Notice from the Korean Food & Drug Administration, No. 2004-196

For the purpose of announcing to the nation the purpose of the amendment and major contents in advance, and for the purpose of hearing the nation's opinion of such announcement, and in the process of amendments to the Standards for Permission and Test Methods for Heavy Metals such as Crude Drugs, the following are announced in accordance with Article 46 of the Law of Administrative Procedure.

Dec. 30, 2004
Director of the Korean Food & Drug Administration

Amendments to the Standards for Permission and Test Methods for Heavy Metals such as Crude Drugs (draft)

In accordance with the first clause of Article 44 of the Drugs, Cosmetics and Medical Instruments Law (including Chinese medicine materials, hereinafter being referred to as such), and for crude drugs (Chinese medicine materials), the standards for permission and test methods for heavy metals in crude drugs and elsewhere are announced as follows:

1. **Range of Application and Standards for Permission**: The crude drugs and crude drugs (Chinese medicine materials). Only in the event the Director of the Korean Food & Drug Administration sets out the items separately such as extract materials • liquid extract materials, the following criteria should be observed.
   a. The vegetable crude drugs should have measurements of lead (Pb) less than 5 mg/kg, arsenic (As) less than 3 mg/kg, hydrargyrum (Hg) less than 0.2mg/kgf, and cadmium (Cd) less than 0.3 mg/kg.
   b. The young antler should have an arsenic level less than 3 mg/kg.
   c. The crude drugs (Chinese medicine materials) (excluding the materials containing mineral crude drugs) using natural medicine as a principle element should have heavy metals totalling less than 30 mg/kg.

2. Test Method

a. Lead, arsenic, cadmium
   Among the general test methods of the Korean Pharmacopoeia Index, it is measured according to the atomic absorbency system of brightness. Crush the sample into small grains, weigh out precisely 0.1～0.5 g, place it in a container for the exclusive use of high-frequency decomposition, and add 12 ml of nitric acid. After adding the nitric acid, cover the container tightly

and remove the generated gas. After removal of the gas, decompose it using the pressurised high-frequency decomposition. Filter the resultant decomposition liquid using the filter paper, dilute with water adequately to the extent of the standard liquid concentration, and prepare the test solution. In the same way, prepare a blank liquid and supplement it. Using the atomic absorbency system of brightness, dilute the standard crude liquid (1000 mg/L) of each heavy metal for atomic absorbency analysis using 0.5mol/L of nitric acid to the appropriate concentration, fill to the measuring line, supplement with the blank liquid, and then measure using the absorbency system of brightness or by the strength of the test solution.

b. Hydrargyrum
This is measured using equipment for hydrargyrum analysis.

c. Total Heavy Metals
Weigh this medicine 1.0 g (or 1.0 ml), concoct and test in accordance with Rule No. 3 of the heavy metal test method, being Rule No. 54 of the General Test Method of the Korean Pharmacopoeia. Place the standard liquid of lead 3.0 ml into the comparison liquid.

Additional Rules

① Commencement date: This notice shall be effective from the date of notice.
② Course of action: If the application was made in the days of the commencement of this notice or in the process of testing, then the former rules shall apply.
③ According to the former notice, the permitted (or reported) items shall be considered as permitted items (or items reported) according to this notice.

Notice from the Korean Food & Drug Administration, No. 2004-197

To announce to the nation the purpose of the amendment and major contents in advance, and then to hear the nation's opinion of such announcement, and in the process of amendments to the Standards for Permission and Test Method of Heavy Metals such as Crude Drugs, the following notices are made in accordance with Article 46 of the Law of Administrative Procedure.

.

Dec. 30, 2004
Director of the Korean Food & Drug Administration

In accordance with the rules in the first clause of Article 44 of the Drugs, Cosmetics and Medical Instruments Law, the following notices are given to obtain the proper quality of the crude drugs (including Chinese medicine materials, hereinafter referred as such) by setting forth the standards for permission and test method of the agricultural chemical residues in the crude drugs (Chinese medicine materials).

1. Range of application
   a. The crude drugs. Mineral crude drugs and animal crude drugs, medicine extracted from the crude materials (extract materials, liquid extract materials, tinctures, etc.) shall only be excluded if such drugs are subjected to direct testing.

2. Object of agricultural chemicals and criteria for permission
   a. The criteria for permission regarding the agricultural chemical residues for all vegetable crude drugs are as follows:

Name of Agricultural Chemical	Criteria for Permission (mg/kg)
Total BHC (total of α,β,r and total δ-BHC)	0.2
Total DDT (total of p,p'-DDD, p,p'-DDE, o,p'-DDT, and p,p'-DDT)	0.1
Aldrin	0.01
Endrin	0.01
Dieldrin	0.01
Methoxychlor	1.0

Name of Agricultural Chemical	Criteria for Permission (mg/kg)
Cypermenthrin	0.5
Endosulfan (total of α,β-endosulfan and endosulfan sulfate)	0.2
Chinomethionate	0.3
Captan	2.0
Quintozene, PCNB	0.1
Chlorothalonil	0.1
Chloropyrifos	0.5
Tolyfluanid	1.0
Procymidone	0.1

b. The criteria for permission for agricultural chemical residues for the individual crude drugs are as follows:

(Unit: mg/kg)

CD: Crude drug CP: Criteria for permission

CD	CP	CD	CP	CD	CP
1) Napropamide					
Limonium Sinuatum	0.1	Peony	0.1	Astragalus membranaceous	0.1
2) Dimethyldithiocarbamates					
Safflower	0.1				
3) Difenoconazole					
Liquorice	0.05				
4) Myclobutanil					
Peony	0.1				
5) Bifenthrin					
Cnidium officinale	0.5	Safflower	0.1		
6) Cyprodinil					
Peony	0.1				
7) Acetamiprid					
Astragalus membranaceous	0.1	Safflower	0.1		
8) Azocyclotin					
Angelica	0.2				
9) Azoxystrobin					
Liquorice	0.05	Angelica	0.1	Astragalus	

					membranaceous	0.1
10) Ethylenebis-dithiocarbamates						
Safflower	0.3					
11) Iminoctadine						
Peony	0.3	Safflower	0.1			
12) Imidacloprid						
Safflower	0.1	Astragalus membranaceous	0.3			
13) Thiamethoxam						
Astragalus membranaceous	0.1					
14) Carbandazim						
Peony	0.1					
15) Chlorfenapyr						
Cnidium officinale	0.05					
16) Tebuconazol						
Angelica	1.0					
17) Triadimenol						
Peony	0.1					
18) Triadimefon						
Peony	0.01					
19) Triforine						
Peony	0.1					
20) Triflumizole						
Astragalus membranaceous	0.1	Peony	1.0			
21) Fenarimol						
Astragalus membranaceous	0.5					
22) Pendimethalin						
Angelica	0.2	Liriope muscari	0.2			
Bupleurum falcatum	0.2	Peony	0.2	Safflower	0.1	
23) Fenpropathrin						
Angelica	0.2					
24) Fosthiazate						
Bupleurum falcatum	0.02					
25) Propineb						
Peony	0.2					
26) Pymetrozine						

Safflower 0.05 Astragalus membranaceous 0.05

27) Fludioxonil

Peony 0.1

c. The following crude drugs are in accordance with the Common Law of Foods No. 3, Common Criteria and Standards for General Foods No. 6; The Application of Criteria and Standards No. 3; Criteria for Permission for Agricultural Chemical Residues of Agricultural Products No. 5; and Criteria for Permission for Agricultural Chemical Residues for Ginseng, of "Notice from the Korean Food & Drug Administration, No. 2004-18 (2004.4.1), Criteria and Standards for Foods":

Crude drug	Name of applicable agricultural product	Crude drug	Name of applicable agricultural product	Crude drug	Name of applicable agricultural product
Nonglutinous rice	Rice	Pine nuts	Pine nuts	Lower part of the green onion	Shallot
Immature grain of wheat	Wheat	White cucumber	Gingko	Garlic	Garlic
Tear-grass	Adlay	Cotton seed	Cotton seed	Ginger, dried ginger	Ginger
Mung bean	Mung bean	Chinese quince	Fruit of the Chinese quince	Burdock	Burdock
Red bean	Red bean	Dried orange peel	Mandarin	Red pepper	Red pepper
Dolichos lablab L		Dried orange peel	Mandarin	Hop	Hop
Dried chestnut	Chestnut	Jujube	Jujube	Fruit of the Chinese matrimony vine	Fruit of the Chinese matrimony vine

Walnut	Walnut	Green Japanese apricot	Plum	Ginseng, red ginseng	Ginseng (dried product)
Bracket fungus	Mushroom (others)	Black sesame (sesame)	Sesame		

d. Regarding the judgment as to whether it was deemed suitable or not during the test of agricultural chemicals, as not set forth in the above a), □ b), the following rules shall apply:

1) The criteria of the clause "PESTICIDE RESIDUES" in the European Pharmacopoeia (EP)

2) If it is not set forth in the criteria, then it shall apply according to the following formula:

$$\frac{\text{ADI} \times \text{M}}{\text{MDD} \times 100}$$

ADI: Acceptable daily intake (mg/kg/day)
M: Average weight of adult (60 kg)
MDD: Daily dosage (kg)

3) In the case of others not applied in the above criteria, the judgment as to whether the agricultural chemicals examined are suitable or not shall be decided by the Director of the Korean Food and Drugs Administration after evaluating the degree of harm, taking into account the quantity of residue and the dosage in the crude drugs.

3. The test method is based on the following test methods, depending on the objects of the agricultural chemicals:

a. Napropamide, DDT, Dielrin, Myclobutanil, Methozychlor, BHC, Bifenthrin, Cypermethrin, Cyprodinil, Acetamiprid, Azoxystrobin, Aldrin, Endosulfan, Endrin, Chinomethionat, Oxythioquinox, Captan, Quintozene (PCNB), Chlorothalonil, Chlorpyrifos, Chlorfenapyr, Tebuconazole, Tolylfluanid, Triadimenol, Triadimefon, Triflumizole, Pendimethalin, Fenpropathrin, Fosthiazate, Procymidone, Pyridaphenthion, Fludioxonil, Fenarimol

1) Equipment: Gas chromatograph [ECD (Electronic Capture Detector)], nitrogen • phosphor (NPD), and MSD (Mass Spectrometer Detector)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues or equivalent

b) Water: Distilled water or equivalent

c) Florisil: The disposable cartridge (capacity 6 ml) filled with the fixed type of florisil (1 g)

d) Filter support material: Celite 545
e) Standard crude solution: After melting the standard product of each agricultural chemical in acetone, make 100 mg/kg.
f) Standard solution: After melting the standard crude solution in acetone separately, mix • dilute to the appropriate concentration.
g) Other reagent: Agricultural chemical residues for testing, or special grade

3) Preparation of test solution

a) Extract: Crush the reagent (500□600 g), weigh approximately 5 g, put 40 ml of water in it, and leave it for four hours. Place 90ml of acetone in it, make it homogeneous for five minutes using a homogenizer, and then filter by decompression using a vacuum pump, a triangle flask with a branch, and a Buchner funnel. Pour the filtered liquid into the separatory funnel with a capacity of 500 ml, and then add 50 ml of saturated saline solution and 100 ml of distilled water. Pour 70 ml of dichloromethane into the liquid, shake it vigorously, and then stabilise and separate the layers. Pour the lower layer (the layer of dichloromethane) into the other separatory funnel with a capacity of 500 ml. Pour 70 ml of dichloromethane into the separatory funnel having the water layer, shake it vigorously to mix, and then stabilise and separate the layers. Collect the lower layer (the layer of dichloromethane) and add this layer to the graduated flask that was collected earlier. Dehydrate the layer of dichloromethane by passing the sodium sulfate through it, put it in the vacuum evaporator, and then graduate it. Liquefy with 4 ml of hexane again, and use it as a test solution.

b) Refining: Place 6 ml of hexane in the florisil cartridge (6 ml, 1 g) in advance, stop it for two minutes, and then make it outflow. Make 6 ml of hexane having 20% of acetone outflow in this cartridge in the same way, and then discard it. After that, place the above graduated solution on the top of the cartridge, let it stay in the column for two minutes, and then dispense it slowly into the test tube by pouring it out. When the cartridge is wet with the solvent, pour out the 5 ml of hexane • dichloromethane • acetone (50:48.5:1.5) and collect it in the same test tube. Using decompression, graduate the solution poured out in the water bath at a temperature of less than 40□, blow the solvent off, liquefy it in the 2 ml of hexane containing 20% of acetone, and then use it as a test solution.

4) Test operation

A) Measuring conditions for the gas chromatograph

(1) Electronic capture detector (GC-ECD)

(a) Column: Place 5% of methyl silicone coated with 0.25μm thickness for gas chromatograph, in the silicic-acid glass capillary column tube with 0.25 mm inside diameter and 30 m length (DB-5 capillary column), and then place 50% of phenyl and 50% of methyl silicone coated with 0.25μm thickness for gas chromatograph, in the silicic-acid glass capillary column with 0.25

mm inside diameter and 30 m length (DB-17 capillary column) or the equivalents to the above.

(b) Carrier gas and flux: Nitrogen, 1.0ml/minute

(c) Oven temperature: Inject the sample at a temperature of 80□ into the oven, keep it for 2 minutes, increase the temperature to 280□ at the rate of 10 □/minute, and then keep it at this level for more than 10 minutes (15 minutes for DB-17).

(d) Injection part: Split mode (10:1), 260□

(e) Detector temperature: 280□

(2) Nitrogen • phosphor detector (GC-NPD)

(a) Column: Place 5% of methyl silicone coated with 0.25 μm thickness for gas chromatograph in the silicic-acid glass capillary column tube with 0.25 mm inside diameter and 30 m length (DB-5 capillary column), and then 50% of phenyl and 50% of methyl silicone coated with 0.25μm thickness for gas chromatograph, in the silicic-acid glass capillary column with the 0.25 mm inside diameter and 30 m length (DB-17 capillary column) or the equivalents to the above.

(b) Carrier gas and flux: Nitrogen, 1.0ml/minute

(c) Column temperature: Inject the sample at a temperature of 80□, keep it for 2 minutes, increase the temperature to 280□ at the rate of 10□/minute, and then keep it at this level for more than 10 minutes (15 minutes for DB-17).

(d) Injection part: 260□, split mode (10:1)

(e) Detector temperature: 280□

(3) Mass spectrometer detector (GC-MSD)

(a) Column: Place 5% of methyl silicone for gas chromatograph in a silicic-acid glass capillary column with 0.25μm thickness, 0.25 mm inside diameter and 30 m length for mass spectrometer detector (DB-5MS capillary column) or its equivalent.

(b) Carrier gas and flux: Helium, 0.9 ml/minute

(c) Oven temperature: Inject the sample at 100□, keep it for 2 minutes, increase the temperature to 280□ at the rate of 10□/minute, and then keep it at this level for more than 15 minutes.

(d) Temperature of the injection part: 260 □, split mode (10:1)

(e) Interface temperature: 280□

(f) Solvent retention time: 5 minutes

(g) Flux under movement: 1.0 ml/minute

B) Qualitative test: Select more than two column fillers, and then inject each standard solution and test solution into the gas chromatograph. In the comparison of each peak obtained from the chromatograph with

the peak of the standard solution, the retention time should the same under any condition of measurement.

※ Note) The elements of each agricultural chemical can be verified from the retention time in the GS-MSD and mass spectrum.

C) Quantitative test: Using the appropriate column filler based on the result from the qualitative test, make the gas chromatography and quantify it according to the peak-height method or the peak-area method.

B. Dithiocarbamates: Dimethyldithiocarbamates, Ethylenebis (dithiocarbamates)s, and Propineb

1) Equipment: A high-performance liquid chromatograph [ultraviolet absorption system of brightness detector (UV detector)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues or equivalent

b) Water: Distilled water or equivalent

c) Standard solution: Dilute each solid standard material (Thiram, Metiram, Propineb) to the proper concentration, treat in the same way as with the process of hydrolysis, and then use it as a standard solution.

d) Other reagent: Agricultural chemical residues for testing or special grade such as methyl iodide, tetrabutyl ammoniumhydrogen sulfate, tetrasodium EDTA, L-cysteine-HCI

3) Preparation of test solution

a) Extract: After crushing the sample (500□600 g), weigh out precisely 20 g of the sample and place it in the triangle flask. Place 80 ml of aqueous solution of 0.45 M NaOH (adjust pH to precisely 9.5□9.6) containing L-cysteine-HCI 0.5 g and 0.25M EDTA, cover with a lid, and then mix it in the shaking machine for ten minutes. Filter the mixture with a glass filter, wash the triangle flask and residues with 10 ml of water several times, and then put this washed liquid together with the remaining liquid. Place 5 ml aqueous solution of 0.41 M tetrabutyl ammoniumhydrogen sulfate and sodium chloride 10 g, shake it well, quickly adjust pH to near 7.0 using 2 M hydrochloric acid, and then pour into a 300 ml separatory funnel.

□ **Note: Because agricultural chemicals such as those of the dithiocarbamates type are quickly discomposed and are not stable in conditions of alkali, the processes of the extraction stage and the adjustment of pH should be performed quickly.**

b) Hydrolysis: Place the mixtures (1:1) 30 ml of dichloromethane and hexane containing 0.05 M methyl iodide in the above separatory funnel, shake vigorously for five minutes, and then leave it. Pour the water layer (the lower layer) into the separatory funnel, place the mixture (1:1) 10 ml of dichloromethane and hexane containing 0.05 M methyl iodide in the separatory funnel, repeat as above, and then place

the solvent layer (the top level) together with the water layer in the aforementioned separatory funnel. Dehydrate it using the appropriate quantity of sodium sulfate, leave it at room temperature for approximately 30 minutes, and place dichloromethane 5 Ml containing 20% 1,2-propanediol. Then, immediately after blowing off the solvent in the water bath at a temperature of less than 30□ under decompression, place 5 ml methanol in the residues, liquefy it, and then use as a test solution.

4) Test operation

a) Measuring conditions for the high-performance liquid chromatograph

(1) Column: Tamp 5μm of the octadecylsilirised silica gel for liquid chromatograph into the stainless-steel pipe with 2□5 mm inside diameter and 20□30 cm length.

(2) Detector and wavelength: Ultraviolet absorption system of brightness detector (272 nm)

(3) Mobile phase: Acetonitrile • water • methanol (25:60:15)

(4) Speed of a funneling fluid: 1 ml/minute

b) Qualitative test: The peak in the chromatograph obtained from the above condition should have the same retention time of the standard solution peak under any condition of measurement.

c) Quantitative test: Depending on the test result obtained under the same conditions with the qualitative test, quantify it according to the peak - height method or the peak-area method.

□ Three peaks can be obtained from the **HPLC** chromatograph. The first is the peak of dimethyldithiocarbamate (thiram), the second is the peak of ethylenebis-dithiocarbamates (metiram), and the third is the peak of propineb.

C. Azocyclotin

1) Equipment: Gas chromatograph [FPD (Flame Photometric Detector)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues for testing or equivalent

b) Water: Distilled water or equivalent

c) Florisil: After heating the florisil (60□100 mesh) for column chromatograph at 130□, cool it in the desiccator. Check whether the appropriate agricultural chemicals are fully collected or not before usage.

d) Filter support material: Celite 545

e) Standard crude solution: Make 100 mg/kg after melting the azocyclotin in hexane.

f) Standard solution: Melt the standard crude solution in hexane, and then dilute it to the proper concentration.

g) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution

a) Extract: Crush the reagent (500□600 g), weigh approximately 50 g of the reagent, and then place 50 ml of distilled water and 10ml of hydrobromic acid into it. Mix it well, add 100 ml of acetone, place it in the homogenizer for 3 minutes. Then, filter by decompression using No. 2 filter paper, pour the filtered residues into the homogenizer again, add 100 ml of acetone. Make it homogenous and put it together with the liquid filtered in the same way. Pour the filtered liquid into the separatory funnel, extract 100 ml of hexane twice, dehydrate by passing the sodium sulfate through it, and graduate with the vacuum evaporator until it is dry.

b) Hydrolysis: After liquefying the graduated liquid with 20 ml of ethyl, add 3 ml of methylmagnesium chloride solution, shake it well, and leave for 10 minutes. After adding 10 ml of distilled water and 1 ml of hydrochloric acid, liquefy completely and pour into the separatory funnel. Wash the flask well with 10 ml of ethyl, pour it into the separatory funnel, and combine it with the previous mixtures. Dispose of the water-soluble layer (the lower level), collect the ethyl level and, after drying with the sodium sulfate, graduate • dry it.

c) Refining: Fill the florisil 10 g with hexane in the glass column with 20 mm inside diameter and 30 mm length. Liquefy the graduated liquid obtained from the above with approximately 10 ml of hexane, put it in the column tube prepared in advance, fraction it to 120 ml by pouring it out with hexane, and then graduate and dry under decompression in the water bath at a temperature of less than 40□. Liquefy with acetone and use it as the test solution after measuring out exactly 10 ml.

4) Test operation

a) Measuring conditions for the gas chromatograph

(1) Column: 10% OV-225 100/120 WHP (19001A-F12) (2 mm x 1.8 m) or equivalent

(2) Temperature of injection hole for test solution and detector temperature: 270□, 220□

(3) Column temperature: 230□

(4) Carrier gas and flux: nitrogen (60 ml/minute), hydrogen (75 ml/minute), air (60 ml/minute)

b) Qualitative test: The peak in the chromatograph obtained from the above conditions should have the same retention time as the standard solution peak under any condition of measurement.

c) Quantitative test: Depending on the test result obtained under the same conditions as the qualitative test, quantify it according to the peak-height method or the peak-area method.

D. Carbendazim

1) Equipment: A high-performance liquid chromatograph [ultraviolet absorption system of brightness detector (UV detector)]
2) Reagent and test solution
   a) Solvent: Agricultural chemical residues or equivalent
   b) Water: Distilled water or equivalent
   c) Florisil: After heating the florisil for column chromatograph at 130□ overnight, cool it in the desiccator.
   d) Standard crude liquid: Melt the carbendazim in methanol and prepare 100 mg/kg.
   e) Standard solution: Dilute the standard crude liquid to the appropriate concentration after melting it in the methanol.
   f) Other reagent: Agricultural chemical residues for testing or special grade
3) Preparation of test solution
   a) Extract: After crushing the reagent (500□600 g), place sodium L-ascorbate 4 g, distilled water 40 ml, methanol 80 ml, and hiprosupercel 5 g in approximately 10 g of crushed reagent, extract by shaking for one hour, filter by decompression using No. 2 filter paper, and then wash the container and residues with methanol 50 ml.
   b) Extract (second): Pour the extracted liquid 1000 ml into the separatory funnel, add distilled water 200 ml and saturated sodium chloride 20 ml, and then adjust it to 0.1 M HCI and pH 2-3. Divide hexane to 70ml twice, then collect the hexane layer and discard it. After adjusting it to pH 6-7, place ethylacetate 100 ml in the water level, divide it twice and then dehydrate it by passing it through the 1 PS filter paper. Graduate the solvent level until approximately 1ml and place in the water bath at 40□, and then dry it with nitrogen gas.
   c) Refining: Fill the florisil (20% deactivated) 5 g with hexane in the glass column with 15 mm inside diameter and 300 mm length. Pour the graduated residues into the 5 ml of mixed liquid of hexane • acetone (7:3), pour out 80 ml of mixed liquid of hexane • acetone (7:3), graduate until approx. 1 ml and place in the water bath (40□), and then dry it with the nitrogen gas. Liquefy the graduated residues with methanol 2 ml, and use it as a test solution.
4) Test operation
   a) Measuring conditions for high-performance liquid chromatograph
      (1) Column: Fill the liquid chromatograph with octadecylsilirized silica 5μm in the stainless-steel pipe with 2□5 mm inside diameter and 20□30 cm length.
      (2) Column temperature: 40□
      (3) Mobile phase: IPS • methanol • acetonitrile (60:35:5)
      (4) Detector wavelength: 285 nm
      (5) Speed of a funneling fluid: 1.0 ml/minute

- IPS: Mix 1-decanesulfonic acid sodium salt 1 g with water 200 ml and phosphoric acid 7 ml, and then pour triethylamine 10 ml into this mixture and adjust it to 1 L.

b) Qualitative test: When tested using the above conditions, the test result should be same as the standard product.

c) Quantitative test: Depending on the test result obtained under the same conditions as the qualitative test, quantify it according to the peak - height method or the peak-area method.

E. Difenoconazole

1) Equipment: Gas chromatograph [nitrogen • phosphor detector (NPD)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues for testing or equivalent

b) Water: Distilled water or equivalent

c) Florisil: Disposable cartridge (capacity 6 ml) filled with the fixed type of florisil (1 g)

d) Filter support material: Celite 545

e) Standard crude solution: Prepare 100 mg/kg after melting difenoconazole in acetone.

f) Standard solution: Melt the standard crude solution in acetone, and then dilute it to the appropriate concentration.

g) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution

a) Extract: After crushing the reagent (500□600 g), add 100 ml of acetone to the crushed reagent 50 g, extract by shaking for 30 minutes, and then filter by decompression by letting it pass through Celite 545. Add saturated sodium chloride 50 ml, extract by dividing hexane 50 ml twice, and dehydrate it by passing the top layer through the sodium sulfate. Then, after evaporating with decompression the water bath less than 40□, melt the dried substance in hexane 5 ml.

b) Refining: Make hexane 5 ml flow at a speed of 2 or 3 drops per second in the florisil cartridge beforehand, and then discard it. Fill the liquid melted from dried substance with hexane 5ml in the cartridge, wash it with hexane • acetone (95:5) 20 ml and discard it, and again pour it out with hexane • acetone (70:30) 40 ml. After decompression-evaporating the liquid poured out in the water bath less than 40□, liquefy the dried substance in acetone 2 ml and use it as a test solution.

4) Test operation

a) Measuring conditions for the gas chromatograph

(1) Column: Place 5% of methyl silicone coated with 0.25μm thickness for gas chromatograph in the silicic-acid glass capillary column tube with 0.25 mm inside diameter and 30 m length (DB-5MS capillary column).

(2) Temperature of injection hole for test solution and detector: 320□

(3) Column temperature: Inject the reagent at 100□, keep it for one minute, and then increase the temperature to 250□ at a rate of 10□/minute and keep it for more than 12 minutes.

(4) Carrier gas and flux: Nitrogen 1.0 ml/minute

b) Qualitative test: The peak in the chromatograph obtained from the above condition should be the same as the retention time of the standard solution peak under any condition of measurement.

c) Quantitative test: Depending on the test result obtained under the same conditions as the qualitative test, quantify it according to the peak - height method or the peak-area method.

F. Imidacloprid

1) Equipment: A high-performance liquid chromatograph [ultraviolet absorption system of brightness detector (UV detector)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues or equivalent

b) Water: Distilled water or equivalent

c) Filter support material: Celite 545

d) Silica gel cartridge: Disposable cartridge (capacity 6 ml) filled with the fixed type of silica gel (1 g) for SPE or equivalent

e) Standard crude liquid: Melt imidacloprid in the mixed liquid of acetonitrile • water (20:80) and prepare 100 mg/kg.

f) Standard solution: Dilute the standard crude liquid to the appropriate concentration after melting in the mixed liquid of acetonitrile • water (20:80).

g) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution

a) Extract: After crushing the reagent (500□600 g), place it in acetone 100 ml, add a small quantity of water and then extract it for 5 minutes. Decompression-filter the extracted liquid using a Buchner funnel, wash the residues with acetone, and then filter by suction. Remove the solvent so that the quantity of the remaining liquid becomes 100ml, and then pour it into 1000 ml separated liquid by filtering. After adding water 300ml, saturated sodium chloride 30ml, divide the hexane by extracting per 50ml twice, discard the hexane layer, and divide the dichloromethane by extracting per 50ml twice. Dry the divided extract liquid by passing it through the sodium sulfate, evaporate it through decompression, and then dry it.

b) Refining: After melting the dried residues with hexane • acetone (70:30) 5 ml, add 4ml of the sample liquid to the silica gel cartridge activated in advance and refine it. Add the liquid of hexane • acetone (70:30) 50 ml developed first and the liquid of hexane • acetone

(60:40) 50 ml developed second in order. Obtain 50 ml of the liquid developed secondly, evaporate with decompression, and then dry it. Melt with 4 ml of acetonitrile and use it as a test solution.

4) Test operation

a) Measuring conditions for the high-performance liquid chromatograph

(1) Column: Tamp octadecylirized silica gel for the liquid chromatograph 5μm in the stainless-steel pipe with 2□5 mm inside diameter and 20□30 cm length.

(2) Detector and wavelength: UV 270 nm

(3) Mobile phase: 35% acetonitrile (0.01 M Na_2 HPO_4 , pH 6.5)

(4) Speed of a funneling fluid: 0.8 ml/minute

(5) Column temperature: 40□

b) Qualitative test: The peak in the chromatograph obtained from the above condition should have the same retention time as the standard solution peak under any condition of measurement.

c) Quantitative test: Depending on the test result obtained under the same conditions as the qualitative test, quantify it according to the peak-height method or the peak-area method.

G. Iminoctadine

1) Equipment: A high-performance liquid chromatograph (fluorescent detector)

2) Reagent and test solution

a) Solvent: Agricultural chemical residues or equivalent

b) Water: Distilled water or equivalent

c) Standard crude liquid: Melt iminoctadine triacetate in distilled water and make 100 mg/kg.

d) Standard solution: Dilute the standard crude liquid to the appropriate concentration after melting it in the distilled water.

e) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution

a) Extract: After crushing the reagent (500□600 g), obtain approximately 200 g, add hydrochloric acid guanidine 20 g, and make it homogeneous by crushing. Obtain approximately 20 g from this, pour it into the precipitated tube, add hydrochloric acid guanidine 3 g, sodium chloride 5 g, triethylamin solution 20 ml, and butanol/hexane (1:1, v/v) 100 ml, and then extract it by crushing for 3 minutes using the high-performance rotary crusher two times consecutively. Centrifuge this extracted liquid at 3,000 rpm for 10 minutes and collect the good-quality liquid. Pour it into separated liquid by filtering, add triethylamin liquid 50 ml, and shake vigorously. Add distilled water 30 ml and 1M phosphoric acid 2ml to this organic solvent layer and, after shaking for 5 minutes, collect the water level

by separation. Try this operation one more time, collect the water level and evaporate with vacuum to the level of 2 ml. Adjust the pH to 6 with 0.1M NaOH.

b) Refining: Flow the SPE cartridge per 5 ml separately with methanol and distilled water and activate it, and pour the graduated liquid adjusted to pH 6 as above into the cartridge. Pour it out with phosphoric acid buffer solution 5 ml and 0.002M hydrochloric acid/MeOH 10 ml, and then discard. After pouring out 10 ml iminoctadine with the 0.1 M hydrochloric acid/MeOH liquid, evaporate with decompression in the water bath at 40□.

4) Test operation

a) Measuring conditions for the high-performance liquid chromatograph

(1) Column: Fill octadecylirized silica gel for liquid chromatograph 5 μm in a stainless-steel pipe with 2□5mm inside diameter and 20□30 cm length.

(2) Detector and wavelength: Fluorescent brightness detector excited wavelength 395nm, fluorescent wavelength 500nm

(3) Mobile phase: Water • 28% ammonia liquid (35:64:1), pH2.5 (adjust to 60% $HClO_4$)

(4) Speed of a funneling fluid: 0.7 ml/minute

b) Qualitative test: The peak in the chromatograph obtained from the above condition should have the same retention time as the standard solution peak under any condition of measurement.

c) Quantitative test: Depending on the test result obtained under the same conditions as the qualitative test, quantify it according to the peak-height method or the peak-area method.

H. Pymetrozine

1) Equipment: A high-performance liquid chromatograph [ultraviolet absorption system of brightness detector (UV detector)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues or equivalent

b) Water: Distilled water or equivalent

c) Cartridge: ENVI-Carb Sep-Pak cartridge, 250 mg

d) Filter support material: Celite 545 (CP grade)

e) Standard crude liquid: After melting pymetrozine in acetonitrile, prepare 500mg/kg and use it as a standard crude liquid.

f) Standard solution: Dilute the standard crude liquid to the appropriate concentration using acetonitrile.

g) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution

a) Extract: After crushing the reagent (500□600 g), obtain approximately 20 g, place methanol 100 ml and distilled water 30 ml in it. Leave it at

room temperature for 2 hours, and then extract it by shaking for 1 hour using the shaking machine. Decompression-filter the extracted liquid using the Celite 545, wash with methanol 100 ml again, after combing it with the filtered liquid adjust the total quantity to 250 ml. Then obtain 100ml from this again, pour it into the separatory funnel (250 ml), and then add hexane 100ml in this liquid. Extract by shaking, carefully remove the hexane layer, collect the filtered layer, and then evaporate with decompression until approximately 5ml is left over.

b) Refining: After placing the above graduated liquid in the ENVI-Carb Sep-Pak cartridge (250 mg) activated in advance with methanol 5ml and distilled water 5 ml, remove any impurities with methanol • distilled water (5:5) 5 ml, methanol • acetonitrile (7:3) 5 ml and ethylacetate 5 ml separately. After drying it for 3 minutes in the vacuum, pour out dichloromethane 30 ml. Vacuum this poured-out liquid in the water bath less than 35□, and then liquefy the residues in the acetonitrile 2 ml. Remove a certain quantity (50μL) and use it as a test solution.

4) Test operation

a) Measuring conditions for the high-performance liquid chromatograph

(1) Column: Fill octadecylirized silica gel for liquid chromatograph 5 μm in the stainless-steel pipe with 2□5 mm inside diameter and 20□30 cm length.

(2) Detector and wavelength: UV 300 nm

(3) Mobile phase: Acetonitrile • distilled water (995/45, v/v)

(4) Speed of a funneling fluid: 1.0 ml/minute

b) Qualitative test: The peak in the chromatograph obtained from the above condition should have the same retention time as the standard solution peak under any condition of measurement.

c) Quantitative test: Depending on the test result obtained under the same conditions with the qualitative test, quantify it according to the peak - height method or the peak-area method.

I. Thiamethoxam

1) Equipment: A high-performance liquid chromatograph [ultraviolet absorption system of brightness detector (UV detector)]

2) Reagent and test solution

a) Solvent: Agricultural chemical residues or equivalent

b) Water: Distilled water or equivalent

c) Silica cartridge: Disposable cartridge (Silica Sep-Pack, capacity 6 ml) filled up with fixed type of silica gel (1 g) for SPE, or equivalent

d) Filter support material: Celite 545

e) Standard crude liquid: After melting thiamethoxam in acetonitrile, prepare 100 mg/kg and use it as standard crude liquid.
f) Standard solution: Dilute the standard crude liquid to the appropriate concentration using acetonitrile.
g) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution
a) Extract: After crushing the reagent (500□600 g), place acetone • ehylacetate (40:60) 100 ml in the 20 g crushed reagent and extract by shaking for 30 minutes with 150 rpm using the shaking machine. By passing the extracted liquid through the Celite 545, make the solvent run off using the vacuum evaporator. Add 10% sodium chloride 50 ml, and then extract the dichloromethane by separating per 50 ml twice. Dehydrate by passing the extracted liquid through the sodium sulfate and, after vacuuming this liquid using the vacuum evaporator, melt the dried substance in hexane • acetone (90:10) 5 ml.
b) Refining: After melting it in the dried substance, fill it in the silica Sep-Pack cartridge, wash it with hexane • acetone (90:10) 10 ml, and pour it out with hexane • acetone (60:40) 5 ml again. After vacuuming the poured-out liquid using the vacuum evaporator, liquefy the dried substance in acetonitrile 2 ml and use it as a test solution.

4) Test operation
a) Measuring conditions for the high-performance liquid chromatograph
(1) Column: Place 5μm of octadecylirized silica gel for liquid chromatograph in a stainless-steel pipe with 2□5 mm inside diameter and 20□30 cm length.
(2) Detector and wavelength: UV 254 nm
(3) Mobile phase: Acetonitrile • distilled water (50:50)
(4) Speed of a funneling fluid: 1.0 ml/minute
(5) Column temperature: 25□
b) Qualitative test: The peak in the chromatograph obtained from the above condition should have the same retention time as the standard solution peak under any condition of measurement.
c) Quantitative test: Depending on the test result obtained under the same conditions with the qualitative test, quantify it according to the peak - height method or the peak-area method.

J. Triforine

1) Equipment: Gas chromatograph [ECD (Electronic Capture Detector)]
2) Reagent and test solution
a) Solvent: Agricultural chemical residues or equivalent
b) Water: Distilled water or equivalent
c) Silica gel: Silica gel (70-230 mesh) for column chromatograph

d) Standard crude solution: After melting triforine in acetone, prepare 100 mg/kg.
e) Standard solution: After melting the standard crude solution in acetone, dilute it to the appropriate concentration.
f) Other reagent: Agricultural chemical residues for testing or special grade

3) Preparation of test solution
a) Extract: After crushing the reagent (500□600 g) well, obtain approximately 20 g, add acetone 200 ml, and make it homogeneous using the homogenizer. Decompression-filter this liquid using a Buchner funnel. Remove the acetone with the rotary vacuum evaporator, pour it into separated liquid by filtering, add 5% sodium chloride 200 ml, combine with benzene layer extracted per 100 ml twice, and then vacuum with the rotary vacuum evaporator.
b) Refining: Place silica gel 5 g activated overnight in the dryer (130 □), and sodium sulfate 2 g in the glass column with 10 mm inside diameter and 40mm length. After fluting with hexane 50 ml, place this graduated liquid in a small quantity of benzene by melting. Flute hexane • acetone (7:3) 150 ml and discard it. Obtain the mixed liquid 100 ml of hexane • acetone (7:3) after fluting, vacuum-adjust the mixed liquid 8ml of methanol • ethylacetate (1:1), or 16 ml, and use it as a standard test solution.

4) Test operation
a) Measuring conditions for the gas chromatograph
(1) Column: 3% SP-2250, 1.2 m (L) x 5 mm (I.D.)
(2) Temperature of injection hole for test solution: 260□
(3) Detector temperature: 270□
(4) Column temperature: 230□
(5) Carrier gas and flux: Nitrogen (adjusted with the speed of triforine flow over approximately 3 minutes)
a) Qualitative test: The peak in the chromatograph obtained from the above condition should have the same retention time as the standard solution peak under any condition of measurement.
b) Quantitative test: Depending on the test result obtained under the same conditions with the qualitative test, quantify it according to the peak-height method or the peak-area method.

Additional rule

□ Commencement date: This announcement shall be effective from the date of announcement.

- Course of action: If the application was made in the days of the commencement of this announcement or in the process of testing, then the former rules shall apply.