SLOVENSKI STANDARD
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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

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This European Standard was approved by CEN on 6 January 2020.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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European foreword

This document (EN 15741:2020) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs: Methods of sampling and analysis”, 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of an identical text or by endorsement, at the latest by September 2020, and conflicting national standards shall be withdrawn at the latest by September 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 15741:2009.

In comparison with the previous edition, the following technical modification has 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 standardization request 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.

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 compound feeds and feed materials (oils and fats, fish meal).

The method is applicable to compound feeds consisting of less than 20 % water 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 o,p'-DDT, p,p'-DDT, p,p'-TDE (p,p'-DDD), and p,p'-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 (µg/kg) noted in Table 1.

Table 1 — Residue compound and range of (µg/kg) collaborative trial

Compound	Range (µg/kg)
all ndl-PCBs	0,7 to 39
aldrin	10 to 34
dieldrin	12 to 97
endrin	13 to 88
cis-chlordane[a]	16 to 24[a]
trans-chlordane	7 to 25
p,p'-DDT[b]	19 to 200[b]
o,p'-DDT	8 to 87
pp'-TDE[c]	9 to 103[c]
pp'-DDE	21 to 264
α-endosulfan	15 to 165
β-endosulfan	26 to 331
endosulfan sulphate[d]	29 to 61[d]
heptachlor	15 to 365
heptachlor epoxide	15 to 382
HCB[e]	8 to 170[e]
α-HCH	21 to 247
β-HCH	6 to 84
γ-HCH[f]	17 to 186[f]
NOTE The following information is to be taken into consideration: a) *Cis*-chlordane has not been fully validated for chicken feed, pig feed and fish oil. b) p,p'-DDT has not been fully validated for pig feed and vegetable oil. c) pp'-TDE has not been fully validated for pig feed and fish meal. d) Endosulfan sulphate has not been fully validated for pig feed and vegetable oil. e) HCB has not been fully validated for fish oil. f) γ-HCH 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.

For those matrix-analyte combinations where the validation data were regarded insufficient, the results obtained with this method can only be regarded as screening results, unless the laboratory performs an in-house validation to show that satisfactory results can be obtained.

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 6 to 29 µg/kg should normally be obtained (see Table 1). For the ndl-PCBs an LOQ of 0,5 to 1,0 µg/kg 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.

<std>EN ISO 6498, *Animal feeding stuffs – Guidelines for sample preparation (ISO 6498)*</std>

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 3 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 6 times the standard deviation of the mean of blank determinations (n > 10).

3.3
feed additives
substances that are added to animal nutrition

Note 1 to entry: see Regulation (EC) No.1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition for more information.

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 ($^{13}C_{12}$-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 ($^{13}C_{12}$-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 ($^{13}C_{12}$-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.

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 5 h, 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 5 min until all lumps have disappeared. Next, shake for 2 h on a mechanical shaker, then store in a tightly stoppered container. Deactivated silica gel is tenable during approximately 2 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 H_2SO_4

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 5 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 5 years if the weight of the solution is carefully controlled.

5.2.12.3 PCB 209 working solution, concentration 1 000 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 5 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), 1 000 ng/ml

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

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

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

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

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

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

Alternatively, use a certified mixture at a concentration of 1 000 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 (±3 °C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

5.4 PCB congeners stock standard solution

5.4.1 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.

Alternatively, use a certified mixture at a concentration of 10 µg/ml.

5.4.2 PCB congeners working standard solution, 2,0 µg/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 (±3 °C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controlled.

5.4.3 PCB congeners work standard solution, 0,2 µg/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 (±3 °C). The solution is tenable under these conditions during at least 5 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-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, α isomer:

1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-ethano-1*H*-indene; α isomer; CAS Number: 5103-71-9.

Chlordane, β isomer:

1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-ethano-1*H*-indene; β isomer; CAS Number: 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.

o,p'-DDT :

1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane; CAS Number: 789-02-6;

p,p'-DDT :

1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; CAS Number: 50-29-3.

pp'-TDE :

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

pp'-DDE :

1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene; CAS Number: 72-55-9.

Endosulfan, α stereoisomer:

6,9-Methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-, 3-oxide, (3α,5aβ, 6α,9α,9aβ); CAS Number: 959-98-8.

Endosulfan, β stereoisomer :

6,9-Methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-, 3-oxide, (3α,5aα,6β,9β,9aα); CAS Number: 33213-65-9.

Endosulfan-sulphate;

6,9-Methano-2,4,3-benzodioxathiepin, 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-, 3,3-dioxide; 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-dd; CAS Number: 72-20-8.

Delta-keto endrin

2,5,7-Metheno-3H-cyclopenta[a]pentalen-3-one,3b,4,5,6,6,6a-hexachlorodecahydro-, 2α, 3aβ,3bβ,4β,5β,6aβ,7α,7aβ,8R*; CAS Number 53494-70-5.

Heptachlor

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

β-Heptachlor epoxide

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.

α-HCH (α-BHC)

α-1,2,3,4,5,6-hexachlorocyclohexane; CAS Number: 319-84-6.

β-HCH (β-BHC)

β-1,2,3,4,5,6-hexachlorocyclohexane; CAS Number: 319-85-7.

γ-HCH (γ-BHC; lindane)

γ-1,2,3,4,5,6-hexachlorocyclohexane; CAS Number: 58-89-9.

Nonachlor, *cis*;

CAS Number 5103-73-1.

Nonachlor, *trans*;

CAS Number 39765-80-5.

Photo heptachlor;

CAS Number 33442-83-0.

Alternatively, use a certified mixture at a precise or certified concentration (approximately 10 µg/ml).

5.5.1 Pesticide stock solution 1, 100 µg/ml

Weigh 10 mg (±0,01 mg) of each individual pesticide (5.5) in separate brown medicine glass bottles of 100 ml and add iso-octane (5.2.7) to achieve a concentration of 100 µg/ml. Store the solutions in a refrigerator at 4 °C (±3 °C). The solutions are tenable under these conditions during at least 5 years if the weight is carefully controlled. Alternatively, use a commercially available standard solution with a precise or certified concentration of approximately 100 µg/ml.

NOTE Dissolve ß-HCH in 10 ml toluene (5.2.8) to achieve complete solvability and dilute further with iso-octane (5.2.7) to achieve a concentration of 100 µg/ml

5.5.2 Pesticide stock solution 2, 2,5 µg/ml

Pipet 2,5 ml of each individual pesticide stock solutions 1 (5.5.1) into a 100,0 ml graduated flask and dilute with hexane (5.2.4) to 100,0 ml. Store the solution 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.

5.5.3 Pesticide working solution, 0,5 µg/ml

Pipet 2,0 ml of pesticide stock solution 2 (5.5.2) into a 10,0 ml graduated flask and dilute with hexane (5.2.4) to 10,0 ml. Store the solution 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.

5.6 Calibration solutions

Prepare calibration mixtures according to Table 2 in a final volume of 1,0 ml of an alkane solvent (5.2.6 or 5.2.7) and store them at 4 °C (±3 °C).

Table 2 — Calibration mixtures

Level	PCB 0,2 µg/ml (5.4.3)		OC 0,5 µg/ml (5.5.3)		OC 5,0 µg/ml (5.5.2)		$^{13}C_{12}$-PCB mix 100 ng/ml (5.3.2)		PCB 209 1 000 ng/ml (5.2.12.3)		Solvent
	µl	ng/ml	µl	ng/ml	µl	ng/ml	µl	ng/ml	µl	ng/ml	µl
1	0	0	0	0	0	n.a.	100	10	50	50	850
2	5	1	20	10	0	n.a.	100	10	50	50	825
3	10	2	50	25	0	n.a.	100	10	50	50	790
4	25	5	250	125	0	n.a.	100	10	50	50	575
5	100	20	0	n.a.	100	500	100	10	50	50	650
6	250	50	0	n.a.	250	1 250	100	10	50	50	350

5.7 Glass vial, 100 ml, including PTFE-lined screwcaps

5.8 Glass wool

Heated at 160 °C – 200 °C during at least 24 h.

5.9 Sodium sulphate, anhydrous

Heated at 160 °C – 200 °C during at least 24 h.

5.10 Helium gas, purity 5,0 or higher.

5.11 Nitrogen gas, purity 5,0 or higher.

5.12 GC sampler vial, 2 ml.

5.13 Glass graduated evaporation tubes, 50 ml

5.14 Chromatographic tubes, glass or PTFE

Chromatographic tube with solvent reservoir.

5.15 Autosampler vial, with limited volume insert

5.16 Glass tubes, approximately 50 ml

5.17 Glass tubes, approximately 4 ml

5.18 Anhydrous sodium sulphate