
**Milk and milk products — Determination
of organochlorine pesticides and
polychlorobiphenyls — Method using
capillary gas-liquid chromatography with
electron-capture detection**

*Lait et produits laitiers — Dosage des pesticides organochlorés et des
polychlorobiphényles — Méthode par chromatographie capillaire en
phase gazeuse-liquide avec détection à capture d'électrons*

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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 8260|IDF 130 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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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.

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 8260|IDF 130 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 Joint IDF-ISO Action Team on *Organic contaminants* of the Standing Committee on *Analytical methods for additives and contaminants* under the aegis of its project leader, Mr. R. de Knecht (NL).

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ISO 8260|IDF 130:2008 cancels and replaces IDF 130A:1991, which has been technically revised.

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Introduction

This International Standard is intended for use in the research, monitoring and control of organochlorine compounds in milk and milk products by isolation of these compounds.

In the past, polychlorinated biphenyls (PCBs) were generally determined by “empirical” methods, most of which used “peak pattern comparison” employing gas-liquid chromatography with electron-capture detection (GLC-ECD) and packed columns. For reference compounds, mixtures produced by perchlorination to decachlorobiphenyl (and GLC-ECD determination) or dechlorination to biphenyl were used. The determination was carried out by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) or by gas-liquid chromatography with flame-ionization detection (GLC-FID).

The aforementioned techniques have three important drawbacks:

- 1) By reducing the information on the PCB pattern to only one number (the “PCB content”), the information on the distribution pattern of the isomers is lost. This information is, however, extremely useful for indicating the sources of contamination and for differentiating between background and recent contamination.
- 2) “PCB contents” determined by the methods mentioned above might be obtained in many different ways as, for most PCB data, what is meant by “PCB content” is not clearly defined. Most reported “PCB contents” cannot, therefore, be compared, and interpretation of the data is difficult. “PCB contents” are not always defined as the sum of the chlorobiphenyl isomers present in the sample, as one would normally expect.
- 3) Chlorobiphenyls are individual chemical compounds which have different properties (e.g. biodegradability, toxicological effects, tendency to accumulate). Therefore, it would be highly desirable to determine these compounds separately.

As a broad concept, the analysis of PCBs needs to be applicable to milk and milk products in different countries throughout the world. To achieve this, the following basic considerations apply:

- a) the need to consider the different situations in different laboratories, for example the equipment available, the level of training of the laboratory personnel, the budget available and the special tasks of the laboratory;
- b) the need to define the purpose of the analysis, for example screening, monitoring and control with respect to legal limits, or research;
- c) the need to determine simultaneously the PCB and organochlorine pesticide (OCP) content;
- d) the need to include, as far as possible, information on the PCB isomer-distribution pattern;
- e) the need to define clearly the contents to be reported;
- f) the need to control carefully the separation of the PCBs from the OCPs in order to avoid interference.

Milk and milk products — Determination of organochlorine pesticides and polychlorobiphenyls — Method using capillary gas-liquid chromatography with electron-capture detection

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This International Standard specifies a method for the determination of the contents of individual organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in milk, evaporated milk, sweetened condensed milk, powdered milk products, butter and butterfat, cheese and other milk products.

The method is capable of determining low levels of specific OCPs down to 5 µg of OCP per kilogram of fat and levels of specific PCBs down to 2,5 µg of PCB per kilogram of fat, using capillary gas-liquid chromatography with electron-capture detection (GLC-ECD).

2 Normative references

[ISO 8260:2008](https://www.iso.org/standards/43ebb315-c340-42c5-8285-4e6481d3c877/iso-8260-2008)

<https://www.iso.org/standards/43ebb315-c340-42c5-8285-4e6481d3c877/iso-8260-2008>

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 14156 | IDF 172, *Milk and milk products — Extraction methods for lipids and liposoluble compounds*

3 Terms and definitions

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

3.1

OCP and PCB contents

mass fractions of organochlorine pesticides and polychlorinated biphenyls as determined by the procedure specified in this International Standard.

NOTE 1 For products containing more than 2 % fat, the organochlorine compound content is expressed in micrograms or milligrams per kilogram of fat.

NOTE 2 For low-fat products containing 2 % fat or less, the organochlorine compound content is expressed in micrograms or milligrams per kilogram of product.

4 Principle

The fat and organochlorine compounds are extracted from a test portion. The organochlorine compounds are isolated by cryogenic extraction and cleaned up in two successive operations using C18 and Florisil SPE (solid-phase extraction) cartridges, respectively.

The eluate is concentrated and dissolved in a suitable volume of *n*-hexane. The organochlorine compounds are identified, and also quantified, by capillary gas-liquid chromatography, using trans-nonachlor as an internal standard.

5 Reagents and materials

All reagents shall be of recognized analytical grade and be suitable for pesticide-residue analysis. Water shall be distilled water or water of at least equivalent purity, suitable for pesticide-residue analysis.

WARNING — Several of the solvents used in this International Standard are highly volatile as well as toxic and/or highly inflammable. Observe current safety precautions for handling, use and disposal.

5.1 Acetonitrile (CH₃CN).

5.2 Methylene chloride (CH₂Cl₂).

5.3 Petroleum ether, with a boiling point of 40 °C to 60 °C, distilled, if necessary, using a Raschig column of length at least 500 mm.

5.4 Diethyl ether [(C₂H₅)₂O].

5.5 *n*-Hexane (C₆H₁₄), distilled, if necessary, using a Raschig column of length at least 500 mm, or, alternatively, iso-octane (C₈H₁₈).

5.6 Sodium sulfate (Na₂SO₄).

5.7 Sodium oxalate (Na₂C₂O₄).

5.8 Acetone (CH₃COCH₃).

5.9 Methanol (CH₃OH).

5.10 Dodecane (C₁₂H₂₆).

5.11 Eluting solvents, as follows:

5.11.1 Acetonitrile/methylene chloride mixture, ratio 3:1.

Mix 3 volumes of acetonitrile (5.1) with 1 volume of methylene chloride (5.2).

5.11.2 Petroleum ether/diethyl ether mixture I, ratio 1:1.

Mix 50 volumes of petroleum ether (5.3) with 50 volumes of diethyl ether (5.4).

5.11.3 Petroleum ether/diethyl ether mixture II, ratio 98:2.

Mix 98 volumes of petroleum ether (5.3) with 2 volumes of diethyl ether (5.4).

5.11.4 Petroleum ether/diethyl ether mixture III, ratio 85:15.

Mix 85 volumes of petroleum ether (5.3) with 15 volumes of diethyl ether (5.4).

5.12 C18 SPE cartridge, of capacity 6 ml, containing 1 g of filter material of particle size 45 µm, pore size 60 Å (e.g. Mega Bond Elut¹).

5.13 Florisil¹ SPE cartridge, of capacity 6 ml, containing 1 g of filter material of particle size 150 µm to 250 µm.

5.14 Internal-standard solutions, as follows:

5.14.1 Trans-nonachlor stock internal-standard solution, $c(\text{C}_{10}\text{H}_5\text{Cl}_9) = 10 \mu\text{g/ml}$.

5.14.2 Working internal-standard solution, containing 1 000 ng/ml of trans-nonachlor.

Pipette 5 ml of trans-nonachlor stock internal-standard solution (5.14.1) into a 50 ml one-mark volumetric flask. Dilute to the mark with *n*-hexane (or iso-octane) (5.5) and mix.

The concentrations given are for guidance only and can be adjusted to suit different requirements.

If the densities of the solution and solvent are known, the solutions can also be prepared gravimetrically.

5.15 OCP and PCB standard solutions, as follows:

5.15.1 Stock standard solutions, containing 10 µg/ml of each compound.

Prepare separate stock standard solutions for each of the following compounds by dissolving 100 µg of each in *n*-hexane (or iso-octane) (5.5) in a 10 ml one-mark volumetric flask, diluting to the mark with *n*-hexane (or iso-octane) and mixing.

HCB (C_6Cl_6); Endrin ($\text{C}_{12}\text{H}_8\text{Cl}_6\text{O}$); α -HCH ($\text{C}_6\text{H}_6\text{Cl}_6$); *pp'*-TDE ($\text{C}_{14}\text{H}_{10}\text{Cl}_4$); β -HCH ($\text{C}_6\text{H}_6\text{Cl}_6$); *op'*-DDT ($\text{C}_{14}\text{H}_9\text{Cl}_5$); γ -HCH ($\text{C}_6\text{H}_6\text{Cl}_6$); *pp'*-DDT ($\text{C}_{14}\text{H}_9\text{Cl}_5$); Heptachlor ($\text{C}_{10}\text{H}_5\text{Cl}_7$); *op'*-dicofol ($\text{C}_{14}\text{H}_9\text{Cl}_5\text{O}$); Aldrin ($\text{C}_{12}\text{H}_8\text{Cl}_6$); Dicofol ($\text{C}_{14}\text{H}_9\text{Cl}_5\text{O}$); Heptachlor epoxide ($\text{C}_{10}\text{H}_5\text{Cl}_7\text{O}$); Oxychlordane ($\text{C}_{10}\text{H}_4\text{Cl}_8\text{O}$); γ -chlordane ($\text{C}_{10}\text{H}_6\text{Cl}_8$); *op'*-DDE ($\text{C}_{14}\text{H}_8\text{Cl}_4$); α -endosulfan ($\text{C}_9\text{H}_6\text{Cl}_6\text{O}_3\text{S}$); α -chlordane ($\text{C}_{10}\text{H}_6\text{Cl}_8$); *pp'*-DDE ($\text{C}_{14}\text{H}_8\text{Cl}_4$); Dieldrin ($\text{C}_{12}\text{H}_8\text{Cl}_6\text{O}$); *op'*-TDE ($\text{C}_{14}\text{H}_{10}\text{Cl}_4$); 2,4,4'-trichlorobiphenyl ($\text{C}_{12}\text{H}_7\text{Cl}_3$, IUPAC No. 28); 2,5,2',5'-tetrachlorobiphenyl ($\text{C}_{12}\text{H}_6\text{Cl}_4$, IUPAC No. 52); 2,4,5,2',5'-pentachlorobiphenyl ($\text{C}_{12}\text{H}_5\text{Cl}_5$, IUPAC No. 101); 2,3',4,4',5-pentachlorobiphenyl ($\text{C}_{12}\text{H}_5\text{Cl}_5$, IUPAC No. 118); 2,4,5,2',4',5'-hexachlorobiphenyl ($\text{C}_{12}\text{H}_4\text{Cl}_6$, IUPAC No. 153); 2,3,4,2',4',5'-hexachlorobiphenyl ($\text{C}_{12}\text{H}_4\text{Cl}_6$, IUPAC No. 138); 2,3,4,5,2',4',5'-heptachlorobiphenyl ($\text{C}_{12}\text{H}_3\text{Cl}_7$, IUPAC No. 180).

NOTE The concentration used here is for guidance only.

If the densities of the solutions and solvent are known, the solutions can also be prepared gravimetrically.

5.15.2 Working standard solutions I (1 µg/ml).

Pipette 5 ml of each of the stock standard solutions (5.15.1) into separate 50 ml one-mark volumetric flasks. Dilute each to the mark with *n*-hexane (or iso-octane) (5.5) and mix.

5.15.3 Working standard solutions II (10 ng/ml).

Pipette 1 ml of each working standard solution I (5.15.2) into separate 100 ml one-mark volumetric flasks. Dilute each to the mark with *n*-hexane (or iso-octane) (5.5) and mix.

NOTE These working solutions are used for identification purposes only.

If the densities of the solution and solvent are known, the solutions can also be prepared gravimetrically.

1) Mega Bond Elut[®] and Florisil[®] are names of suitable products available commercially. This information is given for the convenience of the users of this International Standard but does not constitute an endorsement by either ISO or IDF of the products named.

5.15.4 Working standard solution III (10 ng of each compound/ml).

Prepare working standard solution III by mixing all the working standard solutions I (5.15.2) and the trans-nonachlor working internal-standard solution (5.14.2), as follows:

Pipette 1 ml of each working standard solution I (5.15.2) into a 100 ml one-mark volumetric flask. Add 1 ml of the trans-nonachlor working internal-standard solution (5.14.2) and mix. Dilute to the mark with *n*-hexane (or iso-octane) (5.5) and mix the solution thoroughly again.

NOTE Working standard solution III is used for quantification purposes.

If the densities of the solutions and solvent are known, the solutions can also be prepared gravimetrically.

6 Apparatus

Usual laboratory equipment and, in particular, the following:

- 6.1 **Analytical balance**, capable of weighing to the nearest 1 mg, with a readability of 0,1 mg.
- 6.2 **Water bath**, capable of maintaining a temperature between 35 °C and 40 °C.
- 6.3 **Water bath**, capable of maintaining a temperature between 40 °C and 60 °C.
- 6.4 **Containers**, of various sizes, with airtight lids, for use in homogenizing samples (see 8.2 to 8.4).
- 6.5 **Refrigerated centrifuge**, capable of producing a radial acceleration of 1 200 *g* at –15 °C, equipped with centrifuge tubes with a volume of at least 5 ml.
- 6.6 **Rotary evaporator**, capable of operating at a temperature of 35 °C to 40 °C, equipped with a vacuum pump, condenser and evaporation flasks.
- 6.7 **Pipettes**, of various sizes.
- 6.8 **Gas chromatograph**, fitted with an electron-capture detector, suitable for the determination of OCPs and PCBs.

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 International Standard. A recommended sampling method is given in ISO 707 | IDF 50.

8 Preparation of sample

8.1 Milk

Adjust the temperature of the sample to between 35 °C and 40 °C using a water bath (6.2). Mix the sample thoroughly, but gently, by repeatedly inverting the sample bottle without causing frothing or churning. Then cool the sample quickly to approximately 20 °C.

8.2 Evaporated milk

Shake and invert the sample in its container. Open the container. Pour the sample slowly into a second container provided with an airtight lid (6.4) and mix by repeated transfer, taking care to incorporate in the sample any fat or other constituents adhering to the wall and ends of the first container. Finally, transfer the sample as completely as possible to the second container. Close the container.

If necessary, in the case of samples in sealed cans for instance, condition the unopened containers in a water bath (6.3) maintained at between 40 °C and 60 °C. Remove and shake the can vigorously every 15 min. After 2 h, remove the can and allow it 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 the sample thoroughly with a spoon or spatula. Use an up-and-down rotary movement so that the top layers and the contents of the lower corners of the container are moved and mixed. Take care to incorporate in the sample any milk adhering to the wall and ends of the container. Transfer the product as completely as possible to a second container provided with an airtight lid (6.4). Close the container.

If necessary, in the case of samples in sealed cans for instance, condition the unopened can in a water bath (6.2) maintained at between 30 °C and 40 °C. Open the can, transfer the contents to a dish large enough to permit stirring thoroughly, and mix until the whole mass is homogeneous.

In the case of a 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 that to the contents of the jar.

8.4 Powdered milk products (standards.iteh.ai)

Thoroughly mix the sample by repeatedly rotating and inverting the container, if necessary after having transferred the whole sample to an airtight container provided with an airtight lid (6.4) and of sufficient capacity.

8.5 Butter and butterfat

Mix the sample with a spoon or spatula.

8.6 Cheese

Grate or mash the cheese, depending on its texture.

8.7 Other milk products

Ensure that the sample is homogeneous.

9 Preparation of test sample

9.1 Extraction of milk

Place 50 ml of prepared sample (see 8.1), 50 ml of methanol (5.9) and 0,5 g of sodium oxalate (5.7) in a 250 ml separation funnel. Shake the funnel and its contents for 1 min. Add 25 ml of diethyl ether (5.4) and shake again for 1 min. Add 25 ml of petroleum ether (5.3) and repeat the shaking for 1 min. Let the funnel stand to allow the phases to separate. In the event of poor phase separation, separate the phases by centrifugation at 1 500 rpm for 5 min.