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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.

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.

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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 Knegt (NL).

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

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

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 (CH_3CN).

5.2 Methylene chloride (CH_2Cl_2).

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 [$(C_2H_5)_2O$].

5.5 *n*-Hexane (C_6H_{14}), distilled, if necessary, using a Raschig column of length at least 500 mm, or, alternatively, **iso-octane** (C_8H_{18}).

5.6 Sodium sulfate (Na_2SO_4).

5.7 Sodium oxalate ($Na_2C_2O_4$).

5.8 Acetone (CH_3COCH_3).

5.9 Methanol (CH_3OH).

5.10 Dodecane ($C_{12}H_{26}$).

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 Elut[1)]).

5.13 Florisil[1)] 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, $c(C_{10}H_5Cl_9) = 10$ µg/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 ($C_{12}H_8Cl_6O$); α-HCH ($C_6H_6Cl_6$); *pp*′-TDE ($C_{14}H_{10}C_{14}$); β-HCH ($C_6H_6Cl_6$); *op*′-DDT ($C_{14}H_9Cl_5$); γ-HCH ($C_6H_6Cl_6$); *pp*′-DDT ($C_{14}H_9Cl_5$); Heptachlor ($C_{10}H_5Cl_7$); *op*′-dicofol ($C_{14}H_9Cl_5O$); Aldrin ($C_{12}H_8Cl_6$); Dicofol ($C_{14}H_9Cl_5O$); Heptachlor epoxide ($C_{10}H_5Cl_7O$); Oxychlordane ($C_{10}H_4Cl_8O$); γ-chlordane ($C_{10}H_6Cl_8$); *op*′-DDE ($C_{14}H_8Cl_4$); α-endosulfan ($C_9H_6Cl_6O_3S$); α-chlordane ($C_{10}H_6Cl_8$); *pp*′-DDE ($C_{14}H_8Cl_4$); Dieldrin ($C_{12}H_8Cl_6O$); *op*′-TDE ($C_{14}H_{10}Cl_4$); 2,4,4′-trichlorobiphenyl ($C_{12}H_7Cl_3$, IUPAC No. 28); 2,5,2′,5′-tetrachlorobiphenyl ($C_{12}H_6Cl_4$, IUPAC No. 52); 2,4,5,2′,5′-pentachlorobiphenyl ($C_{12}H_5Cl_5$, IUPAC No. 101); 2,3′,4,4′,5-pentachlorobiphenyl ($C_{12}H_5Cl_5$, IUPAC No. 118); 2,4,5,2′,4′,5′-hexachlorobiphenyl ($C_{12}H_4Cl_6$, IUPAC No. 153); 2,3,4,2′,4′,5′-hexachlorobiphenyl ($C_{12}H_4Cl_6$, IUPAC No. 138); 2,3,4,5,2′,4′,5′-heptachlorobiphenyl ($C_{12}H_3Cl_7$, 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.

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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

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.

Transfer the organic phase to a conical flask of sufficient capacity and the aqueous phase to another separation funnel. Extract the aqueous phase twice with 50 ml of petroleum ether/diethyl ether solution I (5.11.2). Add both extracts to the conical flask containing the organic phase. Dry the combined extracts by adding about 10 g of sodium sulfate to the conical flask and mixing. Filter the organic phase into an evaporation flask (see 6.6). Evaporate the solvents on the rotary evaporator (6.6) maintained at between 35 °C and 40 °C.

9.2 Extraction of sweetened condensed milk, powdered milk products, butter, butterfat and cheese

Separate the fat from the sample as described in ISO 14156 | IDF 172.

10 Procedure

10.1 General

The method requires that users have experience in capillary gas chromatography. Special consideration shall be given to problems associated with carrier gas impurities, septum and column bleeding, and the injection technique, as well as lack of inertness of the column in the low-picogram range.

10.2 Blank test

In the case of milk, sweetened condensed milk, powdered milk products and cheese, prepare a blank following the procedure outlined in 9.1 or 9.2. In the case of milk, replace the sample by the same amount of water. In the case of sweetened condensed milk, powdered milk products and cheese, follow the procedure outlined in 9.2 but omitting the sample. Dissolve the residue in 3 ml of acetonitrile/methylene chloride mixture (5.11.1) and transfer the solution to a centrifuge tube. Add 100 µl of working internal-standard solution (5.14.2) and mix. Proceed as in 10.3 by centrifuging the tube but omitting the heating of the centrifuge tube.

10.3 Cryogenic extraction

Weigh 0,5 g of the milk fat obtained in 9.1 into a centrifuge tube (see 6.5). Add 100 µl of working internal-standard solution (5.14.2) and 3 ml of acetonitrile/methylene chloride mixture (5.11.1). Mix vigorously. Centrifuge the tube and its contents at a radial acceleration of 1 200 g at approximately −15 °C for 20 min. Transfer the supernatant layer to a separate tube.

Then slowly heat the bottom of the centrifuge tube in a water bath set at 40 °C (6.2) to melt the fat. Repeat the extraction with another 3 ml of acetonitrile/methylene chloride mixture (5.11.1) and repeat the centrifugation. Add the supernatant layer to that already transferred to a separate tube. Evaporate the organic phase at approximately 35 °C under nitrogen until about 2 ml to 3 ml remains. This solution is solution A.

10.4 Clean-up

10.4.1 Clean-up on a C18 SPE cartridge

Prepare a C18 SPE cartridge (5.12) by eluting twice with 5 ml of petroleum ether (5.3), then 5 ml of acetone (5.8) and finally 5 ml of methanol (5.9), each time stopping when the liquid meniscus reaches the upper frit. Discard the eluted solutions.

Add solution A (see 10.3) to the cartridge. Elute the solution until the liquid meniscus reaches the upper frit and leave for 3 min. Then elute the solution with 10 ml of acetonitrile (5.1) using a flow rate of 1 drop every 3 s. Collect the eluted solution in a suitable container. Evaporate this solution under nitrogen at about 35 °C. Watch the evaporation carefully, as continuing to heat when dry might lead to losses in the compounds to be determined. Dissolve the residue in 2 ml to 3 ml of *n*-hexane (5.5) and mix to obtain solution B.

10.4.2 Clean-up on a Florisil SPE cartridge

Prepare the Florisil SPE cartridge (5.13) by eluting with 10 ml of *n*-hexane (5.5) until the liquid meniscus reaches the upper frit.

Add solution B (10.4.1) and allow to stand for 3 min. Then elute solution B with 10 ml of petroleum ether/diethyl ether solution II (5.11.3) using a flow rate of 1 drop/s. Collect the eluted fraction in an evaporation flask (see 6.6).

Then elute with 12 ml of petroleum ether/diethyl ether solution III (5.11.4) using a flow rate of 1 drop every 3 s. Collect the eluted fraction in the same flask as the first fraction and mix.

Add 100 µl of dodecane (5.10) to the flask and mix. Evaporate the contents of the flask on the rotary evaporator (6.6). Dissolve the final extract thus obtained in $\leqslant$ 5 ml of *n*-hexane (or iso-octane) (5.5) to obtain solution C for GC analysis.

It is important that the same solvents are used for the standard solutions and for the test sample.

10.5 Gas chromatography

10.5.1 Conditions

Optimize the gas-chromatographic conditions carefully to achieve good resolution of the compounds to be determined. The following conditions are given as example. The conditions are specific and have to be determined for each instrument and column used.

EXAMPLE

— CPSil5 column, using helium as carrier gas at a pressure of 16 kPa (23 psi);

— initial oven temperature 100 °C, maintained for 2 min and then increased at 7 °C/min to 220 °C, this temperature being maintained for 10 min and finally increased at 3 °C/min to a final temperature of 285 °C;

— injector, having an initial temperature of 50 °C, capable of increasing that temperature at a rate of 150 °C/min to a final temperature of 250 °C and holding that temperature for 52 min;

— detector, capable of maintaining a temperature of 320 °C, using nitrogen at 25 ml/min as make-up gas;

— injection volume 1 µl.

NOTE An example of a chromatogram is given in Annex B. It is advisable to run, with each series of determinations, an internal-standard solution for calibration as well as a blank and a recovery test.

Important results can be confirmed by carrying out additional measurements using a second column with a different polarity (preferably in parallel) or by using mass-spectrometric detection. These procedures have been shown to be good practise in GC-ECD measurements for the confirmation of results by eliminating co-eluting substances.

10.5.2 Identification and quantitative determination

When the order of elution of the organochlorine compounds is not known, first inject the working standard solutions II (5.15.3) and measure the retention time of each of the compounds concerned.

Then inject working standard solution III (5.15.4). Measure the peak heights and retention times of the relevant OCPs and PCBs. Calculate the response factor (see 11.1) and the relative retention time (see 11.5) of each compound.

Inject the test solution, solution C (see 10.4.2). Measure the peak heights and retention times of the relevant OCPs and PCBs. Calculate the relative retention time (see 11.5) and the OCP or PCB content (see 11.3).

Identify the OCPs and PCBs by comparing the relative retention times of (OCB-PCB) working standard solution III (5.15.4) with those measured for the test solution.

If, based on operator experience or the criteria used by the operator, a test sample contains significant amounts of OCPs or PCBs, confirm the identity of each, preferably using mass-spectrographic detection or a second column with a different polarity.

10.6 Blank result

The blank value determined in the blank test (see 10.2) run in parallel shall not exceed 2 µg/kg. If a higher value is obtained, check the procedure and the purity of the chemicals.

11 Calculation and expression of results

11.1 Calculation of the response factor

For each organochlorine compound, calculate the response factor, r_f, using the following equation:

$$r_f = \frac{c_a \times h_i}{c_i \times h_a}$$

where

c_a is the concentration, in nanograms per millilitre, of the organochlorine compound in working standard solution III (5.15.4);

c_i is the concentration, in nanograms per millilitre, of the trans-nonachlor working internal-standard solution (5.14.2);

h_i is the peak height for the trans-nonachlor internal standard in working standard solution III (see 10.5.2);

h_a is the peak height for the organochlorine compound in working standard solution III (see 10.5.2).

11.2 Expression of the response factor

Express the response factor to two decimal places.

11.3 Calculation of the organochlorine compound content

For each organochlorine compound, calculate the content of the compound, w_p, expressed in micrograms per kilogram of fat or product (see 3.1), using the following equation:

$$w_p = \frac{h_s \times c_{s,i}}{h_{s,i} \times m} \times V \times r_f$$

where

h_s is the peak height for the organochlorine compound in the test solution (see 10.5.2);

$h_{s,i}$ is the peak height for the internal standard in the test solution (see 10.5.2);

$c_{s,i}$ is the concentration, in nanograms per millilitre, of the working internal-standard solution (5.14.2) added to the fat sample (see 10.3);

m is the mass, in grams, of the fat sample (see 10.3);

V is the volume, in millilitres, of working internal-standard solution (5.14.2) added to the fat sample (see 10.3);

r_f is the response factor for the organochlorine compound.

11.4 Expression of the result

Give the test result to the nearest whole number when expressed in micrograms per kilogram. When the result is expressed in milligrams per kilogram, give it to two decimal places.

11.5 Relative retention time

For each organochlorine compound, calculate the relative retention time, r_{rt}, using the following equation:

$$r_{rt} = \frac{r_t}{r_{ti}}$$

where

r_t is the retention time of the organochlorine compound (see 10.5.2);

r_{ti} is the retention time of the internal standard (see 10.5.2).

11.6 Expression of the retention time

Express the retention time to three decimal places.

12 Precision

12.1 General

The values given for the repeatability and reproducibility limits are expressed with respect to a 95 % probability level and may not be applicable to concentration ranges and matrices other than those given.

Details of an interlaboratory trial carried out in accordance with ISO 5725-2 on the precision of the method are reported in Annex A.

12.2 Repeatability

The absolute difference between two individual test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will be greater than the values for each compound given in Annex A in not more than 5 % of cases.

12.3 Reproducibility

The absolute difference between two individual test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will be greater than the values for each compound given in Annex A in not more than 5 % of cases.

13 Test report

The test report shall specify:

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

b) the sampling method used, if known;

c) the method used, with a reference to this International Standard;

d) all operational details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test results;

e) the test results obtained or, if the repeatability has been checked, the final quoted results obtained.

Annex A
(informative)

Interlaboratory trial

An international collaborative trial involving 15 laboratories was carried out by the French organization AFSSA on test samples of milk [4]. The results obtained were subjected to statistical analysis in accordance with ISO 5725-2 to give the precision data shown in Table A.1.

The number of laboratories removed as outliers is indicated in parentheses. Exclusion was made on the basis of an internal-variance test. PCB 118 was not present in the samples. Spiking was carried out at a level of 40 µg/kg.

Table A.1 — Interlaboratory study results

Compound	Assigned value [a] µg/kg of fat	Mean recovery %	Number of labs [b]	s_r	RSD_r %	s_R	RSD_R %
α-HCH	39	98	15 (3)	3,4	8,7	17,6	45
β-HCH	33	83	14 (2)	3,1	9,4	10,7	33
HCB	27	68	14 (0)	5,2	19,2	12,7	46
γ-HCH	40	100	15 (2)	3,4	8,5	17,4	43
Heptachlor	36	90	15 (2)	4,1	11,4	12,5	35
Aldrin	31	78	15 (1)	2,7	8,7	11,8	38
HEP epoxide	45	113	15 (1)	3,9	8,7	18,6	42
Oxychlordane	38	95	13 (0)	3,4	12,9	19,9	50
γ-Chlordane	37	93	15 (1)	5,1	13,8	14,8	39
op′-DDE	34	85	14 (1)	5,1	15,0	14,1	41
α-Endosulfan	38	95	15 (0)	5,2	13,7	16,5	43
α-Chlordane	39	98	15 (0)	1,5	3,8	16,6	43
pp′-DDE	36	90	15 (0)	5,7	15,8	15,8	44
Dieldrin	41	103	15 (0)	5,7	13,9	16,4	40
op′-TDE	41	103	14 (0)	4,9	12,0	16,8	39
Endrin	42	105	15 (0)	6,0	14,3	16,0	39
pp′-TDE	41	103	15 (1)	5,2	12,7	19,4	43
op′-DDT	36	90	13 (2)	4,1	11,4	11,9	33
pp′-DDT	39	98	14 (1)	5,5	14,1	14,6	42
op′-Dicofol	45	113	7 (1)	9,1	20,2	30,4	67
Dicofol	45	113	9 (0)	5,5	12,2	11,5	67
PCB 28	45	113	15 (0)	6,8	15,1	20,8	46
PCB 52	38	95	14 (0)	6,3	16,6	17,2	46
PCB 101	32	80	15 (1)	3,1	17,5	11,2	35
PCB 153	30	75	15 (1)	4,3	14,3	11,0	37
PCB 138	26	65	15 (0)	3,7	14,2	12,8	42
PCB 180	26	65	14 (1)	6,2	23,8	11,1	43
Mean	37	98		4,7 [c]		15,6 [c]	

[a] The spiking value was 40 µg/kg of fat.

[b] The total number of participating laboratories was 15. The number of outliers is indicated in parentheses.

[c] The ratio s_R/s_r is >3 due to the complexity of the method and differences in operator experience [4].