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

Part 1: General considerations and extraction methods

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.

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 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.

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 heptachlorepoxide; 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-know 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 °C, they are generally stable for 1 to 2 years. Stock solutions of concentration 1 mg/ml, kept in a refrigerator at about 4 °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-Danish[1)] (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 °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-Snyder[1)] 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 min^{-1} to 4 000 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 (^{3}H, ^{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 ^{3}H detectors if temperatures above 225 °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 Q[2)], Chromosorb W-HP[2)], Anachrom Q[2)] 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 L[2)]

— methylsilicones: DC-11[2)], DC-200[2)], OV-1[2)], QC-101[2)], SP-2100[2)], SE-30[2)]

— methylphenylsilicones: OV-17[2)], OV-61[2)], OV-25[2)], SP-2250[2)], SE-52[2)]

— trifluoropropylmethylsilicones: QF-1[2)], OV-210[2)], SP-2401[2)]

— phenylcyanopropylmethylsilicones: OV-225[2)], XE-60[2)]

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 (± 0,5 % to ± 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.

2) 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.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 3890|IDF 75. A recommended sampling method is given in ISO 707|IDF 50[1].

8 Preparation of test sample

8.1 Milk

Adjust the temperature of the test sample to between 35 °C and 40 °C, by means of a water bath if necessary. Mix the test sample thoroughly, but gently, by repeatedly inverting the test sample bottle without causing frothing or churning, and cool quickly to approximately 20 °C.

8.2 Evaporated milk

Shake and invert the container. Open the container, pour the test sample slowly into a second container (provided with an airtight lid) and mix by repeated transfer, taking care to incorporate into the test sample any fat or other constituent adhering to the wall and ends of the first container. Finally, transfer the test sample as completely as possible to the second container.

For test samples in sealed cans, condition the unopened container in a water bath at 40 °C to 60 °C, if necessary. Remove and shake the can vigorously every 15 min. Remove the can after 2 h and allow to cool to room temperature. Remove the lid entirely and thoroughly mix the contents by stirring with a spoon or spatula.

8.3 Sweetened condensed milk

Open the container and mix its contents thoroughly with a spoon or spatula. Use an up-and-down rotary movement in such a way that the top layers and the contents of the lower corners of the container are moved and mixed. Take care to incorporate into the test sample any milk adhering to the wall and ends of the container. Transfer the test sample as completely as possible to a second container (provided with an airtight lid). Close the container.

For test samples in sealed cans, condition the unopened can in a water bath at between 30 °C and 40 °C, if necessary. Open the can, transfer to a dish large enough to permit stirring thoroughly, then mix until the whole mass is homogeneous.

For a test sample in a collapsible tube, open the tube and transfer the contents to a jar. Then cut open the tube, scrape out all material adhering to the interior and add this to the contents of the jar.

8.4 Powdered milk products

Thoroughly mix the test sample by repeatedly rotating and inverting the container. If necessary, transfer all of the test sample to an airtight container of sufficient capacity.

8.5 Butter and butterfat

Heat the test sample to about 60 °C until the fat separates. Decant through a plug of glass wool into a preheated glass funnel.

8.6 Cheese

Separate the fat from the test sample as specified in ISO 3890-2|IDF 75-2.

8.7 Other milk products

Ensure that the test sample is homogeneous.

9 Procedure

9.1 General

Operators shall thoroughly familiarize themselves with the method before starting regulatory analyses. Run reagent blanks until the reagents are found to be satisfactory.

Also carry out “spiked” recovery experiments at levels appropriate to the maximum permitted level until they are found to be satisfactory (see Clause 13). Follow exactly the same procedure for each analysis without introducing any variation.

If it is not possible to complete the analyses in one day and it is necessary to interrupt them for the night, store the sample extract in the form of a solution in an anhydrous solvent in a well-stoppered vessel in a refrigerator at between 0 °C and 5 °C. Do not interrupt the extraction, column chromatography, etc.

Always consider the use of other, more up-to-date or new techniques leading to the same or improved results.

9.2 Extraction

Weigh a specified amount of test samples, preferably in whole grams (± 1 % mass fraction). Allow frozen material to thaw before maceration as in some cases frozen samples can give problems in extraction. Each period of maceration shall be of at least 2 min.

9.3 Clean-up

Carry out separations in a separating funnel for at least 2 min each, with vigorous shaking and occasional release of pressure by opening the stopcock with the funnel inverted. If vigorous shaking produces very stable emulsions, gentle shaking for longer periods may be preferable. Emulsions may be broken up by adding 1 ml to 2 ml of saturated sodium chloride or sodium sulfate solution, by warming under the hot water tap or by centrifuging (see Clause 6). When separating layers, leave any emulsified interface with the portion to be re-extracted or discarded. The rate of elution of chromatographic columns shall be specified; it is generally in the range 1 ml/min to 5 ml/min.

At this stage of the procedure, the addition of a known quantity of the volatile pentachlorobenzene (or 1,7-dibromoheptane) and a less volatile internal standard (e.g. mirex, 1,2,3,4-tetrachloronaphthalene or isodrin) is advised. Use pentachlorobenzene as an indicator for possible losses of pesticides during the evaporation step by comparison of its peak height (area) with the peak height (area) of the less volatile internal standard. The internal standard may be used for identification (relative retention time) and quantification purposes.

Evaporation of organic solvent solutions shall not be allowed to go to complete dryness unless so specified.

10 Preliminary tests

Inject into the gas chromatograph (see 6.2) an appropriate volume of between 1,0 µl and 10 µl, depending on the system, of the purified extracts obtained according to the analytical method used. The chromatogram obtained shall enable both the nature and the approximate concentration of the compounds present in the extracts to be established.

11 Quantitative determination

If the preliminary tests indicate that the residue approaches or exceeds the permitted level, check the results by using at least one further GLC column of different polarity. If the results obtained using this column are in agreement, examine at least two new sample extracts.

For the quantitative determination, prepare two standard solutions (see 5.2.6) of the organochlorine compounds identified (see Clause 10) in the solvent to be used for the final extract, normally light petroleum or *n*-hexane. Their concentrations shall encompass the probable concentration expected in the final extracts (see also Clause 10). Then inject equal volumes of the final extracts obtained and of the two standard solutions into the gas chromatograph (see 6.2). Injection of the purified portions of the sample extract shall be preceded and followed by injection of the two standard solutions. Measure peak heights or peak areas.

The results obtained from any two injections of the same standard solution shall agree to within about 5 %. Inclusion of an internal standard may be useful (see 9.3).

12 Confirmatory tests

Use the procedures for the confirmation of the identity of observed organochlorine compounds, particularly in those cases in which it would appear that the maximum residue limit (MRL) has been exceeded. The methods specified in ISO 3890-2⏐IDF 75-2 allow the residue to be identified from the retention times of the compounds on the GLC columns. At least two columns of different polarities shall be used. Additionally, procedures such as glass-capillary chromatography, thin layer chromatography, mass spectrometry, P-value (partition coefficient), the GLC of oxidation and other conversion products, and similar techniques are of value. See also ISO 3890-2⏐IDF 75-2.

13 Evaluation of results

13.1 Calculation of results

Calculate the concentration of organochlorine compounds in the test sample from the ratio of the chromatograms of the sample and the standard or standard series. Express this concentration on a sample basis or fat basis (see 13.2.1) according to procedure, product and amount of test sample. Recoveries shall be at least 80 % or the result shall be rejected.

13.2 Presentation and expression of results

13.2.1 Normally, the organochlorine pesticide content of milk products is expressed on a fat basis. However, for milk products with a low fat content, it is better to express results on sample basis because the fat content of these low-fat dairy products varies widely depending upon which method is used to extract the fat (see Annex A).

13.2.2 It is advisable to determine the fat content by an appropriate method (see Bibliography) and to report the results together with the organochlorine pesticide content. Furthermore, state how the organochlorine pesticide content has been expressed, i.e. in milligrams per kilogram on a fat basis or on a sample basis.

NOTE A cut-off point of 2 % mass fraction fat is a practical and workable compromise.

13.2.3 Where no residue approaches or exceeds the permitted level, report the value found from a single determination.

13.2.4 If one or more residues approach or exceed the permitted level, proceed as follows.

a) State the mean mass fraction and range for each organochlorine compound. Do not correct the mean mass fraction for the percentage recovery of the organochlorine compound.

b) State the mean percentage mass fraction recovery and the limit of determination for each relevant organochlorine compound.

c) Give details of the repeatability normally obtained in terms of the range of differences between results measured from data obtained in the laboratory from the analysis of spiked samples of the same product, or those with incurred residues.

d) Give details of the reproducibility normally obtained at the mean mass fraction measured in the sample, extrapolated from the data given in 13.2.2.

14 Precision

14.1 Evaluation of precision

Evaluate the precision of the analytical method in accordance with the requirements of ISO 5725-1 and ISO 5725-2. Some general criteria, based on experience, are given in 14.2 and 14.3 as guidance for the analyst.

14.2 Repeatability

Each laboratory should periodically determine its own repeatability by analysing samples which have been spiked with appropriate organochlorine compounds, or preferably by using samples with “incurred” residues, at concentrations near to the maximum permitted levels. Such samples should be of the same product as the test samples and should be introduced as routine samples, if possible, without any indication being given as to their special nature.

The difference between the maximum and minimum of three test results shall be less than the values given in Table 2. Determine intermediate values by interpolation from a log-log graph.

Table 2 — Repeatability

Residue level mg/kg	**Difference** mg/kg
0,01	0,005
0,1	0,025
1	0,125
NOTE In this example, 0,01 mg/kg is near the limit of determination.	

14.3 Reproducibility

Use Table 3, which is based on experimental evidence. Determine intermediate values by interpolation from a log-log graph.

Table 3 — Reproducibility

Residue level mg/kg	**Difference** mg/kg
0,01	0,01
0,1	0,05
1	0,25
NOTE In this example, 0,01 mg/kg is near the limit of determination.	

14.4 Limit of determination

Theoretically, the limit of determination of the product concerned is defined as that mass fraction, in milligrams per kilogram, of the organochlorine compound which would correspond, on a chromatogram of an extract of the said product, to the lowest measurable peak of height, h, given by the following equation:

$$h = h_{B1} + 2W_{B1}$$

where

h_{B1} is the numerical mean value of the height of the peak for the blank above the normal baseline at the corresponding retention time;

W_{B1} is the numerical value of the mean amplitude of background noise of the blank at the corresponding retention time (W_{B1} may be determined graphically as being the mean background noise width of the blank).

The limit of determination depends on the degree of purification, the nature of the substrate and the GLC conditions (particularly the type and temperature of the column, the carrier gas and the sensitivity of the detector). Since these conditions cannot be specified exactly, the limit of determination shall be established for each procedure and in each laboratory. As a general rule, the limit of determination for a pesticide residue should be less than 10 % of its maximum residue limit. If, however, the maximum residue limit is 0,05 mg/kg or less, a limit of determination of 20 % of this value is sufficient, except where the maximum residue limit is set at or about the level of determination.

15 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, together with reference to this part of ISO 3890⏐IDF 75;

d) all operating details not specified in this part of ISO 3890⏐IDF 75, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the corrections made, if a value of more than 2,5 mg is obtained in the blank test for the method;

f) the test result(s) obtained; and if the repeatability has been checked, the final result obtained.

Annex A
(normative)

Extraction of fat and organochlorine compounds and determination of fat content

A.1 General

Organochlorine compounds are associated with the fat phase and are usually expressed, for high-fat (more than 2 % mass fraction) products, in milligrams per kilogram of fat. In such cases, it is not necessary to determine the fat content of the sample, but to measure the organochlorine residues in a known mass of extracted fat. If the fat content of the sample is low (less than 2 % mass fraction), organochlorine levels are reported on the sample basis and it is then necessary to determine the percentage of fat in the sample.

A.2 Determination of fat content

Carry out the determination of fat in low-fat (less than 2 % mass fraction) samples in accordance with either the International Standard appropriate to the product concerned (see Bibliography) or a method known to give comparable results.

The Soxhlet extraction method is not suitable for measurement of fat in milk powders. Instrumental infrared absorption methods of analysis are becoming widely used for milk and milk products and may be used for measurement of fat in low-fat samples.

A.3 Extraction of fat and organochlorine compounds

Extraction methods include:

a) Soxhlet extraction, for non-liquid milk products;

b) column extraction, for all milk products;

c) AOAC extraction method, for milk and liquid products.

CAUTION — Evaporation of organic solvent solutions should not be allowed to go to complete dryness as this may result in loss of organochlorine compounds.

A.4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

A.4.1 Celite 545[3)].

Before use, heat at 600 °C for 4 h. Store in an airtight bottle.

A.4.2 Sodium sulfate (Na_2SO_4), anhydrous.

Before use, heat at 600 °C for 6 h and allow to cool in a desiccator.

A.4.3 Sea sand, acid-washed [e.g. Merck No. 7712[3)]].

Before use, heat at 600 °C for 5 h and allow to cool in a desiccator.

A.4.4 Light petroleum, boiling range 40 °C to 60 °C.

Before use, reflux over sodium hydroxide pellets and distil.

A.4.5 Methanol (CH_3OH) or **ethanol** (CH_3CH_2OH).

A.4.6 Diethyl ether ($C_2H_5OC_2H_5$), peroxide-free.

Redistil before use.

A.4.7 *n*-Hexane [$CH_3(CH_2)_4CH_3$].

Redistil over sodium hydroxide pellets before use.

A.4.8 Acetone (CH_3COCH_3).

Redistil over glass beads before use.

A.4.9 Sodium oxalate ($Na_2C_2O_4$) or **potassium oxalate** ($K_2C_2O_4$).

A.5 Apparatus

Usual laboratory apparatus and, in particular, the following.

A.5.1 Drying oven, capable of being maintained at 102 °C ± 2 °C and at 250 °C ± 25 °C.

A.5.2 Centrifuge, explosion-proof type, provided with glass tubes of capacity 200 ml to 300 ml, and capable of rotating at a frequency of 2 000 min^{-1} to 4 000 min^{-1}.

A.5.3 Soxhlet extraction apparatus, comprising the following:

a) round-bottomed flask, of capacity 500 ml;

b) extraction chamber, of capacity approximately 200 ml;

c) reflux condenser;

d) heat source (e.g. heating mantle).

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3) 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.

A.5.4 Sand, **oil** or **steam baths**, with a capacity of 400 W.

A.5.5 Cylinder, containing dry inert gas, fitted with a cylinder gauge with PTFE line.

A.5.6 Pipettes.

A.5.7 Balance, capable of weighing to the nearest 0,001 g in the range of 0,01 g to 1 000 g.

A.5.8 Rotary evaporator, with evaporation flasks of capacity 500 ml.

A.5.9 Device for shredding foodstuffs of animal origin (e.g. blender, mixer, ball mill).

A.5.10 Extraction column, comprising a glass tube of internal diameter 12 mm and of total length 300 mm, having a capillary exit construction and a top section of length 100 mm and internal diameter 50 mm ± 1 mm.

A.5.11 Watch glasses, of diameter 100 mm.

A.5.12 Mortar, with pestle.

A.5.13 Fluted filter paper, of diameter approximately 300 mm, solvent-washed.

A.5.14 Extraction thimbles (optional), pre-extracted with solvent of highest purity and kept in all-glass chamber under hexane.

NOTE The use of extraction thimbles often results in the presence of impurities in the sample extracts (interference peaks in the gas chromatogram).

A.5.15 Cotton and **glass wool**, chemically pure.

Before use, extract with hexane/acetone and store in a flask under hexane.

A.5.16 Glass funnels, with long and short stems.

A.5.17 Separating funnels.

A.5.18 Glass beads and **glass rods**, solvent-washed.

A.5.19 Beakers, of various sizes.

A.5.20 Volumetric flasks.

A.5.21 Scalpels and **forceps**.

A.5.22 Water pump.

A.6 Extraction procedures

A.6.1 Soxhlet extraction

Heat the 500 ml round-bottomed flask (A.5.3) containing five glass beads (A.5.18) in the drying oven (A.5.1) to 102 °C for 30 min. Cool to room temperature and weigh. Repeat drying until a constant mass is obtained, i.e. until two consecutive weighings differ by no more than 0,01 g.

Weigh the sample directly on a watch glass (A.5.11). For cheese, shred the sample well. Place the sample in the mortar (A.5.12) and grind well in a mixture of sea sand (A.4.3) and sodium sulfate (A.4.2) (1 + 1 parts by mass) to yield a dry powder. Celite 545 (A.4.1) may also be used. The amount of sodium sulfate (A.4.2) and

sea sand (A.4.3) required depends on the quantity and water content of the foodstuff. Transfer the powder quantitatively to a filter paper (A.5.13).

Wipe the mortar, pestle and watch glass with a wad of cotton wool (A.5.15) moistened with light petroleum (A.4.4). Put the cotton wool also in the filter paper (A.5.13) and insert the latter (closed up) into the chamber of the Soxhlet extraction apparatus.

Then fill the weighed flask (A.5.3) with 250 ml of light petroleum (A.4.4) and extract the sample for 6 h under reflux. Remove the solvent in the rotary evaporator (A.5.8) at about 50 °C under reduced pressure using the water pump (A.5.22) and weigh the flask. Repeat this procedure until a constant mass is obtained, i.e. until two consecutive weighings differ by no more than 0,01 g.

A.6.2 Column extraction

Mix a sufficient quantity of sample thoroughly in the mortar (A.5.12) or ball mill (A.5.9) with sufficient sea sand (A.4.3) and sodium sulfate (A.4.2) mixture (1 + 1 parts by mass) to yield a dry product. When extracting milk powders, reconstitute them thoroughly (9 + 1 parts by mass) with distilled water before mixing. Transfer this mixture to an extraction column (A.5.10) previously filled with a small plug of glass wool (A.5.15) and a 20 mm layer of sodium sulfate (A.4.2). Elute the dry column with a mixture of *n*-hexane (A.4.7) and acetone (A.4.8) (2 + 1 parts by volume). The quantity of solvent depends on the mass and nature of the sample. Collect the eluate overnight and concentrate it in a rotary evaporator (A.5.8) as specified in A.6.1.

A.6.3 AOAC extraction method

A.6.3.1 Shake 100 ml of milk with 100 ml of methanol (A.4.5) and 1 g of sodium oxalate (A.4.9) for 1 min in a 500 ml separating funnel (A.5.17). Add 50 ml of diethyl ether (A.4.6) and shake again for 1 min. Repeat with 50 ml of light petroleum (A.4.4).

A.6.3.2 Alternatively, the quantities of sample and reagents may be reduced to half the stated values, adding 50 ml of a mixture (1 + 1 parts by volume) of diethyl ether (A.4.6) and light petroleum (A.4.4). In this case, the mixture should be mixed vigorously for 2 min. Proceed in the same way in the following step.

A.6.3.3 After separating the phases by centrifuging for 5 min at a rotational frequency of 1 500 min^{-1}, transfer the organic phase into another separating funnel (A.5.17) and extract the aqueous phase twice with 50 ml portions of a mixture (1 + 1 parts by volume) of diethyl ether (A.4.6) and light petroleum (A.4.4). Wash the combined solvent phases with 400 ml of water and discard the aqueous layer. Dry the solvent solutions over sodium sulfate (A.4.2) and evaporate to constant mass in the weighed flask using the rotary evaporator (A.5.8).

A.6.4 Extraction for butter

Heat the sample to about 50 °C and decant through a dry, warm filter. Dissolve the butterfat in the appropriate solvent.

Annex B
(informative)

Analysis in the presence of polychlorinated biphenyls (PCBs)

B.1 General

Residues of polychlorinated biphenyls (a mixture of isomers obtained by chlorination of biphenyl) are found frequently in food, mainly food of animal origin, as well as in some packaging materials. As far as extraction and separation are concerned, they show a great similarity to the organochlorine pesticide compounds. In a gas chromatogram, peaks of PCBs can be superimposed on peaks of certain organochlorine pesticide compounds (particularly those of the DDT group) and this makes their quantification rather difficult. As an example, for the more highly chlorinated biphenyls, a PCB content of 1 mg/kg can simulate a residue level of 0,1 mg/kg *p*,*p′*-DDT.

B.2 Separation of PCBs from organochlorine pesticide compounds by column chromatography or preparation of derivatives

Several methods have been developed in order to separate PCBs from organochlorine pesticide compounds, either on silica gel or on Florisil[4)], or on aluminium oxide/charcoal or on charcoal alone. The PCBs are separated from the organochlorine pesticide compounds by the use of solvents having different polarity. The PCBs are eluted first, followed by the elution of the organochlorine pesticides with a more polar solvent. The separation from *p*,*p′*-DDE is sometimes a problem. Traces of polar solvents or variations in activity of the silica gel can lead to a co-elution of a part of the *p*,*p′*-DDE with the PCBs.

PCBs and aldrin are eluted first from the silica gel column by light petroleum, the other organochlorine pesticide compounds being subsequently eluted with a solvent mixture combining acetonitrile/hexane/dichloromethane (1 + 90 + 80 parts by volume).

In cases where the presence of *p*,*p′*-DDE causes difficulties in quantification, the separation of this DDT-metabolite can be achieved by derivatization. Chromium trioxide (CrO_3) in glacial acetic acid oxidizes *p*,*p′*-DDE to *p*,*p′*-dichlorobenzophenone. The PCBs remain essentially unaffected. Treatment of an extract with an alcoholic alkali prior to the chromic acid anhydride oxidation can confirm *p*,*p′*-DDT in the presence of PCBs. In this procedure *p*,*p′*-DDT is first converted by alkali to *p*,*p′*-DDE which is subsequently oxidized to *p*,*p′*-dichlorobenzophenone.

Capillary column gas chromatography can obviate the need for separation by column chromatography and improve the quantification of organochlorine pesticide compounds in the presence of PCBs.

4) 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.

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