INTERNATIONAL STANDARD

ISO 3890-1

First edition
2000-07-01

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

Reference number
ISO 3890-1:2000(E)

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Printed in Switzerland

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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 3890-1 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*

Annex A forms a normative part of this part of ISO 3890. Annex B is for information only.

INTERNATIONAL STANDARD **ISO 3890-1:2000(E)**

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 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 describes general considerations and extraction methods for the determination of residues of organochlorine pesticides in milk and milk products.

Annex A specifies a method for high-fat products.

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

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

Table 1 — Application of methods to various compounds

Method	α-HCH	ß-HCH	γ-HCH	Aldrin/ dieldrin	Heptachlor Heptachlor-epoxide	DDT DDE TDE isomers	Chlordane Oxy-chlordane	Endrin	Delta-keto-endrin	HCB
A	+	+	+	+	+	+	+	+		–
B	+	+	+	+	+	+	+	+		–
C	+	+	+	+	+	+	+	+		+
D	+	+	+	+	+	+	+	+		+
E	+	+	+	+	+	+	+	+		+
F	+	+	+	+	+	+	+	+	+	+
G	+	+	+	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+	+		+

Key: + applicable

– not applicable

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 3890. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 3890 are encouraged to investigate the possibility of applying the most recent editions of the normative documents 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 3890-2, *Milk and milk products — Determination of residues of organochlorine compounds (pesticides) — Part 2: Crude extract purification and confirmation test methods.*

ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions.*

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.*

3 Term and definition

For the purposes of this part of ISO 3890, the following term and definition applies.

3.1
contents of organochlorine compounds
mass fraction of substances determined using the procedures specified in this part of ISO 3890

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.

4.1 Extraction

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.

4.2 Clean-up

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.

4.3 Determination

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

4.4 Confirmation

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 with capillary columns. In the case of relatively high levels of PCBs, it is recommended to determine PCBs according to IDF 130A [14].

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 used reagents 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 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 involved in 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.

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 involved in 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 involved in the method used and test by GLC. 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 % purity to prepare standard solutions for pesticide residue analysis.

If stored at –20 °C, they are generally stable for at least 1 or 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 diluted solutions freshly each day.

NOTE Changes in volume by solvent evaporation, for example through the pores between a glass stopper and the neck of a flask, might 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 gas-liquid chromatography 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. polyvinyl chloride (PVC)] in vessels for storing standards as they may lead to contamination. Glass or polytetrafluoroethylene (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 [1]) which may be used with or without its fractionating column and which is heated on a steam bath. Secondly, 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 (that is 80 mm to 90 mm long) are required for final concentrations. These may be fitted with micro-Snyder columns[1)] (see reference [2]). 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.

[1)] These are examples of suitable products 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 these products.

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 4000 min^{-1} are sometimes required.

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 manufacturers' 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 Gaschrom Q, Chromosorb W-HP, Anachrom Q in 60/80, 80/100 and 100/120 mesh ranges have been successfully employed.)1)

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
  - methylsilicones: DC-11, DC-200, OV-1, QC-101, SP-2100, SE-30
  - methylphenylsilicones: OV-17, OV-61, OV-25, SP-2250, SE-52
  - trifluoropropylmethylsilicones: QF-1, OV-210, SP-2401
  - phenylcyanopropylmethylsilicones: OV-225, XE-60

Deposit stationary phases on the support with care; the ratio depends on the support/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 in the case of 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 EC-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.

7 Sampling

Sampling is not part of the method specified in this part of ISO 3890. A recommended sampling method is given in ISO 707 [3].

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

8 Preparation of test sample

8.1 Milk

Adjust the temperature of the test sample to 35 °C to 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 in 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.

In the case of test samples in sealed cans, condition the unopened container in a water bath at between 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 in 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.

In the case of 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.

In the case of 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. Transfer all of the test sample to an airtight container of sufficient capacity, if necessary for this.

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.

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.

NOTE The use of other, more up-to-date or new techniques leading to the same or even improved results should always be considered.

9.2 Extraction

Weigh a specified amount of test samples, preferably in whole grams (± 1 %). 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 is usually specified but shall generally be in the range of 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 clause 10 too). Then inject equal volumes of the final extracts obtained and of the two standard solutions into the gas chromatograph (see 6.2). It is essential that the injection of the purified portions of the sample extract is 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 described in ISO 3890-2 permit 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, TLC, mass spectrometry, P-value, the GLC of oxidation and other conversion products, and similar techniques are of value. See also ISO 3890-2.

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 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 (see clause 9).

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, in the case of 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 for fat extraction).

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 % 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 In cases where one or more residues approach or exceed the permitted level, proceed as follows.

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

b) State the mean percentage 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 concentration 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 concentration of the organochlorine compound (in milligrams per kilogram) 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 must 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 specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, together with reference to this part of ISO 3890;
- all operating details not specified in this part of ISO 3890, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- the corrections made, if a value of more than 2,5 mg is obtained in the blank test for the method;
- 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 products (more than 2 %), 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 %), 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 %) samples out 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

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

A.4.1 Celite 545[2)]

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

A.4.2 Sodium sulfate, anhydrous (Na_2SO_4).

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

A.4.3 Sea sand, acid-washed (e.g. Merck No. 7712)[2)].

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 approximate 200 ml;

c) reflux condenser;

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

[2)] Celite 545 and Merck No. 7712 are examples of suitable products 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 these products.

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 a 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 weighing differ by no more than 0,01 g.

Weigh the sample directly on a watch glass (A.5.11). In the case of cheese, shred the sample well. Place the sample in the mortar (A.5.12) and grind well in a mixture of sand (A.4.3) and sodium sulfate (A.4.2) (1:1 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 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).