

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

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

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English Version

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

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

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

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

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## Foreword

This document (EN 15742:2009) 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of an identical text or by endorsement, at the latest by August 2009, and conflicting national standards shall be withdrawn at the latest by August 2009.

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## 1 Scope

This European Standard specifies a gas chromatographic method with electron capture detection (ECD) for the determination of organochlorine pesticides (OC's) and polychlorinated biphenyls (PCBs) in animal feeding stuffs.

The method is applicable to animal feeding stuffs with a water content up to about 20 wt% and oil/fatty samples containing residues of one or more of the following OC's, PCBs, toxaphene and some of their isomers and degradation products:

- Aldrin;
- Dieldrin;
- Chlorocamphene (Toxaphene);
- Chlordane (= sum of Chlordane isomers and Oxychlordane);
- DDT (= sum of isomers *op'*-DDT, *pp'*-DDT, *pp'*-TDE (*pp'*-DDD), and *pp'*-DDE);
- Endosulfan (sum of  $\alpha$ -/ $\beta$ -isomers and Endosulfan-sulphate);
- Endrin;
- Heptachlor (= sum of Heptachlor and  $\beta$ -Heptachlorepoxyde);
- Hexachlorobenzene (HCB);
- Hexachlorocyclohexane isomers  $\alpha$ -HCH ( $\alpha$ -BHC),  $\beta$ -HCH ( $\beta$ -BHC),  $\gamma$ -HCH ( $\gamma$ -BHC or lindane);
- PCB 28, 52, 101, 138, 153 and 180 ("Indicator PCBs") and PCB 198, 209.

The limit of quantification for the mentioned organochlorine pesticides and PCBs is 5 ng/g in general. However, 10 ng/g applies for Heptachlor, Aldrin, Endrin, Dieldrin, and Endosulfan ( $\alpha$ -,  $\beta$ - and sulphate). Individual laboratories are responsible to ensure that the equipment they used will achieve these limits of quantifications.

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6498, *Animal feeding stuffs – Preparation of test samples*

## 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 cleanup the analytes are measured using GC-ECD. Identification is done on the basis of comparing retention times on capillary columns of different polarity. Quantification is done using the internal standard method.

## 5 Reagents and materials

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.**

### 5.1 Cyclohexane

### 5.2 Ethylacetate

### 5.3 Hexane

### 5.4 Dichloromethane

### 5.5 Iso-octane

### 5.6 Toluene

### 5.7 Hexane/toluene = 3+7, parts by volume

Mix 30 ml of hexan (5.3) with 70 ml of toluene (5.6) thoroughly. Store at room temperature in a tightly closed glass bottle.

### 5.8 Sodium Sulphate, anhydrous

Heated at 160°C during at least 24 h.

### 5.9 Ethylacetate/Cyclohexane = 1+1, parts by volume

Mix 500 ml of ethylacetate (5.2) with 500 ml of cyclohexane (5.1) thoroughly. Store at room temperature in a tightly closed glass bottle.

### 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 Internal standard (PCB 198)

### 5.12 Internal Standard (PCB 209)

### 5.13 OC-pesticide reference standards

Each with a purity 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-dimethanonaphthalene)  
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

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

(p,p'-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

[(1R,4S,4aS,5S,6S,7R,8R,8aR)-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)

(1,2,3,4,5,6-hexachlorocyclohexane)

CAS Number: 58-89-9

Or a Certified Mixture at a concentration of 10  $\mu$ g/ml.

#### 5.14 PCBs reference standards

Each with a purity not less than 99%.

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 Chlorocamphene (Toxaphene)**

Technical mixture.

### **5.16 Stock solutions, 100 µg/ml**

Weigh 5 -10 mg ( $\pm 0,01$  mg) of each compound (5.11, 5.12, 5.13, 5.14 and 5.15) in separate brown medicine glass bottles of 100 ml and add iso-octane (5.5) to achieve a concentration of 100 µg/ml. Store the solutions 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.

Dissolve  $\beta$ -HCH in 10 ml toluene (5.6), to achieve complete solvability and dilute further with iso-octane (5.5) to achieve a concentration of 100 µg/ml.

### **5.17 Mixed stock solutions**

#### **5.17.1 Mixed stock solution OC (without Endosulfan and Toxaphene)**

Pipet of each OC-stock solution (5.16) the indicated volume (Table 1) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. The achieved concentration is given in Table 1. Transport this solution to a brown medicine glass bottle of 100 ml and store it 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.

#### **5.17.2 Mixed stock solution Endosulfan**

Pipet of each Endosulfan-stock solution (5.16) the indicated volume (Table 1) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. The achieved concentration is given in Table 1. Transport this solution to a brown medicine glass bottle of 100 ml and store it 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.

**Table 1 — Concentration of OCs in Mixed stock solution (5.17) and mixed standard solution (5.18)**

Compound	Pipet volume (ml)	Mixed stock solution (5.17 1&2) (µg/ml)	Mixed standard solution (5.18.1 1&2) (µg/ml)
Aldrin	2,0	2,0	0,10
Dieldrin	2,0	2,0	0,10
α-Chlordane	1,0	1,0	0,05
γ-Chlordane	1,0	1,0	0,05
Oxychlordane	1,0	1,0	0,05
o,p'-DDT	4,0	4,0	0,20
p,p'-DDT	4,0	4,0	0,20
p,p'-TDE	4,0	4,0	0,20
p,p'-DDE	4,0	4,0	0,20
α-Endosulfan	2,0	2,0	0,10
β-Endosulfan	2,0	2,0	0,10
Endosulfan-sulphate	1,0	1,0	0,05
Endrin	1,0	1,0	0,05
Heptachlor	2,0	2,0	0,10
Heptachlor epoxide	2,0	2,0	0,10
HCB	1,0	1,0	0,05
α-HCH	2,0	2,0	0,10
β-HCH	1,0	1,0	0,05
γ-HCH (Lindane)	1,0	1,0	0,05

**5.17.3 Mixed stock solution Toxaphene, 10,0 µg/ml**

Pipet 10,0 ml of the Toxaphene-stock solution (5.16) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.17.4 Mixed stock solution PCBs, 1,0 µg/ml**

Pipet 1,0 ml of each stock solution PCBs (5.16) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.17.5 Internal standard stock solution PCB 198, 2,0 µg/ml**

Pipet 2,0 ml of the PCB 198-stock solution (5.16) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.17.6 Internal standard stock solution PCB 209, 2,0 µg/ml**

Pipet 2,0 ml of the PCB 209-stock solution (5.16) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.18 Mixed standard solution**

**5.18.1 Mixed standard solution OCs**

Pipet 5,0 ml of the mixed stock solution OCs (5.17.1) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. The achieved concentration is given in Table 1. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.18.2 Mixed standard solution Endosulfan**

Pipet 5,0 ml of the mixed stock solution Endosulfan (5.17.2) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. The achieved concentration is given in Table 1. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.18.3 Mixed standard solution Toxaphene, 0,5 µg/ml**

Pipet 5,0 ml of the mixed stock solution Toxaphene (5.17.3) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

**5.18.4 Mixed standard solution PCBs, 0,05 µg/ml**

Pipet 5,0 ml of the mixed stock solution PCBs (5.17.4) in a volumetric flask of 100 ml. Fill up to 100 ml with iso-octane (5.5) and mix. Transport this solution to a brown medicine glass bottle of 100 ml and store it in a refrigerator at 4°C (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

## 5.19 Internal standard solutions

### 5.19.1 PCB 198, 0,1 µg/ml

Pipet 5,0 ml PCB 198 standard solution (5.17.5) in a volumetric flask of 100 ml. Fill up with ethylacetat/cyclohexane (5.9) and mix. Transport this solution to a brown medicine glass bottles of 100 ml and store it in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years.

### 5.19.2 PCB 209, 0,1 µg/ml

Pipet 5,0 ml PCB 209 standard solution (5.17.6) in a volumetric flask of 100 ml. Fill up with ethylacetat/cyclohexane (5.9) and mix. Transport this solution to a brown medicine glass bottles of 100 ml and store it in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 5 years.

## 5.20 GC-standard solutions

### 5.20.1 GC-standard OCs, Endosulfan, Toxaphene and PCB

Prepare calibration mixtures according to Table 2 for OCs, Endosulfan, Toxaphene and PCB each in a final volume of 50,0 ml Ethylacetate/cyclohexane (5.9). Store in a refrigerator at 4°C ( $\pm 3^\circ\text{C}$ ). The solution is tenable under these conditions during at least 1 year.

**Table 2 — Calibration mixtures for OCs, Endosulfan, Toxaphene and PCB, each in a final volume of 50,0 ml Ethylacetate/cyclohexane**

Level	PCB 198 (5.19.1)	PCB 209 (5.19.2)	Pipet from OC (5.18.1) or Endosulfan (5.18.2) or Toxaphene (5.18.3) or PCB (5.18.4) solution
	[ml]	[ml]	[ml]
1	2,50	2,50	0
2	2,50	2,50	0,50
3	2,50	2,50	1,00
4	2,50	2,50	2,00
5	2,50	2,50	4,00

### 5.21 Blanc animal feeding stuff

The sample should be free of residues of OCs and PCBs and interfering impurities.

### 5.22 Glass wool



## 6 Apparatus

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

### 6.1 Analytical balance, accuracy 0,01 mg

### 6.2 Analytical balance, accuracy 10 mg

### 6.3 Polypropylene tubes, 50 ml

### 6.4 Mechanical shaker

### 6.5 Evaporation system, equipped with 10 ml graduated glass tubes and nitrogen gas, for example a turbovab evaporator

### 6.6 GPC cleanup system: HPLC pump, an automatic injection system, a GPC-column and a fraction collector

Equilibrate the GPC-system (6.6) 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.

#### 6.6.1 HPLC-pump

The HPLC pump shall be capable of maintaining a flow-rate of 1,0 ml/min Ethylacetate/Cyclohexane = 1+1, parts by volume (5.9).

#### 6.6.2 Automated injection system

The automated injectionsystem shall be capable of performing a series of unattended injections of a volume of 500  $\mu$ l.

#### 6.6.3 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).

#### 6.6.4 Fraction collector

### 6.7 GC-ECD

The GC-ECD consists of an automatic injection system, a gaschromatograph equipped with a splitless injector, a capillary column, an electron capture detector (ECD), and a computer with appropriate software.

The total chromatographic system should be adjusted and optimised according to the manufacturers instructions.

#### **6.7.1 Automated Injection System**

The autosampler shall be capable of injecting 2 µl.

#### **6.7.2 Gaschromatograph**

The gaschromatograph shall be capable of working with capillary columns. The use of two capillary columns coated with non-polarity and a mid-range polarity stationary phase (dimensions: 25 - 30m x 0,20 – 0,40 mm, filmthickness 0,10 µm – 0,40 µm) is recommended. The column flow is kept constant at 1,0 – 2,0 ml/min.

The injector temperature is 220°C. Injection volume 2 µl splitless.

The oven temperature program starts at an initial temperature of 80°C where it is kept for 1 min. After this the temperature is ramped with 5°C/min to a final temperature of 250°C where it is kept for 40 min. Finally, the GC is cooled to 80°C.

### **6.8 Nitrogen gas**

Purity 5,0 or better.

### **6.9 Helium gas**

Purity 5,0 or better.

### **6.10 Centrifuge**

## **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, oilseeds and oilseed meals, 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) If blank materials is available: animal feed (n=1), blank oil (n=1).
- 3) Blank animal feed spiked with organochlorine pesticides (n=2), blank oil spiked with organochlorine pesticides (n=2).
- 4) Blank animal feed spiked with Endosulfan (n=2), blank oil spiked with Endosulfan (n=2).
- 5) Blank animal feed spiked with Toxaphene (n=2), blank oil spiked with Toxaphene (n=2).
- 6) Blank animal feed spiked with PCBs (n=2), blank oil spiked with PCBs (n=2).
- 7) All samples.

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

## **9.2 Extraction**

### **9.2.1 Animal feed**

#### **9.2.1.1 Spiking**

Weigh 8,0 g ( $\pm$  0,10 g) of the blank animal feed (in 9-fold) (5.21) in a 50 ml tube polypropylene tube (6.3) for spiking. Add to two blank animal feed samples 100  $\mu$ l mixed stock solution OC (5.17.1), to two blank animal feed samples 100  $\mu$ l mixed stock solution Endosulfan (5.17.2), to two blank animal feed samples 100  $\mu$ l mixed stock solution Toxaphene (5.17.3), and to two blank animal feed samples 100  $\mu$ l mixed stock solution PCB (5.17.4). Let the solvent evaporate.

#### **9.2.1.2 Samples**

Weigh 8,0 g ( $\pm$  0,10 g) of each sample.

#### **9.2.1.3 Extraction**

Add 15 g sodium sulphate (5.8) to all samples and mix thoroughly. Fortify the sample with 400  $\mu$ l internal standard stock solution PCB 198 (5.17.5) = 100 ng/g. Add 25 ml ethylacetate (5.2) and extract during 18 h using a mechanical shaker (6.4). Centrifuge (6.10) during 10 min at 3 000 rpm. Take 10,0 ml of the upper solvent layer and evaporate (6.5) in a graduated tube to 2,0 ml.

### **9.2.2 Oil samples**

Weigh 1,0 g ( $\pm$  0,10 g) of the oil sample into a glass tube of 15 ml. Add 1 000  $\mu$ l internal standard PCB 198 (5.19.1) = 100 ng/g oil. Add 3,00 ml ethylacetate/cyclohexane = 1+1, parts by volume mixture (5.9) and vortex for 5 min.

## **9.3 Clean-up procedure**

### **9.3.1 Gel permeation chromatography clean-up (Remark 1)**

Inject 0,5 ml ( $\equiv$  0,8 g feed or 0,1 g oil) from the prepared samples (9.2) into the GPC-system (6.6). Collect the fraction eluting containing the compounds of interest (guideline: 16 and 26 min). The collected GPC fractions are concentrated in an evaporation system (40°C, N<sub>2</sub>) to a volume of approximately 0,5 ml ( $\equiv$  0,8 g feed or 0,1 g oil). Transfer the extract into a 4 ml glass tube and rinse the evaporation tube with 1 ml of hexane (5.3), and combine the solvent with the first fraction into the 4 ml glass tube.

### 9.3.2 Column chromatography on partially deactivated Silica

Pack the chromatographic tube in the following order: glass wool plug (5.22), 4,0 g of deactivated silica gel (5.10), 5 mm to 10 mm layer of sodium sulfate (5.8), glass wool plug (5.22). Before use, rinse the column with 10 ml of hexane (5.3) 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 (9.3.1) on to the pre-washed silica gel column. Elute 6 times with 3 ml eluant (n-hexane:toluene 3:7). Collect eluate and concentrate in an evaporation system (40°C, N<sub>2</sub>) to a volume of approximately 0,5 ml. Add 0,5 ml internal standard PCB 209 (5.19.2) and adjust volume to 1,0 ml with ethylacetate/cyclohexane = 1+1, parts by volume (5.9). Inject 2 µl onto GC-ECD.

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 If the final solution is too concentrated it can be diluted with ethylacetate/cyclohexane.

## 9.4 Gas chromatography

### 9.4.1 Preparation of the system

Equilibrate the gas chromatographic system under the recommended operating conditions (6.7).

### 9.4.2 Determination on GC column 1 (mid range-polarity)

Stabilize the system before use with two injections of the OC GC standard solution (5.20.1 -level 3). Inject further two injections each of the OC (5.20.1 level 1-5), Endosulfan (5.20.1 level 1-5), Toxaphene (5.20.1 level 1-5) and PCB GC standard solution (5.20.1 level 1-5).

Analyse all samples, Inject after each 10 samples the OC (5.20.1 level 3), Endosulfan (5.20.1 level 3), Toxaphene (5.20.1 level 3) and PCB GC standard solution (5.20.1 level 3).

### 9.4.3 Determination on GC column 2 (non-polarity) for confirmation compounds

Stabilize the system before use with two injections of the OC GC standard solution (5.20.1 level 3). Inject further two injections each of the OC (5.20.1 level 1-5), Endosulfan 5.20.1 level 1-5), Toxaphene (5.20.1 level 1-5) and PCB GC standard solution (5.20.1 level 1-5).

Analyse all samples, Inject after each 10 samples the OC (5.20.1 level 3), Endosulfan (5.20.1 level 3), Toxaphene (5.20.1 level 3) and PCB GC standard solution (5.20.1 level 3).

Limitations in the degree of confirmation should be acknowledged when reporting the results.

## 10 Calculation and expression of results

### 10.1 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.



## 10.2 Identification and confirmation

The compounds of interest are identified on two retention times (two capillary GC columns with different stationary phases).

## 10.3 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.

Concentration of all compounds shall be calculated for both GC columns. The concentration based on signal without co-eluting compounds is dominant. 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 component of interest;

$A_{is}$  is the area internal standard PCB 198;

$A_{rs}$  is the area 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:

$$\overline{RRF_{(n)}} = \frac{1}{4} \times \sum_{i=2}^5 RRF_i(n) \quad (3)$$

where

$n$  is the component of interest;



i is the calibration level (2 thru 5).

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

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

where

m is the internal standard (PCB 198);

i is the calibration level (2 thru 5);

Calculation concentration component of interest

The concentration component of interest is calculated by:

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

where

C<sub>x</sub> is the concentration component of interest in ng/g;

A<sub>x</sub> is the area of component of interest in the sample extract;

A<sub>is</sub> is the area of used internal standard (PCB 198) in the sample extract;

Q<sub>is</sub> is the amount of internal standard (PCB 198) ng/ml;

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

#### 10.4 Recovery

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

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

where

A<sub>is</sub> is the area of internal standard (PCB 198) in the sample;

A<sub>rs</sub> is the area of of PCB 209 in the sample;

Q<sub>is</sub> is the amount of internal standard (PCB 198) ng/ml;

Q<sub>rs</sub> 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 for the internal standards PCB 198 can be calculated from the, against PCB 209 corrected area, of the PCB 198 compared to the, against PCB 209 corrected average area in the calibration standard. An acceptable value for this recovery should be between 40% and 150%.

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 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]).

## 10.5 Identification and confirmation

The minimum acceptable retention time of the OC or PCB under investigation should be twice the retention time corresponding to the void volume of the column. The ratio of the chromatographic retention time of the analyt to that of the internal standard (PCB 198), the relative retention time, shall correspondent to that of the calibration solution at a tolerance  $\pm$  of 0,5%.

## 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 10 laboratories participate on GC-ECD 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-ECD 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 10 individual participating laboratories.

Table 3 — Average coefficients of variation [%]

GC-ECD	Average coefficient of variation [%]								
	A	B	C	D	F	G	H	Oil B	Oil S
PCB 28	#####	#####	6	8	#####	#####	#####	#####	5
PCB 52	#####	#####	#####	#####	#####	6	6	#####	11
PCB 101	#####	#####	8	6	#####	6	4	#####	8
PCB 138	#####	#####	6	6	18	6	4	#####	5
PCB 153	#####	#####	#####	#####	8	#####	#####	#####	4
PCB 180	#####	#####	6	5	#####	5	3	#####	5
Aldrin	#####	8	#####	5	#####	7	5	#####	6
Dieldrin	#####	#####	#####	#####	#####	8	7	#####	8
o,p'-DDT	#####	11	#####	4	#####	10	7	#####	#####
p,p'-DDT	#####	10	#####	7	#####	#####	#####	#####	#####
p,p'-TDE	#####	8	#####	6	#####	#####	#####	#####	#####
p,p'-DDE	#####	#####	#####	#####	0	#####	#####	#####	5
α-Endosulfan	#####	6	#####	6	#####	#####	#####	#####	#####
β-Endosulfan	#####	7	#####	15	#####	#####	#####	#####	#####
Endrin	#####	#####	#####	#####	#####	8	13	#####	#####
Heptachlor	#####	#####	#####	#####	#####	#####	#####	#####	#####
HCB	#####	7	#####	7	#####	9	6	#####	#####
α-HCH	#####	#####	#####	#####	#####	#####	#####	#####	6
β-HCH	#####	#####	#####	#####	#####	#####	#####	#####	6
γ-HCH	#####	8	#####	5	#####	6	5	#####	#####

### 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 10 participating laboratories. Secondly these values are compared with the Horwitz coefficient of variation. Both values are listed in the table below. The Horwitz coefficient of variation is given between brackets using the Horwitz formula  $2c^{(-0.1505)}$ .

Table 4 — Coefficient of variation and Horwitz variation at assigned concentrations

GC-ECD	Coefficient of variation and (Horwitz CV at assigned concentration)								
	A	B	C	D	F	G	H	Oil B	Oil S
PCB 28	#####	#####	39 (27)	39 (24)	#####	#####	#####	#####	49 (23)
PCB 52	#####	#####	#####	#####	#####	48 (27)	43 (24)	#####	38 (23)
PCB 101	#####	#####	46 (26)	40 (24)	#####	58 (28)	31 (26)	#####	34 (23)
PCB 138	#####	#####	39 (26)	47 (24)	51 (36)	54 (30)	37 (27)	#####	33 (23)
PCB 153	#####	#####	#####	#####	48 (40)	#####	#####	#####	34 (24)
PCB 180	#####	#####	40 (27)	49 (24)	#####	34 (28)	38 (25)	#####	60 (23)
Aldrin	#####	37 (26)	#####	40 (24)	#####	69 (29)	36 (26)	#####	52 (24)
Dieldrin	#####	#####	#####	#####	#####	66 (29)	58 (26)	#####	53 (23)
o,p'-DDT	#####	42 (25)	#####	24 (23)	#####	73 (28)	57 (26)	#####	#####
p,p'-DDT	#####	41 (25)	#####	56 (23)	#####	#####	#####	#####	#####
p,p'-TDE	#####	60 (25)	#####	56 (22)	#####	#####	#####	#####	#####
p,p'-DDE	#####	#####	#####	#####	0 (38)	#####	#####	#####	36 (23)
α-Endosulfan	#####	32 (26)	#####	33 (23)	#####	#####	#####	#####	#####
β-Endosulfan	#####	45 (26)	#####	59 (24)	#####	#####	#####	#####	#####
Endrin	#####	#####	#####	#####	#####	34 (29)	43 (26)	#####	#####
Heptachlor	#####	#####	#####	#####	#####	#####	#####	#####	#####
HCB	#####	52 (27)	#####	60 (24)	#####	53 (29)	45 (26)	#####	#####
α-HCH	#####	#####	#####	#####	#####	#####	#####	#####	52 (25)
β-HCH	#####	#####	#####	#####	#####	#####	#####	#####	76 (23)
γ-HCH	#####	49 (26)	#####	56 (23)	)	61 (29)	45 (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-ECD method based on spiked concentration in the samples.**

MS   ECD	Accuracy – GC-ECD					
	B	C	D	G	H	Oil S
PCB 28		62	58			97
PCB 52				93	90	108
PCB 101		79	79	102	91	97
PCB 138		82	79	113	109	100
PCB 153						92
PCB 180		90	89	102	100	102
Aldrin	73		75	82	80	67
Dieldrin				77	78	66
o,p'-DDT	105		92	91	93	
p,p'-DDT	105		104			
p,p'-TDE	105		107			
p,p'-DDE						92
α-Endosulfan	84		88			
β-Endosulfan	72		70			
Endosulfan-sulphate						
Endrin				89	85	
HCB	64		64	77	78	
α-HCH						71
β-HCH						110
γ-HCH	77		81	84	91	

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**Egne notater/Notes:**