

# **Foderstoffer – Bestemmelse af OC-pesticider og PCB ved GC/MS**

Animal feeding stuffs – Determination of  
OC-pesticides and PCB's by GC/MS

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English Version

## Animal feeding stuffs - Determination of OC-pesticides and PCB's by GC/MS

Aliments des animaux - Détermination des pesticides organochlorés (OC) et des polychlorobiphényles (PCB) par GC/MS

Futtermittel - Bestimmung der OC-Pestizide und PCB's mittels GC/MS-Verfahren

This European Standard was approved by CEN on 24 January 2009.

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## Foreword

This document (EN 15741:2009) has been prepared by Technical Committee CEN/TC 327 "Animal feeding stuffs", the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2009, and conflicting national standards shall be withdrawn at the latest by August 2009.

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### 3 Terms and definitions

For the purpose of this document, the following terms and definitions apply.

#### 3.1

##### **limit of detection**

smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty

**NOTE** The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations ( $n > 10$ ).

#### 3.2

##### **limit of quantification**

lowest content of the analyte which can be measured with reasonable statistical certainty

**NOTE** If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to 6 times the standard deviation of the mean of blank determinations ( $n > 10$ ).

#### 3.3

##### **feed Additives**

substances are feed additives when they comply with the definition of feed additives given in the Regulation 1831/2003

### 4 Principle

A test portion of animal feeding stuff is fortified with internal standard (PCB 198), and is extracted with ethylacetate. The extract is concentrated and subsequently purified by:

- Gel permeation chromatography (GPC), with cyclohexane/ethylacetate as eluting solvent
- chromatography on partially deactivated silica gel.

The collected fraction containing the compounds of interest is concentrated and re-dissolved in a solution containing another internal standard (PCB 209) as a reference standard. After concentration an aliquot of the extract is injected into a GC-MS, using a splitless injector (an alternative here is PTV injection, see Note below).

**NOTE** In case more sensitivity is necessary or less volume reduction is wanted, injection of a larger volume by PTV is possible (an example is described in Annex B).

### 5 Reagents and materials

#### 5.1 General

Use only reagents of recognized analytical grade and with a purity suitable for OC and PCB residue analysis. Check the purity of the reagents by performing a blank test under the same conditions as used in the method. The chromatogram should not show any interfering impurity at the retention time of compounds of interest.

**WARNING** — The use of this European Standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

## 5.2 Ethylacetate

## 5.3 Cyclohexane

## 5.4 Ethylacetate/Cyclohexane = 1+1 parts by volume

Mix 500 ml of ethylacetate (5.2) with 500 ml of cyclohexane (5.3) and mix thoroughly. Store at room temperature in a tightly closed glass bottle.

## 5.5 Hexane

## 5.6 Decane

## 5.7 Hexane/Decane = 95+5 part by volume

Mix 950 ml of hexane (5.5) with 50 ml of decane (5.6) and mix thoroughly. Store at room temperature in a tightly closed glass bottle.

## 5.8 Iso-octane

## 5.9 Toluene

## 5.10 Silica gel, deactivated with 3,5% water

Heat silica gel 60 (63µm to 200µm = 70 mesh to 230 mesh), at 130°C for at least 5 h, allow to cool in a desiccator, and store in a tightly stopped container in the desiccator. To 96,5 g dried silica gel in a 300 ml Erlenmeyer flask with a ground joint, add 3,5 ml water dropwise from a burette, with continuous swirling. Immediately stopper the flask with a ground stopper and shake vigorously for 5 min until all lumps have disappeared. Next shake for 2 h on a mechanical shaker, and then store in a tightly stoppered container. Deactivated silica gel is tenable during approximately 2 weeks if carefully stored.

## 5.11 Hexane/toluene = 3+7 parts by volume

Mix 30 ml of n-hexane (5.5) with 70 ml of toluene (5.9) and mix thoroughly. Store at room temperature in a tightly closed glass bottle.

## 5.12 Internal standard (PCB 209)

### 5.12.1 PCB 209 Stock solution 1, 100 µg/ml

Weigh 5-10 mg ( $\pm 0,01$  mg) of PCB 209 (5.12) in a brown medicine glass bottle of 100 ml and add iso-octane (5.8) to achieve a concentration of 100 µg/ml. Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled. Or use a commercially available standard solution of 100 µg/ml.

### 5.12.2 PCB 209 Stock solution 2, 10,0 µg/ml

Dilute 10,0 ml of PCB 209 Stock solution 1 (5.12.1) to 100,0 ml with hexane (5.5). Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

### 5.12.3 PCB 209 Work solution, concentration 1 000 ng/ml

Dilute 10 ml of PCB 209 Stock solution 2 (5.12.2) to 100,0 ml with hexane (5.5). Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.



### 5.13 Internal standard (PCB 198)

#### 5.13.1 PCB 198 Stock solution 1, 100 µg/ml

Weigh 5-10 mg ( $\pm 0,01$  mg) of PCB 198 (5.13) in a brown medicine glass bottle of 100 ml and add iso-octane (5.8) to achieve a concentration of 100 µg/ml. Store the solutions in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solutions are tenable under these conditions during at least 5 years if the weight is carefully controlled. Or use a commercially available standard solution of 100 µg/ml.

#### 5.13.2 PCB 198 Stock solution 2, 5,0 µg/ml

Pipet 5,0 ml of PCB 198 Stock solution 1 (5.13.1) to graduated flask of 100,0 ml with hexane (5.5). Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

#### 5.13.3 PCB 198 Work solution, 1 000 ng/ml

Pipet 2,0 ml of the PCB 198 Stock solution 2 (5.13.2) into a 10,0 ml graduated flask and dilute with hexane (5.5) to 10,0 ml. Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

### 5.14 PCB congeners Stock standard solution, 10 µg/ml

PCB 28 (2,4,4' trichlorobiphenyl); CAS Number: 7012-37-5

PCB 52 (2,2',5,5' tetrachlorobiphenyl); CAS Number: 35693-99-3

PCB 101 (2,2',4,5,5' pentachlorobiphenyl); CAS Number: 37680-73-2

PCB 138 (2,2',3',4,4',5 hexachlorobiphenyl); CAS Number: 35065-28-2

PCB 153 (2,2',4,4',5,5' hexachlorobiphenyl); CAS Number: 35065-27-1

PCB 180 (2,2',3,4,4',5,5' heptachlorobiphenyl); CAS Number: 35065-29-3

Or a Certified Mixture at a concentration of 10 µg/ml.

### 5.15 PCB congeners Work standard solution, 2,0 µg/ml

Dilute 2,0 ml of PCB congeners Stock standard solution (5.14) to 10,0 ml with hexane (5.5). Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

### 5.16 OC-pesticide reference standards, as follows:

Each with a purity of not less than 99%.

Aldrin

((1R,4S,4aS,5S,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-imethanonaphthalene);  
CAS Number: 309-00-2

Dieldrin

((1R,4S,4aS,5R,6R,7S,8S,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7epoxy-1,4:5,8-dimethanonaphthalene)  
CAS Number: 60-57-1

## EN 15741:2009 (E)

### Chlordane

(1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-ethano-1*H*-indene);  $\alpha$  and  $\beta$  isomer;  
CAS Numbers: 5103-71-9 and 5103-74-2

### Oxychlordane

(4,7-Methanoindan, 1,2,4,5,6,7,8,8-octachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-, exo,endo-);  
CAS Number: 27304-13-8

### op'-DDT

[o,p'-(1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane)]  
CAS Number: 789-02-6

### pp'-DDT

[p,p'-(1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane)]  
CAS Number: 50-29-3

### pp'-TDE

(pp'-DDD) [p,p'-1,1-dichloro-2,2-bis(4-chlorophenyl) ethane]  
CAS Number: 72-54-8

### pp'-DDE

[p,p'-(1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene)]  
CAS Number: 72-55-9

### Endosulfan

(6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide);  
two stereoisomers,  $\alpha$ , (I), CAS Number: 959-98-8 and  $\beta$ , (II)  
CAS Number: 33213-65-9.

Endosulfan-sulphate; CAS Number: 1031-07-8

### Endrin

[(1*R*,4*S*,4a*S*,5*S*,6*S*,7*R*,8*R*,8a*R*)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene]  
CAS Number: 72-20-8

### Heptachlor

(1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene)  
CAS Number: 76-44-8

### $\beta$ -Heptachlorepoxyde

(1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene(exo))  
CAS Number: 1024-57-3

### HCB

(hexachlorobenzene)  
CAS Number: 118-74-1

### $\alpha$ -HCH ( $\alpha$ -BHC)

( $\alpha$ -1,2,3,4,5,6-hexachlorocyclohexane)  
CAS Number: 319-84-6

### $\beta$ -HCH ( $\beta$ -BHC)

( $\beta$ -1,2,3,4,5,6-hexachlorocyclohexane)  
CAS Number: 319-85-7

### $\gamma$ -HCH ( $\gamma$ -BHC; lindane)

( $\gamma$ -1,2,3,4,5,6-hexachlorocyclohexane)  
CAS Number: 58-89-9

Or a Certified Mixture at a concentration of 10 µg/ml.

### 5.17 Pesticide Stock solution 1, 100 µg/ml

Weigh 5-10 mg ( $\pm 0,01$  mg) of each individual pesticide (5.16) in separate brown medicine glass bottles of 100 ml and add iso-octane (5.8) to achieve a concentration of 100 µg/ml. Store the solutions in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solutions are tenable under these conditions during at least 5 years if the weight is carefully controlled. Or use a commercially available standard solution of 100 µg/ml.

NOTE Dissolve  $\beta$ -HCH in 10 ml toluene (5.9, to achieve complete solvability) and dilute further with iso-octane (5.8) to achieve a concentration of 100 µg/ml.

### 5.18 Pesticide Stock solution 2, 5,0 µg/ml

Mixture of all individual pesticide stock solutions 1 (5.17).

Pipet 5,0 ml of each individual pesticide stock solutions 1 (5.17) into one 100,0 ml graduated flask and dilute with hexane (5.5) to 100,0 ml. Store the solution in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

### 5.19 Calibration solutions

Prepare calibration mixtures according to Table 1 in a final volume of 10,0 ml hexane/decane = 95+5 (5.7) and store them at 4°C  $\pm 3^\circ\text{C}$ .

**Table 1 — Calibration mixtures**

| Level | PCB 2,0 µg/ml (5.15) |       | OC 5,0 µg/ml (5.18) |       | PCB 198 5,0 µg/ml (5.13.2) |       | PCB 209 10 µg/ml (5.12.2) |       |
|-------|----------------------|-------|---------------------|-------|----------------------------|-------|---------------------------|-------|
|       | µl                   | ng/ml | µl                  | ng/ml | µl                         | ng/ml | µl                        | ng/ml |
| 1     | 0                    | 0     | 0                   | 0     | 1 000                      | 500   | 500                       | 500   |
| 2     | 20                   | 4     | 20                  | 10    | 1 000                      | 500   | 500                       | 500   |
| 3     | 50                   | 10    | 50                  | 25    | 1 000                      | 500   | 500                       | 500   |
| 4     | 125                  | 25    | 250                 | 125   | 1 000                      | 500   | 500                       | 500   |
| 5     | 500                  | 100   | 1 000               | 500   | 1 000                      | 500   | 500                       | 500   |
| 6     | 1 250                | 250   | 2 500               | 1 250 | 1 000                      | 500   | 500                       | 500   |

### 5.20 Glass vial, 100 ml, with teflon-lined screwcaps

### 5.21 Glass wool

Heated at 160°C during at least 24 h.

### **5.22 Sodiumsulphate, anhydrous**

Heated at 160°C during at least 24 h.

### **5.23 Helium gas**

Purity 5,0 or better.

### **5.24 Nitrogen gas**

Purity 5,0 or better.

### **5.25 GC sampler vial, 2 ml**

### **5.26 Glass graduated evaporation tubes, 50 ml**

### **5.27 Chromatographic tubes, glass or teflon**

Chromatographic tube with solvent reservoir.

### **5.28 Autosampler vial, with limited volume insert**

### **5.29 Glass tubes, approximately 15 ml**

### **5.30 Glass tubes, approximately 4 ml**

## **6 Apparatus**

### **6.1 General**

All technical descriptions are examples of possible system setups and parameters and have to be scaled or adopted to the user's equipment.

### **6.2 Analytical balance, accuracy 0,01 mg**

### **6.3 Analytical balance, accuracy 10 mg**

### **6.4 Mechanical shaker**

### **6.5 The GPC cleanup system, consisting of an HPLC pump, an automatic injection system, a GPC-column and a fractioncollector**

Equilibrate the GPC-system under the recommended operating conditions and check the GPC column performance as subscribed in EPA method 3640 [3].

In case the recovery of  $\beta$ -HCH and  $\gamma$ -Chlordane in the GPC control with a standard solution is too low the start of the collection time of the OC/PCB fraction is too late. In case the recovery of HCB is too low the end time of the OC/PCB fraction is too early.

#### **6.5.1 HPLC-pump**

The HPLC pump shall be capable of maintaining a flow-rate of 1,0 ml/min Ethylacetate/Cyclohexane 1/1 (5.4).



### 6.5.2 Automated injection system

The automated injection system shall be capable of performing a series of unattended injections of a volume of 500 µl out of 2 ml GC sampler vial (5.28).

### 6.5.3 GPC-column

The GPC-column shall be capable of performing a separation as specified by criteria in EPA Method 3640 [3].

For example: length 45 cm, internal diameter 10 mm, stationary phase Bio Beads SX-3. The OC/PCB containing fraction elutes between 16 min - 26 min. (guideline, should be tested before use).

### 6.5.4 Fraction collector

## 6.6 Evaporation system, equipped with 50 ml graduated glass tubes and nitrogen gas, for example turbovab evaporator

## 6.7 Evaporation block, equipped with heater and nitrogen gas for example a Pierce evaporation apparatus

The evaporation block shall be able to contain 4 ml glass tubes (5.30).

## 6.8 Gaschromatograph-Mass spectrometer

### 6.8.1 General

The gaschromatograph shall be capable of working with capillary columns. The use of a capillary column coated with a mid-range polarity stationary phase (dimensions 30m x 0,25 mm, filmthickness 0,10 µm) is recommended. The column flow is kept constant at 1,3 ml/min.

The temperature program starts at an initial temperature of 80°C where it is kept for 3 min. After this the temperature is ramped with 5°C/min to a final temperature of 280°C where it is kept for 10 min. Finally, the GC is cooled to 80°C.

### 6.8.2 Mass-spectrometer

The mass-spectrometer shall be capable of monitoring in full scan mode or selected ion monitoring (SIM), and should be tuned according to the manufacturer's description.

#### 6.8.2.1 SIM mode operation

The selected mass fragments are shown in the Table 2: OC-pesticides and Table 3: PCBs. At least one quantifier ion and two qualifier ions have to be measured. Depending on the retention time of the components, SIM windows are composed, preferably with less than 20 fragments.

Table 2 — Selected ions for OC-Pesticides

| Compound                    | Fragment Quantifier [m/z] | Fragment Qualifier 1 [m/z] | Fragment Qualifier 2 [m/z] | Fragment Qualifier 3 [m/z] |
|-----------------------------|---------------------------|----------------------------|----------------------------|----------------------------|
| Aldrin                      | 263                       | 293                        | 66                         |                            |
| Dieldrin                    | 263                       | 277                        | 79                         |                            |
| $\alpha$ -Chlordane (cis)   | 373                       | 371                        | 375                        |                            |
| $\gamma$ -Chlordane (trans) | 373                       | 371                        | 375                        |                            |
| Oxychlordane                | 387                       | 389                        | 185                        |                            |
| o,p'-DDT                    | 235                       | 354                        | 237                        | 165                        |
| p,p'-DDT                    | 235                       | 354                        | 237                        | 165                        |
| p,p'-TDE                    | 235                       | 237                        | 165                        |                            |
| p,p'-DDE                    | 246                       | 318                        | 248                        |                            |
| $\alpha$ -Endosulfan        | 195                       | 241                        | 339                        |                            |
| $\beta$ -Endosulfan         | 195                       | 241                        | 339                        |                            |
| Endosulfan-sulphate         | 387                       | 385                        | 272                        |                            |
| Endrin                      | 263                       | 281                        | 81                         |                            |
| Heptachlor                  | 272                       | 274                        | 100                        |                            |
| Heptachlorepoxyde           | 351                       | 353                        | 81                         |                            |
| HCB                         | 284                       | 282                        | 249                        |                            |
| $\alpha$ -HCH               | 183                       | 219                        | 217                        |                            |
| $\beta$ -HCH                | 183                       | 219                        | 217                        |                            |
| $\gamma$ -HCH               | 219                       | 217                        | 183                        |                            |



**Table 3 — Selected ions for PCBs**

| Compound | Fragment Quantifier [m/z] | Fragment Qualifier 1 [m/z] | Fragment Qualifier 2 [m/z] |
|----------|---------------------------|----------------------------|----------------------------|
| PCB 28   | 256                       | 258                        | 186                        |
| PCB 52   | 292                       | 290                        | 220                        |
| PCB 101  | 326                       | 324                        | 254                        |
| PCB 153  | 360                       | 358                        | 290                        |
| PCB 138  | 360                       | 358                        | 290                        |
| PCB 180  | 394                       | 396                        | 324                        |
| PCB 198  | 430                       | 428                        |                            |
| PCB 209  | 498                       | 500                        |                            |

#### 6.8.2.2 Fullscan mode operation

The mass-range is scanned from 50 amu - 550 amu at three scans per second. The scan rate may vary but there have to be at least ten scans per peak.

## 7 Sampling

The sample should be truly representative and not been damaged or changed during transport or storage. Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in ISO 6497 [1].

## 8 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

Dry or low moisture products such as cereals and cereal products, mixed feeds, and hay should be ground carefully so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

## 9 Procedure

### 9.1 General

Analyse in each series the following samples:

- 1) Chemical blanc;
- 2) Blanc animal feed (n=1), blanc oil (n=1);
- 3) Blanc animal feed spiked (n=2), blanc oil spiked (n=2);
- 4) All samples.

NOTE Any blank feed sample proven to be blank in a previous run can be used for quality control.

## **9.2 Test portions of animal feed stuff and oil samples**

### **9.2.1 Test portion of 8 gram animal feed**

Weigh 8,0 g ( $\pm 0,10$  g) of the prepared test sample into a 100 ml glass vial. Fortify the sample with 800  $\mu$ l PCB 198 Work solution (5.13.3). (100 ng/g feed).

NOTE If fat content is higher than 10 wt% the amount of sample must be proportionally less.

### **9.2.2 Test portion 1 gram oil**

Weigh 1,0 g ( $\pm 0,10$  g) of the oil sample into a glass tube of 15 ml (5.29). Fortify the sample with 100  $\mu$ l PCB 198 Work solution (5.13.3). (100 ng/g oil).

Add 3,9 ml ethylacetate/cyclohexane (1:1 v/v) mixture (5.4) and vortex for 5 min.

## **9.3 Extraction**

Add 50 ml ethylacetate (5.2) to the sample, close the vial with a teflon-lined screwcap and shake it during minimal 18 h using a mechanical shaker (6.4). Filter the extract over anhydrous sodiumsulphate (5.22) (approximately 10 gr in a glassfunnel with some glasswool (5.21)). Measure 25 ml filtrate, bring into a graduated tube (5.29) and evaporate (40°C, N<sub>2</sub>) to a final volume of <1,0 ml. Make the volume up to 2,0 ml using cyclohexane/ethylacetate (5.4).

## **9.4 Cleanup**

### **9.4.1 Gel permeation chromatography clean-up**

#### **9.4.1.1 Preparation GPC system**

Equilibrate the GPC-system under the recommended operating conditions (6.5) and check the GPC column performance as subscribed in EPA method 3640 [3].

#### **9.4.1.2 Purification**

Inject 0,5 ml ( $\equiv$  1 g feed or 0,1 g oil) from the prepared samples (9.2.2 and 9.3) into the GPC-system. Collect the fraction eluting containing the compounds of interest (guideline: 16 and 26 min) directly into 15 ml evaporation tubes (5.29). The collected GPC fractions are concentrated in an evaporation system (40°C, N<sub>2</sub>, (6.7)) to a volume of approximately 0,5 ml. Transfer the extract into a 4 ml glass tube (5.30). Rinse the evaporation tube with 1 ml of hexane (5.5), and combine the solvent with the first fraction into the 4 ml glass tube.

### **9.4.2 Column chromatography on partially deactivated silica**

Pack the chromatographic tube (5.27) in the following order: glass wool plug (5.21), 4,0 g of deactivated silica gel (5.10), 5 mm to 10 mm layer of sodium sulfate (5.22), glass wool plug (5.21). Before use, rinse the column with 10 ml of n-hexane (5.5) and discard the eluate. As soon as the hexane has drained to the top of the silica gel, pipette the n-hexane solution derived from the evaporation of the sample solution (9.4.1.2) on to the pre-washed silica gel column. Elute 6 times with 3 ml eluant (n-hexane:toluene) (5.11). Collect eluate and concentrate in an evaporation system (40°C, N<sub>2</sub>, (6.7)) to a volume of approximately 0,5 ml. Transfer the extract into a 4 ml glass tube (5.30), and add 10  $\mu$ l decane (5.6). Rinse the evaporation tube (5.30) with 2 ml of hexane (5.5), and combine the solvent with the first fraction into the 4 ml glass tube. Evaporate the solvent gently in an evaporation block (40°C, N<sub>2</sub>, (6.7)) until only the decane remains. Add 100  $\mu$ l PCB 209 work solution (5.12.3) and 90  $\mu$ l hexane (5.5) to the residue, and mix thoroughly. Finally, transfer the extract into the autosampler vial, using a limited volume insert (5.28).

Low recoveries for endosulfan (alpha, beta) and/or dieldrin can be due to an insignificant elution. If so the number of eluting cycles shall be increased.

NOTE The final concentration is 5 g feed per ml. If necessary the final solution can be diluted with hexane (5.5).

## 9.5 Gas chromatography

### 9.5.1 Preparation of the system

Equilibrate gas chromatographic system under the recommended operating conditions (6.8).

Tune and Calibrate the MS system.

### 9.5.2 Checking Instrument settings

Inject calibration level 5 (5.19), and check peakshape and retention times for all compounds of interest. Modify the SIM window if necessary for start/ending time in the MS page of the instrument method.

### 9.5.3 Checking sensitivity of the system

Inject Calibration level 2 (5.19). All compounds of interest should be detectable with a signal-to-noise (s/n) ratio of six or higher. If not, appropriate action has to be taken, for example by cleaning the ion source (when a dirty ion source is present) or by retuning the MSD or by changing the analytical column (bad peak shape).

### 9.5.4 Determination

Inject 2 µl of the calibration standard solutions (5.19, 1 to 6) and an equal volume of the sample extracts, using a splitless injector.

Identify the individual pesticides/PCB peaks on basis of retention time and ion ratio.

Determine the amount of pesticides/PCB by comparing the size of the sample peaks with those of the known amount of the corresponding pesticide/PCB peaks in the calibration standard solutions (5.19, 1 to 6). Calibration is based on internal standard principle.

## 10 Calculation and expression of results

### 10.1 General

Calculations are performed using data acquisition software. Settings are as below:

Use a five-point internal standard calibration, force the intercept through zero, and calculate the correlation coefficient  $r^2$ .

Before processing all data, the retention times of all compounds of interest are checked and, if necessary, modified in the processing method.

After processing of the data, every result is manually checked for correct integration.

### 10.2 Calibration criteria

Criterion for Correlation coefficient:  $>0,995$ .

The results should fit within the calibration curve. When a result exceeds the thresholds of the calibration curve the sample should be diluted and reanalysed until it fits within the calibration curve.

### 10.3 Identification and confirmation

The compounds of interest are identified on retention time and mass ratio (SIM) or mass spectrum (full scan). The relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense ion, shall correspond to those of the calibration standard, either from calibration standard solutions or from spiked samples, at comparable concentrations, measured under the same conditions within the following tolerances (Table 4. Relative Intensities):

**Table 4 — Relative intensities**

| Relative intensity (% of base peak) | Tolerances |
|-------------------------------------|------------|
| > 50%                               | ± 10%      |
| > 20% to 50%                        | ± 15%      |
| > 10% to 20%                        | ± 20%      |
| < 10%                               | ± 50%      |

The minimum acceptable retention time of the OC or PCB under investigation is twice the retention time corresponding to the void volume of the column. The ratio of the chromatographic retention time of the analyte to that of the internal standard (PCB 198), the relative retention time, shall correspond to that of the calibration solution at a tolerance ± of 0,5%.

### 10.4 Calculation

For all calibration levels per component of interest the Relative Response Factor (RRF) is calculated.

For the pesticides and the PCBs this is done in relation with PCB 198.

For PCB 198 this is done in relation with PCB 209.

Components of interest:

$$RRF_{(n)} = \frac{A_x \times Q_{is}}{Q_x \times A_{is}} \quad (1)$$

Internal standards:

$$RRF_{(m)} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs}} \quad (2)$$

where

$A_x$  is the Area of the Quantifier Ion of component of interest;

$A_{is}$  is the Area of the Quantifier Ion of used internal standard (PCB 198);

$A_{rs}$  is the Area of the Quantifier Ion of PCB 209;

$Q_{is}$  is the Amount of internal standard PCB 198 ng/ml;



$Q_{rs}$  is the Amount of PCB 209 ng/ml;

$Q_x$  is the Amount of component of interest ng/ml.

Consequently the averaged relative response factor is calculated:

$$\overline{RRF}_{(n)} = \frac{1}{5} \times \sum_{i=2}^6 RRF_i(n) \quad (3)$$

where

$n$  is the Component of interest;

$i$  is the Calibration level (2 thru 6) (level 1 is blanc).

Consequently the averaged relative response factor is calculated for internal standard (PCB 198):

$$\overline{RRF}_{(m)} = \frac{1}{5} \times \sum_{i=2}^6 RRF_i(m) \quad (4)$$

where

$m$  is the Internal standard (PCB 198);

$i$  is the Calibration level (2 thru 6) (level 1 is blanc).

Calculation concentration component of interest.

The concentration component of interest is calculated by:

$$C_x = \frac{A_x \times Q_{is}}{A_{is} \times W \times \overline{RRF}_{(n)}} \quad (5)$$

where

$C_x$  is the Concentration component of interest in ng/g;

$A_x$  is the Area of the Quantifier Ion of component of interest in the sample extract;

$A_{is}$  is the Area of the Quantifier Ion of used internal standard (PCB 198) in the sample extract;

$Q_{is}$  is the Amount of internal standard (PCB 198) ng/ml;

$W$  is the Weight of injected sample amount equivalent g/ml.

## 10.5 Recovery

The recovery for the used internal standard (PCB 198) is calculated by:

$$\text{percentage recovery (\%)} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs} \times \overline{RRF}_{(m)}} \times 100 \quad (6)$$

where

$A_{is}$  is the Area of the Quantifier Ion of used internal standard (PCB 198) in the sample;

$A_{rs}$  is the Area of the Quantifier Ion of PCB 209 in the sample;

$Q_{is}$  is the Amount of internal standard (PCB 198) ng/ml;

$Q_{rs}$  is the Amount of PCB 209 ng/ml.

The collected data make it possible to check a series of recovery values.

- 1) The injection efficiency can be calculated from the area for PCB 209 in the sample extract compared to the average area of the calibration levels. The injection efficiency should be between 60% and 150%.
- 2) The recovery percentage of the internal standard PCB 198 in a sample can be calculated as follows: first, the relative area of PCB 198 is calculated by determining the ratio between the areas of PCB 198 and PCB 209 of the sample of interest. Then the ratio is calculated for PCB 198 and PCB 209 in a solvent standard. Finally, the calculated PCB ratio in the sample is divided by the PCB ratio in the solvent standard and multiplied by 100%.

NOTE During evaporation the more volatile compounds can be lost, easily recognised by the profile, leading to a low recovery for the lighter (and more volatile) compounds, and a higher recovery for the heavier (and less volatile) compounds.

- 3) The recovery percentage for the native PCBs and pesticides (trueness) can be calculated by comparing the calculated result for the fortified blanc sample with the theoretical value (fortified amount). The trueness should be between 80% and 110% for the PCB's and between 70% and 120% for pesticides (see decision 2002/657/EC [7]).

## 11 Precision

### 11.1 Interlaboratory test

An interlaboratory comparison was organized by RIKILT, Institute of Food Safety in the Netherlands. This international laboratory ring trial aimed at the determination of organochlorines and indicator PCBs in animal feed and oil (compounds of interest: Aldrin, Dieldrin, Chlordane, DDT, Endosulfan, Endrin, Heptachlor, Hexachlorobenzene, Hexachlorocyclohexane and PCB 28, 52, 101, 138, 153, 180). This paragraph describes the results of the interlaboratory comparison of 2007.

For this interlaboratory ring trial four cattle feed, two oil samples, a chicken feed, a pig feed and a fish meal sample were taken into account. Unfortunately, no samples were available which contain incurred residues of, for the scope of the method, representative analytes. Therefore the contaminated samples were artificially spiked on an appropriate level of 5-100 ng/g. For this interlaboratory 8 laboratories participate on GC-MS method. The relative standard deviation and the Horwitz coefficient of variation and the 'HorRat' were calculated [9]. The averages and standard deviation of each individual compound were calculated over participating laboratories using the GC-MS method.

### 11.2 Repeatability and precision within participating laboratories

The repeatability of all compound-matrix combinations is given as the average variation coefficient [%] which is calculated over the 8 individual participating laboratories, see Table 5.



Table 5 — Average coefficients of variation [%]

| GC-MS        | Average coefficient of variation [%] |       |       |       |       |       |       |       |       |
|--------------|--------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
|              | A                                    | B     | C     | D     | F     | G     | H     | Oil B | Oil S |
| PCB 28       | #####                                | ##### | 11    | 6     | ##### | ##### | ##### | ##### | 14    |
| PCB 52       | #####                                | ##### | ##### | ##### | ##### | 6     | 6     | ##### | 15    |
| PCB 101      | #####                                | ##### | 8     | 5     | ##### | 9     | 4     | ##### | 9     |
| PCB 138      | #####                                | ##### | 4     | 5     | 22    | 6     | 4     | ##### | 6     |
| PCB 153      | #####                                | ##### | ##### | ##### | 17    | ##### | ##### | ##### | 6     |
| PCB 180      | #####                                | ##### | 9     | 8     | ##### | 6     | 4     | ##### | 4     |
| Aldrin       | #####                                | 8     | ##### | 8     | ##### | 6     | 6     | ##### | 14    |
| Dieldrin     | #####                                | ##### | ##### | ##### | ##### | 7     | 9     | ##### | 6     |
| o,p'-DDT     | #####                                | 6     | ##### | 5     | ##### | 6     | 7     | ##### | ##### |
| p,p'-DDT     | #####                                | 10    | ##### | 7     | ##### | ##### | ##### | ##### | ##### |
| p,p'-TDE     | #####                                | 5     | ##### | 4     | ##### | ##### | ##### | ##### | ##### |
| p,p'-DDE     | #####                                | ##### | ##### | ##### | 22    | ##### | ##### | ##### | 6     |
| α-Endosulfan | #####                                | 8     | ##### | 10    | ##### | ##### | ##### | ##### | ##### |
| β-Endosulfan | #####                                | 3     | ##### | 11    | ##### | ##### | ##### | ##### | ##### |
| Endrin       | #####                                | ##### | ##### | ##### | ##### | 10    | 8     | ##### | ##### |
| HCB          | #####                                | 8     | ##### | 12    | ##### | 7     | 4     | ##### | ##### |
| α-HCH        | #####                                | ##### | ##### | ##### | ##### | ##### | ##### | ##### | 17    |
| β-HCH        | #####                                | ##### | ##### | ##### | ##### | ##### | ##### | ##### | 12    |
| γ-HCH        | #####                                | 7     | ##### | 9     | ##### | 7     | 10    | ##### | ##### |

### 11.3 Reproducibility and precision between participating laboratories

The reproducibility of all compound-matrix combinations is given as the average variation coefficient [%] which is calculated over all 8 participating laboratories. Secondly, these values are compared with the Horwitz coefficient of variation. Both values are listed in Table 6. The Horwitz coefficient of variation is given between brackets using the Horwitz formula  $2c^{(-0.1505)}$ .

Table 6 — Coefficient of variation and Horwitz variation at assigned concentrations

| GC-MS        | CV and (Horwitz CV at assigned concentration) |            |            |            |             |            |            |       |            |
|--------------|---|------------|------------|------------|-------------|------------|------------|-------|------------|
|              | A   | B          | C          | D          | F           | G          | H          | Oil B | Oil S      |
| PCB 28       | #####   | #####      | 59<br>(27) | 39<br>(24) | #####       | #####      | #####      | ##### | 37<br>(24) |
| PCB 52       | #####   | #####      | #####      | #####      | #####       | 52<br>(26) | 44<br>(24) | ##### | 38<br>(24) |
| PCB 101      | #####   | #####      | 35<br>(26) | 14<br>(23) | #####       | 19<br>(28) | 22<br>(25) | ##### | 26<br>(24) |
| PCB 138      | #####   | #####      | 36<br>(26) | 26<br>(24) | 77<br>(40)  | 21<br>(30) | 13<br>(27) | ##### | 40<br>(24) |
| PCB 153      | #####   | #####      | #####      | #####      | 87<br>(39)  | #####      | #####      | ##### | 33<br>(24) |
| PCB 180      | #####   | #####      | 32<br>(26) | 32<br>(24) | #####       | 70<br>(27) | 70<br>(25) | ##### | 47<br>(23) |
| Aldrin       | #####   | 50<br>(27) | #####      | 50<br>(24) | #####       | 34<br>(29) | 57<br>(26) | ##### | 46<br>(23) |
| Dieldrin     | #####   | #####      | #####      | #####      | #####       | 4<br>(28)  | 18<br>(26) | ##### | 35<br>(23) |
| o,p'-DDT     | #####   | 75<br>(25) | #####      | 55<br>(22) | #####       | 56<br>(29) | 60<br>(26) | ##### | #####      |
| p,p'-DDT     | #####   | 50<br>(25) | #####      | 26<br>(23) | #####       | #####      | #####      | ##### | #####      |
| p,p'-TDE     | #####   | 69<br>(25) | #####      | 46<br>(23) | #####       | #####      | #####      | ##### | #####      |
| p,p'-DDE     | #####   | #####      | #####      | #####      | 21<br>(38)  | #####      | #####      | ##### | 23<br>(23) |
| α-Endosulfan | #####   | 41<br>(27) | #####      | 47<br>(24) | #####       | #####      | #####      | ##### | #####      |
| β-Endosulfan | #####   | 19<br>(26) | #####      | 31<br>(24) | #####       | #####      | #####      | ##### | #####      |
| Endrin       | #####   | #####      | #####      | #####      | #####       | 37<br>(28) | 51<br>(25) | ##### | #####      |
| HCB          | #####   | 81<br>(28) | #####      | 81<br>(25) | 70<br>(45)) | 75<br>(30) | 90<br>(27) | ##### | #####      |
| α-HCH        | #####   | #####      | #####      | #####      | #####       | #####      | #####      | ##### | 62<br>(24) |
| β-HCH        | #####   | #####      | #####      | #####      | #####       | #####      | #####      | ##### | 30<br>(22) |
| γ-HCH        | #####   | 10<br>(27) | #####      | 51<br>(24) | #####       | 70<br>(29) | 64<br>(26) | ##### | #####      |

## 12 Test report

In the test report the following data is reported:

- 1 Information about the animal feed samples and the oil sample used for this ring trial;
- 2 Spiking levels of all compound/matrix combinations;
- 3 Statistical calculation on suspected values and outliers;
- 4 Z-score information;
- 5 Assigned values and 95% confidence interval;

- 6 Calculations on coefficient of variations;
- 7 Horwitz coefficient of variations;
- 8 “HorRat” coefficient;
- 9 Accuracy.

## **13 Important considerations**

### **13.1 Consideration 1**

It is possible to use alternative extraction techniques (e.g. accelerated solvent extraction ASE). The suitability shall be proven.

### **13.2 Consideration 2**

It is possible to use different amounts of silica with different deactivation status. The suitability shall be proven.

## Annex A

(informative)

### Results of interlaboratory tests

Table A.1 — Accuracy of the GC-MS method based on spiked concentration in the samples

| MS                  | Accuracy – GC-MS |    |     |     |     |       |
|---------------------|------------------|----|-----|-----|-----|-------|
|                     | B                | C  | D   | G   | H   | Oil S |
| PCB 28              |                  | 56 | 62  |     |     | 92    |
| PCB 52              |                  |    |     | 96  | 93  | 92    |
| PCB 101             |                  | 78 | 85  | 102 | 101 | 99    |
| PCB 138             |                  | 83 | 84  | 105 | 107 | 91    |
| PCB 153             |                  |    |     |     |     | 92    |
| PCB 180             |                  | 92 | 92  | 111 | 111 | 97    |
| Aldrin              | 64               |    | 69  | 75  | 76  | 73    |
| Dieldrin            |                  |    |     | 93  | 80  | 68    |
| o,p'-DDT            | 113              |    | 112 | 85  | 85  |       |
| p,p'-DDT            | 106              |    | 102 |     |     |       |
| p,p'-TDE            | 104              |    | 100 |     |     |       |
| p,p'-DDE            |                  |    |     |     |     | 81    |
| α-Endosulfan        | 57               |    | 72  |     |     |       |
| β-Endosulfan        | 77               |    | 68  |     |     |       |
| Endosulfan-sulphate |                  |    |     |     |     |       |
| Endrin              |                  |    |     | 109 | 121 |       |
| HCB                 | 50               |    | 52  | 66  | 63  |       |
| α-HCH               |                  |    |     |     |     | 81    |
| β-HCH               |                  |    |     |     |     | 132   |
| γ-HCH               | 70               |    | 70  | 87  | 75  |       |

## Annex B

### Description of PTV injection system (in case this is available).

#### B.1 Sample preparation procedure

The sample preparation is similar to the preparation described in Clause 9 of the method. The difference is the end volume of the extract that will be injected. Instead of the 2 µl injected into a splitless injector, the example described below is for 50 µl LV PTV (large volume PTV). To achieve the same sensitivity compared to splitless injection a 25 fold larger end volume can be used (this would be 5 ml instead of 200 µl used when injecting splitless). Achieving more sensitivity can be accomplished through evaporating more solvent.

#### B.2 PTV injection conditions using 50 µl injection (extracts are dissolved in Hexane/Decane (95/5 (5.6))).

Initial temperature (Base)(°C): 80°C

Mode: PTV Large Volume

Splitflow (ml/min): 100

Splitless time (min): 2

Solvent Valve temp. (°C): 150

Surge Pressure (kPa): 150

Surge duration (min): 1

Evaporation phase: on

Cleaning phase: on

Ramped pressure: off

Backflush: off

Injection time (min): 0,2 (depending on injection speed; in this example 5 µl/ sec).

Vent flow (ml/min): 50

Evaporation rate (°C/sec): 10

Evaporation temp (°C): 80

Evaporation time (min): 0,8

Transfer rate (°C/sec): 10

Transfer temp (°C): 280

Transfer time (min): 30

Clean rate (°C/sec): 10

Clean temp (°C): 340

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|                      |  |
|----------------------|--|
| Clean time (min):    | 2 (this stage is positioned after the components of interest have eluted from the column). |
| Clean flow (ml/min): | 100  |



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