
Krma: metode vzorčenja in analize - Določevanje OCP in PCB z GC/MS

Animal feeding stuffs: Methods of sampling and analysis - Determination of OCPs and PCBs by GC/MS

Futtermittel: Probenahme- und Untersuchungsverfahren - Bestimmung von OCP und PCB mittels GC/MS

Aliments des animaux : Méthodes d'échantillonnage et d'analyse - Détermination des pesticides organochlorés (POC) et des polychlorobiphényles (PCB) par GC-MS

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**Animal feeding stuffs: Methods of sampling and analysis -
Determination of OCPs and PCBs by GC/MS**

Futtermittel: Probenahme- und
Untersuchungsverfahren

Aliments des animaux: Méthodes d'échantillonnage et
d'analyse

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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prEN 15741:2018 (E)**European foreword**

This document (prEN 15741:2018) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, the secretariat of which is held by NEN.

This document is currently submitted to the CEN Enquiry.

This document will supersede EN 15741:2009.

In comparison with the previous edition, the following technical modifications have been made:

Regarding non dioxin-like PCBs (ndl-PCBs), this document contains two approaches that can be followed. Method 1 concerns the original extraction and clean-up methods of the previous edition of this standard, but combined with more sensitive detection approaches. In method 2, the extraction and clean-up methods have been modified in order to increase the test portion. The detection of method 2 concerns the original detection method of the previous edition of this standard.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

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Introduction

WARNING — The use of this document 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.

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1 Scope

This document specifies a gas chromatographic mass spectrometric (GC-MS) method for the determination of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in animal feeding stuffs and oil.

The method is applicable to animal feeding stuffs consisting of less than 20 % by mass and oil/fatty samples containing residues of one or more of the following OCPs and PCBs and some of their isomers and degradation products:

- aldrin;
- dieldrin;
- chlordane, as the sum of chlordane isomers and oxychlordane;
- dichlorodiphenyltrichloroethane (DDT), as the sum of isomers *op'*-DDT, *pp'*-DDT, *pp'*-TDE (*pp'*-DDD), and *pp'*-DDE;
- endosulfan, as the sum of α -/ β -isomers and endosulfan-sulphate;
- endrin, as the sum of endrin and delta-keto-endrin;
- heptachlor, as the sum of heptachlor and heptachlor epoxide;
- hexachlorobenzene (HCB);
- hexachlorocyclohexane isomers α -HCH (α -BHC), β -HCH (β -BHC), γ -HCH (γ -BHC or lindane);
- photo heptachlor;
- *cis*- and *trans*-nonachlor;
- non dioxin-like PCBs (ndl-PCBs), as the sum of PCB 28, 52, 101, 138, 153 and 180.

The method has been fully validated by a collaborative trial for the substances and corresponding ranges (ng/g) noted in Table 1.

Table 1 — Residue compound and range of (ng/g) collaborative trial

Compound	Range (ng/g)
all ndl-PCBs	0,7 - 39
aldrin	10 - 34
dieldrin	12 - 97
endrin	13 - 88
cis-chlordane ^a	7 - 24a
trans-chlordane ^b	7 - 25b
pp'-DDT	19 - 199
op'-DDT	8 - 87
pp'-TDE	7 - 103
pp'-DDE	21 - 263
alpha-endosulfan	15 - 165
beta-endosulfan	26 - 331
endosulfan sulphate ^c	38 - 61c
heptachlor	15 - 365
heptachlor epoxide	15 - 382
HCB ^d	8 - 170d
alpha-HCH	21 - 247
beta-HCH	6 - 84
gamma-HCH	17 - 186
NOTE The following information is to be taken into consideration: a) Cis-chlordane has not been fully validated for fish oil. b) Trans-chlordane has not been fully validated for vegetable oil. c) Endosulfan sulphate has not been fully validated for pig feed, vegetable oil and fish oil. d) HCB has not been fully validated for fish oil.	

The method has not been fully validated for oxychlordane, endrin ketone, cis- and trans-nonachlor and photo heptachlor in all matrices.

The method is not applicable to chlorocamphene (toxaphene), a complex mixture of polychlorinated camphenes. Chlorocamphene has a very distinctive chromatographic profile and is easily recognizable by GC/ECD. Positive identification of the toxaphene isomers can be performed by negative chemical ionization mass spectrometry (NCI-MS), electron impact tandem mass spectrometry (EI MS × MS) or electron impact high resolution mass spectrometry (EI-HRMS), which is not within the scope of this method.

A limit of quantification (LOQ) for the mentioned organochlorine pesticides of 5 ng/g should normally be obtained. However, 10 ng/g applies for heptachlor aldrin, endrin, dieldrin, and endosulfan (α -, β - and sulphate). For the ndl-PCBs an LOQ of 0,5 to 1,0 ng/g should be obtained. The LOQs mentioned apply to the individual compounds (i.e. not the sum of two or more compounds). Individual laboratories are responsible for ensuring that the equipment that they used will achieve these LOQs. On customers' demand the standard may be applied to solely the analysis of PCBs or OCPs.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

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 1 to entry: 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 1 to entry: 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 six times the standard deviation of the mean of blank determinations ($n > 10$).

3.3

feed additives

substances that comply with the definition of feed additives given in the Regulation (EC) No.1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition

4 Principle

4.1 General

In order to check for the presence of organochlorine pesticides (OCPs) a test portion of animal feeding stuff is fortified with internal standard ($^{13}\text{C}_{12}$ -PCB mix) and extracted with ethyl acetate. The extract is concentrated and subsequently purified by:

- gel permeation chromatography (GPC), with a mixture of cyclohexane/ethyl acetate 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. In case more sensitivity is necessary or less volume reduction is wanted, injection of a larger volume by means of a Programmed Temperature Vaporizer (PTV) injector is possible. An example is described in Annex B.

For ndl-PCBs, two approaches can be followed.

4.2 Ndl-PCBs; method 1

A test portion of animal feeding stuff is fortified with internal standard ($^{13}\text{C}_{12}$ -PCB mix), and is extracted with ethyl acetate. The extract is concentrated and subsequently purified by:

- gel permeation chromatography, with a mixture of cyclohexane /ethyl acetate 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-MS or GC-HRMS using a splitless injector. As an alternative, injection by means of a PTV injector is possible. An example is described in Annex B.

4.3 Ndl-PCBs; method 2

A test portion of animal feeding stuff is fortified with internal standard ($^{13}\text{C}_{12}$ -PCB mix), and is extracted with ethyl acetate. The extract is concentrated and subsequently purified by:

- concentrated sulfuric acid;
- gel permeation chromatography (GPC), with a mixture of cyclohexane/ethyl acetate 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. As an alternative, injection by means of a PTV injector 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.

5.2 Chemicals

5.2.1 Ethyl acetate

5.2.2 Cyclohexane

5.2.3 Ethyl acetate/Cyclohexane = 1+1 parts by volume

Mix 500 ml of ethyl acetate (5.2.1) with 500 ml of cyclohexane (5.2.2) and mix thoroughly. Store at room temperature in a tightly closed glass bottle.

prEN 15741:2018 (E)**5.2.4 Hexane****5.2.5 Decane****5.2.6 Hexane/Decane = 95+5 part by volume**

Mix 950 ml of hexane (5.2.4) with 50 ml of decane (5.2.5) and mix thoroughly. Store at room temperature in a tightly closed glass bottle.

5.2.7 Iso-octane**5.2.8 Toluene****5.2.9 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 five hours, allow to cool in a desiccator, and store in a tightly stopped container in the desiccator. Add 3,5 ml water dropwise from a burette, with a continuous swirling, to 96,5 g dried silica gel in a 300 ml Erlenmeyer flask with a ground joint. Immediately stopper the flask with a ground stopper and shake vigorously for five minutes until all lumps have disappeared. Next, shake for two hours on a mechanical shaker, then store in a tightly stoppered container. Deactivated silica gel is tenable during approximately two weeks if carefully stored.

5.2.10 Hexane/toluene = 3+7 parts by volume

Mix 30 ml of n-hexane (5.2.4) with 70 ml of toluene (5.2.8) and mix thoroughly. Store at room temperature in a tightly closed glass bottle.

5.2.11 Concentrated H₂SO₄**5.2.12 Internal standard (PCB 209)****5.2.12.1 PCB 209 stock solution 1, 100 µg/ml**

Weigh 10 mg (±0,01 mg) of PCB 209 (5.2.12) in a brown medicine glass bottle of 100 ml and add iso-octane (5.2.7) to achieve a concentration of 100 µg/ml. Store the solution in a refrigerator at 4°C (±3°C). The solution is tenable under these conditions during at least five years if the weight of the solution is carefully controlled. Alternatively, use a commercially available standard solution of 100 µg/ml.

5.2.12.2 PCB 209 stock solution 2, 10,0 µg/ml

Dilute 10,0 ml of PCB 209 Stock solution 1 (5.2.12.1) to 100,0 ml with hexane (5.2.4). Store the solution in a refrigerator at 4°C (±3°C). The solution is tenable under these conditions during at least five years if the weight of the solution is carefully controlled.

5.2.12.3 PCB 209 working solution, concentration 1000 ng/ml

Dilute 10 ml of PCB 209 Stock solution 2 (5.2.12.2) to 100,0 ml with hexane (5.2.4). Store the solution in a refrigerator at 4°C (±3°C). The solution is tenable under these conditions during at least five years if the weight of the solution is carefully controlled.

5.3 Internal standards (^{13}C mass labelled PCBs)

5.3.1 Internal standards (^{13}C mass labelled PCBs), 1000 ng/ml

$^{13}\text{C}_{12}$ PCB 28 (2,4,4' trichlorobiphenyl, $^{13}\text{C}_{12}$); CAS Number: 208263-76-7;

$^{13}\text{C}_{12}$ PCB 52 (2,2',5,5' tetrachlorobiphenyl, $^{13}\text{C}_{12}$); CAS Number: 208263-80-3;

$^{13}\text{C}_{12}$ PCB 101 (2,2',4,5,5' pentachlorobiphenyl, $^{13}\text{C}_{12}$); CAS Number: 104130-39-4;

$^{13}\text{C}_{12}$ PCB 138 (2,2',3',4,4',5 hexachlorobiphenyl, $^{13}\text{C}_{12}$); CAS Number: 208263-66-5;

$^{13}\text{C}_{12}$ PCB 153 (2,2',4,4',5,5' hexachlorobiphenyl, $^{13}\text{C}_{12}$); CAS Number: 185376-58-3;

$^{13}\text{C}_{12}$ PCB 180 (2,2',3,4,4',5,5' heptachlorobiphenyl, $^{13}\text{C}_{12}$); CAS Number: not available.

Alternatively, use a certified mixture at a concentration of 1000 ng/ml.

5.3.2 Internal standards (^{13}C mass labelled PCBs), 100 ng/ml

Dilute 1,0 ml of internal standards (^{13}C mass labelled PCBs) (5.3.1) to 10,0 ml with hexane (5.2.4). Store the solution in a refrigerator at 4°C ($\pm 3^{\circ}\text{C}$). The solution is tenable under these conditions during at least five years if the weight is carefully controlled.

5.4 PCB congeners stock standard solution

5.4.1 PCB congeners stock standard solution, 10 $\mu\text{g}/\text{ml}$

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.

Alternatively, use a certified mixture at a concentration of 10 $\mu\text{g}/\text{ml}$.

5.4.2 PCB congeners working standard solution, 2,0 $\mu\text{g}/\text{ml}$

Dilute 2,0 ml of PCB congeners stock standard solution (5.4.1) to 10,0 ml with hexane (5.2.4). Store the solution in a refrigerator at 4°C ($\pm 3^{\circ}\text{C}$). The solution is tenable under these conditions during at least five years if the weight is carefully controlled.

5.4.3 PCB congeners work standard solution, 0,2 $\mu\text{g}/\text{ml}$

Dilute 1,0 ml of PCB congeners stock standard solution (5.4.2) to 10,0 ml with hexane (5.2.4). Store the solution in a refrigerator at 4°C ($\pm 3^{\circ}\text{C}$). The solution is tenable under these conditions during at least five years if the weight is carefully controlled.

5.5 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.