SLOVENSKI STANDARD
SIST EN 15741:2009

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Krma - Določ evanje OC-pesticidov in PCB z GC/MS

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

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

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

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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of an identical text or by endorsement, at the latest by August 2009, and conflicting national standards shall be withdrawn at the latest by August 2009.

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1 Scope

This European Standard specifies a gas chromatographic/mass spectrometric method for the determination of organochlorine pesticides (OC's) and polychlorinated biphenyls (PCBs) in animal feeding stuffs and oil.

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 and PCBs and some of their isomers and degradation products:

— Aldrin;

— Dieldrin;

— 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 α-/β-isomers and Endosulfan-sulphate);

— Endrin;

— Heptachlor (= sum of Heptachlor and β-Heptachlorepoxide);

— Hexachlorobenzene (HCB);

— Hexachlorocyclohexane isomers α-HCH (α-BHC), β-HCH (β-BHC), γ-HCH (γ-BHC or lindane);

— PCB 28, 52, 101, 138, 153 and 180 ("Indicator PCBs") and PCB 198, 209.

The method is not yet applicable to Chlorocamphene (Toxaphene), a complex mixture of polychlorinated camphenes. Chlorocamphene has a very distinctive chromatographic profile and is easily recognisable by GC/ECD. Positive identification of the toxaphene isomers can be performed by negative chemical ionisation mass spectrometry (NCI-MS), electron impact tandem mass spectrometry (EI-MSxMS) or electron impact high resolution mass spectrometry (EI-HRMS) [1], which is not within the scope of this method.

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 (α-, β- 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 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 (± 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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled. 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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled.

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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled.

5.13 Internal standard (PCB 198)

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

Weigh 5-10 mg (± 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 (± 3°C). The solutions are tenable under these conditions during at least 5 years if the weight is carefully controled. 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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled.

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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled.

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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled.

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

Chlordane
(1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-ethano-1*H*-indene); α and β 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, α, (I), CAS Number: 959-98-8 and β, (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

β-Heptachlorepoxide
(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

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

5.17 Pesticide Stock solution 1, 100 µg/ml

Weigh 5-10 mg (± 0,01 mg) of each individual pesticide (5.16) in seperate 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 (± 3°C). The solutions are tenable under these conditions during at least 5 years if the weight is carefully controled. Or use a commercially available standard solution of 100 µg/ml.

NOTE Dissolve ß-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 (± 3°C). The solution is tenable under these conditions during at least 5 years if the weight is carefully controled.

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 ± 3°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.