene column of 12–13 mm in inner diameter packed with 200 mg of divinylbenzen-*N*-vinylpyrrolidone copolymer, or a column equivalent to the specified one in separation capability.

Water Water produced for liquid chromatography.

3. Reference standard

Chloramphenicol: This product contains not less than 99% chloramphenicol.

Decomposition point: 208°C.

4. Procedure

a. Extraction

i. Honey

Weigh 5.00 g of the test sample, previously homogenized, and dissolve it in 20 ml of water.

ii. Royal jelly

Weigh 1.00 g of the test sample, previously homogenized, add 60 ml of methanol/1% metaphosphoric acid (3:2), and homogenize the mixture again. Filter the homogenized sample by suction into a rotary vacuum evaporator through a filter paper covered with a 2-mm thick layer of diatomaceous earth. Wash the residue on the filter paper with 15 ml of methanol/1% metaphosphoric acid (3:2), and filter the washings by suction. Combine the filtrates into the evaporator, and evaporate the mixture to 2 ml at 45ºC or lower.

iii. Foods other than those given in sections i and ii

Weigh 5.00 g of the test sample, previously ground. For muscle, the fat layer should be removed as much as possible before grinding. To the sample measured out, add 100 ml of methanol/1% metaphosphoric acid (3:2), and homogenize the mixture again. Filter the homogenized sample by suction into a rotary vacuum evaporator through a filter paper covered with a 2-mm thick layer of diatomaceous earth. Wash the residue on the filter paper with 10 ml of methanol/1% metaphosphoric acid (3:2), and filter the washings by suction. Combine the filtrates into the evaporator, and evaporate the mixture to 30 ml at 45ºC or lower.

b. Clean-up

i. Honey

Pour 5 ml of methanol and 5 ml of water into a divinylbenzene-*N*-vinylpyrrolidine copolymer cartridge column (60 mg), and discard

the effluent. Pour the sample solution obtained by the extraction method described in 4-a. into the column, then pour 5 ml of 20% (vol) methanol. Discard the effluent. Pour 6 ml of 60% (vol) methanol, and collect the eluate into a rotary vacuum evaporator. Remove the methanol and water at 45°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (3:7), and use the obtained solution as the test solution.

ii. Royal jelly

Pour 10 ml of methanol and 10 ml of water into a divinylbenzene-*N*-vinylpyrrolidine copolymer cartridge column (200 mg), and discard the effluent. Pour the sample solution obtained by the extraction method described in 4-a. into the column, then pour 4 ml of water and 4 ml of 5% (vol) methanol into the column. Discard the effluent. Pour 10 ml of 60% (vol) methanol, and collect the eluate into a rotary vacuum evaporator. Remove the methanol and water at 45°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (3:7), and use the obtained solution as the test solution.

iii. Foods other than those given in sections i and ii

Pour 5 ml of methanol and 5 ml of water into a divinylbenzene-*N*-vinylpyrrolidine copolymer cartridge column (60 mg), and discard the effluent. Pour the sample solution obtained by the extraction method described in 4-a. into the column, then pour 10 ml of water. Discard the effluent. Pour 10 ml of methanol, and collect the eluate into a rotary vacuum evaporator. Remove the methanol at 40°C or lower. Dissolve the residue in 1.0 ml of acetonitrile/water (3:7), and use the obtained solution as the test solution.

5. Determination

a. Qualitative tests

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

Testing conditions

Column packing: Octadecylsilanized silica gel (2–5 μm in particle size)

Column: A stainless steel tube (2.0–6.0 mm in inner diameter, 100–250 mm in length)

Column temperature: 40°C

Mobile phase: Acetonitrile/10 mmol/l ammonium formate (3:7). The flow rate should be adjusted so that chloramphenicol flows out in approximately 5 minutes.

b. Quantitative tests

Determine the quantity from the test results obtained under the conditions specified for the 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:

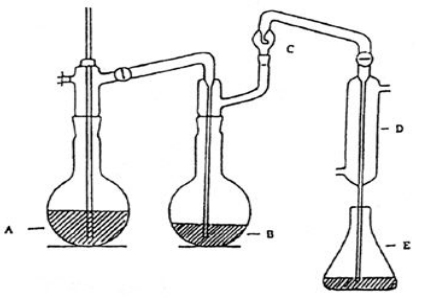
A: 1,000-ml round bottom flask (for steam generation)

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

Apricot, mume plum, cherry, Japanese plum (including prune) and nectarine	With the peduncles and the seeds removed
Peach	With the skins and the seeds removed
Orange (including navel orange), grapefruit, citrus *natsudaidai* (whole) , lime and lemon	Whole fruit
Citrus *natsudaidai* (pulp) and *unshu* orange (pulp)	With the peels removed
Citrus *natsudaidai*, peels	With the calyxes removed
Other citrus fruits	Whole fruit
Pear, Japanese pear, quince and apple	With the blossom scars, the cores and the peduncles removed
Loquat	With the peduncles, the skins and the seeds removed
Avocado and mango	With the seeds removed
Kiwifruit	With the skins removed
Guava	With the calyxes removed
Date	With the calyxes and the seeds removed
Pineapple	With the tops removed
Passion fruit and papaya	Whole fruit
Banana	With the pedicels removed
Strawberry, cranberry, huckleberry, blackberry and blueberry	With the calyxes removed
Raspberry	Whole fruit
Other berries	With the calyxes removed
Japanese persimmon	With the calyxes and the seeds removed
Watermelon, *makuwauri* melon and melons	With the rinds removed
Grape	With the peduncles removed
Other fruits	Edible portions
Turnip (roots) and Japanese radish (roots, including radish)	With the dirt lightly rinsed off with water

Turnip (leaves), watercress, kale, Japanese radish (leaves, including radish) and brussels sprouts	With the decayed leaves removed
Cauliflower and broccoli	With the leaves removed
Cabbage and Chinese cabbage	A sample consisting of one portion from each of four heads, with each head equally cut into four portions, without the decayed outer leaves and the cores.
Kyona and *komatsuna* (Japanese mustard spinach)	With the roots and the decayed leaves removed
Horseradish	Roots with the dirt lightly rinsed off with water
Qing-geng-cai and other cruciferous vegetables	Edible portions
Sweet potato, konjac, taro, potato, yam and other potatoes	With the dirt lightly rinsed off with water
Pumpkin (including squash), cucumber (including gherkin) and oriental pickling melon (vegetable)	With the vines removed
Other cucurbitaceous vegetables	Edible portions
Artichoke, endive and chicory	With the decayed leaves removed
Burdock and salsify	A sample thinly sliced then ground with a meat grinder, the leaves having been removed and the dirt having been lightly rinsed off with water
Shungiku	With the roots and the decayed leaves removed
Lettuce (including cos lettuce and leaf lettuce)	With the decayed outer leaves and the cores removed
Other composite vegetables	Edible portions
Shiitake mushroom, button mushroom and other mushrooms	Edible portions
Celery, parsley and *mitsuba*	With the roots and the decayed leaves removed
Carrot and parsnip	With the dirt lightly rinsed off
Other umbelliferous vegetables	Edible portions

Tomato, egg plant and pimiento (sweet pepper)	With the calyxes removed
Other solanceous vegetables	Edible portions
Asparagus	Stems
Onion, garlic, welsh (including leek) and multiplying onion	With the outer skins and the root hair removed
Nira and other liliaceous vegetables	Edible portions
Green soybeans, kidney beans (with pods, immature) and peas (with pods, immature)	With the pedicels removed
Okra	With the calyxes removed
Sugarcane	With the husks removed
Ginger	With the leaves removed, and with the dirt lightly rinsed off with water
Sugar beet	With the dirt lightly rinsed off with water
Spinach	With red roots left on, and with the root hair and the decayed leaves removed
Bamboo shoots and other vegetables	Edible portions
Sesame seeds, rapeseeds, sunflower seeds, safflower seeds, cotton seed 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

Should be performed according to 5 (3).

(4) Amitrol analytical method

Should be performed according to 5 (5).

(5) Analytical method for aldrin, endrin and dieldrin

1. Equipment

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

2. Reagents/Test solutions

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

Reagents designated as “special grade” in this section must meet the requirements for “special grade” specified in the Japan Industrial Standards for the reagents.

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

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

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

being allowed to cool in a desiccator.

Diatomaceous earth: Diatomaceous earth for chemical analysis is used.

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

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

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

3. Reference material

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

Melting point: 102-104°C

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

Decomposition point: 200°C

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

Melting point: 177-179°C

4. Preparation of test solutions

a. Extraction methods

i. Cereal grains, legumes/pulses and seeds

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

The concentrated solution is transferred to a 300-ml separating

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

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

ii. Fruit, vegetables, matcha

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

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

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

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

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

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

Thirty grams of sodium chloride and 100 ml of *n*-hexane are also

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

b. Clean-up

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

5. Determination

a. Qualitative tests

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

Testing conditions 1

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

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

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

Inlet temperature: 230°C

Detector: Should be operated at 300°C

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

Testing conditions 2

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

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

Inlet temperature: 230°C

Detector: Should be operated at 300°C

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

b. Quantitative tests

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

c. Confirmation tests

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

(6) Captafol analytical method

Should be performed according to 5 (6).

(7) Quinoxaline-2-carboxylic acid analytical method

Should be performed according to 5 (7).

(8) Cyhexatin analytical method

Should be performed according to 5 (4).

(9) Daminozide analytical method

Should be performed according to 5 (13).

(10) Analytical method for triazophos and parathion

1. Equipment

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

2. Reagents/Test solutions

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

Reagents designated as “special grade” in this section must meet the requirements for “special grade” specified in the Japan Industrial Standards for the reagents.

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

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

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

Diatomaceous earth: Diatomaceous earth for chemical analysis is used.

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

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

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

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

3. Reference material

Triazophos: This product should consist of 98% or more triazophos.
Melting point: 0-5°C

Parathion: This product should consist of 97% or more parathion.
Boiling point: 375°C

4. Preparation of test solutions

a. Extraction methods

i. Cereal grains and legumes/pulses

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

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

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

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

ii. Fruit and vegetables

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

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

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

b. Clean-up

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

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

5. Determination

a. Qualitative tests

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

Testing conditions 1

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

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

Inlet temperature: 230°C

Detector: Should be operated at 280°C

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

Testing conditions 2

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

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

Inlet temperature: 230°C

Detector: Should be operated at 280°C

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

b. Quantitative tests

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

height or peak area method.

c. Confirmation tests

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

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

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

(2) Samples

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

Other legumes/pulses	Seeds without the pods
Apricot, mume plum, cherry, Japanese plum (including prune) and nectarine	With the peduncles and the seeds removed
Peach	With the skins and the seeds removed
Orange (including navel orange), grapefruit, citrus *natsudaidai* (whole) , lime and lemon	Whole fruit
Citrus *natsudaidai* (pulp) and *unshu* orange (pulp)	With the peels removed
Citrus *natsudaidai*, peels	With the calyxes removed
Other citrus fruits	Whole fruit
Pear, Japanese pear, quince and apple	With the blossom scars, the cores and the peduncles removed
Loquat	With the peduncles, the skins and the seeds removed
Avocado and mango	With the seeds removed
Kiwifruit	With the skins removed
Guava	With the calyxes removed
Date	With the calyxes and the seeds removed
Pineapple	With the tops removed
Passion fruit and papaya	Whole fruit
Banana	With the pedicels removed
Strawberry, cranberry, huckleberry, blackberry and blueberry	With the calyxes removed
Raspberry	Whole fruit
Other berries	With the calyxes removed
Japanese persimmon	With the calyxes and the seeds removed
Watermelon, *makuwauri* melon and melons	With the rinds removed
Grape	With the peduncles removed
Other fruits	Edible portions

Turnip (roots) and Japanese radish (roots, including radish)	With the dirt lightly rinsed off with water
Turnip (leaves), watercress, kale, Japanese radish (leaves, including radish) and brussels sprouts	With the decayed leaves removed
Cauliflower and broccoli	With the leaves removed
Cabbage and Chinese cabbage	A sample consisting of one portion from each of four heads, with each head equally cut into four portions, without the decayed outer leaves and the cores
Kyona and *komatsuna* (Japanese mustard spinach)	With the roots and the decayed leaves removed
Horseradish	Roots with the dirt lightly rinsed off with water
Qing-geng-cai and other cruciferous vegetables	Edible portions
Sweet potato, konjac, taro, potato, yam and other potatoes	With the dirt lightly rinsed off with water
Pumpkin (including squash), cucumber (including gherkin) and oriental pickling melon (vegetable)	With the vines removed
Other cucurbitaceous vegetables	Edible portions
Artichoke, endive and chicory	With the decayed leaves removed
Burdock and salsify	A sample thinly sliced then ground with a meat grinder, the leaves having been removed and the dirt having been lightly rinsed off with water
Shungiku	With the roots and the decayed leaves removed
Lettuce (including cos lettuce and leaf lettuce)	With the decayed outer leaves and the cores removed
Other composite vegetables	Edible portions
Shiitake mushroom, button mushroom and other mushrooms	Edible portions
Celery, parsley and *mitsuba*	With the roots and the decayed leaves removed

Carrot and parsnip	With the dirt lightly rinsed off
Other umbelliferous vegetables	Edible portions
Tomato, egg plant and pimiento (sweet pepper)	With the calyxes removed
Other solanceous vegetables	Edible portions
Asparagus	Stems
Onion, garlic, welsh (including leek) and multiplying onion	With the outer skins and the root hair removed
Nira and other liliaceous vegetables	Edible portions
Green soybeans, kidney beans (with pods, immature) and peas (with pods, immature)	With the pedicels removed
Okra	With the calyxes removed
Sugarcane	With the husks removed
Ginger	With the leaves removed, and with the dirt lightly rinsed off with water
Sugar beet	With the dirt lightly rinsed off with water
Spinach	With red roots left on, and with the root hair and the decayed leaves removed
Bamboo shoots and other vegetables	Edible portions
Sesame seeds, rapeseeds, sunflower seeds, safflower seeds, cotton seed 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) Analytical method for aldrin, endrin and dieldrin

Should be performed according to 6 (5).

(4) Clenbuterol 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 benzenesulfonil 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

Clenbuterol hydrochloride: This product should consist of 98% or more clenbuterol hydrochloride.

Melting point: 174-176°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 then crushed and centrifuged as described 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 the acetonitrile layer is transferred to a 100-ml separating funnel, where 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 also 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 the 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) is 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, and this 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 clenbuterol flows out in approximately 10 minutes.

b. Quantitative tests

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

(5) Dexamethasone 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.

Octadecylsilane-bonded silica gel minicolumn (360 mg): A polyethylene column with an inner diameter of 8-9 mm packed with 360 mg of octadecylsilane-bonded silica gel or one with the same separation characteristics is used.

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.

Water: Water produced for liquid chromatography is used.

Methanol: Methanol produced for liquid chromatography is used.

Phosphate buffer solution (pH 5.0):

First solution: An amount of 27.2 g of monopotassium dihydrogen monophosphate is measured, which is dissolved in water to make a 1,000-ml solution.

Second solution: An amount of 3.48 g of dipotassium hydrogen orthophosphate is measured, which is dissolved in water to make a 100-ml solution.

The first solution is combined with the second solution to adjust the pH to 5.0.

3. Reference material

Dexamethasone: This product should consist of 99% or more dexamethasone.

Melting point: 262-264°C

4. Preparation of test solutions

a. Extraction methods

After homogenizing, a sample of 5.00 g is measured out, to which 30 ml of 95% acetonitrile solution is added. The mixture is finely crushed again before being centrifuged at 2,500 rpm for five minutes and the acetonitrile layer is collected. Thirty ml of 95% acetonitrile solution is added to the residue, and then it is finely crushed and centrifuged as described above before collecting the acetonitrile layer.

b. Clean-up

i. Florisil for column chromatography

Eight grams of florisil for column chromatography suspended in

acetonitrile is poured into a chromatograph tube (inner diameter: 15 mm and length: 300 mm). The acetonitrile is then spilt out until only a small amount remains on the packing of the column, into which 100 ml of acetonitrile is poured and the effluent is discarded. The solution obtained by the method described in “a. Extraction methods” and, subsequently, 30 ml of acetonitrile are also poured into the column. The eluate is transferred to a 300-ml separating funnel. Fifty ml of *n*-hexane is then added to the funnel before being vigorously shaken using a shaker for three minutes and left to stand. The acetonitrile layer is then collected in a rotary vacuum evaporator, and the acetonitrile is removed at 40°C or lower. The residue is dissolved in 4 ml of phosphate buffer solution (pH 5.0), to which 6 ml of water is added.

ii. Octadecylsilane-bonded silica gel column chromatography

Ten ml of methanol, 10 ml of water and 2 ml of phosphate buffer solution (pH 5.0) are added to the octadecylsilane-bonded silica gel minicolumn (360 mg) in that order. The effluent is discarded. The solution obtained in “i. Florisil for column chromatography” is poured into this column. Subsequently, 5 ml of phosphate buffer solution (pH 5.0) and 10 ml of 25% methanol solution are also poured into the column in that order. The effluent is discarded. Ten ml of 60% acetonitrile solution is poured into the column. The eluate is collected in a rotary vacuum evaporator, and the acetonitrile and water are removed at 40°C or lower. The residue is dissolve in 0.5 ml of 10% acetonitrile solution, and this 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, formic acid and water (1,200:1:800) is used. The flow rate should be adjusted so that dexamethasone flows out in approximately 7-10 minutes.

b. Quantitative tests

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

(6) Analytical method for triazophos and parathion

Should be performed according to 6 (10).

(7) Analytical method for alpha-trenbolone and beta-trenbolone

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.

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.

Hydroxypropyl dextran for column chromatography: Dextran (particle size: 25-100 μm) chemically bonded with a hydroxypropyl group produced for column chromatography is used.

Dichloromethane: Dichloromethane (special grade).

3. Reference material

Alpha-trenbolone: This product should consist of 94% or more alpha-trenbolone.

Melting point: 110°C

Beta-trenbolone: This product should consist of 99% or more beta-trenbolone.

Melting point: 186°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 being chopped.

Twenty ml of a mixture of acetonitrile and methanol (4:1) is added. The mixture is finely crushed before being centrifuged at 2,600 rpm for five minutes. The supernatant liquid is collected in a 100-ml separating funnel. Twenty ml of a mixture of acetonitrile and methanol (4:1) is added to the precipitate, and the mixture is then centrifuged under the above conditions. The mixed layer of acetonitrile and methanol is combined in the separating funnel, to which 20 ml water-saturated *n*-hexane is also added before shaking vigorously for five minutes. The shaken mixture is then left to stand and the mixed layer of acetonitrile and methanol is transferred to a rotary vacuum evaporator. Forty ml each of 5% sodium sulfate solution and dichloromethane are also added before shaking vigorously for five minutes. The shaken mixture is then left to stand and the layer of dichloromethane is transferred to a rotary vacuum evaporator. Fifteen ml of dichloromethane is added to the aqueous layer, and after repeating the above procedure, the dichloromethane layer is combined in the rotary vacuum evaporator, and the dichloromethane is removed at 40°C or lower. The residue is dissolved in 1 ml of a mixture of *n*-hexane and benzene (3:1).

b. Clean-up

i. Weak basic anion exchange resin column chromatography

A weak basic anion exchange resin minicolumn (500 mg) is injected with 6 ml of a mixture of *n*-hexane and benzene (3:1) 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) is also added to the column and the effluent is discarded. Three 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 solvent is evaporated to near dryness under nitrogen at 40°C or lower. The residue is dissolved in 0.5 ml of a mixture of benzene

and methanol (17:3).

ii. Hydroxypropyl dextran for column chromatography

A polyethylene column with an inner diameter of 6 mm is packed with hydroxypropyl dextran for column chromatography suspended in a mixture of benzene and methanol (17:3) before being left to stand for 12 hours so as to obtain a 120-mm long layer of hydroxypropyl dextran for column chromatography. The mixture of benzene and methanol (17:3) is then spilt out until only a small amount remains on the packing of the column. The solution obtained in “i. Weak basic anion exchange resin column chromatography” is poured into this column. Ten ml of a mixture of benzene and methanol (17:3) is also added to the column and 2.0 ml of the first effluent is discarded. The next eluate is collected in a rotary vacuum evaporator, and the solvent is evaporated to near dryness under nitrogen at 40°C or lower. The residue is dissolved in 0.5 ml of a mixture of acetonitrile and water (1:1), and this 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 340 nm.

Mobile phase: A mixture of acetonitrile and water (5:6) is used. The flow rate should be adjusted so that alpha-trenbolone flows out in approximately 10 minutes.

b. Quantitative tests

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

c. Confirmation tests

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

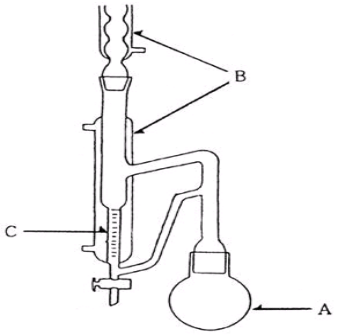
(8) Ethylene dibromide analytical method

1. Equipment

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

The Dean Stark distillation apparatus is roughly as shown in the following figure:

A: Distillation flask
B: Cooling tube
C: Distillation trap



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.

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.

Silicone for defoaming: Silicone produced for defoaming is used.

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

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

3. Reference material

Ethylene dibromide: This product should consist of 99% or more ethylene dibromide.

Boiling point: 131.5°C

4. Preparation of test solutions

a. Extraction methods

About one kg of a sample is chopped into small pieces of equal size. Of the chopped sample, 100 g is measured out to be transferred into a 1,000-ml distillation flask. Two hundred ml of water and 10 ml of *n*-hexane are also added to the flask. A few drops of defoaming silicone and boiling stones are then added before the flask is attached to the Dean Stark distillation apparatus to be heated and refluxed for one hour. After cooling, most of the water in the distillation trap is removed. The *n*-hexane layer is then filtered into a 10-ml measuring flask through liquid phase separation filter paper. The inside of the trap is washed with a small amount of *n*-hexane to obtain the washings, which are filtered through the filter paper as stated above. The filtrate is combined with the *n*-hexane layer to make exactly a 10-ml solution.

b. Clean-up

The solution obtained by the method described in “a. Extraction methods” is transferred to a 10-ml test tube with a glass stopper, to which approximately 1 g of florisil for column chromatography is added. The mixture is vigorously shaken before being left to stand at room temperature for about 15 minutes. Then, about five ml of supernatant liquid is collected in another test tube with a glass stopper, and this 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: 0.32 mm and length: 30 m) coated with 6% cyanopropylphenyl-methyl silicone for gas chromatography to a thickness of 1.8 μm is used.

Column temperature: The column temperature is held at 50°C for two

minute, followed by an increase of 5°C every minute until reaching 110°C, after which the temperature is increased by 30°C every minute until reaching 260°C, where it is held for five minutes.

Inlet temperature: 250°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 ethylene dibromide flows out in approximately eight minutes.

b. Quantitative tests

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

c. Confirmation tests

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

8. In cases where a substance used as an ingredient of an agricultural chemical (excluding substances determined by the Minister of Health, Labour and Welfare as having no potential to cause damage to human health under Paragraph 3 of Article 11 of the Law), for which no compositional standards are stipulated in 5 to 7, is the same as the substance naturally contained in the food, the amount of the substance contained in the food shall not exceed the amount normally contained in the food. This, however, does not apply to foods containing substances that may cause damage to human health at the amount normally contained.

9. In cases where substances used as ingredients of agricultural chemicals and other chemical substances that are listed in the first column of the following table are contained in a food, the amount of the substances shall not exceed the amount stipulated in the third column of the same table according to the food categories shown in the second column of the same table.

The maximum residue limits of substances used as ingredients of agricultural chemicals in foods (excluding foods listed in the second

column of the table in 6 (1) and the second column of the table in 7 (1)):

Column 1	Column 2	Column 3
AZINPHOS-METHYL	Other spices, dried	0.5 ppm
ACEPHATE	Other spices, dried (except the fruit of *Sansho* (Japanese pepper))	0.2 ppm
AMITRAZ	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the Japanese Agricultural Standards (JAS) for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
ALDICARB	Peanut oil (limited to refined peanut oil and peanut salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.01 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.01 ppm
IPRODIONE	Other spices, dried (limited to seeds)	0.05 ppm
	Other spices, dried (limited to roots or rhizomes)	0.1 ppm
IMIDACLOPRID	Wheat flour (except whole grain)	0.03 ppm
	Wheat bran	0.3 ppm
ETHION	Other spices, dried (limited to fruits)	5 ppm
	Other spices, dried (limited to seeds)	3 ppm

	Other spices, dried (limited to roots or rhizomes)	0.3 ppm
ETHEPHON	Raisin	5 ppm
	Fig, dried	10 ppm
ENDOSULFAN	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Other spices, dried (limited to fruits)	5 ppm
	Other spices, dried (limited to seeds)	1 ppm
	Other spices, dried (limited to roots or rhizomes)	0.5 ppm
CARBARYL	Rice bran	170 ppm
	Milled rice	1 ppm
	Wheat flour (except whole grain)	0.2 ppm
	Wheat germ	1 ppm
	Wheat bran	2 ppm
	Corn oil (except edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
	Soybean oil (except edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.2 ppm
	Tomato juice	3 ppm
	Tomato paste	10 ppm
	Edible olive oil (limited to virgin oil)	25 ppm

	Sunflower oil (except edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
QUINTOZENE	Other spices, dried (limited to fruits)	0.02 ppm
	Other spices, dried (limited to seeds)	0.1 ppm
	Other spices, dried (limited to roots or rhizomes)	2 ppm
GLYPHOSATE	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
GLUFOSINATE	Sunflower oil (except edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Rapeseed oil (except refined rapeseed oil and rapeseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
KRESOXIM-METHYL	Raisin	2 ppm
	Edible olive oil (limited to virgin olive oil)	0.7 ppm

CLETHODIM	Soybean oil (limited to edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Soybean oil (except edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	1 ppm
	Sunflower oil (except edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
	Cottonseed oil (limited to refined cottonseed oil and cotton seed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Cottonseed oil (except refined cottonseed oil and cotton seed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Rapeseed oil (limited to refined rapeseed oil and rapeseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Rapeseed oil (except refined rapeseed oil and rapeseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm

CHLORDANE	Milled rice	0.02 ppm
	Soybean oil (limited to edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.02 ppm
	Soybean oil (except edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Linseed oil (except refined linseed oil)	0.05 ppm
CHLORPYRIFOS	Wheat flour (except whole grain)	0.1 ppm
	Corn oil (limited to edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.2 ppm
	Soybean oil (limited to edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm

	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Other spices, dried (limited to fruits)	1 ppm
	Other spices, dried (limited to seeds)	5 ppm
	Other spices, dried (limited to roots or rhizomes)	1 ppm
CHLORPYRIFOS-METHYL	Wheat flour (except whole grain)	2 ppm
	Wheat bran	20 ppm
	Other spices, dried (limited to fruits)	0.3 ppm
	Other spices, dried (limited to seeds)	1 ppm
	Other spices, dried (limited to roots or rhizomes)	5 ppm
CHLORMEQUAT	Wheat flour (limited to whole grain)	5 ppm
	Wheat flour (except whole grain)	2 ppm
	Wheat bran	10 ppm
	Rye flour (limited to whole grain)	4 ppm
	Rye flour (except whole grain)	3 ppm
	Rye bran	10 ppm
	Rapeseed oil (except refined rapeseed oil and rapeseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
FENBUTATIN OXIDE	Plum, dried	10 ppm
	Raisin	20 ppm

DICHLORVOS and NALED (as total)	Wheat flour (limited to whole grain)	2 ppm
	Wheat flour (except whole grain)	1 ppm
	Wheat bran	10 ppm
	Wheat germ	10 ppm
	Other spices, dried	0.1 ppm
DIQUAT	Milled rice	0.2 ppm
	Wheat flour (limited to whole grain)	2 ppm
	Wheat flour (except whole grain)	0.5 ppm
	Wheat bran	5 ppm
	Vegetable oil (except refined vegetable oil)	0.05 ppm
DICOFOL	Plum, dried	3 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Other spices, dried (limited to fruits)	0.1 ppm
	Other spices, dried (limited to seeds)	0.05 ppm
	Other spices, dried (limited to roots or rhizomes)	0.1 ppm
DISULFOTON	Other spices, dried	0.05 ppm

CYHALOTHRIN	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.02 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.02 ppm
DIPHENYLAMINE	Apple juice	0.5 ppm
CYPRODINIL	Wheat bran	2 ppm
	Plum, dried	5 ppm
	Raisin	5 ppm
CYPERMETHRIN	Other spices, dried (limited to fruits)	0.1 ppm
	Other spices, dried (limited to roots or rhizomes)	0.2 ppm
	Vegetable oil (limited to refined vegetable oil)	0.5 ppm
DIMETHIPIN	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
DIMETHOATE	Edible olive oil (except virgin oil)	0.05 ppm

	Other spices, dried (limited to fruits)	0.5 ppm
	Other spices, dried (limited to seeds)	5 ppm
	Other spices, dried (limited to roots or rhizomes)	0.1 ppm
BROMIDE	Wheat flour (limited to whole grain)	50 ppm
	Peach, dried	50 ppm
	Plum, dried	20 ppm
	Raisin	100 ppm
	Date, dried	100 ppm
	Fig, dried	250 ppm
	Fruits (dried fruits except peach, plum, grape, date and fig)	30 ppm
	Other herbs, dried	400 ppm
SPINOSAD	Wheat bran	2 ppm
	Raisin	1 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.01 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.01 ppm
DIAZINON	Plum, dried	2 ppm
	Other spices, dried (limited to fruits)	0.1 ppm

	Other spices, dried (limited to seeds)	5 ppm
	Other spices, dried (limited to roots or rhizomes)	0.5 ppm
THIODICARB and METHOMYL	Wheat flour (except whole grain)	0.03 ppm
	Wheat germ	2 ppm
	Wheat bran	3 ppm
	Corn oil (limited to edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.02 ppm
	Soybean oil (limited to edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.2 ppm
	Soybean oil (except edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.2 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.04 ppm
TEBUCONAZOLE	Raisin	3 ppm
TEBUFENOZIDE	Raisin	2 ppm
DELTAMETHRIN and TRALOMETHRIN (as total)	Wheat flour (limited to whole grain)	2 ppm
	Wheat flour (except whole grain)	0.3 ppm
	Wheat bran	5 ppm

TERBUFOS	Rapeseed oil (except refined rapeseed oil and rapeseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
PARAQUAT	Sunflower oil (limited to edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Sunflower oil (except edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
PARATHION	Other spices, dried (limited to fruits)	0.2 ppm
	Other spices, dried (limited to seeds)	0.1 ppm
	Other spices, dried (limited to roots or rhizomes)	0.2 ppm
PARATHION-METHYL	Raisin	1 ppm
	Other spices, dried (limited to fruits)	5 ppm
	Other spices, dried (limited to seeds)	5 ppm
	Other spices, dried (limited to roots or rhizomes)	3 ppm
BIORESMETHRIN	Wheat flour (limited to whole grain)	1 ppm

	Wheat flour (except whole grain)	1 ppm
	Wheat germ	3 ppm
	Wheat bran	5 ppm
BIFENTHRIN	Wheat flour (limited to whole grain)	0.5 ppm
	Wheat flour (except whole grain)	0.2 ppm
	Wheat bran	2 ppm
PIPERONYL BUTOXIDE	Wheat flour (limited to whole grain)	30 ppm
	Wheat flour (except whole grain)	10 ppm
	Wheat germ	90 ppm
	Wheat bran	80 ppm
	Corn oil (except edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	80 ppm
	Tomato juice	0.3 ppm
	Juice of citrus fruits	0.05 ppm
	Fruits, dried	0.2 ppm
PYRIPROXYFEN	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.01 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.01 ppm

PIRIMICARB	Other spices, dried (limited to seeds)	5 ppm
PIRIMIPHOS-METHYL	Wheat bran	15 ppm
	Other spices, dried (limited to fruits)	0.5 ppm
	Other spices, dried (limited to seeds)	3 ppm
PYRETHRINS	Fruits, dried	0.2 ppm
VINCLOZOLIN	Other spices, dried	0.05 ppm
FAMOXADONE	Wheat bran	0.2 ppm
	Raisin	5 ppm
FENAMIPHOS	Peanut oil (except refined peanut oil and peanut salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
FENITROTHION	Wheat flour (limited to whole grain)	5 ppm
	Wheat bran	20 ppm
	Other spices, dried (limited to fruits)	1 ppm
	Other spices, dried (limited to seeds)	7 ppm
	Other spices, dried (limited to roots or rhizomes)	0.1 ppm
FENTHION	Edible olive oil (limited to virgin oil)	1 ppm
PHENTHOATE	Other spices, dried (limited to seeds)	7 ppm

FENVALERATE	Wheat flour (limited to whole grain)	2 ppm
	Wheat flour (except whole grain)	0.2 ppm
	Wheat bran	5 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
FENPROPATHRIN	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	3 ppm
FLUDIOXONIL	*Nira*, dried	50 ppm
	Basil, dried	50 ppm
FLUSILAZOLE	Raisin	1 ppm
FLUTOLANIL	Rice bran	10 ppm
	Milled rice	1 ppm
PROCHLORAZ	Wheat bran	7 ppm
	Sunflower oil (limited to edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	1 ppm

PROCYMIDONE	Sunflower oil (limited to edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
PROPARGITE	Corn flour	0.2 ppm
	Corn oil (limited to edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
	Corn oil (except edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.7 ppm
	Peanut oil (limited to refined peanut oil and peanut salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.3 ppm
	Peanut oil (except refined peanut oil and peanut salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.3 ppm
	Orange juice	0.3 ppm
	Apple juice	0.2 ppm
	Grape juice	1 ppm
	Raisin	12 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable	0.2 ppm

	Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	
PROFENOFOS	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
HEPTACHLOR	Soybean oil (limited to edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.02 ppm
	Soybean oil (except edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.5 ppm
PERMETHRIN	Wheat flour (limited to whole grain)	2 ppm
	Wheat flour (except whole grain)	0.5 ppm
	Wheat germ	2 ppm
	Wheat bran	5 ppm
	Soybean oil (except edible soybean oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
	Sunflower oil (limited to edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	1 ppm

	Sunflower oil (except edible sunflower oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	1 ppm
	Cottonseed oil (limited to refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.1 ppm
	Other spices, dried	0.05 ppm
PENCONAZOLE	Raisin	0.5 ppm
PHOSALONE	Other spices, dried (limited to fruits)	2 ppm
	Other spices, dried (limited to seeds)	2 ppm
	Other spices, dried (limited to roots or rhizomes)	3 ppm
PHORATE	Peanut oil (limited to refined peanut oil and peanut salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Peanut oil (except refined peanut oil and peanut salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.05 ppm
	Other spices, dried (limited to fruits)	0.1 ppm
	Other spices, dried (limited to seeds)	0.5 ppm
	Other spices, dried (limited to roots or rhizomes)	0.1 ppm
MALATHION	Tomato juice	0.01 ppm

	Other spices, dried (limited to fruits)	1 ppm
	Other spices, dried (limited to seeds)	2 ppm
	Other spices, dried (limited to roots or rhizomes)	0.5 ppm
METHAMIDOPHOS	Other spices, dried (except the fruit of *Sansho* (Japanese pepper))	0.1 ppm
METALAXYL and MEFENOXAM	Other spices, dried (limited to seeds)	5 ppm
METHIDATHION	Edible olive oil (limited to virgin oil)	2 ppm
	Cottonseed oil (except refined cottonseed oil and cottonseed salad oil that meet the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	2 ppm
METHOXYFENOZIDE	Plum, dried	2 ppm
	Raisin	3 ppm
METHOPRENE	Wheat flour (limited to whole grain)	5 ppm
	Wheat flour (except whole grain)	2 ppm
	Wheat bran	10 ppm
	Corn oil (limited to edible corn oil that meets the JAS for Edible Vegetable Fats and Oils, and other edible oils that meet standards equivalent to or stricter than JAS)	0.2 ppm
HYDROGEN PHOSPHIDE	Vegetables, dried	0.01 ppm
	Fruits, dried	0.01 ppm

10. In addition to the provisions of 6 or 9, when food products are manufactured or processed using foods for which compositional standards are specified in 6 through 9, the foods used must comply with the standards given in 6 through 9.