INTERNATIONAL STANDARD

ISO 3890-2

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Milk and milk products — Determination of residues of organochlorine compounds (pesticides) —

Part 2: Test methods for crude extract purification and confirmation

Lait et produits laitiers — Détermination des résidus de composés organochlorés (pesticides) —

Partie 2: Méthodes d'essai pour la purification des extraits bruts et tests de confirmation

Reference number
ISO 3890-2:2000(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this part of ISO 3890 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 3890-2 was prepared by Technical Committee ISO/TC 34, *Agricultural food products*, Subcommittee SC 5, *Milk and milk products*, in collaboration with the International Dairy Federation (IDF) and AOAC International, and will also be published by these organizations.

ISO 3890 consists of the following parts, under the general title *Milk and milk products — Determination of residues of organochlorine compounds (pesticides)*:

— *Part 1: General considerations and extraction methods*

— *Part 2: Test methods for crude extract purification and confirmation*

Milk and milk products — Determination of residues of organochlorine compounds (pesticides) —

Part 2: Test methods for crude extract purification and confirmation

WARNING — The use of this part of ISO 3890 may 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 standard to establish safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 3890 specifies test methods for the purification of the crude extracts obtained by the general methods given in ISO 3890-1. It also gives recommended methods for the determination of the residues of organochlorine compounds in milk and milk products, together with confirmatory tests and clean-up procedures.

2 Normative reference

The following normative document contains provisions which, through reference in this text, constitute provisions of this part of ISO 3890. For dated references, subsequent amendments to, or revisions of, this publication do not apply. However, parties to agreement based on this part of ISO 3890 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 document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 3890-1:2000, *Milk and milk products — Determination of residues of organochlorine compounds (pesticides) — Part 1: General considerations and extraction methods.*

3 Method A: Liquid-liquid partitioning with acetonitrile and clean-up on a Florisil column (see reference [3])

3.1 Principle

The organochlorine compounds, together with the fat, are extracted from the sample by one of the procedures described in ISO 3890-1. The extract is concentrated almost to dryness, then redissolved in light petroleum and the organochlorine compounds are partitioned into acetonitrile. After mixing the acetonitrile with an excess of water, the organochlorine compounds are partitioned into light petroleum. This organic phase is purified chromatographically on a Florisil column using light petroleum/diethyl ether as eluting solvent. The eluates are concentrated then examined by GLC.

A special method is described for cheese.

3.2 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

3.2.1 Light petroleum, boiling range 40 °C to 60 °C.

Distil over potassium hydroxide or sodium hydroxide pellets.

3.2.2 Acetonitrile (CH_3CN), saturated with light petroleum.

To purify, mix 4 l of acetonitrile with 1 ml of orthophosphoric acid and 30 g of phosphorus pentoxide in a round-bottomed glass flask. Add glass beads and distil at a temperature of between 81 °C and 82 °C. Do not allow the temperature to exceed 82 °C.

Mix the purified acetonitrile with light petroleum until phase separation just occurs.

3.2.3 Diethyl ether ($C_2H_5OC_2H_5$), peroxide-free.

Distil and stabilize with 2,0 % of its volume of absolute ethanol (C_2H_5OH).

3.2.4 Eluting solvent A: mixture of diethyl ether (3.2.3) and light petroleum (3.2.1) (6:94 by volume).

Dry over 10 g to 25 g of anhydrous sodium sulfate (3.2.6).

3.2.5 Eluting solvent B: mixture of diethyl ether (3.2.3) and light petroleum (3.2.1) (15:85 by volume).

Dry over 10 g to 25 g of anhydrous sodium sulfate (3.2.6).

3.2.6 Sodium sulfate (Na_2SO_4), granular, anhydrous.

Heat at 500 °C ± 25 °C for 4 h. Cool and store in a stoppered bottle.

3.2.7 Adsorbent: Florisil (Floridin Co[1)]), 60 to 100 mesh.

Activate by heating at 650 °C ± 25 °C for 4 h and immediately pour the adsorbent into well-stoppered bottles and store in the dark. Before use, heat to 130 °C for at least 5 h.

The adsorbent should be stored either at 130 °C ± 2 °C or at room temperature in a desiccator. In the latter case it should, however, be heated to 130 °C ± 2 °C every 2 days.

Each batch of adsorbent should be checked from time to time as follows.

Pass 1 ml of a standard hexane solution containing 0,1 mg/l of lindane, heptachlor epoxide, aldrin and dieldrin, and 0,3 mg/l of endrin through the adsorption column (see ISO 3890-1:2000, 9.3). Elute and concentrate as described in 3.4.3. Determine by gas chromatography.

The adsorbent is satisfactory if lindane, heptachlor, aldrin and heptachlor epoxide are found quantitatively in the eluting solvent A (3.2.4) and dieldrin and endrin in the eluting solvent B (3.2.5).

3.2.8 Sodium chloride solution (NaCl), 2 % solution.

Heat solid sodium chloride at 500 °C ± 25 °C for 4 h before making up the solution.

3.2.9 Ethanol (C_2H_5OH), absolute.

3.2.10 Sodium oxalate ($Na_2C_2O_4$) or **potassium oxalate** ($K_2C_2O_4$)

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1) Floridin Co is an example of suitable a product available commercially. This information is given for the convenience of users of this part of ISO 3890 and does not constitute an endorsement by ISO of this product.

3.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

3.3.1 Chromatographic columns, of internal diameter 20 mm and length 300 mm, and with PTFE stopcocks and sintered glass discs or glass wool plugs.

3.3.2 Rotary evaporator (Kuderna-Danish[2)] or equivalent), with flask of capacity 500 ml, and with graduated tube attached.

3.3.3 High-speed blender

3.3.4 Separating funnels, of capacities 125 ml and 1 000 ml.

3.4 Procedure

3.4.1 Extraction of fat and organochlorine compounds

3.4.1.1 General methods.

See ISO 3890-1:2000, annex A.

3.4.1.2 Special method for cheese

Place enough diced sample (to provide 3 g of fat), about 2 g of sodium or potassium oxalate (3.2.10) and 100 ml of ethanol (3.2.9) in a high-speed blender (3.3.3) and blend for 2 min to 3 min. (If experience with the product indicates that emulsions will not be broken by centrifuging, add 1 ml of water per 2 g of sample before blending.) Pour the homogenized slurry into a 500 ml centrifuge bottle, add 50 ml of diethyl ether (3.2.3), and shake vigorously for 1 min. Add 50 ml of light petroleum (3.2.1) and shake vigorously for 1 min to 2 min (or divide between two 250 ml bottles and extract each by shaking vigorously for 1 min to 2 min with 25 ml of light petroleum. Proceed as in ISO 3890-1:2000, A.6.3.3.

3.4.2 Liquid-liquid partitioning

Weigh, to the nearest 0,01 g, 1 g to 3 g of the extracted fat into a 125 ml separating funnel (3.3.4) and dissolve in 15 ml of light petroleum (3.2.1). Add 30 ml of acetonitrile saturated with light petroleum (3.2.2) and shake vigorously for 1 min to 2 min. After phase separation, run the lower acetonitrile layer into a 1 000 ml separating funnel (3.3.4) containing 700 ml of sodium chloride solution (3.2.8) and 100 ml of light petroleum (3.2.1). Vigorously shake the light petroleum layer left in the 125 ml separating funnel three times with 30 ml portions of the acetonitrile (3.2.2).

Combine the acetonitrile extracts in the 1 000 ml separating funnel and then shake carefully. Drain the lower, aqueous phase into a second 1000 ml separating funnel and shake it for 12 s with 100 ml of light petroleum. Combine the light petroleum phases from the two 1 000 ml separating funnels. Wash twice with 100 ml portions of water or the sodium chloride solution (3.2.8). Dry over sodium sulfate (3.2.6) and filter into the 500 ml rotary evaporator flask (3.3.2) with attached graduated tube. Rinse the sodium sulfate three times with 10 ml portions of light petroleum (3.2.1). Then concentrate the light petroleum solution to 10 ml using the rotary evaporator (3.3.2).

3.4.3 Clean-up on Florisil

Add to a chromatographic column (3.3.1) a 100 mm layer of adsorbent (3.2.7). Cover with a 10 mm layer of sodium sulfate (3.2.6) and rinse with 40 ml to 50 ml of light petroleum (3.2.1). Pipette 10 ml of the light petroleum concentrate (3.4.2) onto the column (3.3.1), rinsing the container twice with approximately 5 ml portions of light petroleum. Elute into an evaporator flask (3.3.2) with attached graduated tube, using 200 ml of the eluting solvent A (3.2.4). The elution rate should not exceed 5 ml/min. Change the receivers and elute in the same way using 200 ml of the eluting solvent B (3.2.5).

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2) Kuderna-Danish is an example of suitable equipment available commercially. This information is given for the convenience of users of this part of ISO 3890 and does not constitute an endorsement by ISO of this equipment.

Concentrate the two eluates separately to the required small volume using the rotary evaporator (3.3.2). Examine each eluate by GLC. Should further purification be necessary, this can be carried out on a second, freshly prepared adsorbent column or as in ISO 3890-1:2000, annex A.

The first eluate contains any HCB, the HCH isomers, heptachlor, heptachlor epoxide, aldrin, DDE, TDE and DDT. The second eluate contains dieldrin and endrin.

3.5 Gas chromatography

See ISO 3890-1:2000, 6.2. For preliminary tests, etc., see ISO 3890-1:2000, clauses 10 to 14.

4 Method B: Liquid-liquid partitioning with dimethylformamide (DMF) and clean-up on an alumina column (see references [4], [5])

4.1 Principle

The organochlorine compounds, together with the fat, are extracted from the test sample by the procedure described in ISO 3890-1:2000, clause A.6, then the residues are partitioned with dimethylformamide. After addition of sodium sulfate solution, the organochlorine compounds are further partitioned into *n*-hexane. The organic phase is purified by chromatography on neutral aluminium oxide using *n*-hexane as the eluting solvent. The eluate is concentrated then examined by GLC.

Special methods are described for milk and butter.

4.2 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

4.2.1 ***n*-Hexane** [$CH_3(CH_2)_4CH_3$], boiling range 68 °C to 70 °C.

Examine for gas chromatographic purity under working column conditions. Distil over potassium hydroxide, if necessary.

4.2.2 **Acetone** (CH_3COCH_3), general-purpose, reagent grade.

4.2.3 **Dimethylformamide (DMF)**

Examine an *n*-hexane extract of a dilute aqueous solution for interference peaks by GLC. Redistil the solvent, if necessary, and collect the fraction with boiling range 152 °C to 154 °C.

4.2.4 ***n*-Hexane**, saturated with dimethylformamide.

4.2.5 **Dimethylformamide**, saturated with *n*-hexane.

4.2.6 **Sand**, acid washed.

Heat at 500 °C for 4 h, then cool and store in a stoppered bottle.

4.2.7 **Sodium sulfate** (Na_2SO_4), granular, anhydrous.

Heat at 500 °C for 4 h, then cool and store in a stoppered bottle.

4.2.8 **Aluminium oxide** (Al_2O_3), neutral, activated.

Heat aluminium oxide to 500 °C for 4 h, then cool. Carefully add 7 parts of water to 93 parts of aluminium oxide (mass fraction) and mix the solid thoroughly in a closed vessel for at least 90 min. Keep the vessel well stoppered and use the aluminium oxide within 10 days.

4.2.9 Sodium sulfate solution, 2 % solution.

4.3 Apparatus

Usual laboratory apparatus and, in particular, the following.

4.3.1 Soxhlet extraction apparatus

4.3.2 Rotary evaporator (Kuderna-Danish[2)] or equivalent), with flask of capacity 500 ml, and with graduated tube attached.

4.3.3 High-speed blender

4.3.4 Chromatographic columns, of internal diameter 12 mm and length 300 mm, with PTFE stopcocks.

4.3.5 Micro-Snyder[3)] columns

4.4 Procedure

4.4.1 Extraction of fat and organochlorine compounds

4.4.1.1 General methods

See ISO 3890-1:2000, annex A.

4.4.1.2 Special methods

a) Milk

Transfer in the following order, 40 ml of well-mixed milk, 80 ml of acetone (4.2.2) and 80 ml of *n*-hexane (4.2.1) to a 250 ml vortex beaker. Homogenize the mixture for 3 min. Transfer it immediately to a 250 ml centrifuge tube, washing the mixer blades with 10 ml of *n*-hexane, then with 5 ml of water, and add the washings to the tube.

Spin the tube in a centrifuge at a rotational frequency of 2 500 min^{-1} for 5 min. Separate the *n*-hexane solvent layer and pass it through a short column of anhydrous sodium sulfate (4.2.7). Wash the contents of the tube with two successive 25 ml portions of *n*-hexane and run the washings through the column. Reduce the combined extracts to about 15 ml in the rotary evaporator (4.3.2). Transfer the solution to a 100 ml separating funnel graduated at 25 ml and adjust the volume to 25 ml. [See also method E, 7.4.1.2 b) for milk.]

b) Butter

Dissolve 5 g of clarified butterfat (melted and decanted through a filter) in 10 ml of *n*-hexane. Transfer the solution to a 100 ml separating funnel using three successive 5 ml portions of *n*-hexane.

4.4.2 DMF-partitioning of fat and organochlorine compounds

Extract the fat from the 25 ml of hexane solution (4.4.1) with 10 ml of dimethylformamide (DMF) saturated with *n*-hexane (4.2.5), by shaking in a separating funnel. After 2 min to 3 min, run the lower DMF layer into a second

3) Micro-Snyder is an example of suitable equipment available commercially. This information is given for the convenience of users of this part of ISO 3890 and does not constitute an endorsement by ISO of this equipment.

100 ml separating funnel (retaining any interfacial emulsion in the first separating funnel). Repeat the extraction of the *n*-hexane solution with two further 10 ml portions of DMF (4.2.5). Combine the DMF extracts and wash them with 10 ml of *n*-hexane saturated with DMF (4.2.4).

Separate the 10 ml of *n*-hexane and wash with a further 10 ml of DMF (4.2.5). Reject the *n*-hexane and add the washings to the original 30 ml of DMF extract in a 500 ml (or preferably 350 ml) separating funnel. Add 6 ml of *n*-hexane (4.2.1) and shake vigorously for 2 min with 200 ml of sodium sulfate solution (4.2.9).

Allow to stand for 20 min to separate. Collect the *n*-hexane phase by gentle swirling. Drain the aqueous layer to waste, dry the separating funnel with filter paper and drain the *n*-hexane into a graduated tube with a ground-glass neck which will hold 15 ml of solvent. Rinse the separating funnel with small quantities of *n*-hexane and add these to the tube.

Fit the tube with a micro-Snyder column (4.3.5) and concentrate the *n*-hexane extract to about 2 ml.

4.4.3 Clean-up on aluminium oxide with *n*-hexane

Pour a slurry of 5 g of prepared aluminium oxide (4.2.8) in *n*-hexane (4.2.1) into a chromatographic column (4.3.4) containing a solvent-washed cotton wool plug (ISO 3890-1:2000, A.5.15). Allow the aluminium oxide to settle and cover it with a 30 mm layer of anhydrous sodium sulfate (4.2.7). Drain the *n*-hexane until the meniscus reaches the top of the sodium sulfate layer. Add the *n*-hexane extract (4.4.2) and wash into the column with 2 ml portions of *n*-hexane.

Elute at a flow rate not exceeding 5 ml/min with 50 ml of *n*-hexane (4.2.1), collecting the eluate in the rotary evaporator (4.3.2). Concentrate the eluate to approximately 5 ml. Detach the graduated tube, fit a micro-Snyder column (4.3.5) and concentrate the eluate to 1 ml.

4.5 Gas chromatography

See ISO 3890-1:2000, 6.2. For preliminary tests, etc., see ISO 3890-1:2000, clauses 10 to 14.

5 Method C: Liquid-liquid partitioning with dimethylformamide (DMF) and clean-up on a Florisil column (see reference [6])

5.1 Principle

The organochlorine compounds, together with the fat, are extracted from the sample by the procedure described in 5.4.1. The extract is concentrated almost to dryness, then dissolved in light petroleum. The organochlorine compounds are partitioned into dimethylformamide. After addition of sodium sulfate solution, the organochlorine compounds are further partitioned into light petroleum.

The organic phase is purified by chromatography on Florisil, using light petroleum/diethyl ether as the eluting solvent. The eluate is concentrated then examined by GLC.

5.2 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

5.2.1 **Light petroleum**, boiling range 30 °C to 40 °C, redistilled.

5.2.2 **Diethyl ether** ($C_2H_5OC_2H_5$), peroxide free.

5.2.3 **Light petroleum**, boiling range 60 °C to 80 °C, redistilled.

5.2.4 **Eluting solvent**, mixture of diethyl ether (5.2.2) and light petroleum (5.2.1) (6:94 by volume).