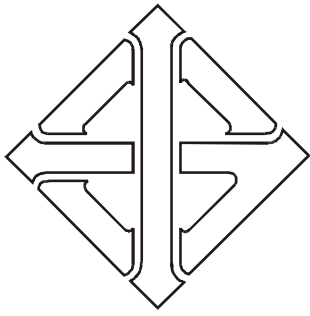




มาตรฐานผลิตภัณฑ์อุตสาหกรรม

THAI INDUSTRIAL STANDARD

มอก. 2322–2549

ISO 14181–2000

อาหารสัตว์–วิธีวิเคราะห์สารตกค้างของสาร
ป้องกันและกำจัดศัตรูพืชกลุ่มออร์กาโนคลอรีน
โดยวิธีก๊าซโครมาโทกราฟี

ANIMAL FEEDING STUFFS–DETERMINATION OF RESIDUES OF
ORGANOCHLORINE PESTICIDES–GAS CHROMATOGRAPHIC METHOD

สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

กระทรวงอุตสาหกรรม

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มาตรฐานผลิตภัณฑ์อุตสาหกรรม
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กระทรวงอุตสาหกรรม ถนนพระรามที่ 6 กรุงเทพฯ 10400
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คณะกรรมการวิชาการคณะที่ 463
มาตรฐานอาหารสัตว์

ประธานกรรมการ

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สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

เนื่องจากการนำวัตถุพิษอาหารสัตว์ประเภทพืชนำมาผสมในสูตรอาหารสัตว์แต่ละชนิดโดยมีสัดส่วนปริมาณที่ไม่เท่ากันเพื่อให้การเลี้ยงสัตว์เกิดประโยชน์สูงสุด จึงจำเป็นต้องตรวจสอบคุณภาพพืชที่นำมาใช้ ซึ่งอาจจะมีการใช้สารป้องกันและกำจัดศัตรูพืชใน กระบวนการผลิตวัตถุพิษอาหารสัตว์ประเภทพืช จึงกำหนดมาตรฐานวิธีวิเคราะห์สารตกค้าง ของสารป้องกันและกำจัดศัตรูพืชกลุ่มออร์กาโนคลอรีน โดยวิธีโครมาโทกราฟีขึ้น

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดขึ้นโดยรับ ISO 14181-2000 Animal feeding stuffs-Determination of residues of organochlorine pesticides-Gas chromatographic method ซึ่งจัดพิมพ์ขึ้นโดยองค์การระหว่างประเทศว่าด้วยการมาตรฐาน (ISO) ในปี 2000 มาใช้ในระดับเหมือนกันทุกประการ (identical) โดยใช้มาตรฐานฉบับภาษาอังกฤษเป็นหลัก

คณะกรรมการวิชาการคณะที่ 463 มาตรฐานอาหารสัตว์ รับผิดชอบงานของคณะอนุกรรมการคณะที่ 10 ภายใต้กรรมการวิชาการคณะที่ 34 ขององค์การระหว่างประเทศว่าด้วยการมาตรฐานเป็นผู้จัดทำมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้จัดทำขึ้นเพื่อให้ทันกับความต้องการของผู้ใช้และจักได้แปลเป็นภาษาไทยในโอกาสอันสมควร หากมีข้อสงสัยโปรดติดต่อสอบถามที่สำนักงานมาตรฐานผลิตภัณฑ์อุตสาหกรรม

คณะกรรมการมาตรฐานผลิตภัณฑ์อุตสาหกรรมได้พิจารณามาตรฐานนี้แล้ว เห็นสมควรเสนอรัฐมนตรีประกาศตาม มาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511



ประกาศกระทรวงอุตสาหกรรม

ฉบับที่ 3667 (พ.ศ. 2550)

ออกตามความในพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม

พ.ศ. 2511

เรื่อง กำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม

อาหารสัตว์-วิธีวิเคราะห์สารตกค้างของสารป้องกันและกำจัดศัตรูพืชกลุ่มออร์กาโนคลอรีน

โดยวิธีก๊าซโครมาโทกราฟี

อาศัยอำนาจตามความในมาตรา 15 แห่งพระราชบัญญัติมาตรฐานผลิตภัณฑ์อุตสาหกรรม พ.ศ. 2511 รัฐมนตรีว่าการกระทรวงอุตสาหกรรมออกประกาศกำหนดมาตรฐานผลิตภัณฑ์อุตสาหกรรม อาหารสัตว์-วิธีวิเคราะห์สารตกค้างของสารป้องกันและกำจัดศัตรูพืชกลุ่มออร์กาโนคลอรีนโดยวิธีก๊าซโครมาโทกราฟี มาตรฐานเลขที่ มอก.2322-2549 ไว้ ดังมีรายการละเอียดต่อท้ายประกาศนี้

ประกาศ ณ วันที่ 29 มกราคม พ.ศ. 2550

โสมิต ปันเปี่ยมรัชฎ์

รัฐมนตรีว่าการกระทรวงอุตสาหกรรม

มาตรฐานผลิตภัณฑ์อุตสาหกรรม อาหารสัตว์-วิธีวิเคราะห์สารตกค้างของสาร ป้องกันและกำจัดศัตรูพืชกลุ่มออร์กาโนคลอรีน โดยวิธีก๊าซโครมาโทกราฟี

บทนำ

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดขึ้นโดยรับมาตรฐาน ISO 14181 : 2000 Animal feeding stuffs-Determination of residues of organochlorine pesticides-Gas chromatographic method มาใช้ในระดับเหมือนกันทุกประการ (identical) โดยใช้ ISO ฉบับภาษาอังกฤษเป็นหลัก

ขอบข่าย

มาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้กำหนดวิธีวิเคราะห์หาสารตกค้างของสารป้องกันและกำจัดศัตรูพืชด้วยวิธีก๊าซโครมาโทกราฟีในอาหารสัตว์ ซึ่งสารป้องกันและกำจัดศัตรูพืช กลุ่มออร์กาโนคลอรีน และไอโซเมอร์บางชนิด และสารที่เกิดจากการสลายตัวได้แก่ ออลดริน, ดีดีอี, ดีดีที, ดีลนริน, เอ็นโดซัลแฟน, เอ็นคริน, เอชซีบี, แอลฟา-บีเอชซี, เบต้า-บีเอชซี, แกมมา-บีเอชซี, เดลต้า-บีเอชซี, ดีลนริน, เฮปตาคลอร์, เฮปตาคลอรีอีพอกไซด์, ทีดีอี และเมทรอกซีคลอร์

ค่าวิเคราะห์ต่ำสุดที่วิเคราะห์ได้ คือ 0.005 ไมโครกรัม/กรัม

เอกสารอ้างอิง

ISO 3696, : 1987 Water for analytical laboratory use – Specification and test method

ISO 6498, Animal feeding stuffs-Preparation of test samples

หลักการ

หลักการในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 3

สารเคมี

สารเคมีที่ใช้ในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 4

เครื่องมือ

เครื่องมือที่ใช้ในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 5

การชักตัวอย่าง

การชักตัวอย่างในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 6

การเตรียมตัวอย่างวิเคราะห์

การเตรียมตัวอย่างในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 7

การวิเคราะห์

การวิเคราะห์ที่ใช้ในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 8

การคำนวณและการแสดงผลลัพธ์

การคำนวณและการแสดงผลลัพธ์ในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 9

การยืนยันผลการจำแนกชนิดและปริมาณ

การยืนยันผลการจำแนกชนิด และปริมาณในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 10

ความเที่ยง

ความเที่ยงในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 11

การรายงานผลวิเคราะห์

การรายงานผลวิเคราะห์ในมาตรฐานผลิตภัณฑ์อุตสาหกรรมนี้ให้เป็นไปตามที่ระบุไว้ใน ISO 14181 : 2000 ข้อ 12

Animal feeding stuffs — Determination of residues of organochlorine pesticides — Gas chromatographic method

1 Scope

This International Standard specifies a gas chromatographic method for the determination of residues of organochlorine pesticides in animal feeding stuffs.

The method is applicable to animal feeding stuffs containing residues of one or more of the following organochlorine pesticides and some of their isomers and degradation products: aldrin, *op*¹-DDE, *pp*¹-DDE, *op*¹-DDT, *pp*¹-DDT, dieldrin, endosulfan, endrin, HCB, α -HCH (α -BHC), β -HCH (β -BHC), γ -HCH (γ -BHC), δ -HCH (δ -BHC), heptachlor, heptachlor epoxide, *op*¹-TDE (*op*¹-DDD), *pp*¹-TDE (*pp*¹-DDD) and methoxychlor.

The lower determination limit for these organochlorine pesticides is 0,005 µg/g. However, the lower determination limit is 0,01 µg/g for *op*¹-DDT and *pp*¹-DDT, and 0,05 µg/g for methoxychlor.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent edition of the normative document indicated below. For undated references, the latest edition of the normative documents referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

ISO 6498, *Animal feeding stuffs — Preparation of test samples*.

3 Principle

A test portion is extracted with acetone. The filtered extract is diluted with water and saturated sodium chloride solution. The pesticides are partitioned into dichloromethane. The concentrated extract is purified on a chromatographic column of 10 % water-deactivated silica gel. The pesticide residues are determined by gas chromatography using an electron capture detector or a mass selective detector.

4 Reagents and materials

Use only reagents of recognized analytical grade and with a purity suitable for pesticide 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.

WARNING — Some of the organic solvents are suspected carcinogens. Use with care.

4.1 Water, complying with at least grade 3 in accordance with ISO 3696.

4.2 Hexane.

4.3 Acetone.

4.4 Dichloromethane.

4.5 Silica gel 60, with a mass fraction of water of 10 %.

Activate silica gel 60, particle size 63 µm to 200 µm, at 130 °C overnight, then cool in a desiccator. After cooling to room temperature, pour the silica gel into an airtight glass container and add sufficient distilled water to bring the final mass fraction of water to 10 %. Shake the container mechanically or by hand vigorously for 30 s and allow to stand for 30 min with occasional shaking. After 30 min the silica gel is ready for use. It should not be stored for more than 6 h.

4.6 Eluting solvent: dichloromethane in hexane, 20 % (by volume).

Mix 1 volume of dichloromethane with 4 volumes of hexane.

4.7 Inert gas, for example nitrogen.

4.8 Sodium sulfate, anhydrous.

4.9 Sodium chloride, saturated solution.

4.10 Pesticide reference standards, as follows:

- aldrin [(1*R*,4*S*,4*aS*,5*S*,8*R*,8*aR*)-1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4:5,8-dimethanonaphthalene];
- *op'*-DDE [*o,p'*-(1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene)];
- *pp'*-DDE [*p,p'*-(1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene)];
- *op'*-DDT [*o,p'*-(1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane)];
- *pp'*-DDT [*p,p'*-(1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane)];
- dieldrin [(1*R*,4*S*,4*aS*,5*R*,6*R*,7*S*,8*S*,8*aR*)-1,2,3,4,10,10-hexachloro-1,4,4*a*,5,6,7,8,8*a*-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene];
- endosulfan (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide);
- endrin [(1*R*,4*S*,4*aS*,5*S*,6*S*,7*R*,8*R*,8*aR*)-1,2,3,4,10,10-hexachloro-1,4,4*a*,5,6,7,8,8*a*-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene];
- HCB (hexachlorobenzene);
- α-HCH (α-BHC) (α-1,2,3,4,5,6-hexachlorocyclohexane);
- β-HCH (β-BHC) (β-1,2,3,4,5,6-hexachlorocyclohexane);
- γ-HCH (γ-BHC; lindane) (γ-1,2,3,4,5,6-hexachlorocyclohexane);
- δ-HCH (δ-BHC) (δ-1,2,3,4,5,6-hexachlorocyclohexane);
- heptachlor (1,4,5,6,7,8,8-heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene);
- heptachlor epoxide (heptachloroepoxy-tetrahydromethanoindene);

- *op'*-TDE (*op*-DDD) [*o,p'*-1,1-dichloro-2,2-bis(4-chlorophenyl) ethane];
- *pp'*-TDE (*pp*-DDD) [*p,p'*-1,1-dichloro-2,2-bis(4-chlorophenyl) ethane];
- methoxychlor [1,1,1-trichloro-2,2-bis(4-methoxyphenyl) ethane].

4.11 Internal standard: mirex (1,3,5-tribromobenzene) or pentachloronitrobenzene.

4.12 Pesticide standard and internal standard solutions.

4.12.1 Stock solutions, of concentration 1 000 µg/ml.

Prepare a stock solution of each pesticide reference standard (4.10) and of the internal standard (4.11) as follows.

Weigh, to the nearest 0,1 mg, a mass of a pesticide reference standard (4.10) or the internal standard (4.11) which will result in a solution with a content of reference standard or internal standard of 1 000 µg/ml. While weighing, observe the cleanness of the standard material. Transfer the weighed mass into a volumetric flask, dissolve in hexane or in an other solvent such as toluene or iso octane. However, dissolve β-HCH in acetone. Dilute to volume with the same solvent and mix well.

These solutions are stable for 6 months when stored at 4 °C in the dark.

4.12.2 Intermediate solutions, of concentration 10 µg/ml.

Pipette 1 ml of each stock solution (4.12.1) into individual 100 ml volumetric flasks. Dilute to volume with hexane. The solutions are stable for at least 3 months when stored at 4 °C in the dark.

NOTE The stability of properly stored pesticide standards is very widely known. Investigations have shown that all neat pesticide standards tested are stable for 15 years when stored at –18 °C and that stock solutions of pesticide standards in toluene of 1mg/ml are stable for at least 3 years when stored at –18 °C.

The following recommended practice may be used for longer storage. Transfer portions of the prepared standard solutions to amber vials with PTFE-lined screwcaps. Weigh the vials and store at –20 °C. When needed, remove a vial from the freezer, bring to room temperature and weigh. If the accumulated loss in mass due to evaporation is 10 % or more of the prefrozen net mass, discard the vial. Weigh and refreeze stock standards and intermediate solutions that are in use for more than 3 months (usually in 25 ml vials). Otherwise the prepared standard solutions (usually in 2 ml vials) may be stored at 4 °C and shall be discarded after 3 months.

4.12.3 Working solution, of concentration 0,05 µg/ml.

Pipette a 0,5 ml of each intermediate solution (4.12.2) into a 100 ml volumetric flask and dilute to volume with hexane. The solution is stable for 1 month when stored at 4°C in the dark (see 4.12.2).

5 Apparatus

Before use, wash all glassware thoroughly with detergent free of interfering substances. Rinse with water, then with acetone and dry.

Avoid the use of plastics containers and do not lubricate the stopcocks with grease, otherwise impurities could be introduced into the solvents.

Usual laboratory equipment and, in particular, the following.

5.1 Separating funnels, of 500 ml capacity with polytetrafluoroethylene (PTFE) stopcocks and stoppers.

5.2 Filtering flasks, of 500 ml capacity.

5.3 Büchner funnel, of porcelain, with internal diameter 90 mm.

5.4 Graduated tubes, of 10 ml capacity, with polytetrafluoroethylene (PTFE) stoppers.

5.5 Glass chromatographic tube, about 300 mm in length, 8 mm to 10 mm internal diameter, with coarse fritted plate of porosity grade P 100 (pore size index 40 μm to 100 μm [2]) or glass wool plug.

5.6 Rotary vacuum evaporator, provided with round-bottom flasks of 100 ml and 500 ml capacities and a water bath at $40\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

5.7 Mechanical shaker or high-speed blender.

5.8 Gas chromatographic system consisting of:

- splitless or on-column injection system;
- column;
- electron capture detector or mass selective detector;
- electrometer;
- mV recorder or integrator;
- data handling software and computer system.

Each injection port, column oven and detector shall be provided with an independent heating device controlled to the nearest $0,1\text{ }^{\circ}\text{C}$.

The chromatographic system shall be adjusted and the parameters optimized according to the characteristics of the instrument used.

5.8.1 Injection device

An autosampler or any other adequate injection device may be used. For manual injections, use a microsyringe of 1 μl to 5 μl capacity with a needle length suitable for the mode of injection (splitless or on-column).

Before injecting the solution into the gas chromatograph, rinse the syringe 10 times with pure solvent, then 5 times with solution. After injection, rinse the syringe 5 times with pure solvent.

5.8.2 Column

The use of capillary columns coated with mid-range polarity stationary phases (e.g. SE-30, SE-54, OV-17, or equivalent) is recommended.

Standard glass columns, of length 2 m to 4 m and of internal diameter 2 mm to 4 mm, packed with a mixture of 2,5 % QF1 + 1 % OV 11 + 0,5 % XE 60 on Chromosorb WHP 0,125 mm to 0,15 mm particle size, or any other stationary phases and inert supports recommended for organochlorine residue analyses could be used as an alternative.

The temperature programme for the column shall be chosen to separate the pesticides mixture specified in clause 1 into individual components (see annex A).

After installing a new column, it shall be conditioned for at least 24 h at a temperature slightly above the maximum proposed operating temperature, with carrier gas flowing through it and the end of the column disconnected from the detector.

5.8.3 Detector

Use an electron capture detector (ECD) operating either in constant current mode or in constant frequency mode at the polarization voltage, width, amplitude or frequency of the pulses at which a maximum of 0,05 ng heptachlor epoxide causes 40 % to 50 % full-scale deflection.

5.8.4 Carrier gas and make-up gas

Pure nitrogen (oxygen-free), pure helium or pure hydrogen, or a mixture of argon and methane [90 + 10, by volume or (95 + 5) % by volume].

5.9 Grinder

6 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 6497 [1].

7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

Grind a portion of the well-mixed laboratory sample (dry or low moisture products such as cereals and cereal products, oilseeds and oilseed meals, mixed feeds, hay, etc.) so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

Chop up the high moisture products (e.g. grasses, silages, etc.) and mix thoroughly to obtain homogeneous samples.

8 Procedure

8.1 General

Carry out steps 8.2 to 8.4 using both the prepared test sample (clause 7) and a blank sample. The blank sample shall be free of residues at or above the limits of detection found from previous determinations. The blank extract is used to prepare the matrix-matched standard solution (see 8.5.3).

8.2 Test portion

Weigh, to the nearest 0,1 g, 50 g of the prepared test sample (clause 7) for dry or low moisture products, or 100 g for high moisture products, into a 1 000 ml conical flask.

8.3 Extraction

Add sufficient water to the test portion so that a total water amount of about 100 g is obtained. Allow the sample to soak for about 5 min. Add 200 ml of acetone. Close the flask tightly and shake for 2 h on a mechanical shaker or homogenize for 2 min in a high-speed blender.

Filter the homogenate with suction through a Büchner funnel (5.3) fitted with filter paper of medium porosity, into a 500 ml filtering flask (5.2). Wash the conical flask or the blender cup and the residue on the filter paper with two 25 ml portions of acetone, collecting the washings in the same filtering flask.

Measure the volume of the filtrate (V_1) and transfer one-fifth of the solution (V_2) to a 500 ml separating funnel. Add 250 ml of water, about 50 ml of saturated sodium chloride solution (4.9) and 100 ml of dichloromethane (4.4) to the separating funnel. Stopper and shake for 2 min.

Allow the phases to separate and draw off the lower phase (dichloromethane) into a second 500 ml separating funnel (5.1). Repeat the extraction twice with 50 ml of dichloromethane then combine the extracts in the same 500 ml separating funnel.

Wash the dichloromethane extract with two 100 ml portions of water, discarding the washings.

Filter the dichloromethane extract through a filter paper containing about 20 g of sodium sulfate (4.8) into a 500 ml flask of a vacuum evaporator. Rinse the separating funnel and the sodium sulfate with two 10 ml portions of dichloromethane and add to the flask.

Concentrate the solution to about 2 ml under vacuum at a temperature not exceeding 40 °C. Transfer the solution to a 10 ml graduated tube using 1 ml to 2 ml of hexane and concentrate under nitrogen to about 1 ml.

Do not allow the solution to dry completely or losses of pesticides may occur because of volatility or poor solubility.

8.4 Column clean-up

8.4.1 Preparation of the column

Transfer 5 g of silica gel (4.5) to a glass chromatographic tube (5.5). Add 5 g of anhydrous sodium sulfate (4.8) on the top of the silica gel. Wash the prepared column with 20 ml of hexane.

NOTE Prepacked silica or florisil cartridges (e.g. Millipore-SEP PAK) may be used instead of a silica gel column, after checking for efficiency and absence of interferences.

8.4.2 Purification

Transfer quantitatively the concentrated extract (8.3) on the top of the column (8.4.1) using 1 ml to 2 ml portions of hexane.

Elute the organochlorine pesticides with 50 ml of eluting solvent (4.6) and collect the eluate in the 100 ml flask of the rotary vacuum evaporator (5.6).

Concentrate the eluate to about 2 ml under vacuum at a temperature not exceeding 40 °C. Add about 10 ml of hexane and concentrate once more to about 1 ml. Repeat two times, leaving about 1 ml of hexane at the final evaporation. Transfer to a 10 ml graduated tube using 1 ml to 2 ml of hexane. Dilute the sample extract to 10 ml with hexane for determination by chromatography.

When an internal standard method is used, add 1 ml of the intermediate solution (4.12.2) of the internal standard (4.11) to the final extract before diluting to 10 ml with hexane. Keep the blank extract to prepare the matrix-matched standard solution (see 8.5).

8.5 Gas chromatography

8.5.1 Preparation of the system

Equilibrate the gas chromatographic system under the recommended operating conditions (5.8).

If the carrier gas volume flow rate through the column is below 25 ml/min, introduce a supplemental contribution of gas at the outlet of the column in order to ensure a sufficient flow of gas through the electron capture detector (make-up gas).

Dry the carrier gas by passing it through 0,5 nm molecular sieve traps, previously activated at 350 °C for 4 h to 8 h, installed in the carrier gas line.

Reactivate the molecular sieves each time a new gas cylinder is assembled and as often as needed.

8.5.2 Checking the linearity of the system

Check the linearity of the system from 0,05 ng to 0,5 ng of heptachlor epoxide.

Prepare working solutions with heptachlor epoxide contents ranging from 0,01 µg/ml to 0,1 µg/ml. Inject 1 µl.

Plot the response factor (area/mass, in nanograms, of heptachlor epoxide injected) versus the mass, in nanograms of heptachlor epoxide injected. The graph shall be straight line parallel to the x-axis. If not, establish the range of concentrations within which the detector response is linear.

8.5.3 Determination

Inject 1 µl to 2 µl of working standard solution (4.12.3), then the same volume of the sample extract (8.4.2). Dilute the sample extract if necessary.

Identify the individual pesticide peaks on the basis of retention times.

Determine the amount of pesticides by comparing the size of the sample peaks with those of the known amount of the corresponding pesticide peak in the working standard solution.

When the results based on solvent standards indicate residue levels at or above 50 % of the appropriate MRL, a matrix-matched solution should be used, prepared by adding to the blank extract (8.4.2) appropriate amounts of intermediate solutions (4.12.2) of those pesticides identified in the sample solution, so that the size of the peaks of this reference solution is within 25 % of the size of the peaks in the sample solution. Bring to 10 ml with hexane. Inject into the gas chromatograph the same volume as for the sample solution.

Determine the amount of pesticides by comparing the size of the sample peaks with those of the known amount of the corresponding pesticide peak in the matrix-matched standard solution.

9 Expression of results

9.1 Calculation

Calculate the residue of each individual pesticide in the test sample by the equation:

$$w = \frac{A \times A_{Si} \times m_s \times V_2 \times V_3}{A_s \times A_i \times m \times V_1 \times V_4}$$

where

w is the content of an individual pesticide residue, in micrograms per gram, in the test sample;

A is the area (or height) of the pesticide peak in the sample solution;

A_s is the area (or height) of the corresponding standard pesticide peak in the working standard solution or in the matrix-matched standard solution;

A_i is the area (or height) of the internal standard peak in the sample solution;

A_{Si} is the area (or height) of the internal standard peak in the working standard solution or in the matrix-matched standard solution;

m_s is the mass, in nanograms, of pesticide in the volume injected into the gas chromatograph;

V_1 is the total volume of filtrate, in millilitres, obtained in the extraction step;

V_2 is the volume of the filtrate, in millilitres, used in the purification step;

V_3 is the final volume, in millilitres, of the test solution, taking into account any further dilution that is necessary;

V_4 is the volume, in microlitres, of the sample solution injected into the gas chromatograph;

m is the mass, in grams, of the test portion.

9.2 Recovery

Verify the performance of the method by recovery experiments made on fortified blank samples at the 0,05 µg/g level.

Add to the test portion of a blank sample a known amount of pesticide solution. Let it stand for 30 min then analyse the fortified test portion together with the original blank sample with no added pesticides.

The recovery (%) is calculated for each pesticide as $100 \times [(\text{analysed amount in the fortified sample minus the original amount in the sample}) / (\text{added amount})]$

The recovery coefficient for each individual pesticide shall be between 70 % and 110 %.

When a residue exceeding a maximum residue limit (MLR) is to be confirmed, the concurrent recovery level should be approximately similar to that of the sample.

10 Confirmation of identity

Analyses for confirmation of the identity and quantity of pesticides shall be performed, particularly when the results obtained correspond to or exceed the maximum residue limits (MRLs), either by chromatography on a second column of significantly different polarity or, where the apparatus is available, by using GC-MS to quantify and confirm the identity of the pesticide.

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are given in annex B. The values derived from this test may not be applicable to concentration ranges and matrices other than those given.

11.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases exceed the repeatability limit r derived from Tables B.1 to B.17.

11.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the reproducibility limit R derived from Tables B.1 to B.17.

12 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- the test result obtained, or the two test results obtained if the repeatability has been checked.

Annex A (informative)

Examples of GC operating conditions for organochlorine pesticides

A.1 Example 1

Column:	fused silica capillary OV-1, length 25 m, internal diameter 0,25 mm, film thickness 0,25 µm
Column oven temperature:	50 °C for 1 min; 30 °C/min to 150 °C; 3 °C/min to 240 °C; 240 °C for 2 min
Injector:	splitless with 45 s delay, 250 °C, or on-column, initial oven temperature
Detector:	ECD, 300 °C or MSD

A.2 Example 2

Column:	fused silica capillary, SE-54 or OV-17, length 25 m, internal diameter 0,25 mm, film thickness 0,25 µm
Column oven temperature:	4 °C/min from 60 °C to 240 °C
Injector:	splitless with 45 s delay, 250 °C, or on-column, ambient temperature
Detector:	ECD, 300 °C or MSD

Annex B (informative)

Results of interlaboratory test

The precision of the method was established by an interlaboratory test organized by the Romanian Standardization institute (IRS) in 1996 and carried out in accordance with ISO 5725-2 [3]. In this test 12 laboratories participated. Samples of the following composition were investigated: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt, with organochlorine pesticide contents of 0,005 µg/g up to and including 0,5 µg/g.

Table B.1 — Statistical results for aldrin

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0056	0,0124	0,045	0,084	0,438
Repeatability standard deviation (s_r), µg/g	0,0089	0,00122	0,0041	0,0054	0,027
Repeatability coefficient of variation, %	16,0	9,9	9,0	6,4	6,1
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0025	0,0034	0,011	0,015	0,076
Reproducibility standard deviation (s_R), µg/g	0,0014	0,00247	0,0068	0,009	0,051
Reproducibility coefficient of variation, %	25,3	19,9	15,1	10,8	11,6
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0039	0,0069	0,019	0,025	0,143
^a 1: sample with a target aldrin content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target aldrin content of 0,01 µg/g; composition as for sample 1; 3: sample with a target aldrin content of 0,05 µg/g; composition as for sample 1; 4: sample with a target aldrin content of 0,1 µg/g; composition as for sample 1; 5: sample with a target aldrin content of 0,5 µg/g; composition: as for sample 1.					

Table B.2 — Statistical results for *op*'DDE

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0068	0,0112	0,0438	0,083	0,427
Repeatability standard deviation (s_r), µg/g	0,0015	0,00148	0,0039	0,0064	0,0292
Repeatability coefficient of variation, %	21,7	13,2	8,9	7,7	6,8
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0042	0,0041	0,011	0,018	0,082
Reproducibility standard deviation (s_R), µg/g	0,0024	0,00247	0,0072	0,0115	0,067
Reproducibility coefficient of variation %	35,7	22,0	16,5	13,9	15,7
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0067	0,0069	0,020	0,032	0,188
^a 1: sample with a target <i>op</i> 'DDE content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target <i>op</i> 'DDE content of 0,01 µg/g; composition as for sample 1; 3: sample with a target <i>op</i> 'DDE content of 0,05 µg/g; composition as for sample 1; 4: sample with a target <i>op</i> 'DDE content of 0,1 µg/g; composition as for sample 1; 5: sample with a target <i>op</i> 'DDE content of 0,5 µg/g; composition as for sample 1.					

Table B.3 — Statistical results for *pp*'DDE

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0065	0,0093	0,043	0,0855	0,435
Repeatability standard deviation (s_r), µg/g	0,0013	0,0012	0,0035	0,0075	0,034
Repeatability coefficient of variation, %	19,5	12,9	8,2	8,7	7,8
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0036	0,0034	0,0098	0,021	0,095
Reproducibility standard deviation (s_R), µg/g	0,002	0,0021	0,0054	0,0135	0,069
Reproducibility coefficient of variation, %	30,8	22,6	12,6	15,8	15,9
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0056	0,0059	0,015	0,038	0,193
^a 1: sample with a target <i>pp</i> 'DDE content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target <i>pp</i> 'DDE content of 0,01 µg/g; composition as for sample 1; 3: sample with a target <i>pp</i> 'DDE content of 0,05 µg/g; composition as for sample 1; 4: sample with a target <i>pp</i> 'DDE content of 0,1 µg/g; composition as for sample 1; 5: sample with a target <i>pp</i> 'DDE content of 0,5 µg/g; composition as for sample 1.					

Table B.4 — Statistical results for *op*¹-DDT

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	9	12	12	12	12
Number of accepted results	18	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,007	0,0094	0,045	0,087	0,46
Repeatability standard deviation (<i>s_r</i>), µg/g	0,00095	0,0013	0,0035	0,0049	0,029
Repeatability coefficient of variation, %	14,3	13,3	7,8	5,7	6,3
Repeatability limit (<i>r</i>) [<i>r</i> = 2,8 <i>s_r</i>], µg/g	0,0027	0,0036	0,010	0,014	0,081
Reproducibility standard deviation (<i>s_R</i>), µg/g	0,0022	0,0030	0,0098	0,013	0,041
Reproducibility coefficient of variation, %	31,6	26,2	18,0	15,0	8,8
Reproducibility limit (<i>R</i>) [<i>R</i> = 2,8 <i>s_R</i>], µg/g	0,0062	0,0084	0,027	0,036	0,115
^a 1: sample with a target <i>op</i> ¹ -DDT content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target <i>op</i> ¹ -DDT content of 0,01 µg/g; composition as for sample 1; 3: sample with a target <i>op</i> ¹ -DDT content of 0,05 µg/g; composition as for sample 1; 4: sample with a target <i>op</i> ¹ -DDT content of 0,1 µg/g; composition as for sample 1; 5: sample with a target <i>op</i> ¹ -DDT content of 0,5 µg/g; composition as for sample 1.					

Table B.5 — Statistical results for *pp*¹-DDT

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	10	12	12	12	12
Number of accepted results	20	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0077	0,0123	0,0495	0,088	0,46
Repeatability standard deviation (<i>s_r</i>), µg/g	0,00118	0,0017	0,0038	0,007	0,036
Repeatability coefficient of variation, %	15,4	14,0	7,7	8,0	7,9
Repeatability limit (<i>r</i>) [<i>r</i> = 2,8 <i>s_r</i>], µg/g	0,0033	0,0048	0,011	0,02	0,100
Reproducibility standard deviation (<i>s_R</i>), µg/g	0,0024	0,0037	0,0135	0,0142	0,047
Reproducibility coefficient of variation, %	31,2	30,0	27,3	16,2	10,2
Reproducibility limit (<i>R</i>) [<i>R</i> = 2,8 <i>s_R</i>], µg/g	0,0067	0,010	0,038	0,040	0,132
^a 1: sample with a target <i>pp</i> ¹ -DDT content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target <i>pp</i> ¹ -DDT content of 0,01 µg/g; composition as for sample 1; 3: sample with a target <i>pp</i> ¹ -DDT content of 0,05 µg/g; composition as for sample 1; 4: sample with a target <i>pp</i> ¹ -DDT content of 0,1 µg/g; composition as for sample 1; 5: sample with a target <i>pp</i> ¹ -DDT content of 0,5 µg/g; composition as for sample 1.					

Table B.6 — Statistical results for dieldrin

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0047	0,0093	0,042	0,080	0,43
Repeatability standard deviation (s_r), µg/g	0,0008	0,0011	0,0041	0,0067	0,0347
Repeatability coefficient of variation, %	17,8	11,8	9,9	8,5	8,0
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0022	0,0031	0,0115	0,019	0,097
Reproducibility standard deviation (s_R), µg/g	0,0014	0,00176	0,0067	0,013	0,078
Reproducibility coefficient of variation, %	30,0	18,9	16,0	16,6	18,3
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0039	0,0049	0,0188	0,036	0,218
^a 1: sample with a target dieldrin content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target dieldrin content of 0,01 µg/g; composition as for sample 1; 3: sample with a target dieldrin content of 0,05 µg/g; composition as for sample 1; 4: sample with a target dieldrin content of 0,1 µg/g; composition as for sample 1; 5: sample with a target dieldrin content of 0,5 µg/g; composition as for sample 1.					

Table B.7 — Statistical results for endrin

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	11	11	11	11	11
Number of accepted results	22	22	22	22	22
Mean organochlorine pesticide content, µg/g	0,0046	0,0096	0,0446	0,084	0,448
Repeatability standard deviation (s_r), µg/g	0,00063	0,00105	0,0044	0,0057	0,036
Repeatability coefficient of variation, %	13,7	10,9	9,9	6,8	8,0
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0018	0,0029	0,0123	0,016	0,100
Reproducibility standard deviation (s_R), µg/g	0,00138	0,002	0,0053	0,0112	0,0494
Reproducibility coefficient of variation, %	30,0	20,8	11,9	13,4	11,0
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0039	0,0056	0,0148	0,031	0,138
^a 1: sample with a target endrin content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target endrin content of 0,01 µg/g; composition: as sample 1; 3: sample with a target endrin content of 0,05 µg/g; composition: as sample 1; 4: sample with a target endrin content of 0,1 µg/g; composition: as sample 1; 5: sample with a target endrin content of 0,5 µg/g; composition: as sample 1.					

Table B.8 — Statistical results for HCB

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0047	0,0091	0,049	0,090	0,45
Repeatability standard deviation (s_r), µg/g	0,00077	0,0009	0,0035	0,0065	0,031
Repeatability coefficient of variation, %	16,5	9,8	7,1	7,2	6,9
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0022	0,0025	0,0098	0,0182	0,087
Reproducibility standard deviation (s_R), µg/g	0,00114	0,00179	0,006	0,0113	0,048
Reproducibility coefficient of variation, %	24,3	19,7	12,3	12,6	10,8
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0032	0,005	0,0168	0,032	0,134
^a 1: sample with a target HCB content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target HCB content of 0,01 µg/g; composition as for sample 1; 3: sample with a target HCB content of 0,05 µg/g; composition as for sample 1; 4: sample with a target HCB content of 0,1 µg/g; composition as for sample 1; 5: sample with a target HCB content of 0,5 µg/g; composition as for sample 1.					

Table B.9 — Statistical results α-HCH

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0067	0,0137	0,055	0,092	0,48
Repeatability standard deviation (s_r), µg/g	0,0007	0,0014	0,0042	0,0064	0,035
Repeatability coefficient of variation, %	10,6	10,3	7,6	6,9	7,3
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0020	0,0039	0,0118	0,018	0,098
Reproducibility standard deviation (s_R), µg/g	0,00155	0,0027	0,0095	0,016	0,055
Reproducibility coefficient of variation, %	23,1	19,9	17,3	17,5	11,5
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0043	0,0076	0,027	0,045	0,154
^a 1: sample with a target α-HCH content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target α-HCH content of 0,01 µg/g; composition as for sample 1; 3: sample with a target α-HCH content of 0,05 µg/g; composition as for sample 1; 4: sample with a target α-HCH content of 0,1 µg/g; composition as for sample 1; 5: sample with a target α-HCH content of 0,5 µg/g; composition as for sample 1.					

Table B.10 — Statistical results for β -HCH

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, $\mu\text{g/g}$	0,0088	0,0127	0,044	0,08	0,404
Repeatability standard deviation (s_r), $\mu\text{g/g}$	0,001	0,00134	0,0026	0,0051	0,0264
Repeatability coefficient of variation, %	11,9	10,6	5,9	6,5	6,5
Repeatability limit (r) [$r = 2,8 s_r$], $\mu\text{g/g}$	0,0028	0,0038	0,0073	0,0143	0,074
Reproducibility standard deviation (s_R), $\mu\text{g/g}$	0,0019	0,00255	0,005	0,011	0,036
Reproducibility coefficient of variation, %	21,9	20,0	11,4	13,8	9,0
Reproducibility limit (R) [$R = 2,8 s_R$], $\mu\text{g/g}$	0,0053	0,0071	0,014	0,031	0,100
^a 1: sample with a target β -HCH content of 0,005 $\mu\text{g/g}$; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target β -HCH content of 0,01 $\mu\text{g/g}$; composition as for sample 1; 3: sample with a target β -HCH content of 0,05 $\mu\text{g/g}$; composition as for sample 1; 4: sample with a target β -HCH content of 0,1 $\mu\text{g/g}$; composition as for sample 1; 5: sample with a target β -HCH content of 0,5 $\mu\text{g/g}$; composition as for sample 1.					

Table B.11 — Statistical results for γ -HCH

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, $\mu\text{g/g}$	0,109	0,127	0,167	0,195	0,55
Repeatability standard deviation (s_r), $\mu\text{g/g}$	0,0069	0,0055	0,008	0,0063	0,030
Repeatability coefficient of variation, %	6,4	4,3	4,8	3,2	5,5
Repeatability limit (r) [$r = 2,8 s_r$], $\mu\text{g/g}$	0,019	0,015	0,022	0,018	0,084
Reproducibility standard deviation (s_R), $\mu\text{g/g}$	0,017	0,0083	0,0168	0,0148	0,051
Reproducibility coefficient of variation, %	15,7	6,6	10,0	7,6	9,3
Reproducibility limit (R) [$R = 2,8 s_R$], $\mu\text{g/g}$	0,048	0,023	0,047	0,041	0,143
^a 1: sample with a target γ -HCH content of 0,005 $\mu\text{g/g}$; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target γ -HCH content of 0,01 $\mu\text{g/g}$; composition as for sample 1; 3: sample with a target γ -HCH content of 0,05 $\mu\text{g/g}$; composition as for sample 1; 4: sample with a target γ -HCH content of 0,1 $\mu\text{g/g}$; composition as for sample 1; 5: sample with a target γ -HCH content of 0,5 $\mu\text{g/g}$; composition as for sample 1.					

Table B.12 — Statistical results for δ -HCH

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, $\mu\text{g/g}$	0,0057	0,01	0,054	0,087	0,475
Repeatability standard deviation (s_r), $\mu\text{g/g}$	0,0007	0,00094	0,003	0,0059	0,036
Repeatability coefficient of variation, %	12,4	9,4	5,5	6,5	7,6
Repeatability limit (r) [$r = 2,8 s_r$], $\mu\text{g/g}$	0,0020	0,0026	0,0084	0,0165	0,100
Reproducibility standard deviation (s_R), $\mu\text{g/g}$	0,0011	0,00195	0,0062	0,0136	0,052
Reproducibility coefficient of variation, %	20,0	19,5	11,5	15,6	10,9
Reproducibility limit (R) [$R = 2,8 s_R$], $\mu\text{g/g}$	0,0031	0,0055	0,017	0,038	0,146
^a 1: sample with a target δ -HCH content of 0,005 $\mu\text{g/g}$; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target δ -HCH content of 0,01 $\mu\text{g/g}$; composition as for sample 1; 3: sample with a target δ -HCH content of 0,05 $\mu\text{g/g}$; composition as for sample 1; 4: sample with a target δ -HCH content of 0,1 $\mu\text{g/g}$; composition as for sample 1; 5: sample with a target δ -HCH content of 0,5 $\mu\text{g/g}$; composition as for sample 1.					

Table B.13 — Statistical results for heptachlor

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, $\mu\text{g/g}$	0,005	0,01	0,046	0,088	0,445
Repeatability standard deviation (s_r), $\mu\text{g/g}$	0,00068	0,001	0,0042	0,0063	0,032
Repeatability coefficient of variation, %	13,5	10,0	9,2	7,2	7,2
Repeatability limit (r) [$r = 2,8 s_r$], $\mu\text{g/g}$	0,0019	0,0028	0,0118	0,0176	0,090
Reproducibility standard deviation (s_R), $\mu\text{g/g}$	0,0011	0,0018	0,0048	0,0109	0,056
Reproducibility coefficient of variation, %	22,2	18,4	10,5	12,3	12,7
Reproducibility limit (R) [$R = 2,8 s_R$], $\mu\text{g/g}$	0,0031	0,0050	0,0134	0,031	0,157
^a 1: sample with a target heptachlor content of 0,005 $\mu\text{g/g}$; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target heptachlor content of 0,01 $\mu\text{g/g}$; composition as for sample 1; 3: sample with a target heptachlor content of 0,05 $\mu\text{g/g}$; composition as for sample 1; 4: sample with a target heptachlor content of 0,1 $\mu\text{g/g}$; composition as for sample 1; 5: sample with a target heptachlor content of 0,5 $\mu\text{g/g}$; composition as for sample 1.					

Table B.14 — Statistical results for heptachlor epoxide

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	12	12	12	12	12
Number of accepted results	24	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0054	0,0116	0,051	0,091	0,454
Repeatability standard deviation (s_r), µg/g	0,00063	0,00114	0,0033	0,005	0,027
Repeatability coefficient of variation, %	11,7	9,8	6,6	5,5	5,9
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0018	0,0032	0,0092	0,014	0,076
Reproducibility standard deviation (s_R), µg/g	0,00114	0,00226	0,0096	0,010	0,042
Reproducibility coefficient of variation, %	21,1	19,5	18,8	10,9	9,3
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0032	0,0063	0,027	0,039	0,213
^a 1: sample with a target heptachlor epoxide content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target heptachlor epoxide content of 0,01 µg/g; composition as for sample 1; 3: sample with a target heptachlor epoxide content of 0,05 µg/g; composition as for sample 1; 4: sample with a target heptachlor epoxide content of 0,1 µg/g; composition as for sample 1; 5: sample with a target heptachlor epoxide content of 0,5 µg/g; composition as for sample 1.					

Table B.15 — Statistical results for *op'*-DDD

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	8	12	12	12	12
Number of accepted results	16	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,0073	0,0095	0,038	0,08	0,40
Repeatability standard deviation (s_r), µg/g	0,00122	0,00126	0,0038	0,006	0,029
Repeatability coefficient of variation, %	16,8	13,3	10,0	7,5	7,3
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0034	0,0035	0,011	0,017	0,081
Reproducibility standard deviation (s_R), µg/g	0,0017	0,0020	0,0047	0,0128	0,061
Reproducibility coefficient of variation, %	23,3	21,6	12,5	16,0	15,2
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0048	0,0056	0,013	0,036	0,171
^a 1: sample with a target <i>op'</i> -DDD content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target <i>op'</i> -DDD content of 0,01 µg/g; composition as for sample 1; 3: sample with a target <i>op'</i> -DDD content of 0,05 µg/g; composition as for sample 1; 4: sample with a target <i>op'</i> -DDD content of 0,1 µg/g; composition as for sample 1; 5: sample with a target <i>op'</i> -DDD content of 0,5 µg/g; composition as for sample 1.					

Table B.16 — Statistical results for *pp'*-DDD

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	8	12	12	12	12
Number of accepted results	16	24	24	24	24
Mean organochlorine pesticide content, µg/g	0,007	0,011	0,049	0,084	0,42
Repeatability standard deviation (s_r), µg/g	0,00145	0,00145	0,0039	0,0068	0,033
Repeatability coefficient of variation, %	20,7	13,2	7,9	8,1	7,8
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	0,0041	0,0041	0,011	0,019	0,092
Reproducibility standard deviation (s_R), µg/g	0,00197	0,00272	0,0064	0,0138	0,054
Reproducibility coefficient of variation, %	27,5	24,7	13,1	16,4	12,9
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	0,0054	0,0076	0,018	0,039	0,151
^a 1: sample with a target <i>pp'</i> -DDD content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target <i>pp'</i> -DDD content of 0,01 µg/g; composition as for sample 1; 3: sample with a target <i>pp'</i> -DDD content of 0,05 µg/g; composition as for sample 1; 4: sample with a target <i>pp'</i> -DDD content of 0,1 µg/g; composition as for sample 1; 5: sample with a target <i>pp'</i> -DDD content of 0,5 µg/g; composition as for sample 1.					

Table B.17 — Statistical results for methoxychlor

Parameter	Sample ^a				
	1	2	3	4	5
Number of laboratories retained after eliminating outliers	—	—	12	12	12
Number of accepted results	—	—	24	24	24
Mean organochlorine pesticide content, µg/g	—	—	0,046	0,091	0,414
Repeatability standard deviation (s_r), µg/g	—	—	0,0049	0,0085	0,036
Repeatability coefficient of variation, %	—	—	10,6	9,4	8,7
Repeatability limit (r) [$r = 2,8 s_r$], µg/g	—	—	0,014	0,024	0,100
Reproducibility standard deviation (s_R), µg/g	—	—	0,0072	0,0129	0,058
Reproducibility coefficient of variation, %	—	—	15,7	14,2	13,9
Reproducibility limit (R) [$R = 2,8 s_R$], µg/g	—	—	0,020	0,036	0,162
^a 1: sample with a target methoxychlor content of 0,005 µg/g; composition: 50 % maize, 20 % barley, 20 % soya bean meal, 3 % fish meal, 3 % fat, 1 % premix, 1,5 % dicalcium phosphate, 1,2 % calcium carbonate and 0,3 % salt; 2: sample with a target methoxychlor content of 0,01 µg/g; composition as for sample 1; 3: sample with a target methoxychlor content of 0,05 µg/g; composition as for sample 1; 4: sample with a target methoxychlor content of 0,1 µg/g; composition as for sample 1; 5: sample with a target methoxychlor content of 0,5 µg/g; composition as for sample 1.					

Bibliography

- [1] ISO 6497, *Animal feeding stuffs — Sampling.*
- [2] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.*
- [3] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.*