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 (HCB)
(g) Hexachlorocyclohexane (BHC) isomers (α-, β- and δ-hexachlorocyclohexane)
(h) Lindane [γ-hexachlorocyclohexane (BHC)]
(i) Quintozene [sum of quintozene (PCNB), pentachloroaniline (PCA) and methyl pentachlorophenyl sulphide (MPCPS)]

(1) **Analysis of pesticide residues** – The analytical procedures must be verifi ed 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 quantifi cation are determined for each targeted pesticide;

(c) the limit of quantifi cation 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%; and

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

(2) **Reagents** – All reagents used should be of pesticide 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.

Method I – Gas chromatography with electron-capture detector (GC-ECD)

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

(a) the repeatability of the method is less than 15% RSD.

(2) **Procedures** – 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 at reduced pressure 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 considered suitable unless the resolution of all adjacent peaks is ≥ 0.85. If necessary, calibrate the column using a solution containing pesticides [at a suitable concentration and in a mixture of dichloromethane and cyclohexane (1:1, v/v)] with the lowest (for example pentachloroaniline) and that with the highest (for example oxychlordane) molecular weights. 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 about 35°C until the solvent has almost completely evaporated. Then dissolve the residue in 1 mL of *n*-hexane **(Solution B)**.

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

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

If necessary, calibrate the column by using a solution in *n*-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 - 15 mL of *n*-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 chromatography 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 *iso*-octane 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 *iso*-octane 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)-methylpolysiloxane 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, the temperature of the transfer line at 270°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

Method II – Gas chromatography with tandem mass spectrometry (GC-MS/MS)

(1) **Analysis of pesticide residues** – The analytical procedures must be verified and satisfy the following additional criterium –

(a) the repeatability of the method is less than 25% RSD.

(2) **Preparation of internal standard solution** –

(a) Internal standard solution – Prepare a solution containing 2,4,5,6-tetrachloro-*m*-xylene and polychlorinated biphenyl congener #138 (PCB 138) in *iso*-octane at a suitable concentration.

(b) Labeled internal standard solution – Prepare a solution containing isotopically labeled internal standards, such as PCB-138-$^{13}C_{12}$, HCB-$^{13}C_6$, aldrin-$^{13}C_{12}$, dieldrin-$^{13}C_{12}$, endrin-$^{13}C_{12}$, α-BHC-$^{13}C_6$, β-BHC-$^{13}C_6$, δ-BHC-$^{13}C_6$, γ-BHC-$^{13}C_6$, heptachlor-$^{13}C_{10}$, PCNB-$^{13}C_6$, *cis*-chlordane-$^{13}C_{10}$, *trans*-chlordane-$^{13}C_{10}$, oxychlordane-$^{13}C_{10}$, *p,p*'-TDE-$^{13}C_{12}$, *p,p*'-DDE-$^{13}C_{12}$, *o,p*'-DDT-$^{13}C_{12}$ and *p,p*'-DDT-$^{13}C_{12}$, in *iso*-octane at a suitable concentration.

(3) **Procedures** – The following three sample preparation procedures are applicable for the quantitative detection of pesticide residues in CMM samples. The sample preparation procedure could be selected according to the matrix of the CMM sample. One may have to modify the procedures for the analysis of some samples.

Sample preparation procedure A

(a) **Soxhlet extraction** – Weigh accurately 5.0 g of the blended sample powder and add an appropriate amount of labeled internal standard solution if available. Add about 20 mL of distilled water, stir and mix well. Allow the mixture to stand overnight at room temperature. Add 3.5 g of super absorbent polymer (acrylate type or equivalent) and 3.5 g of celite, agitate or stir vigorously for 3 min. Allow the mixture to stand for at least 4 h. Transfer the mixture to a cellulose extraction thimble and add 10 g of anhydrous sodium sulphate. Mix well. Add 300 mL of a mixture of *n*-hexane and acetone (1:1, v/v) to a 500-mL round-bottomed flask. Perform the soxhlet extraction for 20 h at 4-6 cycles per hour. Cool down to room temperature. Evaporate the extract to near dryness at reduced pressure 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, 70 g in weight, 78 cm in length and 2.5 cm internal diameter, 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 considered suitable unless the resolution of all adjacent peaks is ≥0.85. If necessary, calibrate the column using a solution containing pesticides [at a suitable concentration and in a mixture of dichloromethane and cyclohexane (1:1, v/v)] with the lowest (for example pentachloroaniline) and that with the highest (for example oxychlordane) molecular weights. 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 at about 800 × *g* for 5 min. Collect 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 at about 35°C until the solvent has almost completely evaporated. Then dissolve the residue in 1 mL of *n*-hexane **(Solution B)**.

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

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

If necessary, calibrate the column by using a solution in *n*-hexane containing suitable concentration 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 – 10 mL of *n*-hexane. Transfer quantitatively solution B onto the florisil column and proceed with the chromatography. Collect the eluate **(Solution C)**.

Sample preparation procedure B

(a) **Ultrasonic extraction** – Weigh accurately 10.0 g of the blended sample powder and add an appropriate amount of labeled internal standard solution if available. Add about 40 mL of distilled water, stir and mix well. Allow the mixture to stand overnight at room temperature. Add 7.5 g of super absorbent polymer (acrylate type) and 7.5 g of celite, agitate or stir vigorously for 3 min. Allow the mixture to stand for at least 4 h. Add 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 at reduced pressure 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, 70 g in weight, 78 cm in length and 2.5 cm internal diameter, 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 considered suitable unless the resolution of all adjacent peaks is ≥0.85. If necessary, calibrate the column using a solution containing pesticides [at a suitable concentration and in a mixture of dichloromethane and cyclohexane (1:1, v/v)] with the lowest (for example pentachloroaniline) and that with the highest (for example oxychlordane) molecular weights. 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 at about 800 × *g* for 5 min. Collect 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 at about 35°C until the solvent has almost completely evaporated. Then dissolve the residue in 1 mL of *n*-hexane **(Solution B)**.

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

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

If necessary, calibrate the column by using a solution in *n*-hexane containing suitable concentration 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 – 10 mL of *n*-hexane. Transfer quantitatively solution B onto the florisil column and proceed with the chromatography. Collect the eluate **(Solution C)**.

Sample preparation procedure C

(a) **Agitation extraction** – Weigh accurately 1.0 g of the blended sample powder, add an appropriate amount of the internal standard solution and labeled internal standard solution. Add distilled water of 1 – 2 times of the sample weight, stir and mix well. Allow the mixture to stand for 15 min. Add 15 mL of a chilled mixture of acetic acid and acetonitrile (1:99, v/v). Shake for 5 min. Add 6 g of anhydrous magnesium sulphate and 1.5 g of anhydrous sodium acetate. Shake for 5 min. Centrifuge at about 3000 × *g* for 5 min (at about 4°C). Collect the supernatant **(Solution A)**.

Note: *For sample with high oil content, store the extract in the refrigerator overnight at -20 °C after the centrifugation, if necessary. Carefully collect the clear supernatant excluding the dispersing solids or top-floating layer from freeze-out for further dSPE cleanup.*

(b) **Clean-up** –

(i) **Dispersive solid-phase extraction (dSPE)** – The chromatographic procedure may be carried out by using –

- a 15 mL dSPE tube, containing 900 mg of anhydrous magnesium sulphate, 150 mg of primary secondary amine (PSA) sorbent, 45 mg of graphite carbon black, 150 mg of ODS and 150 mg of silica gel or equivalent.

Purification of the test solution – Transfer solution A to a dSPE tube. Shake for 2 min. Centrifuge at about 3000 × *g* for 5 min. Pipette 4.5 mL of the supernatant and evaporate the solvent to near dryness under gentle stream of nitrogen. Dissolve the residue in 4.5 mL of *n*-hexane **(Solution B)**.

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

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

If necessary, calibrate the column by using a solution in *n*-hexane containing suitable concentration 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 – 10 mL of *n*-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-MS/MS using suggested internal standard or labeled internal standard (Table 3). Another internal standard or labeled internal standard may be needed if interferences occur.

Use the gas chromatography coupled with tandem mass spectrometer that satisfies with all of the following criteria -

- The RRT of the analytes in sample shall agree within ± 0.5 % with that of the reference RRT obtained from calibration standards.
- At least two multiple reaction monitoring (MRM) transitions shall be monitored and calculate relative intensity between two MRM transitions (MRM_{AR}) and the absolute percentage deviation of MRM relative intensity between calibration standard and sample (D_{MRM}, %) according to the following equations:

$$MRM_{AR} = \frac{Area_{MRM2}}{Area_{MRM1}}$$

Where MRM with larger area count ($Area_{MRM1}$) is selected as the denominator
MRM with smaller area count ($Area_{MRM2}$) is selected as the nominator

$$D_{MRM}\,(\%) = \frac{|MRM_{ARsample} - MRM_{ARcalibration}|}{MRM_{ARcalibration}} \times 100\%$$

Where $MRM_{ARsample}$ refers to the relative intensity for the sample solution
$MRM_{ARcalibration}$ refers to the average relative intensity for the calibration standards

- Deviation of each of the relative intensity of the characteristics multiple reaction monitoring (MRM) of the analytes in sample from that of calibration standard (D_{MRM}, %) shall be within ± 30%.

Calibration curve: Prepare at least five standard solutions in *iso*-octane containing all the targeted pesticides with respective suggested internal standard or labeled internal standard at concentrations suitable for plotting calibration curves. The analytes should be calibrated with the respective internal standard solution or labeled internal standard suggested in Table 3.

Sample solution: Concentrate solution C obtained in sample preparation procedure A or B in a stream of nitrogen to almost dryness and dissolve the residues with 1 mL of suggested internal standard solution; concentrate solution C obtained in sample preparation procedure C in a stream of nitrogen to almost dryness and dissolve the residue with 0.2 mL of *iso*-octane [Notes 1 and 2].

Note 1: *The concentration of the internal standard in the sample solution should be the 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 **gas chromatographic with tandem mass spectrometer procedure** may be carried out by using –

- a capillary column (0.25 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick; and

- helium as the carries gas at a flow of 1.5 mL/min; and

- electron impact ionization mode; and

- argon as the collision gas; and

- a triple quadrupole mass spectrometer capable of operating in a MRM scan mode (suggested MRM for the targeted pesticides and internal standards are listed in Table 4 for reference); and

- a device allowing split/splitless injection. Maintain the temperature of the column at 95°C for 2 min, raise to 130°C at a rate of 30°C/min and afterward to 250°C at a rate of 5°C/min and further to raise to 300°C at a rate of 10°C/min, then maintain at this temperature for 8 min. Maintain the temperature of the injector port at 250°C and both temperatures of the transfer line and ion source at 300°C.

In the prescribed conditions, inject 1 µL or other appropriate volume of each solution and record the chromatograms. Calculate the content of each targeted pesticide from its concentration according to the following equations:

$$C_{sample} = C_{sln} \times \frac{V}{W} \times D$$

Where C_{sample} refers to the concentration of analyte in sample, in mg/kg

C_{sln} refers to the concentration of analyte in sample solution, in mg/L

V refers to the final sample volume, in mL

W refers to the weight of sample, in g

D refers to the dilution factor (if any)

$$C_{sln} = \frac{AR - c}{m}$$

Where AR refers to the relative peak area ratio corresponding to analyte and internal standard or labeled internal standard in sample solution

c refers to the y-intercept of calibration curve of the respective analyte standard

m refers to the slope of calibration curve of the respective analyte standard

Table 3 Suggested respective internal standard or labeled internal standard for each analyte

Analyte	**Internal Standard**
α- and β- Hexachlorocyclohexane, lindane and hexachlorobenzene	2,4,5,6-tetrachloro-*m*-xylene
Aldrin, dieldrin, endrin, *cis*-chlordane, *trans*-chlordane, heptachlor, *cis*-heptachlor epoxide, δ-hexachlorocyclohexane, methyl pentachlorophenyl sulphide, oxychlordane, quintozene, pentachloroaniline, *p,p*'-DDE, *p,p*'-TDE, *o,p*'-DDT, and *p,p*'-DDT	PCB 138
Analyte	**Labeled Internal Standard**
Aldrin	endrin-$^{13}C_{12}$ or aldrin-$^{13}C_{12}$
Dieldrin	endrin-$^{13}C_{12}$ or dieldrin-$^{13}C_{12}$
Endrin	endrin-$^{13}C_{12}$
α- Hexachlorocyclohexane	γ-BHC-$^{13}C_6$ or *α*-BHC-$^{13}C_6$
β- Hexachlorocyclohexane	γ-BHC-$^{13}C_6$ or β-BHC-$^{13}C_6$
δ- Hexachlorocyclohexane	γ-BHC-$^{13}C_6$ or δ-BHC-$^{13}C_6$
Lindane	γ-BHC -$^{13}C_6$
Hexachlorobenzene	HCB-$^{13}C_6$
Heptachlor	heptachlor-$^{13}C_{10}$
cis-Heptachlor epoxide	
Quintozene	PCNB-$^{13}C_6$
Methyl pentachlorophenyl sulphide	
Pentachloroaniline	
p,p'-DDE	*p,p*'-DDE-$^{13}C_{12}$
p,p'-TDE	*p,p*'-TDE-$^{13}C_{12}$
o,p'-DDT	*o,p*'-DDT-$^{13}C_{12}$
p,p'-DDT	*p,p*'-DDT-$^{13}C_{12}$
cis-Chlordane	*cis*-chlordane-$^{13}C_{10}$
trans-Chlordane	*trans*-chlordane-$^{13}C_{10}$
Oxychlordane	oxychlordane-$^{13}C_{10}$

Table 4 Suggested MRM parameters of the targeted pesticides and internal standards

Pesticide		Precursor ion *(m/z)*	Product ion *(m/z)*	Collision energy (eV)	Remark
1	Aldrin	262.8	192.9	32	#
		262.7	227.9	20	
		293.0	186.0	35	
2	α-Hexachlorocyclohexane	180.8	145.0	12	#
		218.8	146.6	20	
		218.8	183.0	8	
3	β- Hexachlorocyclohexane	180.9	145.0	14	#
		218.7	146.6	18	
		218.7	183.0	8	
4	*cis*-Chlordane	236.9	118.9	25	
		372.8	265.8	20	#
		409.8	374.8	5	
5	δ-Hexachlorocyclohexane	182.8	146.7	14	
		218.7	183.0	8	#
		218.8	146.6	20	
6	Dieldrin	262.8	192.9	30	#
		262.8	227.9	16	
		276.9	241.0	10	
7	Endrin	243.0	173.0	25	
		262.8	190.9	30	
		262.8	192.9	30	#
8	Lindane	180.9	109.0	26	
		180.9	145.0	14	#
		218.7	183.0	8	
9	Hexachlorobenzene	248.8	213.9	14	
		283.8	213.8	28	
		283.8	248.8	18	#
10	Heptachlor	99.8	65.0	12	
		271.8	236.9	12	#
		273.9	238.9	15	

	Pesticide	Precursor ion *(m/z)*	Product ion *(m/z)*	Collision energy (eV)	Remark
11	*cis*-Heptachlor epoxide	354.7	264.9	12	
		352.9	262.9	16	#
		352.9	281.9	10	
12	*o,p*'-DDT	235.0	165.1	22	#
		235.0	199.1	10	
		199.1	163.1	30	
13	Oxychlordane	184.9	84.9	26	
		184.9	121.0	12	#
		386.8	322.8	15	
14	*p,p*'-TDE	235.0	165.1	20	#
		235.0	199.1	14	
		236.8	165.0	20	
15	*p,p*'-DDE	246.0	176.1	28	#
		248.0	176.0	28	
		317.8	248.0	18	
16	*p,p*'-DDT	199.1	163.1	30	
		235.0	165.1	22	#
		235.0	199.1	15	
17	Pentachloroaniline	262.8	191.9	20	#
		264.8	202.8	20	
		264.8	229.3	12	
18	Methyl pentachlorophenyl sulphide	262.8	192.9	28	
		295.7	245.9	30	
		295.7	262.9	12	#
19	Quintozene	213.8	141.9	28	
		213.8	178.9	14	#
		294.8	236.9	14	
20	*trans*-Chlordane	271.7	236.9	12	#
		372.8	265.8	20	
		409.8	374.8	5	

Internal Standard or Labeled Internal Standard	**Precursor ion** *(m/z)*	**Product ion** *(m/z)*	**Collision energy** (eV)	**Remark**
Polychlorinated biphenyl congener #138	289.9	218.0	30	
	357.9	287.8	25	
	359.8	289.8	25	#
2,4,5,6-Tetrachloro-*m*-xylene	207.0	136.0	20	#
	207.0	172.0	10	
	244.0	209.0	10	
Hexachlorobenzene -$^{13}C_6$	254.9	220.0	30	
	287.8	218.0	20	
	289.9	219.9	20	#
Aldrin-$^{13}C_{12}$	98.1	70.1	10	#
	268.0	198.0	30	
	270.0	200.0	15	
Dieldrin-$^{13}C_{12}$	85.1	55.0	10	
	86.1	57.1	10	#
	269.9	199.9	5	
Endrin-$^{13}C_{12}$	254.0	184.1	25	#
	267.9	198.0	30	
	269.9	200.0	30	
α- Hexachlorocyclohexane -$^{13}C_6$	187.0	151.0	15	#
	189.0	153.0	10	
	225.0	189.0	5	
β- Hexachlorocyclohexane -$^{13}C_6$	112.0	50.0	20	
	187.0	151.0	15	#
	189.0	153.0	10	
δ- Hexachlorocyclohexane -$^{13}C_6$	187.0	151.0	15	#
	189.0	153.0	15	
	223.0	187.0	5	
Lindane -$^{13}C_6$	187.0	151.0	15	#
	223.0	187.0	5	
	225.0	189.0	5	
Heptachlor-$^{13}C_{10}$	105.1	70.1	10	#
	241.9	170.0	30	
	276.9	241.9	10	

Internal Standard or Labeled Internal Standard	Precursor ion *(m/z)*	Product ion *(m/z)*	Collision energy (eV)	Remark
Quintozene -$^{13}C_6$	148.0	113.0	10	
	219.9	185.0	20	
	254.9	220.0	15	#
p,p'-DDE-$^{13}C_{12}$	258.1	188.1	30	#
	260.0	188.1	30	
	328.0	258.1	20	
p,p'-TDE-$^{13}C_{12}$	211.1	175.1	30	
	247.0	177.1	25	#
	249.1	177.1	20	
o,p'-DDT-$^{13}C_{12}$	211.1	175.1	30	
	247.0	177.1	25	#
	249.1	177.1	25	
p,p'-DDT-$^{13}C_{12}$	211.1	175.1	30	
	247.1	177.1	20	#
	249.1	177.1	20	
cis-Chlordane-$^{13}C_{10}$	241.9	170.0	30	#
	382.9	276.0	20	
	384.9	276.0	20	
	384.9	311.0	20	
trans-Chlordane-$^{13}C_{10}$	241.9	145.9	30	
	382.9	276.0	20	#
	384.9	276.0	20	
Oxychlordane-$^{13}C_{10}$	120.0	55.1	20	
	154.0	125.0	5	#
	190.0	125.0	10	
Polychlorinated biphenyl congener #138-$^{13}C_{12}$	300.0	230.1	30	
	369.9	300.0	30	
	371.9	302.0	30	#

 # denotes MRM transition commonly used for quantification. Alternative MRM transitions may be used if background / matrix interference is encountered.

Limits – The amount of pesticide residues in CMM samples should comply with the limits listed in Table 5 below, unless in the case of a CMM of mineral origin or as otherwise specified.

Table 5 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