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

**Part 1:
General considerations and extraction
methods**

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

Partie 1: Considérations générales et méthodes d'extraction

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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 2.

The main task of technical committees is to prepare International Standards. 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 document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 3890-1|IDF 75-1 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

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ISO 3890|IDF 75 consists of the following parts, under the general title *Milk and milk products — Determination of residues of organochlorine compounds (pesticides)*:

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— *Part 1: General considerations and extraction methods*

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

This second edition of ISO 3890-1|IDF 75-1 cancels and replaces the first edition (ISO 3890-1:2000), of which it constitutes a minor revision.

Foreword

IDF (the International Dairy Federation) is a non-profit organization representing the dairy sector worldwide. IDF membership comprises National Committees in every member country as well as regional dairy associations having signed a formal agreement on cooperation with IDF. All members of IDF have the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of Standing Committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of the IDF National Committees casting a vote.

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

ISO 3890-1|IDF 75-1 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by IDF and ISO.

All work was carried out by the former Joint ISO-IDF Group of Experts (E12 — *Pesticide residues*) which is now part of the Joint ISO-IDF Action Team on *Organic contaminants and veterinary residues* of the Standing Committee on *Analytical methods for additives and contaminants*.

ISO 3890|IDF 75 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*

This edition of ISO 3890-1|IDF 75-1, together with ISO 3890-2|IDF 75-2, cancels and replaces IDF 75C:1991, of which it constitutes a minor revision.

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

Part 1: General considerations and extraction methods

WARNING — The use of this part of ISO 3890|IDF 75 may involve hazardous materials, operations and equipment. This part of ISO 3890|IDF 75 does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this part of ISO 3890|IDF 75 to establish health and safety practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 3890|IDF 75 describes general considerations and specifies extraction methods for the determination of residues of organochlorine pesticides in milk and milk products.

A method for high-fat products is specified in Annex A.

Guidance is given on the conduct of analyses in the presence of polychlorinated biphenyls (PCBs) in Annex B.

The methods are applicable to: α -HCH; β -HCH; γ -HCH; aldrin/dieldrin; heptachlor and heptachlorepoxyde; isomers of DDT, DDE, TDE; chlordane and oxychlordane; and endrin. Certain methods are applicable to δ -ketoendrin and HCB.

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 3890-2|IDF 75-2, *Milk and milk products — Determination of residues of organochlorine compounds (pesticides) — Part 2: Test methods for crude extract purification and confirmation*

ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

ISO 5725-2, *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*

3 Terms and definitions

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

3.1 contents of organochlorine compounds

mass fraction of substances determined using the procedures specified in this part of ISO 3890|IDF 75

NOTE It is expressed in milligrams per kilogram, either on a fat basis or on a product basis (for low-fat products).

4 Principle

NOTE The methods are based on a four-stage process; two stages may sometimes be combined, in whole or in part.

Residues from the sample substrate are extracted by appropriate solvents, so as to obtain the maximum efficiency of extraction of the residues and minimum co-extraction of any substances which may give rise to interference in the determination.

Interfering materials are removed from the extract to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

The content of organochlorine compounds is determined by gas-liquid chromatography (GLC) with electron-capture detection.

The identity of the observed pesticide residues is confirmed, particularly in those cases where it would appear that the maximum permitted level has been exceeded.

Interference of PCBs and pesticides is a well-known problem in packed columns and to a lesser extent in capillary columns. For relatively high levels of PCBs, determination of PCBs according to ISO 8260|IDF 130^[8] is recommended.

The applicability of the various methods is given in Table 1.

5 Requirements for reagents and materials

5.1 General.

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity. Redistil water and solvents used and check their purity (see 5.2). The limit of the impurity of each of the reagents used shall not exceed the limit of determination defined in 14.4. The total impurity of all reagents used in the method, however, may exceed that limit. Purify and periodically activate adsorbents according to the requirements of the different analytical methods. Check their purity (see 5.2.5).

Every precaution shall be taken to avoid possible contamination of water, solvents, adsorbents, etc. by plastic or rubber materials.

Store all purified reagents, adsorbents, etc. in glass bottles with glass stoppers or with polytetrafluoroethylene (PTFE) wads in the caps. Do not leave them exposed to the atmosphere after purification. Acetone-washed aluminium foil provides suitable protection in many situations.

5.2 Check for purity of reagents.

5.2.1 Solvents. Concentrate solvents by the factor required by the method to be used. Test for purity by GLC (see 6.2). The chromatogram shall not show any interfering impurity whose concentration exceeds the limit of determination defined in 14.4. Extract or concentrate acetonitrile, dimethylformamide (DMF) and methylene chloride in the same volumes as used in the method and examine the resulting solution by gas chromatography.

Table 1 — Application of methods to various compounds

Method	α -HCH ^a	β -HCH ^a	γ -HCH ^a	Aldrin/ dieldrin	Heptachlor Heptachlor- epoxide	DDT ^b DDE ^c TDE ^d isomers	Chlordane Oxy- chlordane	Endrin	δ - Keto- endrin	HCB ^e
A	+	+	+	+	+	+	+	+	–	–
B	+	+	+	+	+	+	+	+	–	–
C	+	+	+	+	+	+	+	+	–	+
D	+	+	+	+	+	+	+	+	–	+
E	+	+	+	+	+	+	+	+	–	+
F	+	+	+	+	+	+	+	+	+	+
G	+	+	+	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+	+	–	+

Key: + applicable
– not applicable

^a HCH = 1,2,3,4,5,6-hexachlorocyclohexane
^b DDT = 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
^c DDE = 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene
^d TDE = 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
^e HCB = hexachlorobenzene

5.2.2 Water. Extract 10 parts by volume of water with 1 part by volume of *n*-hexane or light petroleum. Separate the organic phase. Concentrate by the factor required by the method used and test for purity by GLC (see 6.2). The chromatogram shall not show any interfering impurity whose concentration exceeds the limits of determination defined in 14.4.

5.2.3 Inorganic salts. Extract inorganic salts (e.g. sodium chloride), after purification according to the requirements of the different analytical methods, and any aqueous solutions used with *n*-hexane or light petroleum. Concentrate the extract by the factor required by the method used and test for purity by GLC (see 6.2). The chromatogram shall not show any interfering impurity whose concentration exceeds the limit of determination defined in 14.4.

5.2.4 Cotton wool, glass wool and quartz wool. Extract these with *n*-hexane and acetone using a Soxhlet extractor, until they are sufficiently free from interfering substances.

5.2.5 Adsorbents. Elute an amount of adsorbent equal to that used in the analytical method with the corresponding type and volume of solvent mixture. Concentrate the eluate as indicated in the analytical method and test for purity by GLC (see 6.2). The chromatogram shall not show any interfering impurity whose concentration exceeds the limit of determination defined in 14.4. Check the activity of adsorbents regularly.

5.2.6 Standard solutions. Use materials of at least 95 % mass fraction purity to prepare standard solutions for pesticide residue analysis.

If stored at $-20\text{ }^{\circ}\text{C}$, they are generally stable for 1 to 2 years. Stock solutions of concentration 1 mg/ml, kept in a refrigerator at about $4\text{ }^{\circ}\text{C}$, are usually stable for 2 to 3 months. Prepare dilute solutions freshly each day.

NOTE Changes in volume by solvent evaporation, e.g. through the pores between a glass stopper and the neck of a flask, can be a source of error.

Store standard solutions in glass bottles in a refrigerator and take every precaution to avoid possible contamination by plastic or rubber materials. Do not expose standard solutions to sunlight or ultraviolet light for extended periods. Mass spectrometry and GLC may be used to examine analytical standards for impurities. Experience has shown that faults introduced in the preparation, handling and storage of standards and standard solutions are a major source of error.

6 Requirements for apparatus

6.1 General. Thoroughly clean all glassware used for residue analysis. Hot chromic/sulfuric acid solution may be used for cleaning. If this solution is used, wash the glassware well afterwards with distilled water and acetone before drying. Immediately before use, rinse the glassware again with the solvent to be used.

Do not use ordinary plastics stoppers [e.g. poly(vinyl chloride) (PVC)] in vessels for storing standards as they may lead to contamination. Glass or PTFE stoppers are necessary. Similarly, do not use separating funnels with plastic stoppers or stopcocks. Wash bottles shall be all glass. Replace ordinary stoppers with glass or PTFE stoppers.

Most methods specify particular chromatographic columns, which shall be specially made and have glass or PTFE stopcocks. The tops of the columns shall have ground-glass joints to permit attachment of a solvent reservoir or pressure adapter. Occasionally, a ground-glass joint below the tap may be useful for applying suction using a suitable Büchner flask.

Two types of solvent evaporators may be used. First, the Kuderna-Danish¹⁾ (or its equivalent) evaporator (see Reference [13]), which may be used with or without its fractionating column and which is heated on a steam bath. Second, the various types of rotary film evaporators (marketed commercially), which require a source of vacuum, preferably a water vacuum pump, and which can be heated to a temperature above $50\text{ }^{\circ}\text{C}$. The effect of the type of solvent evaporator on the loss of volatile pesticides should be checked periodically. A "keeper" (propylene glycol, *n*-undecane or hexadecane) may be used to minimize loss of pesticides.

If homogenizers are used, take care to ensure that they are kept free of contamination. Check bottom-drive macerators for leaks around the drive. The various seals can be a source of contamination.

Tapered tubes fitted with 14 mm standard ground-glass joints and having a capacity of about 15 ml (i.e. 80 mm to 90 mm long) are required for final concentrations. These may be fitted with micro-Snyder¹⁾ columns (see Reference [14]). Solutions are often reduced to a final small volume by passing a stream of air or nitrogen over them. Do not use rubber or PVC tubing for this purpose: PTFE or nylon tubing usually presents the least danger of contamination.

It may be necessary to extract filter papers with solvent.

Steam baths and water baths are also required with adequate support for the apparatus used in them.

Centrifuges capable of handling several hundred millilitres of emulsion at rotational frequencies of $2\ 000\ \text{min}^{-1}$ to $4\ 000\ \text{min}^{-1}$ are sometimes required.

1) Example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 3890|IDF 75 and does not constitute an endorsement by ISO or IDF of this product.

6.2 Gas-liquid chromatography apparatus. A suitable GLC system shall be used, preferably equipped with separate heaters for the injector, detector and column oven. The facility to inject directly on to the GLC column is generally an advantage. Although the choice of the different parts of the GLC system is a matter for the experience of the analyst, the following recommendations are made.

- a) Electron-capture detectors (^3H , ^{63}Ni) have proved to be most useful for the determination of organochlorine compounds. Adjust the detectors according to the manufacturer's instructions. Check the variations in detector sensitivity periodically by verifying the linearity of the calibration graphs using standard solutions of pesticides (see 5.2.6). Do not use ^3H detectors if temperatures above $225\text{ }^\circ\text{C}$ are required.
- b) Fused silica or glass columns of length between 1,5 m and 3 m and of internal diameter 2 mm to 6 mm are preferred.
- c) Use good quality, suitable support materials. [Support materials such as Gas Chrom Q²⁾, Chromosorb W-HP²⁾, Anachrom Q²⁾ in 60/80, 80/100 and 100/120 mesh ranges have been successfully employed.]
- d) A variety of stationary phases and stationary phase mixtures have been used successfully depending upon the amount and type of organochlorine pesticide, including, for example:
 - hydrocarbon: Apiezon L²⁾
 - methylsilicones: DC-11²⁾, DC-200²⁾, OV-1²⁾, QC-101²⁾, SP-2100²⁾, SE-30²⁾
 - methylphenylsilicones: OV-17²⁾, OV-61²⁾, OV-25²⁾, SP-2250²⁾, SE-52²⁾
 - trifluoropropylmethylsilicones: QF-1²⁾, OV-210²⁾, SP-2401²⁾
 - phenylcyanopropylmethylsilicones: ~~ISO OV-2252²⁾~~, XE-60²⁾

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Deposit stationary phases on the support with care, the ratio depends on the support and phase combination chosen. In all cases, condition newly filled columns for at least 24 h at a temperature near the maximum compatible with the type of stationary phase used. Test their efficiency and selectivity at the required operating temperature using standard mixtures of organochlorine compounds.

Capillary gas chromatography is an important technique with a separation power superior to that of packed columns. The capillary technique is recommended especially for complex extracts. Care shall be taken, however, to use capillaries with inactive glass walls, otherwise, at the picogram level, compounds of interest will be lost due to adsorption on the glass surface. To avoid that problem, it is recommended to use fused silica columns.

Use pure, dry nitrogen (oxygen-free, when using an electron-capture detector) or an argon/methane mixture (when using a pulsed electrochemical detector) as carrier gas for packed columns, with a flow rate depending on the size and type of columns used. Control the flow rates according to the column and detector characteristics. Generally, ensure that gas flow rates are controlled as accurately as possible ($\pm 0,5\%$ to $\pm 1,0\%$ of the flow rate). Install molecular sieve filters in all supply circuits and regenerate them periodically. To summarize, make sure that the GLC conditions (i.e. column length, stationary phase type, injector, detector, column temperatures, gas flow rates, etc.) are such that separation of the organochlorine compounds likely to be present is as complete as possible.

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