BRITISH STANDARD

BS EN
1528-4 : 1997

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

Part 4. Determination, confirmatory tests, miscellaneous

The European Standard EN 1528-4 : 1996 has the status of a British Standard

ICS 67.040

Committees responsible for this British Standard

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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

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Contents

National foreword

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Cross-references

Publication referred to	Corresponding British Standard
EN 1528-1 : 1996	BS EN 1528-1 : 1997 *Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) Part 1 : General*
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*

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN title page, pages 2 to 10, an inside back cover and a back cover.

EUROPEAN STANDARD
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Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) — Part 4: Determination, confirmatory tests, miscellaneous

Aliments gras — Dosage des pesticides et des polychlorobiphényls (PCB) — Partie 4: Détermination, essais de confirmation, divers

Fettreiche Lebensmittel — Bestimmung von Pestiziden und polychlorierten Biphenylen (PCB) — Teil 4: Verfahren zur Bestimmung und Absicherung, Verschiedenes

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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 CEN/TC 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 Part of EN 1528 gives guidance on some recommended techniques for the determination of pesticides and polychlorinated biphenyls (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.

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.

EN 1528-1 : 1996	*Fatty food — Determination of pesticides and polychlorinated biphenyls (PCBs) — Part 1 : General*
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*

3 General

The methods described in this Part of EN 1528 permit the residues present to be provisionally identified and quantified, by gas chromatographic methods using selective detectors.

All positive results require confirmation of identity and quantity.

The procedures listed for confirmation such as alternative GC columns, alternative GC detectors, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), column fractionation, derivatization, spectral measurements, etc., are all of value. Results obtained using mass spectrometry (MS) present definitive evidence for confirmation/identification purposes.

4 Determination

4.1 Gas chromatography

4.1.1 *General*

A suitable GC system, preferably equipped with separate heaters for injector, detector and column ovens, should be used. 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 manufacturer's instructions. Variations in detector sensitivity should be checked periodically by verifying the linearity of the calibration curves using standard solutions of pesticides.

The quantification unit of the gas chromatographic system needs to include an integration system which permits the calculation 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.

4.1.2 *Columns*

Either packed or capillary columns may be used.

When packed columns are to be used, then glass columns of lengths between 1,5 m and 3 m and of internal diameter (i.d.) 2 mm to 6 mm, are recommended, however, they are not suitable for the separation of PCB congeners.

A robust, inert support should be used. Materials such as Gaschrom Q, Chromosorb W/HP, Anachrom Q in 125 μm to 150 μm (100 to 120 mesh), 150 μm to 190 μm (80 mesh to 100 mesh) or 190 μm to 250 μm (60 to 80 mesh) ranges have been successfully employed.[1)]

[1)] Gaschrom Q, Chromosorb W/HP, Anachrom Q, Apiezon L, DC-11 ... Carbowax 20 M are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

A variety of stationary phases and stationary phase mixtures have been used successfully for a variety of residue analyses. For example, the following types are most frequently used

– Hydrocarbon: Apiezon L;

– Methylsilicones: DC-11, DC-200, OV-1, OV-101, SP-2100, SE-30;

Methylphenylsilicones: OV-17, OV-25, OV-61, SP-2250, SE-52, SE-54;

– Trifluoropropylmethylsilicones: QF-1, OV-210, SF-2401;

Phenylcyanopropylmethylsilicones: DB-1301, DB-1701, OV-225, XE-60;

– Polyethylene glycol: Carbowax 20 M[1)].

Stationary phases should be coated onto the support with care, the ratio depending on the support/phase combination chosen. Newly filled columns should be conditioned for at least 24 h at a temperature near the maximum recommended operating temperature with the type of stationary phase used, and should then be tested for their efficiency and selectivity at the required operating temperature using standard mixtures of pesticides. The end of the column should always be disconnected from the detector during conditioning.

Pure, dry nitrogen (oxygen-free, especially when using an electron capture detector (ECD)), or an argon/methane mixture (in the case of a pulsed ECD), should be used as carrier gas for packed columns. The flow rate depends on the size and type of column used. Generally, gas flow rates should be controlled as accurately as possible. Molecular sieve filters should be installed for all gas supplies and regenerated regularly.

Finally, GC conditions (column length, stationary phase type, injector, detector and column temperatures, gas flow rates, etc.) should be such that the separation of the pesticides and PCBs likely to be present is as complete as possible.

Capillary GC has a separation power superior to that of packed columns. This technique is recommended especially in the case of complex extracts.

Fused silica columns having an internal diameter of 0,20 mm to 0,35 mm and a length of between 20 m and 60 m have proved particularly satisfactory because of their separation efficiency, service life and mechanical properties. Wide-bore columns having an internal diameter of 0,5 mm to 0,8 mm may also be useful in some cases. The following stationary phases are frequently used as coatings:

– SE-30 (equivalent to OV-1, DB-1, CP Sil 5, BP-1, SPB-1, etc.);

– SE-54 (equivalent to DB-5, CP Sil 8, BP-5, SPB-5, etc.);

– OV-17 (equivalent to OV-11, OV-22, SP-2250, DC-710, DB 608, etc.);

– DB 1301(equivalent to DB-624);

– DB-1701(equivalent to OV-1701, CP Sil 19-CB, BP-10, SPB-7, etc.);

– OV 225 (equivalent to DB-225, SIL 43-CB, SPB-2330, etc.);

– WAX (equivalent to DB-WAX, CP-WAX-52-CB, Carbowax 20 M, etc.)[2)]

A test for separation efficiency of capillary columns, is given in **7.2** of EN 1528-1 : 1996.

4.1.3 *Injection techniques*

Various injection techniques are useful such as:

a) Grob splitless injection.

b) On-column injection.

c) Programmed Temperature Vaporization (PTV) injection.

The applicability of these techniques depends on the apparatus used and on special requirements.

4.2 Preliminary tests

Determine the linear dynamic range of detector response under the actual GC conditions used by injecting dilute standard solutions.

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

4.3 Determination

Make sure that all measurements are performed within the linear dynamic range of the system.

Prepare at least two standard solutions of the pesticides or PCB congeners identified in the solvent to be used for the final extract (usually light petroleum or*n*-hexane). Their concentrations should encompass the probable concentration expected in the final extracts. Then inject equal volumes of the final extracts obtained and of the two or more standard solutions into the gas chromatograph. It is essential that the injection of the purified portions of the sample extracts is preceded and followed by injection of the standard solutions.

Measure the peak areas or peak heights. The results obtained from any two injections of the same standard

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[2)] SE-30 ... Carbowax 20 M are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

solution should not differ more than approximately 5 % from each other. Inclusion of an internal standard is useful (see clause **4** of EN 1528-3 : 1996).

It is necessary to ensure that the standard materials and samples are dissolved in the same solvent, otherwise varying evaporation profiles will result, which could lead to changes in the retention times and peak areas or heights. For example, increases in peak heights of 35 % have been observed for PCB congeners on changing from iso-octane to toluene.

The contents of individual PCB congeners should not be added together to obtain the total PCBs since such a value is meaningless. There is also no point in carrying out other extrapolations to a fictitious total content of PCBs (e.g. calculated as Clophen® A 60 [3)] since these are generally based on the incorrect assumption that the PCB distribution pattern in the sample is exactly the same as that of the industrial PCB commercial product.

A determination is only possible if the mean of recoveries from multiple determinations for the substance concerned is in the range 70 % to 110 % for individual determinations. Compliance with this condition has to be checked periodically by repeated measurements of recovery from samples containing known additions of the relevant standard material.

5 Confirmatory tests [1]

5.1 General

When analyses are performed for regulatory purposes it is especially important that confirmatory tests are carried out before reporting adversely on samples containing residues of pesticides not usually associated with that commodity or where maximum residue limits (MRLs) appear to have been exceeded. Contamination of samples with non-pesticidal chemicals occurs from time to time, and in some chromatographic methods these compounds can have similar properties to pesticides and could therefore be misidentified as such. Examples in gas chromatography include the responses of ECD to phthalate esters and of phosphorus-specific detectors to compounds containing sulfur.

Confirmatory tests can be divided into two types: quantitative tests are necessary when MRLs appear to be exceeded, whilst qualitative confirmation of identity is also needed in these cases and when atypical residues are encountered. Qualitative tests can involve chemical reactions or separations where some loss of the residue occurs. Particular problems occur in confirmation when MRLs are set at or about the limit of determination.

The need for confirmatory tests can depend upon the type of sample or its known history. In many substrates, certain residues are nearly always found. For a series of samples of similar origin it could only be necessary to confirm the identity of residues in the initial samples. Similarly, when it is known that a particular pesticide has been applied to the sample material there could be little need for confirmation of identity, although a random proportion of samples should be confirmed. Where control samples are available, these should be used to check the presence of possible interfering substances.

In quantitative confirmation at least one alternative procedure should be used and the lower result reported. In qualitative confirmation, an alternative technique using different physiochemical properties is desirable.

The necessary steps to positive identification are a matter of judgement for the analyst and particular attention should be paid to the choice of a method which will eliminate the effect of interfering compounds. The chosen method will depend upon the availability of suitable apparatus and expertise within the testing laboratory.

As guidance to the analyst a number of alternative procedures for confirmation are given in **5.2** to **5.9**.

5.2 Alternative GC columns

The results obtained in the primary analysis should be quantitatively and qualitatively confirmed using at least one alternative column containing a stationary phase of different polarity. The quantitative results obtained should be within 20 % of the primary analysis and the lower figure should be reported, since the higher figure could have been enhanced by interference from co-extracted material. Further quantitative confirmation is required if the results differ by more than 20 %, except when the MRL is set at or about the limit of determination when a variation of up to 100 % would be acceptable.

In choosing the alternative column material, consideration should be given to separating any other pesticide or PCB residues or interfering compounds known to have retention times on the primary column identical to that of the residue detected. The alternative column may be a packed column or, preferably, a capillary column whose differing resolving power can be utilized. Whilst the use of an alternative gas chromatographic column might not always give positive confirmation, it will often quickly disprove a suspected identity. In either case, further confirmation is required to identify the residue.

[3)] Clophen® A 60 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 this product.

5.3 Alternative GC detectors

When pesticides containing several chemical elements are present, detectors showing enhanced response to these elements may be used. Detectors such as flame photometric (sulfur, phosphorus and tin), alkali flame ionization (phosphorus and nitrogen) and coulometric/conductivity (nitrogen, sulfur and halogens) can give valuable additional information on residues. The sulfur/phosphorus response ratio obtained by using a flame photometric detector can give useful information in the case of phosphorothioates.

5.4 Thin layer chromatography (TLC)

In some instances, confirmation of gas chromatographic findings is most conveniently achieved by TLC. Identification is based on two criteria, R_f value and visualization reaction. The scientific literature contains numerous references to the technique. An IUPAC Report on Pesticides [2] reviews the technique and serves as a convenient introduction. The quantitative aspects of thin layer chromatography are, however, limited. A further extension of this technique involves the removal of the area on the plate corresponding to the R_f of the compound of interest followed by elution from the layer material and further chemical or physical confirmatory analysis.

A solution of the standard pesticide should always be spotted on the plate alongside the sample extract to obviate any problems of non-repeatability of R_f. Over-spotting of extract with standard pesticide can also give useful information. The advantages of TLC are speed, low cost and applicability to heat sensitive materials; disadvantages include (usually) lower sensitivity than GC and frequent need for a more efficient clean-up. In some countries problems can be encountered when high humidity or high temperature cause lack of repeatability.

5.5 High performance liquid chromatography (HPLC)

HPLC can often be used advantageously for the confirmation of residues initially found by gas chromatography or by other techniques and can be in certain circumstances the preferred quantitative technique. Post- or pre-column derivatization, and/or use of different detectors, are further options available to the analyst, especially when heat-sensitivity or low volatility make the compound to be analysed less amenable to gas chromatography.

5.6 Column fractionation

The order of elution from chromatographic columns used for cleaning up sample extracts can help to verify the identity of a compound. Thus an element of confirmation can be built in to the extraction and clean-up procedure.

5.7 Derivatization

5.7.1 *Chemical reactions*

Small scale chemical reactions resulting in degradation, addition or condensation products of pesticides, followed by re-examination of the products by chromatographic techniques, have frequently been used. The reactions result in products possessing different retention times and/or detector response from those of the parent compound. A sample of standard pesticide should be treated alongside the suspected residue so that the results from each can be directly compared. A fortified extract should also be included to prove that the reaction has proceeded in the presence of co-extracted sample material. A review of chemical reactions which have been used for confirmatory purposes has been published [3]. Chemical reactions have the advantages of being fast and easy to carry out, but it is possible that specialized reagents will need to be purchased and/or purified.

5.7.2 *Physical reactions*

A useful technique is the photochemical alteration of a pesticide residue to give one or more products with a reproducible chromatographic pattern [4]. A sample of standard pesticide and fortified extract should always be treated in an exactly similar manner. Samples containing more than one pesticide residue can give problems in the interpretation of results. In such cases pre-separation of specific residues may be carried out using TLC, HPLC or column fractionation prior to reaction.

5.7.3 *Other methods*

Many pesticides are susceptible to degradation/transformation by enzymes. In contrast to normal chemical reactions, these processes are very specific and generally consist of oxidation, hydrolysis or de-alkylation. The products possess different chromatographic characteristics from the parent pesticide and may be used for confirmatory purposes if compared with reaction products using standard pesticides.

5.8 Mass spectrometry (MS)

Results obtained using MS present definitive evidence for confirmation/identification purposes [5], [6]. Where the apparatus is available it is usually the confirmatory technique of choice. There are two principal methods of introducing samples into the instrument. The preferred method utilizes gas chromatographic separation prior to introduction into the mass spectrometer. This allows full mass spectral analysis of the peak observed during the primary analysis. Alternatively, samples may be introduced using the direct insertion probe technique. This method may be used in conjunction with TLC or HPLC when these have been used as initial confirmatory procedures. Residues separated by these techniques are isolated and subjected to MS.

To increase sensitivity, particularly with fast scanning

quadruple instruments, techniques known as single and multiple ion detection have been used. A sufficient number of fragment ions have to be selected to ensure unambiguous identification. Increased sensitivity with respect to the molecular ion can be obtained by using chemical ionization in place of electron-impact. As mass spectrometers are generally sensitive at the nanogram level some extracts from primary gas chromatographic analysis might require concentration before mass spectrometric analysis, particularly when electron-capture detectors have been used for quantification. In some cases additional clean-up will be necessary, particularly if full spectra are to be obtained.

Problems can be encountered with heat sensitive compounds during MS and particular care has to be taken when coupling gas chromatographs to mass spectrometers. As there is almost no differential response to compounds in MS, complications can arise in the presence of co-eluting contaminants.

5.9 Spectral measurements

At present little use is made of infra-red, Raman or nuclear magnetic resonance spectroscopy in pesticide residue analysis. Instrumental techniques using multiple reflection cells, microcells, microprobes, laser light, Fourier Transform NMR, etc. are being developed. These improve the quality of spectra and enhance the sensitivity and it is possible that the application of these techniques will be enlarged as postcolumn detection methods for identification of compounds isolated by chromatographic techniques.

6 Additional clean-up procedure for large quantities of fats using Calflo E®[4] [7]

6.1 General

The more commonly used methods employed for the determination of non polar organochlorine and organophosphorus compounds in fats and fatty products permit the use of only a small amount of sample with the result that sensitivity is limited. More polar lipophilic pesticides usually cannot be determined by these methods, or at least not without interferences, because they cannot be separated sufficiently from fats and other lipids.

The principle of the method described herein is the removal of the bulk of lipids from a fatty solution by a suitable adsorbent. The yielded extracts are then further processed by method B or D (see clauses **6** and **8** of EN 1528-3 : 1996). In this way, the fat sample mass can be raised to 30 g and sensitivity can be improved significantly. Additionally, more polar compounds and metabolites can be determined substantially better than before if subsequent processing is performed using method B.

6.2 Principle

The acetonic solution of the fat to be analysed is mixed thoroughly with the suspension of a synthetic calcium silicate (trade name Calflo E®[4]). The mixture is filtered twice, the volume of the filtrate is measured, and the solution is rotary-evaporated to dryness.

6.3 Reagents and materials

All reagents and materials used shall be suitable for the analysis of residues of pesticides and PCBs and shall be in accordance with clause **4** of EN 1528-1 : 1996. If purification is necessary, the procedures given in annex A of EN 1528-3 : 1996 are appropriate.

6.3.1 *Acetone.*

6.3.2 *Acetonitrile.*

6.3.3 *Iso-octane.*

6.3.4 *Calflo E®*, dried overnight at 130 °C.

6.3.5 *Celite® 545*[4], dried overnight at 130 °C.

6.4 Apparatus

Usual laboratory apparatus and, in particular, the following.

6.4.1 *High-speed blender*, fitted with a leak proof glass jar and an explosion proof motor; or homogenizer.

6.4.2 *Beaker*, capacity 100 ml.

6.4.3 *Büchner porcelain funnel*, 12 cm diameter, with vacuum filtration flask.

6.4.4 *Graduated cylinder*, of capacity 200 ml, high form.

6.4.5 *Round-bottomed flask*, capacity 500 ml and 250 ml, with ground joint.

6.4.6 *Rotary evaporator*, with round-bottomed flask, capacity 500 ml, with a water bath capable of being controlled between 20 °C and 50 °C.

6.4.7 *Fluted filter papers*, 20 cm diameter, exhaustively extracted with acetone.

6.4.8 *Round filter papers*, 12 cm diameter, fast or slow flow rate.

6.5 Procedure

Dissolve 5 g to 30 g of fat in 25 ml of acetone (**6.3.1**) in a beaker. Stir the fat solution into a blender jar containing 200 ml of acetonitrile (**6.3.2**), 20 g of Calflo E® (**6.3.4**) and 10 g of Celite® 545 (**6.3.5**). Rinse

[4] Calflo E® and Celite® 545 are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

the beaker several times with small aliquot portions of acetonitrile (total amount of 25 ml). Blend the resulting 250 ml (V_2) intensively for 2 min, and filter the suspension with suction through a paper filter in a Büchner porcelain funnel. Apply gentle suction only, to ensure that no more than minimal portions of the filtrate can evaporate. Then filter the filtrate through a dry fluted filter paper covered with 3 g of Calflo E® into a graduated cylinder. Measure the volume of the filtrate (V_1), and transfer it, rinsing with acetone, into a round-bottomed flask. Add 2 ml of iso-octane (**6.3.3**), and rotary-evaporate at a bath temperature of 40 °C to a volume of 0,5 ml to 1 ml. Remove the last traces of solvent with a gentle stream of nitrogen at room temperature.

NOTE 1. With sample masses of 10 g to 30 g of different fats and oils, residues after evaporation were found to amount from 0,1 g to 0,7 g. Somewhat larger amounts of lipids were obtained from milk fat and natural olive oil than from refined edible fats so that for these it is best to use sample masses of only 10 g to 15 g. Using wool wax sample masses of 4 g to 10 g, residues after evaporation amounted to 1 g to 2 g.

NOTE 2. To avoid loss of filtrate due to evaporation, filtration with suction should be limited in time before the filter content becomes dry, even if it means obtaining filtrate volumes of only 100 ml to 150 ml.

NOTE 3. Exploratory experiments have shown that dry, fatty foods (dry egg yolk, cocoa powder) and feeds as well as oilseeds (rape, poppy seeds, groundnuts) can be cleaned up directly by the procedure described. The samples are homogenized in the blender jar with 25 ml of acetone, 225 ml of acetonitrile, 20 g of Calflo E® and 10 g of Celite® 545. The suspension is then cleaned up as described in **6.5**. The sample mass has to be adjusted according to the fat content and the amount of lipid left in the extract.

6.6 Evaluation of the fat sample weight

The residue after evaporation, derived from step **6.5**, is equivalent to only a portion (G_{corr}) of the fat sample mass. G_{corr} in g fat is calculated from equation 1:

$$G_{corr} = \frac{G \cdot V_1}{V_2} \qquad ...(1)$$

where

G is the sample mass, in grams;

V_1 is the volume of filtrate after clean-up, in millilitres;

V_2 is the initial volume (here: 250 ml), in millilitres.

6.7 Further processing

For further processing of the residue after evaporation derived from **6.5**, methods B and D (see clauses **6** and **8** of EN 1528-3 : 1996) have proven suitable, see table 1. When samples need to be analysed only for organochlorine and non polar organophosphorus compounds, it is advantageous to use method D since it involves little work and time. By using method B, the more polar organophosphorus compounds and several metabolites can be determined additionally. Further, the extracts are cleaned up more intensively so that method B is appropriate also for the analysis of materials from which Calflo E® removes only a portion of the lipids.

Table 1. Organochlorine and organophosphorus compounds including several metabolites recovered at levels exceeding 70 % (marked thus +) or not recovered (marked thus —) in recovery experiments following further extract clean-up by method B or D

Compound	B	D	Compound	B	D
aldrin (HHDN)	+	+	azinphos-ethyl	+	—
γ-chlordane	+	+	carbophenothion	+	+
chlorfenson	+		chlorfenvinphos	+	—
o, p′-TDE (DDD)	+	+	diazinon	+	—
o, p′-TDE (DDD)	+	+	dioxathion	+	
o, p′-DDE	+	+	ethion	+	—
p, p′-DDE	+	+	fenchlorphos	+	+
o, p′-DDT	+	+	malathion	+	—
p, p′-DDT	+	+	parathion-ethyl	+	—
dieldrin (HEOD)	+	+	parathion- methyl	+	
α-endosulfan	+	+	phosalone	+	—
β-endosulfan	+	—			
endosulfan sulphate	+	—			
endrin	+	+			
fenson	+				
α-HCH	+	+			
β-HCH	+	+			
γ-HCH (lindane)	+	+			
δ-HCH	+	+			
heptachlor	+	+			
heptachlor epoxide	+	+			
hexachlorobenzene	+	+			
methoxychlor	+				
PCB	+				
quintozene	+	+			
tetrasul	+				
camphechlor (toxaphene)	+				

Recoveries of the compounds to be analysed can be determined only in conjunction with further extract clean-up. In an interlaboratory study performed by nine laboratories, recovery experiments were run in which control samples of refined sunflower oil were spiked with different pesticides and metabolites at concentrations ranging from 0,02 mg/kg to 16 mg/kg. Table 1 lists those compounds for which total recoveries of between 70 % and 100 % (in most cases ranging from 80 % to 95 %) were obtained by the reported procedure in conjunction with either method B or D.

Annex A (informative)
Bibliography

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Annex B (informative)
Typical GC operating conditions

B.1 Organochlorine pesticides

B.1.1 *Operating conditions 1*

Column	Fused silica capillary DB-5 (30 m long, 0,25 mm i.d.; film thickness 0,23 μm)
Column temperature	110 °C isothermal for 2 min programmed to rise at 6 °C/min from 110 °C to 245 °C isothermal at 245 °C for 2 min
Detector	Electron capture detector, temperature 350 °C
Injector	Programmable temperature vaporizer (PTV)
PTV program	Time (min)

minus 0,15	Split open
minus 0,10	PTV temperature 40 °C
0,20	Split close
0,25	PTV temperature 250 °C
2,00	Split open
4,00	PTV temperature 40 °C

Split flow rate	50 ml/min

B.1.2 *Operating conditions 2*

Column	Fused silica capillary DB-1701 (30 m long, 0,53 mm i.d.; film thickness 1,0 μm)
Column temperature	80 °C isothermal for 1 min programmed to rise at 30 °C/min from 80 °C to 150 °C and at 5 °C/min from 150 °C to 280 °C
Detector	Electron capture detector, temperature 280 °C
Injector	Programmable temperature vaporizer (PTV)
PTV program	Time (min)

minus 0,15	PTV temperature 40 °C
minus 0,10	Split open
0,20	Split close
0,25	PTV temperature 250 ºC
2,00	Split open
4,00	PTV temperature 40 ºC

B.2 PCB indicator congeners

B.2.1 *Operating conditions 1*

Column	Fused silica capillary CP Sil 8 (50 m long, 0,34 mm i.d.; film thickness 0,24 μm)
Column temperature	90 °C isothermal for 4 min, programmed to rise at 35 °C/min from 90 °C to 160 °C isothermal at 160 °C for 1 min programmed to rise at 3 °C/min from 160 °C to 244 °C, isothermal at 244 °C for 10 min
Injector	Temperature 250 °C, splitless injection (1 min)
Detector	Electron capture detector, temperature 350 °C
Gas flow rates	Helium carrier, $1,5 \times 10^5$ Pa inlet pressure Nitrogen purge gas, 35 ml/min

B.2.2 *Operating conditions 2*

Column	Fused silica capillary DB-1 (25 m long, 0,32 mm i.d.; film thickness 1 μm)
Column temperature	90 °C isothermal for 3 min programmed to rise at 35 °C/min from 90 °C to 160 °C isothermal at 160 °C for 1 min programmed to rise at 2 °C/min from 160 °C to 220 °C and at 5 °C/min from 220 °C to 240 °C isothermal at 240 °C for 10 min
Injector	Temperature 250 °C, splitless injection (1 min)
Detector	Electron capture detector, temperature 300 °C
Gas flow rates	Helium carrier, $0,8 \times 10^5$ Pa inlet pressure Nitrogen purge gas, 35 ml/min

B.3 Organophosphorus pesticides

B.3.1 *Operating conditions 1*

Column	Fused silica capillary DB-1 (30 m long, 0,25 mm i.d.; film thickness 0,25 μm)
Column temperature	Programmed to rise at 50 °C/min from 50 °C to 150 °C and at 10 °C/min from 150 °C to 250 °C
Detector	Thermionic detector in P- or N/P-mode, temperature 275 °C
Injector	Temperature 250 °C

B.3.2 *Operating conditions 2*

Column	Fused silica capillary DB-1301 (30 m long, 0,25 mm i.d.; film thickness 0,25 μm)
Column temperature	Programmed to rise at 50 °C/min from 60 °C to 150 °C at 4 °C/min from 150 °C to 200 °C and at 12 °C/min from 200 °C to 275 °C isothermal at 275 °C for 2 min
Detector	Thermionic detector in P- or N/P-mode, temperature 280 °C
Injection	On-column, ambient temperature

B.3.3 *Operating conditions 3*

Column	Fused silica capillary DB-5 (30 m long, 0,53 mm i.d.; film thickness 1,5 μm)
Column temperature	Programmed to rise at 5 °C/min from 150 °C to 250 °C
Detector	Flame Photometric Detector, P-filter, temperature 250 °C
Injector	Temperature 250 °C

List of references

See national foreword.

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