BRITISH STANDARD

BS EN
1528-1 : 1997

Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs)

Part 1. General

The European Standard EN 1528-1 : 1996 has the status of a British Standard

ICS 67.040

Committees responsible for this British Standard

The preparation of this British Standard was entrusted to Technical Panel AW/-/3, Food analysis — Horizontal methods, upon which the following bodies were represented:

Association of Public Analysts
Department of Trade and Industry (Laboratory of the Government Chemist)
Food and Drink Federation
Institute of Food Science and Technology
Ministry of Agriculture Fisheries and Food
Royal Society of Chemistry

This British Standard, having been prepared under the direction of the Consumer Products and Services Sector Board, was published under the authority of the Standards Board and comes into effect on 15 June 1997

Amendments issued since publication

Amd. No.	Date	Text affected

The following BSI references relate to the work on this standard:
Committee reference AW/-/3
Draft for comment 94/505374 DC

ISBN 0 580 27379 2

Contents

National foreword

This British Standard has been prepared by Technical Panel AW/-/3 and is the English language version of EN 1528-1: 1996 *Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) Part 1: General* published by the European Committee for Standardization (CEN). EN 1528-1 was produced as a result of international discussions in which the United Kingdom took an active part.

Cross-references

Publication referred to	Corresponding British Standard
EN 1528-2 : 1996	BS EN 1528-2 : 1997 *Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) Part 2 : Extraction of fat, pesticides and PCBs, and determination of fat content*
EN 1528-3 : 1996	BS EN 1528-3 : 1997 *Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) Part 3 : Clean-up methods*
EN 1528-4 : 1996	BS EN 1528-4 : 1997 *Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) Part 4 : Determination, confirmatory tests, miscellaneous*

The Technical Commmittee has reviewed the provisions of ISO 1750, to which normative reference is made in the text, and has decided that they are acceptable for use in conjunction with this standard.

ISO 5725 : 1986, to which informative reference is made in the text, has been superseded by ISO 5725-1 : 1994, ISO 5725-2 : 1994, ISO 5725-3 : 1994, ISO 5725-4 : 1994 and ISO 5725-6 : 1994 which are identical with BS ISO 5725 *Accuracy (trueness and precision) of measurement methods and results*, BS ISO 5725-1 : 1994 *General principles and definitions*, BS ISO 5725-2 : 1994 *Basic method for the determination of repeatability and reproducibility of a standard measurement method*, BS ISO 5725-3 : 1994, *Intermediate measures of the precision of a standard measurement method*, BS ISO 5725-4 : 1994 *Basic method for the determination of the trueness of a standard measurement method*, and BS ISO 5725-6 : 1994 *Use in practice of accuracy values*.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN title page, pages 2 to 12, an inside back cover and a back cover.

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 1528-1

November 1996

ICS 67.040

Descriptors: Food products, edible fats, chemical analysis, determination of content, pesticides, polychlorobiphenyl, gas chromatography, generalities

English version

Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) — Part 1 : General

Aliments gras — Dosage des pesticides et des polychlorobiphényles (PCB) — Partie 1 : Généralités

Fettreiche Lebensmittel — Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) — Teil 1 : Allgemeines

This European Standard was approved by CEN on 1996-10-27. CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Foreword

This European Standard has been prepared by Technical Committee 275, Food analysis, horizontal methods', the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 1997, and conflicting national standards shall be withdrawn at the latest by May 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

This European Standard consists of the following Parts.

– Part 1 *General* presents the scope of the standard and describes general considerations with regard to reagents, apparatus, gas chromatography etc., applying to each of the analytical methods selected.

– Part 2 *Extraction of fat, pesticides and PCBs, and determination of fat content* presents a range of analytical procedures for extracting the fat portion containing the pesticide and PCB residues from different groups of fat containing foodstuffs.

– Part 3 *Clean-up methods* presents the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid—liquid partition, adsorption or gel permeation column chromatography.

– Part 4 *Determination, confirmatory tests, miscellaneous* gives guidance on some recommended techniques for the determination of pesticides and PCBs in fatty foodstuffs and on confirmatory tests, and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

Contents

Introduction

This European Standard comprises a range of multi residue methods of equal status: no single method can be identified as the prime method because, in this field, methods are continuously developing. The methods selected for inclusion in this standard have been validated and are widely used throughout Europe. Any variation in the methods used should be shown to give comparable results.

1 Scope

This European Standard specifies methods for the determination of residues of pesticides and polychlorinated biphenyls (PCBs) in fatty food.

Each method described in this European Standard is suitable for identifying and quantifying a definite range of those non polar organochlorine and/or organophosphorus pesticides which occur as residues in fats and oils as well as in the fat portion of fat containing foodstuffs, both of either animal or vegetable origin. The PCB indicator congeners usually selected for the enforcement of maximum residue limits (MRLs) are determined along with the organochlorine pesticides.

This European Standard contains the following clean-up methods that have been subjected to interlaboratory studies and are adopted throughout Europe.

– Method A. Liquid—liquid partitioning with acetonitrile and clean-up on a Florisil ®[1)] column (AOAC) [1].

– Method B. Liquid—liquid partitioning with dimethylformamide and clean-up on a Florisil® column (Specht) [2].

– Method C. Column chromatography on activated Florisil® (AOAC) [3].

– Method D. Column chromatography on partially deactivated Florisil® (Stijve) [4].

– Method E. Column chromatography on partially deactivated aluminium oxide (Greve & Grevenstuk) [5].

– Method F. Gel permeation chromatography (GPC) (AOAC) [6].

– Method G. Gel permeation chromatography (GPC) and column chromatography on partially deactivated silica gel (Specht) [7].

– Method H. High performance gel permeation chromatography (HPGPC) (MAFF) [8].

The applicability of the eight methods A to H for residue analysis of organochlorine pesticides, PCB indicator congeners, and organophosphorus pesticides, respectively, is given in table A.1. Where no + sign is shown, there are no data available in literature, but this does not necessarily exclude the applicability.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

ISO 1750	*Pesticides and other agrochemicals — Common names*
EN 1528-2 : 1996	*Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) — Part 2 : Extraction of fat, pesticides and PCBs, and determination of fat content*
EN 1528-3 : 1996	*Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) — Part 3 : Clean-up methods*
EN 1528-4 : 1996	*Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) — Part 4 : Determination, confirmatory tests, miscellaneous*

NOTE. See also international standards concerning the determination of fat content, product sampling and preparation of test sample.

3 Principle

3.1 General

The methods described in this European Standard are based on a four stage process (in some cases two stages may be combined, in whole or in part) as described in **3.2** to **3.5**.

3.2 Extraction

Extraction of the residues from the sample matrix by the use of appropriate solvents, so as to obtain the maximum efficiency of extraction of the residue and minimum co-extraction of any substances which can give rise to interferences in the determination.

NOTE. Methods for extraction of fat are recommended which are simultaneously applicable for the extraction and determination of fat and the residue analysis in the fat portion.

3.3 Clean-up

Maximum removal of interfering substances with minimal loss of analyte from the sample extract, so as to obtain a solution of the extracted residue in a solvent which is suitable for quantitative examination by the selected method of determination.

[1)] Florisil® is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named.

3.4 Determination

Gas chromatography (GC) with various detectors, e.g. electron-capture detector (ECD), the thermionic detector (P- or N/P- mode), the flamephotometric detector (FPD), the Hall detector or mass spectrometry (MS) as appropriate.

3.5 Confirmation

Procedures to confirm the identity and quantity of observed residues, particularly in those cases where it would appear that the maximum residue limit has been exceeded.

4 Reagents

4.1 General

Unless otherwise stated, use reagents of the highest purity (i.e. for residue analysis) and only distilled or demineralized water if possible; if this is not possible, redistil the water, solvents, and reagents used as described in annex B and check their purity (see **4.2**). Note that ion-exchange resins used for demineralized water can be a source of interferences. Purify and periodically activate adsorbents according to the requirements of the different analytical methods; check their purity (see **4.2**).

Take every precaution to avoid possible contamination of water, solvents, adsorbents etc. from plastics and rubber materials. If an interference is encountered in a reagent blank determination, then check the purity of all reagents used.

4.2 Check for purity of reagents

4.2.1 *Solvents*

Concentrate solvents by the factor involved in the respective method to be used. Test for purity by GC under the same conditions as used in the method. The chromatogram should not show any interfering impurity. Extract or concentrate acetonitrile, dimethylformamide and dichloromethane in the same volume as used in the method and examine the resulting solution as above by GC.

4.2.2 *Water*

Extract 10 parts by volume of water with one part by volume of *n*-hexane or light petroleum, dichloromethane or any other non water miscible solvent used in the method. Separate the organic phase, concentrate by the factor involved in the respective method and test for purity by GC under the same conditions as used in the method. The chromatogram should not show any interfering impurity.

4.2.3 *Inorganic salts*

Extract inorganic salts, for example sodium chloride, after purification according to annex B or the requirements of the different analytical methods and any aqueous solution used, with *n*-hexane or light petroleum, dichloromethane or any other non water miscible solvent used in the method. Concentrate the extract by the factor involved in the respective method and test by GC under the same conditions as used in the method. The chromatogram should not show any interfering impurity.

4.2.4 *Adsorbents*

Elute an amount of adsorbent equal to that used in the analytical method with the corresponding type and volume of solvent or solvent mixture. Concentrate the eluate as indicated in the analytical method and test for purity by GC. The chromatogram should not show any interfering impurity. Check the activity of adsorbents regularly, for example as described in **5.3.9** of EN 1528-3 : 1996.

4.2.5 *Standard materials and solutions*

Use materials of at least 95 % purity and traceable quality as standards for residue analysis.

Ensure dilute solutions are prepared and checked frequently, and that standard solutions are stored in glass bottles in a refrigerator and every precaution is taken to avoid possible contamination from plastics or rubber materials. Ensure that the standard solutions are not directly exposed to sunlight or ultraviolet light. Examine analytical standards for impurities.

When stored at −20 °C, standard materials are generally stable for at least a year or two. To allow equilibration, allow the standards to come up to room temperature before the containers are opened. Stock solutions of concentration 1 mg/ml, if kept in a spark proof refrigerator (at about 4 °C), are usually stable for 2 to 3 months.

NOTE 1. Changes in volume due to solvent evaporation, for example through the space between a glass stopper and the neck of a flask, can be a source of error.

NOTE 2. Experience has shown that errors introduced in the preparation, handling and storage of standards and standard solutions are major sources of inaccuracies. Experiences obtained by other national, European and international bodies should be observed [9], [10].

4.3 Safety aspects associated with reagents

4.3.1 *General*

The analysis of pesticide and PCB congener residues in a food matrix includes the use of several hazardous chemicals.

The list given in **4.3.2** shows some appropriate safety precautions which shall be observed at all times.

4.3.2 *Pesticides and PCBs*

Many pesticides are extremely toxic by various routes of exposure, especially in their concentrated forms. As an example, the family of organophosphorus pesticides is consistently highly toxic, not only by oral ingestion, but dermally and by inhalation as well. When working with standard materials, standard solutions, etc., the following minimal precautions shall be observed at all times. Consult safety data sheets or labels for additional information.

a) Perform all laboratory sampling, mixing, weighing, etc., under an effective fume removal device in an area having good forced ventilation of non recirculated air; or wear a respirator of the proper type. If the respirator is used, replace cartridges as recommended, since using a contaminated respirator could be worse than wearing no respirator at all.

b) Keep pesticides and PCBs off the skin. Wear clean protective clothing and non permeable gloves (such as polyethylene gloves) as necessary. Wash hands thoroughly with soap and water to avoid contaminating food.

c) Clearly label all containers with the name and concentration of the appropriate pesticide.

d) Study and have readily available information on symptoms of poisoning and first aid treatment for each type of pesticide being handled.

e) Consult a physician about preventive measures and antidotes for use in emergencies when pesticide poisoning is suspected.

f) Follow your organization's procedures when disposing of waste pesticides. The manufacturer can be contacted for advice on disposal problems.

g) Do not enter laboratories working with pesticide residues or other laboratories after handling pesticide formulations until protective clothing and gloves have been removed and hands thoroughly washed with soap and water.

4.3.3 *Hazardous reagents*

Do not let vapours concentrate to a flammable level in the work area, since it is impossible to eliminate all chance of sparks from static electricity, even though electrical equipment is earthed. Use an effective fume removal device to remove these vapours as they are released.

Vapours from certain volatile solvents are highly toxic. Several of these solvents are readily absorbed through skin. Use an effective fume removal device to remove vapours of these solvents as they are released.

A list of some hazardous reagents is given in table 1.

5 Apparatus

5.1 Glassware: general

Clean glassware shall be used for residue analysis. Hot detergent solution may be used for cleaning but afterwards the glassware shall be well rinsed with distilled water and acetone before drying. When a washing machine is used, rinse the glassware after use with acetone then with water. Wash it in the machine with a non chlorinated detergent, rinse with water and dry. In both cases, verify that the detergent does not leave any interfering impurity. It is also advisable to rinse glassware again with the solvent to be used immediately before use.

Common laboratory glassware or equipment such as beakers, round-bottomed flasks, watchglasses, pipettes, filter paper, glass wool, etc. is not listed in the apparatus subclause of each method in detail.

5.2 Special glassware

5.2.1 *Tapered tubes*, suitable for evaporation, fitted with 14 mm ground-glass joints and having a capacity of about 15 ml, 80 mm to 90 mm long, are required for final concentrations. These are preferably calibrated and may be fitted with micro-Snyder columns [11].

5.2.2 *Chromatographic tubes*, specially made and with glass or polytetrafluoroethylene (PTFE) stopcocks are specified in most methods. The tops of the columns should have ground-glass joints to permit attachment of a solvent reservoir or pressure adaptor.

5.3 Auxiliary materials

If necessary, wash filter papers, glass rods and glass beads with pure solvent prior to use. Extract cotton wool, glass wool, and quartz wool with *n*-hexane and acetone or with any other suitable solvent using a Soxhlet extractor, until sufficiently free from interfering substances.

Solutions are often reduced to a final small volume by passing a stream of nitrogen over them. Rubber or polyvinyl chloride (PVC) tubing shall not be used for this purpose. Polytetrafluoroethylene (PTFE) or nylon tubing usually presents the least risk of contamination.

Do not use ordinary plastics, for example PVC stoppers, in vessels for storing standard materials and solutions as they may lead to contamination. Glass or PTFE stoppers are necessary. Similarly, do not use separating funnels with plastic stoppers or stopcocks. Replace ordinary plastic stoppers with glass or PTFE stoppers.

Table 1. Hazardous reagents

Name of reagent	Problem	Comment	Solution
Acetone	Highly flammable	Forms explosive peroxides with oxidizing agents	Use an effective fume removal device
Acetonitrile	Toxic	Avoid contact with skin and eyes	Use an effective fume removal device
Cyclohexane	Highly flammable		Use an effective fume removal device
Dichloromethane	Toxic	Avoid contact with eyes. Avoid breathing the vapours	Use an effective fume removal device
Diethyl ether	Unstable peroxides can form upon long standing or exposure to sunlight in bottles. Extremely flammable	Store protected from light.	Use an effective fume removal device. See also note on peroxides
Dimethylformamide	Toxic. Flammable	Avoid contact with skin and eyes. Can react vigorously with halogenated hydrocarbons	Use an effective fume removal device
Ethanol	Flammable		Use an effective fume removal device when heating or evaporating
Ethyl acetate	Flammable, especially when being evaporated	Irritating to eyes and respiratory tract	Use an effective fume removal device
n-hexane	Highly flammable		Use an effective fume removal device
Iso-octane	Highly flammable		Use an effective fume removal device
Light petroleum	Extremely flammable		Use an effective fume removal device
Methanol	Flammable. Toxic	Avoid contact with eyes. Avoid breathing the vapours. Can react vigorously with sodium or potassium hydroxide plus chloroform	Use an effective fume removal device

NOTE. Peroxides form in diethyl ether, dioxane, and other ethers during storage. They are explosive and have to be destroyed chemically before distillation or evaporation. Exposure to light increases peroxide formation in ethers. Filtration through activated aluminium oxide is reported to be effective in removing peroxides.

5.4 Solvent evaporators

5.4.1 *General*

Solvent evaporators shall have a thermostable water bath, capable of being controlled between ambient temperature and 100 °C and preferably a controller for the vacuum.

The effect of the solvent evaporator on the loss of volatile residues should be checked periodically. A keeper (e.g. propylene glycol, *n*-undecane or hexadecane) may be used to minimize losses of pesticides in certain cases.

Solvent evaporators (see **5.4.2** to **5.4.4**) may be used for concentrating large solvent volumes. For small volumes, the use of a gentle stream of pure, dry nitrogen is more advisable.

5.4.2 *Kuderna-Danish evaporator* [12] (or equivalent) with or without fractionating column, which is heated on a thermostable water bath.

5.4.3 *Rotary film evaporator* (commercially available), which requires a source of vacuum, and can be heated up to a temperature of 50 °C.

5.4.4 *Rotary vacuum evaporator* (commercially available), rotating at speeds up to 1300 min^{-1}, which requires a source of vacuum and which shall have a thermostable water bath.

5.5 Homogenizers

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

5.6 Centrifuges

If required, explosion proof centrifuges may be used, in which centrifuge tubes with several hundred millilitres of emulsion can be spun at rotational frequencies of 2000 min^{-1} to 4000 min^{-1} or more.

5.7 Gas chromatography

Gas chromatography apparatus as described in clause **4** of EN 1528-1 : 1996 shall be used.

6 Procedure

6.1 General

Operators should thoroughly familiarize themselves with the method before starting regulatory analyses. Reagent blanks shall be performed and established as being satisfactory. Also, spiked recovery experiments over a broad range of levels including the maximum residue limit should be carried out and found to be satisfactory (see clause **9**). Additionally, an appropriate reference material should be analysed whenever it is available. Exactly the same procedure should be followed for each analysis and no variations shall be introduced.

Part 7 of CODEX Recommendations for methods of analysis for pesticide residues should be observed [9].

6.2 Small scale procedures

If, in some cases, extractions of fats and residues are to be carried out only with small amounts of sample, solvents and materials (small scale procedure), choose a compatible small scale procedure for clean-up.

If, however, the results obtained by a small scale procedure show that the residues approach or exceed the maximum residue limit, choose the second extraction and clean-up procedure for confirmation with larger amounts of sample, solvents and materials.

6.3 Preparation of the test sample

Carry out the preparation of test samples immediately on their arrival. If this is not possible, store the samples in suitable, well closed containers under deep freeze conditions of at least −18 °C. Ensure that samples delivered to the laboratory which are wholly or extensively spoiled are not used for analysis.

It is not always possible to complete analyses in a day and sometimes it is necessary to store sample extracts overnight. In such a case, ensure that the sample extracts in the form of a solution in an anhydrous solvent are stored either:

a) in a refrigerator (at about 4 °C) in a well stoppered vessel in the dark; or

b) in the dark under deep freeze conditions of at least −18 °C.

Where sample extracts are stored overnight, check sample extracts to ensure that they are stable during overnight storage.

Do not interrupt clean-up steps, such as column chromatography, etc. Specify which parts of the food have been analysed, and indicate what proportion these parts are of the whole sample.

6.4 Preparation of the test portion

Weigh out the specified amount of samples, preferably in whole grams (±1 %). Ensure that frozen material is allowed to thaw before homogenization.

NOTE. In some cases, frozen samples can give problems with extraction. Each period of homogenization should be for at least 2 min.

Where samples of fresh milk or other liquid dairy products can be analysed within a few days, either store in a refrigerator at about 4 °C, thus avoiding the separation of fat upon thawing, or thoroughly mix and divide the laboratory sample into a suitable number of test portions before freezing.

6.5 Extraction

The residues to be analysed in this European Standard are associated with the fat portion of the sample.

The efficiency of the extraction of fat depends on the polarity of the extraction solvent and the nature of the substrate.

NOTE. EN 1528-2 : 1996 presents a range of analytical procedures for extracting the fat portion containing the pesticide and PCB residues from different groups of fat containing foodstuffs.

6.6 Clean-up

In addition to the residues, the extracts obtained in accordance with EN 1528-2 : 1996 or EN 1528-3 : 1996 contain material, including fats and other lipids, which could interfere in the analysis. To purify the crude extracts or the fats and oils to be analysed, several methods can be used, including partitioning, adsorbent column chromatography and gel permeation chromatography.

NOTE. EN 1528-3 : 1996 presents the details of methods A to H for the clean-up of fats and oils or the isolated fat portion, respectively, using techniques such as liquid-liquid partition, adsorption or gel permeation column chromatography.

7 Determination

7.1 Gas chromatography

For determination of residues, GC is used in most cases.

A suitable GC system, preferably equipped with separate heaters for injector, detector and column ovens, should be used. The facility to inject directly onto the GC column is generally of advantage. Although the choice of the different parts of the GC system is a matter for the experience of the analyst, the following general recommendations are made.

The detectors should be properly adjusted, according to the manufacturers' instructions. Variations in detector sensitivity should be checked periodically by verifying the linearity of the calibration curves using standard solutions of pesticides.

The gas chromatographic apparatus shall include an integration system which permits the measurement not only of peak heights but also of peak areas.

It has been found in practice that equivalent results can be achieved despite the adoption of different GC conditions and different makes of instruments. On the other hand, specifying standard GC parameters does not in any way guarantee that the quality of the results generated will be identical.

For typical GC conditions, see annex B of EN 1528-4 : 1996.

7.2 Test for separation efficiency

The separation of 2, 4, 4′-trichlorobiphenyl (No. 28) from 2, 4′, 5 -trichlorobiphenyl (No. 31), which improves as the polarity of the stationary phase increases, can be regarded as a measure of the separation efficiency of the GC system. Steps should also be taken to ensure that there is no interference between 2, 2′, 4 , 5, 5′ - pentachlorobiphenyl (No. 101) and o, p′-DDE or alpha-endosulfan, between 2, 2′, 3, 4, 4′, 5′ -hexachlorobiphenyl (No. 138) and p, p′-DDT, and between 2, 2′, 4, 4′, 5, 5′-hexachlorobiphenyl (No. 153) and endosulfan sulfate.

The chemical names and numbers of some PCB indicator congeners are given in table 2.

If required, other peaks also obtained, e.g. for congeners No. 105, No. 118 and No. 156 may be evaluated quantitatively.

If camphechlor is present in the sample, it is only possible to prevent interference with the PCB determination after separation by column chromatography as specified in **11.5.3** in EN 1528-3 : 1996.

If aldrin, dieldrin, endrin, heptachlor or heptachlor epoxide are to be determined in the presence of PCBs, fractionation on a mini-silica gel column according to **11.5.3** in EN 1528-3 : 1996 is also recommended.

Table 2 PCB indicator congeners

Chemical name	Number
1) 2, 4, 4′-trichlorobiphenyl	28
2) 2, 2′, 5, 5′-tetrachlorobiphenyl	52
3) 2, 2′, 4, 5, 5′-pentachlorobiphenyl	101
4) 2, 2′, 3, 4, 4′, 5′-hexachlorobiphenyl	138
5) 2, 2′, 4, 4′, 5, 5′-hexachlorobiphenyl	153
6) 2, 2′, 3, 4, 4′, 5, 5′-heptachlorobiphenyl	180

8 Confirmatory tests

Analyses for confirmation of the identity and quantity of observed residues should be performed, particularly in those cases in which it would appear that the maximum residue limit has been exceeded.

The methods described in this European Standard permit the residue to be identified from the retention times of the compounds on the GC columns; at least two columns of different polarities should be used. The procedures listed in EN 1528- 4 : 1996 as glass-capillary GC, thin layer chromatography (TLC), the GC of oxidation and other conversion products and similar techniques are of value. Results obtained using MS present the most definitive evidence for confirmation/identification purposes.

NOTE. EN 1528-4 : 1996 *Determination, confirmatory tests, miscellaneous* gives guidance on some recommended techniques for the determination of pesticides and PCBs in fatty foodstuffs and on confirmatory tests, and lists a clean-up procedure for the removal of the bulk of lipids when analysing large quantities of fat.

9 Evaluation

9.1 Calculation

Calculate the concentration of residues of pesticides or PCB congeners in the sample from the ratio of the chromatograms of sample and standard or standard series. Express this concentration on the whole product or on the fat (see clause **11**) according to the requirements of the analysis. The mean of recoveries from replicate determinations should fall within the range 70 % up to 110 %.

NOTE. When working near the limit of determination, this range may not be achieved.

9.2 Precision

9.2.1 *General*

The precision of the analytical method should be evaluated in accordance with the requirements of ISO 5725 [13]. Some general criteria, based on experience, are given in **9.2.2** to **9.2.3** as guidance for the analyst.

9.2.2 *Repeatability conditions*

Each laboratory should periodically determine if its results under repeatability conditions are acceptable by analysing samples which have been spiked with appropriate standard materials at suitable concentrations, e.g. near to the maximum residue limits, or preferably by using samples with incurred residues.

Repeatability conditions are defined as conditions under which mutually independent test results are obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

9.2.3 *Reproducibility conditions*

Reproducibility conditions are defined as conditions under which test results are obtained with the same method on identical test material in different laboratories with different operators using different equipment.

Table 3 shows examples of acceptable differences of test results under reproducibility conditions [14].

Table 3. Examples of acceptable differences of test results under reproducibility conditions

Residue level mg/kg	**Difference** ($\pm$) mg/kg
0,01	0,01
0,1	0,05
1,0	0,25

(In this example 0,01 mg/kg is near the limit of determination.)

Determine intermediate values by interpolation from a log-log graph.

10 Practical limit of determination

Theoretically, the practical limit of determination in the sample concerned is defined as that concentration of the pesticide or PCB congener residue (in milligrams per kilogram) which would correspond, on a chromatogram of an extract of the sample, to the lowest measurable peak area or height, with an acceptable degree of confidence in the result.

The practical limit of determination depends on the degree of purification, the nature of the substrate and the GC conditions (particularly the type and temperature of the column, the carrier gas and the sensitivity of the detector). Since these conditions cannot be laid down exactly, the practical limit of determination should be established for each method and in each laboratory. In general, the practical limit of determination for a residue should be at least one-tenth of its maximum residue limit. If, however, the maximum residue limit is 0,05 mg/kg or less, a practical limit of determination one-fifth of this value is sufficient, except where the maximum residue limit is set at or about the level of determination.

11 Expression of results

Express the pesticide or PCB congener content according to current legislation. Do not correct the mean concentration for the percentage recovery of the residue.

Where no residue approaches or exceeds the maximum residue limit, report the value found from a single determination.

In cases where one or more residues approach or exceed the maximum residue limit proceed as follows.

a) State the mean concentration and range for each residue. If blank values did occur, they should be reported separately without correcting the mean concentrations of the residue.

b) State the mean percentage recovery and practical limit of determination for each relevant residue.

12 Test report

The test report shall contain at least the following data:

– all information necessary for the identification of the sample;

– a reference to the European standard in question or to the method used;

– the results and the units in which the results have been expressed;

– any particular points observed in the course of the test;

– any operations not specified in the method or regarded as optional.

Annex A (normative)
Applicability of methods

Table A.1 Applicability of methods A to H according to reference given in literature

Compound[2)]	Method							
	A	B	C	D	E	F	G	H
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
Organochlorine pesticides								
aldrin (HHDN)	+	+		+	+	+	+	+
cis-chlordane		+		+	+	+	+	+
trans-chlordane		+		+	+	+	+	+
o, p′-TDE (DDD)	+			+		+	+	+
p, p′-TDE (DDD)	+	+	+	+	+	+	+	+
o, p′-DDE	+				+		+	+
p, p′-DDE	+	+	+	+	+	+	+	+
o, p′-DDT	+	+		+	+	+	+	+
p, p′-DDT	+	+	+	+	+	+	+	+
dieldrin (HEOD)	+	+	+	+	+	+	+	+
α-endosulfan					+		+	
β-endosulfan							+	
endrin	+	+		+	+	+	+	+
hexachlorobenzene (HCB)		+		+	+	+	+	+
α-HCH	+	+		+	+	+	+	+
β-HCH	+	+		+	+	+	+	+
γ-HCH (lindane)	+	+		+	+	+	+	+
δ-HCH	+	+		+			+	+
heptachlor	+	+		+	+	+	+	+
heptachlor epoxide	+	+		+	+		+	+
methoxychlor	+	+		+	+	+	+	
mirex	+					+	+	+
oxychlordane		+		+	+		+	+
camphechlor (toxaphene)				+	+	+	+	
PCB indicator congeners	+	+	+	+	+		+	+

[2)] For the full chemical names and structures, see ISO 1750 Pesticides and other agrochemicals — Common names.

Table A.1 Applicability of methods A to H according to reference given in literature *(continued)*

Compound[2)]	Method							
	A	B	C	D	E	F	G	H
	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]
Organophosphorus pesticides								
bromophos		+		+			+	+
bromophos-ethyl							+	+
carbophenothion		+					+	+
chlorfenvinphos		+					+	+
chlorpyrifos				+			+	+
chlorpyrifos-methyl								+
crotoxyphos								+
diazinon	(+)	+					(+)	+
dichlorvos								+
ethion	(+)			+			+	+
famphur								+
fenitrothion							+	+
fenchlorphos (ronnel)	(+)	+		+			+	
fenthion								+
iodofenphos				+			+	+
malathion	(+)	+					+	+
phosmet								+
pirimiphos-methyl							+	+
parathion	(+)	+					+	
parathion-methyl	(+)						+	
phenkapton				+				
tetrachlorvinphos							+	

Key: + applicable, (+) validated for special cases, see [1].

[2)] For the full names and structures, see ISO 1750 Pesticides and other agrochemicals — Common names.

Annex B (informative)
Purification of some solvents and reagents

Acetone	Distilled over glass beads.
Acetonitrile	4000 ml of acetonitrile are mixed with 1 ml of orthophosphoric acid and 30 g of phosphorus pentoxide in a round-bottomed glass flask. Glass beads are added and the mixture is distilled at 81 °C to 82 °C (do not allow the temperature to exceed 82 °C).
Diethyl ether	Distilled over glass beads.
Dimethylformamide	Distilled over glass beads.
Ethanol	Distilled over glass beads.
Light petroleum	Distilled over potassium hydroxide or sodium hydroxide pellets.
Methanol	Distilled over glass beads.
n-hexane	Distilled over sodium hydroxide pellets.
Sodium chloride	Heated at 500 °C for at least 4 h and cooled in a desiccator.
Sodium sulfate	Heated at 500 °C for at least 4 h and cooled in a desiccator.

Annex C (informative)
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List of references

See national foreword.

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