Appendix VI: Determination of Pesticide Residues

Pesticide is a synthetic chemical, a natural or biological substance, or a mixture thereof, used for prevention, termination and/or control of diseases, pests, grass or other living things which are hazardous to agriculture and forestry; or for regulation of the growth of plants and pests in an intended way.

The targeted pesticides for the analysis of pesticide residues in CMM are listed as follows –

(a) Aldrin and Dieldrin (sum of)
(b) Chlordane (sum of *cis*-, *trans*- and oxychlordane)
(c) Dichlorodiphenyltrichloroethane (DDT) [sum of p,p'-DDT, o,p'-DDT, p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) and p,p'-dichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-TDE)]
(d) Endrin
(e) Heptachlor (sum of heptachlor and heptachlor epoxide)
(f) Hexachlorobenzene
(g) Hexachlorocyclohexane isomers (α-, β- and δ-hexachlorocyclohexane)
(h) Lindane (γ-hexachlorocyclohexane)
(i) Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)

Method –

(1) **Analysis of pesticide residues** – The analytical procedures must be verified and satisfy with all of the following criteria –

(a) the method is suitable for the analysis of the targeted pesticides;

(b) the limits of detection and quantification are determined for each targeted pesticide;

(c) the limit of quantification for each targeted pesticide is 0.02 mg/kg. Except for *cis*-chlordane, *trans*-chlordane and oxychlordane, each of which is set at 0.01 mg/kg;

(d) the recovery for each targeted pesticide is between 70 and 120%;

(e) the repeatability of the method is less than 15% RSD; and

(f) a linear response is obtained from the analytical detector within the calibration range.

(2) **Reagents** – All reagents used should be of analytical grade or equivalent and free from any contaminant which may interfere with the analysis. Suitable blank tests should be conducted to demonstrate no occurrence of contamination of the pesticide residues.

(3) **Apparatus** – All apparatus to be used should be thoroughly cleaned to ensure that they are free from any pesticides. Soak the apparatus in a solution of phosphate-free detergent for at least 16 h, then rinse them with a large quantity of distilled water and wash them with acetone.

(4) **Preparation of test sample** – Take a representative CMM sample and cut it into pieces, if necessary, before grinding. Powder the sample before the analysis. Whenever possible, the quantity of the sample to be powdered should be of at least five times as much as those needed for the analysis.

(5) **Procedure** – The following procedures are applicable for the quantitative detection of pesticide residues in CMM samples. It may have to modify the procedures for the analysis of some samples. Wherever possible, it is necessary to use a second capillary column with different polarities and/or MS to confirm the analytical results.

(a) **Extraction** – Weigh accurately 10.0 g of the blended sample powder, add about 4.0 g of anhydrous sodium sulphate and about 100 mL of ethyl acetate. Sonicate in pulse mode by using an ultrasonic processor for 3 min. Allow the solids to settle and then filter the supernatant solution and collect the filtrate. Repeat the extraction twice each with 50 mL of ethyl acetate. Combine the filtrates and the washings and then evaporate to near dryness in a rotary evaporator at about 35°C. Dissolve the residue in 10 mL of a mixture of dichloromethane and cyclohexane (1:1, v/v) **(Solution A)**.

(b) **Clean-up** –

(i) **Gel permeation chromatography** – The chromatographic procedure may be carried out by using –

- a Bio-beads S-X3 glass column, 60 g in weight and 43 cm in length, or equivalent; and
- a mixture of dichloromethane and cyclohexane (1:1, v/v) as the mobile phase.

Performance of the column – Inject a solution containing corn oil (about 25 mg/mL), bis(2-ethylhexyl)phthalate (about 1 mg/mL), methoxychlor (about 0.2 mg/mL) and perylene (about 0.02 mg/mL) and proceed with the chromatography. The column is not suitable unless the resolution of any adjacent peaks is ≥ 0.85. If necessary, calibrate the column using a solution containing the pesticides [at a suitable concentration and in a mixture of dichloromethane and

cyclohexane (1:1, v/v)] with the lowest molecular weight (for example pentachloroaniline) and that with the highest molecular weight (for example oxychlordane). Determine which fractions of the eluate contain the target pesticides.

Purification of the test solution – To 10 mL of solution A, add about 1.0 g of anhydrous sodium sulphate, centrifuge the mixture and get the supernatant layer. Inject an appropriate volume of the extract and proceed with the chromatography. Collect the fraction as determined above. Concentrate the solution in a rotary evaporator on a water bath at a temperature below 35°C until the solvent has almost completely evaporated. Then dissolve the residue in 1 mL of hexane **(Solution B)**.

(ii) **Solid phase extraction** – The chromatographic procedure may be carried out by using –

- a florisil solid phase extraction column, 75-150 μm in diameter and 1000 mg in weight, or equivalent; and
- a solution of diethyl ether in hexane (15%, v/v) as the eluting solvent.

If necessary, calibrate the column by using a solution in hexane containing suitable concentrations of the targeted pesticides. Determine the fractions of the targeted pesticides from the eluate.

Pack about 10 mm of anhydrous sodium sulphate on the top of the florisil column. Condition the column with about 5 mL of hexane. Transfer quantitatively solution B onto the florisil column and proceed with the chromatography. Collect the eluate **(Solution C)**.

(c) **Quantitative and qualitative analysis** – Examined by GC using 2,4,5,6-tetrachloro-m-xylene as an internal standard. Another internal standard may be needed if interferences occur.

Use the gas chromatograph that satisfies with all of the following criteria –

- the *R* value of any analyte peak with the adjacent peak: > 1.5;
- the *n* value: ≥ 100000 for the peak of α-hexachlorocyclohexane; and
- the RSD of the peak area: ≤ 5%.

Solution (1): Prepare at least five standard solutions in isooctane containing 2,4,5,6-tetrachloro-m-xylene and all the targeted pesticides at concentrations suitable for plotting calibration curves.

Solution (2): Concentrate solution C in a stream of nitrogen to almost dryness and dilute to 1 mL with isooctane containing 2,4,5,6-tetrachloro-m-xylene as an internal standard [Notes 1 and 2].

Note 1: *The concentration of the internal standard in the test solution should be same as those in the standard solutions.*

Note 2: *The sulphuric acid treatment in combination with copper powder treatment may prove useful to remove certain matrix interference arisen from the sample matrix. However, this treatment will destroy or remove certain targeted pesticides such as aldrin, dieldrin, endrin, heptachlor epoxide, methyl pentachlorophenyl sulphide and pentachloroaniline.*

The **chromatographic procedure** may be carried out by using –

- a capillary column (0.25 mm × 30 m) of which the internal wall is covered with (14%-cyanopropylphenyl)-methylpolysiloxan in a layer about 0.25 μm thick;
- a second capillary column of different polarities (0.25 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick;
- nitrogen as the carrier gas;
- an electron-capture detector; and
- a device allowing split/splitless injection. After maintaining the temperature of the column at 100°C for 2 min, raise it to 165°C at a rate of 10°C/min and maintain at this temperature for 10 min. Raise the temperature to 230°C at a rate of 3°C/min and afterward to 280°C at a rate of 15°C/min, then maintain at this temperature for 10 min. Maintain the temperature of the injector port at 210°C and the temperature of the detector at 300°C.

In the prescribed conditions, inject 1 μL or other appropriate volume of each solution and record the chromatograms. The reference RRTs of the targeted pesticides obtained are listed in Table 1. Calculate the content of each targeted pesticide from its peak area and concentration.

The results obtained can be confirmed by GC-MS.

The **chromatographic procedure** may be carried out by using –

- a capillary column (0.25 mm × 30 m) of which the internal wall is covered with (35%-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick;

- helium as the carrier gas;

- a mass selective detector capable of operating in a scan mode or selective ion mode (*m/z* of the monitoring ions for the targeted pesticides are listed in Table 2 for reference); and

- a device allowing split/splitless injection. Maintain the temperature of the column at 100°C for 2 min, raise to 160°C at a rate of 15°C/min and afterward to 270°C at a rate of 5°C/min, then maintain at this temperature for 10 min. Maintain the temperature of the injector port at 250°C and the temperature of the ion source at 230°C.

In the prescribed conditions, inject 1 μL or other appropriate volume of each solution and record the chromatograms. The reference RRTs of the targeted pesticides obtained are listed in Table 2.

Table 1 The reference RRTs of the targeted pesticides obtained by GC

Pesticide	**RRT [column used: 0.25 mm × 30 m, (14%-cyanopropylphenyl)-methylpolysiloxane of 0.25-μm thick]**
Hexachlorobenzene	1.24
α-Hexachlorocyclohexane	1.55
Quintozene	1.64
Lindane	1.83
Heptachlor	1.94
Pentachloroaniline	2.01
Aldrin	2.09
Methyl pentachlorophenyl sulphide	2.10
β-Hexachlorocyclohexane	2.32
Oxychlordane	2.41
δ-Hexachlorocyclohexane	2.43
Heptachlor epoxide	2.50
trans-Chlordane	2.67
cis-Chlordane	2.71
p,p'-DDE	2.76
Dieldrin	2.82
Endrin	2.92
o,p'-DDT	2.98
p,p'-TDE	3.15
p,p'-DDT	3.21

Table 2 The reference RRTs and the monitoring ions of the targeted pesticides obtained by GC-MS

Pesticide	**RRT**	**Primary Ion,** *m/z*	**Secondary Ion,** *m/z*
Hexachlorobenzene	1.18	284	286, 282
α-Hexachlorocyclohexane	1.22	181	183, 217
Quintozene	1.32	237	249, 214
Lindane	1.35	183	217, 221
β-Hexachlorocyclohexane	1.45	181	183, 217
Heptachlor	1.48	272	274, 270
Pentachloroaniline	1.49	265	267, 263
δ-Hexachlorocyclohexane	1.55	181	183, 217
Aldrin	1.58	263	265, 261
Methyl pentachlorophenyl sulphide	1.63	296	246, 263
Oxychlordane	1.74	185	387, 237
Heptachlor epoxide	1.79	353	355, 351
trans-Chlordane	1.87	373	375, 377, 371
cis-Chlordane	1.91	373	375, 377, 371
p,p'-DDE	2.00	246	316, 248
Dieldrin	2.03	263	261, 265
Endrin	2.14	263	265, 281
o,p'-DDT	2.17	235	237, 165
p,p'-TDE	2.20	235	237, 165
p,p'-DDT	2.30	235	237, 165

Limits – The amount of pesticide residues in CMM samples should comply with the limits listed in Table 3 below, unless in the case of a CMM of mineral origin or as otherwise specified.

Table 3 The maximum permitted limits of pesticide residues in CMM samples

Pesticide	Limit (Not more than)
Aldrin and Dieldrin (sum of)	0.05 mg/kg
Chlordane (sum of *cis*-, *trans*- and oxychlordane)	0.05 mg/kg
DDT (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-TDE)	1.0 mg/kg
Endrin	0.05 mg/kg
Heptachlor (sum of heptachlor and heptachlor epoxide)	0.05 mg/kg
Hexachlorobenzene	0.1 mg/kg
Hexachlorocyclohexane isomers (α-, β- and δ- hexachlorocyclohexane)	0.3 mg/kg
Lindane (γ-hexachlorocyclohexane)	0.6 mg/kg
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0 mg/kg