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BSI Standards Publication

Sludge, treated biowaste and soil — Determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)

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National foreword

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A list of organizations represented on this committee can be obtained on request to its secretary.

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Sludge, treated biowaste and soil - Determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)

Boues, bio-déchets traités et sols - Détermination des biphényles polychlorés (PCB) par chromatographie en phase gazeuse-spectrométrie de masse (CG-SM) et chromatographie en phase gazeuse avec détection par capture d'électrons (CG-DCE)

Schlamm, behandelter Bioabfall und Boden - Bestimmung von polychlorierten Biphenylen (PCB) mittels Gaschromatographie mit massenspektrometrischer Detektion (GC-MS) und Gaschromatographie mit Elektroneneinfangdetektion (GC-ECD)

This European Standard was approved by CEN on 24 May 2012.

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 16167:2012) has been prepared by Technical Committee CEN/TC 400 “Project Committee - Horizontal standards in the fields of sludge, biowaste and soil”, 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of an identical text or by endorsement, at the latest by February 2013, and conflicting national standards shall be withdrawn at the latest by February 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

The preparation of this document by CEN is based on a mandate by the European Commission (Mandate M/330), which assigned the development of standards on sampling and analytical methods for hygienic and biological parameters as well as inorganic and organic determinants, aiming to make these standards applicable to sludge, treated biowaste and soil as far as this is technically feasible.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Polychlorinated biphenyls (PCB) have been widely used as additives in industrial applications where chemical stability has been required. This stability on the other hand creates environmental problems when PCBs are eventually released into the environment. Since some of these PCB compounds are highly toxic, their presence in the environment (air, water, soil, sediment and waste) is regularly monitored and controlled. At present determination of PCB is carried out in these matrices in most of the routine laboratories following the preceding steps for sampling, pretreatment, extraction, clean-up by measurement of specific PCB by means of gas chromatography in combination with mass spectrometric detection (GC-MS) or gas chromatography with electron capture detector (GC-ECD).

This European Standard was developed in the European project 'HORIZONTAL'. It is the result of a desk study “3-12 PCB” and aims at evaluation of the latest developments in assessing PCBs in sludge, soil, treated biowaste and neighbouring fields. Taken into account the different matrices and possible interfering compounds, this European Standard does not contain one single possible way of working. Several choices are possible, in particular relating to clean-up. Detection with both MS-detection and ECD-detection is possible. Two different extraction procedures are described and 11 clean-up procedures. The use of internal and injection standards is described in order to have an internal check on choice of the extraction and clean-up procedure. The method is as far as possible in agreement with the method described for PAHs (see CEN/TS 16181). It has been tested for ruggedness.

This European Standard is applicable and validated for several types of matrices as indicated in Table 1 (see also Annex A for the results of the validation).

Table 1 — Matrices for which this European Standard is applicable and validated

Matrix	Materials used for validation
Sludge	Municipal sewage sludge
Biowaste	Compost

WARNING — Persons using this European Standard should be familiar with usual laboratory practice. This European 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.

IMPORTANT — It is absolutely essential that tests conducted according to this European Standard be carried out by suitably trained staff.

1 Scope

This European Standard specifies a method for quantitative determination of seven selected polychlorinated biphenyls (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153 and PCB180) in sludge, treated biowaste and soil using GC-MS and GC-ECD (see Table 2).

Table 2 — Target analytes of this European Standard

Target analyte		CAS-RN[a]
PCB28	2,4,4'-trichlorobiphenyl	7012-37-5
PCB52	2,2',5,5'-tetrachlorobiphenyl	35693-99-3
PCB101	2,2',4,5,5'-pentachlorobiphenyl	37680-37-2
PCB118	2,3',4,4',5-pentachlorobiphenyl	31508-00-6
PCB138	2,2',3,4,4',5'-hexachlorobiphenyl	35056-28-2
PCB153	2,2',4,4',5,5'-hexachlorobiphenyl	35065-27-1
PCB180	2,2',3,4,4',5,5'-heptachlorobiphenyl	35065-29-3
[a] CAS-RN Chemical Abstracts Service Registry Number.		

The limit of detection depends on the determinants, the equipment used, the quality of chemicals used for the extraction of the sample and the clean-up of the extract.

Under the conditions specified in this European Standard, limit of application of 1 µg/kg (expressed as dry matter) can be achieved.

Sludge and treated biowaste may differ in properties and also in the expected contamination levels of PCBs and presence of interfering substances. These differences make it impossible to describe one general procedure. This European Standard contains decision tables based on the properties of the sample and the extraction and clean-up procedure to be used.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15934, *Sludge, treated biowaste, soil and waste — Calculation of dry matter fraction after determination of dry residue or water content*

EN 16179, *Sludge, treated biowaste and soil — Guidance for sample pretreatment*

EN ISO 5667-15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples (ISO 5667-15)*

EN ISO 16720, *Soil quality — Pretreatment of samples by freeze-drying for subsequent analysis (ISO 16720)*

EN ISO 22892, *Soil quality — Guidelines for the identification of target compounds by gas chromatography and mass spectrometry (ISO 22892)*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 18512, *Soil quality — Guidance on long and short term storage of soil samples*

3 Terms and definitions

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

3.1
polychlorinated biphenyl
PCB
biphenyl substituted by one to ten chlorine atoms

[SOURCE: EN 15308:2008, 3.1]

3.2
congener
member of the same kind, class or group of chemicals, e.g. anyone of the two hundred and nine individual PCB

Note 1 to entry: The IUPAC congener numbers are for easy identification; they do not represent the order of chromatographic elution.

[SOURCE: EN 15308:2008, 3.2]

3.3
critical pair
pair of congeners that will be separated to a predefined degree (e.g. R = 0,5) to ensure chromatographic separation meets minimum quality criteria

[SOURCE: EN 15308:2008, 3.6]

4 Principle

Due to the horizontal character of this European Standard, different procedures for different steps (modules) are allowed. Which modules should be used depends on the sample. A recommendation is given in this European Standard. Performance criteria are described and it is the responsibility of the laboratories applying this European Standard to show that these criteria are met. Using of spiking standards (internal standards) allows an overall check on the efficiency of a specific combination of modules for a specific sample. But it does not necessarily give the information upon the extensive extraction efficiency of the native PCB bonded to the matrix.

After pretreatment according to the methods referred to in 9.2, the test sample is extracted with a suitable solvent.

The extract is concentrated by evaporation: If necessary, interfering compounds are removed by a clean-up method suitable for the specific matrix. The eluate is concentrated by evaporation.

The extract is analyzed by gas chromatography. The various compounds are separated using a capillary column with a stationary phase of low polarity. Detection occurs with mass spectrometry (MS) or an electron capture detector (ECD) (see 8.2.1).

PCBs are identified and quantified by comparison of relative retention times and relative peak heights (or peak areas) with respect to internal standards added. The efficiency of the procedure depends on the composition of the matrix that is investigated.

5 Interferences

5.1 Interference with sampling and extraction

Use sampling containers of materials (preferably of steel, aluminium or glass) that do not change the sample during the contact time. Avoid plastics and other organic materials during sampling, sample storage or extraction. Keep the samples from direct sunlight and prolonged exposure to light.

During storage of the samples, losses of PCBs may occur due to adsorption on the walls of the containers. The extent of the losses depends on the storage time.

5.2 Interference with GC

Substances that co-elute with the target PCB may interfere with the determination. These interferences may lead to incompletely resolved signals and may, depending on their magnitude, affect accuracy and precision of the analytical results. Peak overlap does not allow an interpretation of the result. Asymmetric peaks and peaks being broader than the corresponding peaks of the reference substance suggest interferences.

Chromatographic separation between the following pairs can be critical. The critical pair PCB28 and PCB31 is used for selection of the capillary column (see 8.2.2). If molecular mass differences are present, quantification can be made by mass selective detection. If not or using ECD, the specific PCB is reported as the sum of all PCBs present in the peak. Typically, the concentrations of the co-eluting congeners compared to those of the target congeners are low. When incomplete resolution is encountered, peak integration shall be checked and, when necessary, corrected.

- PCB28 – PCB31
- PCB52 – PCB73
- PCB101 – PCB89 / PCB90
- PCB118 – PCB106
- PCB138 – PCB164 / PCB163

Presence of considerable amounts of mineral oil in the sample may interfere with the quantification of PCB in GC-MS. In presence of mineral oil, GC-ECD may be preferred or mineral oil can be removed using clean-up procedure G (see 10.4.8) using DMF/*n*-hexane.

Presence of tetrachlorobenzyltoluene (TCBT)-mixtures may disturb the determination of the PCB with GC-ECD.

6 Safety remarks

PCBs are highly toxic and shall be handled with extreme care. Avoid contact with solid materials, solvent extracts and solutions of standard PCB with the body shall not be allowed to occur. It is strongly advised that standard solutions are prepared centrally in suitably equipped laboratories or are purchased from suppliers specialised in their preparation.

Solvent solutions containing PCB shall be disposed of in a manner approved for disposal of toxic wastes.

For the handling of hexane precautions shall be taken because of its neurotoxic properties.

National regulations shall be followed with respect to all hazards associated with this method.

7 Reagents

7.1 General

All reagents shall be of recognised analytical grade. The purity of the reagents used shall be checked by running a blank test as described in 10.1. The blank shall be less than 50 % of the lowest reporting limit.

7.2 Reagents for extraction

7.2.1 Acetone (2-propanone), $(CH_3)_2$ CO.

7.2.2 *n*-heptane, C_7H_{16}.

7.2.3 Petroleum ether, boiling range 40 °C to 60 °C.

Hexane-like solvents with a boiling range between 30 °C and 69 °C are allowed.

7.2.4 Anhydrous sodium sulfate, Na_2SO_4. The anhydrous sodium sulfate shall be kept carefully sealed.

7.2.5 Distilled water or water of equivalent quality, H_2O.

7.2.6 Sodium chloride, NaCl, anhydrous.

7.2.7 Keeper substance. High boiling compound, i.e. octane, nonane.

7.3 Reagents for clean-up

7.3.1 Clean-up A using aluminium oxide

7.3.1.1 Aluminium oxide, Al_2O_3.

Basic or neutral, specific surface 200 m^2/g, activity Super I according to Brockmann.

7.3.1.2 Deactivated aluminium oxide

Deactivated with approximately 10 % water.

Add approximately 10 g of water (7.2.5) to 90 g of aluminium oxide (7.3.1.1). Shake until all lumps have disappeared. Allow the aluminium oxide to condition before use for some 16 h, sealed from the air, use it for maximum two weeks.

NOTE The activity depends on the water content. It can be necessary to adjust the water content.

7.3.2 Clean-up B using silica gel 60 for column chromatography

7.3.2.1 Silica gel 60, particle size 63 µm to 200 µm.

7.3.2.2 Silica gel 60, water content: mass fraction $w(H_2O)$ = 10 %.

Silica gel 60 (7.3.2.1), heated for at least 3 h at 450 °C, cooled down in a desiccator and stored containing magnesium perchlorate or a suitable drying agent. Before use heat at least for 5 h at 130 °C in a drying oven. Then allow cooling in a desiccator and add 10 % water (mass fraction) in a flask. Shake for 5 min intensively by hand until all lumps have disappeared and then for 2 h in a shaking device. Store the deactivated silica gel in the absence of air, use it for maximum of two weeks.

7.3.3 Clean-up C using gel permeation chromatography (GPC)

7.3.3.1 Bio-Beads®[1)] S-X3.

7.3.3.2 Ethyl acetate, $C_4H_8O_2$.

7.3.3.3 Cyclohexane, C_6H_{12}.

Preparation of GPC, for example: Put 50 g Bio-Beads® S-X3 (7.3.3.1) into a 500 ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane (7.3.3.3) and ethyl acetate (7.3.3.2) 1:1 (volume) in order to allow the beads to swell; after swirling for a short time until no lumps are left, maintain the flask closed for 24 h. Drain the slurry into the chromatography tube for GPC. After approximately three days, push in the plungers of the column so that a filling level of approximately 35 cm is obtained. To further compress the gel, pump approximately 2 l of elution mixture through the column at a flow rate of 5 ml · min^{-1} and push in the plungers to obtain a filling level of approximately 33 cm.

7.3.4 Clean-up D using Florisil®[2)]

7.3.4.1 Florisil®, baked 2 h at 600 °C. Particle size 150 µm to 750 µm.

7.3.4.2 Iso-octane, C_8H_{18}.

7.3.4.3 Toluene, C_7H_8.

7.3.4.4 Iso-octane/Toluene 95/5.

7.3.5 Clean-up E using silica H_2SO_4/silica NaOH

7.3.5.1 Silica, SiO_2, particle size 70 µm to 230 µm, baked at 180 °C for a minimum of 1 h, and stored in a pre-cleaned glass bottle with screw cap that prevents moisture from entering.

7.3.5.2 Silica, treated with sulfuric acid.

Mix 56 g silica (7.3.5.1) and 44 g sulfuric acid (7.3.8.1).

7.3.5.3 Sodium hydroxide solution, c(NaOH) = 1 mol/l.

7.3.5.4 Silica, treated with sodium hydroxide.

Mix 33 g silica (7.3.5.1) and 17 g sodium hydroxide (7.3.5.3).

7.3.5.5 *n*-hexane, C_6H_{14}.

7.3.6 Clean-up F using benzenesulfonic acid/sulfuric acid

7.3.6.1 3 ml silica gel column, of adsorbent mass 500 mg, particle size 40 µm.

7.3.6.2 3 ml benzenesulfonic acid column, of adsorbent mass 500 mg, particle size 40 µm.

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1) Bio-Beads® 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. Equivalent products may be used if they can be shown to lead to the same results.

2) Florisil® is a trade name for a prepared diatomaceous substance, mainly consisting of anhydrous magnesium silicate. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

7.3.7 Clean-up G using DMF/hexane partitioning

7.3.7.1 Dimethylformamide(DMF), C_3H_7NO.

7.3.8 Clean-up H using concentrated sulfuric acid

7.3.8.1 Sulfuric acid, H_2SO_4 of purity 96 % to 98 % (mass fraction).

7.3.9 Clean-up I using TBA sulfite reagent

7.3.9.1 Tetrabutylammonium reagent (TBA sulfite reagent)

Saturate a solution of tetrabutylammonium hydrogen sulfate in a mixture of equal volume of water and 2-propanol, $c((C_4H_9)_4NHSO_4)$ = 0,1 mol/l, with sodium sulfite.

NOTE 25 g of sodium sulfite should be sufficient for 100 ml of solution.

7.3.9.2 2-Propanol, C_3H_8O.

7.3.9.3 Sodium sulfite, Na_2SO_3.

7.3.10 Clean-up J using pyrogenic copper

WARNING — Pyrogenic copper is spontaneously inflammable. Suitable precautions shall be taken.

7.3.10.1 Copper(II)-sulfate pentahydrate, $CuSO_4 \cdot 5\ H_2O$.

7.3.10.2 Hydrochloric acid, c(HCl) = 2 mol/l.

7.3.10.3 Zinc granules, Zn, particle size 0,3 mm to 1,4 mm.

7.3.10.4 Anionic detergent aqueous solution (e.g. 35 g/100 ml, n-dodecane-1-sulfonic acid sodium salt ($CH_3(CH_2)_{11}SO_3Na$).

NOTE Other commercially available detergents may also be suitable.

7.3.10.5 Deoxygenated water

7.3.10.6 Pyrogenic copper

Dissolve 45 g copper(II)-sulfate pentahydrate (7.3.10.1) in 480 ml water containing 20 ml hydrochloric acid (7.3.10.2) in a 1 000 ml beaker.

Take 15 g of zinc granules size (7.3.10.3), add 25 ml water and one drop of anionic detergent solution (7.3.10.4) in another 1 000 ml beaker.

Stir with a magnetic stirrer at a high speed to form a slurry. Then whilst stirring at this high speed, carefully add the copper(II)-sulfate solution drop by drop using a glass rod.

Hydrogen is liberated and elemental pyrogenic copper is precipitated (red coloured precipitate).

Stirring is continued until the hydrogen generation almost ceases. Then the precipitated copper is allowed to settle. The supernatant water is carefully removed and the product washed with deoxygenated water (7.3.10.5) three times, to eliminate residual salts.

Then the water is carefully replaced with 250 ml acetone (7.2.1) (whilst continuously stirring the mixture). This operation is repeated twice more to ensure elimination of water.

Then the above procedure is repeated three times with 250 ml hexane (7.3.5.5), to ensure elimination of the acetone.

Carefully transfer the copper with hexane into an Erlenmeyer flask and store under hexane. The flask shall be sealed to prevent ingress of air and stored in an explosion-proof refrigerator 2 °C to 8 °C.

The shelf life of the pyrogenic copper is at least two months. The clean-up efficiency then declines. The copper changes colour as the clean-up efficiency decreases.

7.3.11 Clean-up K using silica/silver nitrate

7.3.11.1 Silver nitrate, $AgNO_3$.

7.3.11.2 Silver nitrate/silica adsorbent

Dissolve 10 g of $AgNO_3$ (7.3.11.1) in 40 ml water and add this mixture in portions to 90 g of silica (7.3.5.1). Shake the mixture until it is homogenous and leave it for 30 min. Put the mixture into a drying oven at (70 ± 5) °C. Within 5 h regular increase the temperature from 70 °C to 125 °C. Activate the mixture for 15 h at 125 °C. Store the mixture in brown glass bottles.

7.4 Gas chromatographic analysis

Operating gases for gas chromatography/ECD or MS, of high purity and in accordance with the manufacturer's specifications.

7.5 Standards

7.5.1 General

Choose the internal standards substances whose physical and chemical properties (such as extraction behaviour, retention time) are similar to those of the compounds to be analysed. $^{13}C_{12}$-PCBs should be used as internal standards for the GC-MS method for evaluation of results. Verify the stability of the internal standards regularly.

NOTE Certified solutions of PCB, and single solid PCB substances with certified purity are available from a limited number of suppliers e.g. Institute for Reference Materials and Measurements (IRMM) B-2440 Geel, Belgium. National Institute of Science and Technology Office of Standard Ref. Data, Washington D.C. 20 234 U.S.A or from other commercial providers.

7.5.2 Calibration standards

The calibration standard should contain the following compounds:

PCB28	2,4,4'-trichlorobiphenyl	(CAS-RN 7012-37-5)
PCB52	2,2',5,5'-tetrachlorobiphenyl	(CAS-RN 35693-99-3)
PCB101	2,2',4,5,5'-pentachlorobiphenyl	(CAS-RN 37680-37-2)
PCB118	2,3',4,4',5-pentachlorobiphenyl	(CAS-RN 31508-00-6)
PCB138	2,2',3,4,4',5'-hexachlorobiphenyl	(CAS-RN 35056-28-2)
PCB153	2,2',4,4',5,5'-hexachlorobiphenyl	(CAS-RN 35065-27-1)
PCB180	2,2',3,4,4',5,5'-heptachlorobiphenyl	(CAS-RN 35065-29-3)

NOTE The numbers 28, 52 etc. correspond to the sequential numbers of chlorobiphenyls according to the IUPAC rules for the nomenclature of organic compounds.

7.5.3 Internal and injection standards

7.5.3.1 General

The PCB congeners to be considered as internal and injection standards are listed below. The internal standard shall be added to the sample. For MS-detection labelled PCB congeners are advised.

When highly contaminated samples are analysed, an aliquot of the extract is often used for further clean-up. This makes the costs of analyses caused by the use of labelled standard very high. In these cases, it is allowed to add the internal standard in two steps. Step 1 addition of unlabelled internal standards to the sample. Step 2 addition of labelled congeners to the aliquot of the extract used for clean-up.

At least three congeners, covering the chromatogram shall be used as internal standard.

Other PCB not present in the sample, or $^{13}C_{12}$-labelled PCBs not used as internal standard, can be used as injection standard.

NOTE 1 Some PCB mixtures contain up to 2,5 % of PCB155.

NOTE 2 PCB30, PCB143 and PCB207 are recommended as internal standards.

NOTE 3 PCB198 or PCB209 are recommended as injection standards for ECD-detection because of lesser interferences.

7.5.3.2 Labelled PCB congeners

PCB28	$^{13}C_{12}$-2,4,4'-trichlorobiphenyl	
PCB52	$^{13}C_{12}$-2,2‘,5,5‘-tetrachlorobiphenyl	
PCB101	$^{13}C_{12}$-2,2‘,4,5,5‘-pentachlorobiphenyl	(CAS-RN 37680-73-2)
PCB118	$^{13}C_{12}$-2,3‘,4,4‘,5-pentachlorobiphenyl	
PCB138	$^{13}C_{12}$-2,2‘,3,4,4‘,5‘-hexachlorobiphenyl	(CAS-RN 35065-28-2)
PCB153	$^{13}C_{12}$-2,2‘,4,4‘,5,5‘-hexachlorobiphenyl	
PCB180	$^{13}C_{12}$-2,2‘,3,4,4‘,5,5‘-heptachlorobiphenyl	

7.5.3.3 Non-labelled PCB congeners

PCB29	2,4,5-trichlorobiphenyl	(CAS-RN 15862-07-4)
PCB30	2,4,6-trichlorobiphenyl	(CAS-RN 35693-92-6)
PCB143	2,2',3,4,5,6'-hexachlorobiphenyl	(CAS-RN 68194-15-0)
PCB155	2,2',4,4',6,6'-hexachlorobiphenyl	(CAS-RN 33979-03-2)
PCB198	2,2',3,3',4,5,5’,6,-octachlorobiphenyl	(CAS-RN 68194-17-2)
PCB207	2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl	(CAS-RN 52663-79-3)
PCB209	2,2',3,3',4,4',5,5’,6,6'-decachlorobiphenyl	(CAS-RN 2051-24-3)

7.5.3.4 PCB congeners for resolution check

PCB28	2,4,4'-trichlorobiphenyl	(CAS-RN 7012-37-5)
PCB 31	2,4',5-trichlorobiphenyl	(CAS-RN 16606-02-3)

7.6 Preparation of standard solutions

7.6.1 Preparation of calibration standard solutions of PCBs

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in *n*-heptane (7.2.2) by weighing approximately 10 mg of each of the calibration standards (7.5.2) to the nearest 0,1 mg and dissolving them in 25 ml of *n*-heptane.

Combine small quantities (2 ml to 10 ml) of these individual primary standard solutions into a mixed standard solution of PCB.

NOTE Because of the dangerous nature of the substances to be used, commercially available - preferably certified - standard solutions or mixed standard solutions are preferred. Avoid skin contact.

The working standard solutions shall be in the same solvent like the extract.

Store the primary and diluted standard solutions in a dark place at a temperature of (5 ± 3) °C. The solutions are stable for at least one year, provided that evaporation of solvent is negligible.

Components present in mixed standard solutions should be completely separated by the gas chromatographic columns used.

7.6.2 Preparation of internal standard solution

Prepare a concentrated primary internal standard solution, containing at least three different components (7.5.3), of about 0,4 mg/ml in *n*-heptane (7.2.2) by weighing approximately 10 mg of each of the chosen internal standards to the nearest 0,1 mg and dissolving them in 25 ml of *n*-heptane. Prepare from this a secondary internal solution with such a concentration that the added amount gives a peak with measurable peak area or peak height in the chromatogram (at least 10 times the detection limit).

If the two step procedure for GC-MS is used, make two different internal standard solutions, one containing the non-labelled compounds. At least two unlabelled congeners shall be used in the first internal standard solution and at least three labelled congeners in the second solution.

7.6.3 Preparation of injection standard solution

Prepare a concentrated primary injection standard solution, containing at least two different components (7.5.3), of about 0,4 mg/ml in *n*-heptane (7.2.2) by weighing approximately 10 mg of each of the chosen injection standards to the nearest 0,1 mg and dissolving them in 25 ml of *n*-heptane. Prepare from this a secondary internal solution which such a concentration that the added amount gives a peak with measurable peak area or peak surface in the chromatogram (at least 10 times the detection limit).

8 Apparatus

8.1 Extraction and clean-up procedures

Usual laboratory glassware.

All glassware and material that comes into contact with the sample or extract shall be thoroughly cleaned.

8.1.1 Sample bottles, made of glass, stainless steel or aluminium, with glass stopper or screw top and polytetrafluoroethylene (PTFE) seal of appropriate volume.

Glass is not appropriate for sludge samples.

WARNING — For safety reasons, biologically active sludge samples shall not be stored in a sealed container.

8.1.2 Shaking device, with horizontal movement (200 strokes to 300 strokes per min).

8.1.3 Water bath, adjustable up to 100 °C.

8.1.4 Separating funnels of appropriate volume.

8.1.5 Conical flasks of appropriate volume.

8.1.6 Soxhlet extraction apparatus, consisting of round bottom flask, e.g. 100 ml, Soxhlet extractors and Soxhlet thimbles, e.g. 27 mm × 100 mm, vertical condensers, e.g. 300 mm, heating device.

8.1.7 Concentrator, Kuderna Danish type.

Other evaporators, e.g. a rotary evaporator, may be used if found to be equally suitable.

8.1.8 Boiling chips, glass or porcelain beads.

8.1.9 Quartz wool or silanized glass wool.

WARNING — Working with quartz wool imposes a risk to health through the release of fine quartz particles. Inhalation of these should be prevented by using a fume cupboard and wearing a dust mask.

8.1.10 Calibrated test tubes, with a nominal capacity of 10 ml to 15 ml and ground glass stopper.

8.1.11 Chromatography tubes. Chromatography column of glass, 5 mm to 10 mm inside diameter, length e.g. 600 mm.

8.2 Gas chromatograph

8.2.1 General

Equipped with a capillary column, mass spectrometric detection (MS) or electron capture detector (ECD) based on ^{63}Ni.

NOTE Working with an encapsulated radioactive source as present in an ECD requires a licence according to the appropriate national regulations.

Using ECD, gas chromatographs equipped with two detectors and with facilities for connecting two capillary columns to the same injection system are very well suited for this analysis; with such apparatus the confirmatory analysis can be performed simultaneously.

8.2.2 Capillary columns, each comprising a 5 % phenyl-methyl silicone stationary phase coated onto fused silica capillary column or an equivalent chemically bonded phase column. The chromatographic peaks of PCB28 and PCB31 shall be resolved sufficiently (resolution at least 0,5) for integrating the PCB28 peak. In general column length should be 25 m to 60 m. Internal diameter 0,18 mm to 0,32 mm and film thickness 0,1 µm to 0,5 µm.

Using ECD-detection, a second column, coated with a moderate polar phase (e.g. CP-Sil 19, OV 1701[3)], etc.), shall be used to confirm the result obtained.

NOTE The retention times for the PCB on different capillary columns are given in Annex B.

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3) CP-Sil 19, OV 1701 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. Equivalent products may be used if they can be shown to lead to the same results.

9 Sample storage and preservation

9.1 Sample storage

The samples shall be analysed as soon as possible after sampling. This applies in particular to the examination of microbiologically active solids.

If necessary, sludge samples shall be stored according to EN ISO 5667-15.

Dried samples can be stored at room temperature in a dark place up to one month. Soil samples shall be stored according to ISO 18512.

9.2 Sample pretreatment

Pretreat samples according to EN 16179, if not otherwise specified, and considering the specific drying procedures as specified in Table 3 to obtain a test sample.

Pretreatment is necessary to reduce the moisture content to enable extraction of the PCBs and to increase the homogeneity.

Complete drying of the sample is essential if Soxhlet is used for extraction or to increase the homogeneity.

Complete drying is also recommended if the sample shall be stored for a long period.

Table 3 — Drying techniques for samples of different matrices for subsequent analyses of PCB

Matrix	Drying technique			
	Air drying	Freeze drying (EN ISO 16720)	Na_2SO_4	No drying
Sludge	x	x	x[a]	
Biowaste (compost, mixed waste)		x	x	x
Soil (e. g. sand, clay)	x	x	x	x

[a] Na_2SO_4 can be used for the preservation of hygroscopic dried sludge.

10 Procedure

10.1 Blank test

Perform a blank test following the applied procedure (selected extraction and clean-up procedure) using the same amount of reagents that are used for the pretreatment, extraction, clean-up and analysis of a sample. Analyse the blank immediately prior to analysis of the samples to demonstrate sufficient freedom from contamination. The blank shall be less than 50 % of the lowest reporting limit.

10.2 Extraction

10.2.1 General

Depending on the test sample (matrix and moisture content), choose a suitable extraction method (see Table 4). Extraction method 1 (10.2.2) or 3 (10.2.4) are recommended if it is important to break up aggregates in the sample to access the PCBs. With wet samples these methods shall be applied in order to eliminate the presence of water. If dissolving of the PCBs is the most important step (waste and organic rich materials) and

the sample is dry, extraction method 2 (10.2.3) using Soxhlet is recommended. For sludge it has been shown that Soxhlet is applicable. In presence of plastics, use of acetone shall be avoided, because the use of acetone leads to a high amount of co-extractives. However, a general rule cannot be given, because samples may contain all: aggregates, organic matter and (plastic) waste.

Other extraction procedures, e.g. ultrasonic extraction, microwave or high pressure liquid extraction may be used provided:

— the laboratory can show that the extraction efficiency is equivalent to one of the extraction procedures 1, 2 or 3 as described in this European Standard, or

— the sample requires another approach as shown by the laboratory and the results of the procedures are in agreement with the performance criteria as described in 10.7.4 and 10.8.6.

NOTE For application of this European Standard for some types of waste, the addition of acetone with Soxhlet extraction has been shown to be effective.

Extraction procedures described in this European standard are suitable to extract up to 20 g of dry sample. If the test sample has a low density (i.e. some wastes) or the sample is homogeneous, depending on the expected PCB content and on the homogeneity of the sample, less sample can be used. In general the following amounts of dry sample can be used: 2 g to 10 g of sewage sludge, 5 g to 20 g of compost or 2 g to 20 g of biowaste. The amount of sample shall be weighed with an accuracy of at least 1 %.

Table 4 — Extraction procedures to be used for different matrices

Moisture status of the test sample	Matrix	Extraction solvent	Extraction technique	Extraction procedure	Remark
Dry	Soil-like materials, sludge, biowaste, compost	Acetone/ petroleum ether	Agitation	Extraction procedure 1 (see 10.2.2)	
	Sludge, biowaste, compost,	Petroleum ether	Soxhlet, pressurised liquid extraction	Extraction procedure 2 (see 10.2.3)	
Wet	Soil-like materials, biowaste, compost	Acetone/ petroleum ether	Agitation	Extraction procedure 1 (see 10.2.2)	Also applicable for field moist samples with dry matter content >75 %
	Soil-like material biowaste, compost	Acetone/ petroleum ether/NaCl	Agitation	Extraction procedure 3 (see 10.2.4)	

10.2.2 Extraction procedure 1: Samples using acetone/petroleum ether and agitation

Place the test sample in a bottle (8.1.1). Add a definite volume of the secondary internal standard solution (7.5.2). Add 50 ml of acetone (7.2.1) to the test sample and extract by shaking thoroughly to break up aggregates for 30 min. Then add 50 ml of petroleum ether (7.2.3) and shake again thoroughly during at least 12 h. Use a horizontal shaking device (8.1.2) and have the solvent movement in the sample bottle as long as possible (horizontal position). After the solids have been settled, decant the supernatant. Wash the solid phase with 50 ml of petroleum ether (7.2.3) and decant again. Collect the extracts in a separating funnel (8.1.4) and remove the acetone by shaking twice with 400 ml of water (7.2.5). Dry the extract over anhydrous sodium sulfate (7.2.4). Rinse the sodium sulfate with petroleum ether (7.2.3) and add the rinsing to the extract.

NOTE 1 Tap water has shown to be applicable for removal of the acetone, because target compounds are not present.

If the sample contains water up to 25 %, the same procedure can be used. If the water content of the sample is greater than 25 % this procedure is less effective and the amount of acetone shall be increased. The ratio acetone : water should be at least 9 : 1. The ratio acetone : petroleum ether should be kept constant to 2 : 1.

The definite amount of the internal standard added in all extraction methods shall have such a quantity that their concentrations in the final extract fall under the working range of the measurement method. Typically the concentration of the individual internal standards in the final extract is 0,1 µg/ml. In order to 'wet' the complete sample, a minimum amount of 100 µl of internal standard is recommended.

NOTE 2 In matrices with a high organic matter content (e.g. some sludges) longer extraction procedures can be necessary. Extraction procedure 2 (see 10.2.3) may be preferred for these samples.

10.2.3 Extraction procedure 2: Samples using Soxhlet

Place the test sample in the extraction thimble (8.1.6). Add the definite amount of the secondary internal standard solution (7.5.3) and approximately 70 ml of the extraction solvent (7.2.2) to the extraction vessel. Extract the sample with the Soxhlet extraction apparatus (8.1.6.). The duration of the extraction should be calculated with a minimum of 100 extraction cycles.

NOTE If the sample is hygroscopic and is not dried just before analysis, add Na_2SO_4 to the test sample to get a free flowing material.

Pressurised liquid extraction may also be used.

10.2.4 Extraction procedure 3: Samples using acetone/petroleum ether/sodium chloride and agitation

Take an amount of sample and put it into a 1 l sample bottle (8.1.1). Add the definite amount of the secondary internal standard solution (7.5.3). If the sample is dry, add 50 ml water. For moist samples, the water quantity to be added is calculated using Formula (1):

$$m_{\mathrm{W}} = 50 - \frac{m_{\mathrm{E}} \cdot m_{\mathrm{H_2O}}}{100} \qquad (1)$$

where

m_{W} is the mass of water to be added, expressed in grams (g);

m_{E} is the mass of the sifted sample, expressed in grams (g);

$m_{\mathrm{H_2O}}$ is the water content of the sample, determined according to EN 15934, expressed in percent (%).

Add 40 g sodium chloride (7.2.6), 100 ml acetone (7.2.1) and 50 ml petroleum ether (7.2.3) to the moistened preparations, close the sample bottle and shake it with a shaking device (8.1.2) for at least 12 h.

The organic phase shall be separated, if necessary, using a centrifuge with sealable centrifuge cups. Collect the extract in a separating funnel of 1 l capacity and remove the acetone by shaking twice with 400 ml of water (7.2.5). Dry the extract over anhydrous sodium sulfate (7.2.4) and transfer the dried extract to the concentrator (8.1.7). Rinse the sodium sulfate with petroleum ether (7.2.3) and add the rinsing to the extract.

10.3 Concentration

Add a boiling chip (8.1.8) to the extract and concentrate the extract to approximately 10 ml by evaporation using a concentrator (8.1.7). Transfer the concentrated extract to a calibrated test tube (8.1.10) and concentrate to 1 ml using a gentle stream of nitrogen or another inert gas at room temperature. If clean-up method H is used, concentration is not necessary. Record the final volume of the extract.

In heavily contaminated samples, an aliquot is used for further clean-up. Establish the fraction f of the extract used for further clean-up. If non-labelled congeners have been used as internal standard added to the sample, add a definite amount of the secondary internal standard solution containing $^{13}C_{12}$-congeners.

To prevent losses of the most volatile PCBs it is not allowed to evaporate till complete dryness. It is advisable to add a small amount (one drop) of keeper substance (7.2.7).

10.4 Clean-up of the extract

10.4.1 General

Clean-up shall be used if compounds are present that can interfere with the PCB congeners of interest in the gas chromatogram or if those compounds can influence the GC-procedure (i.e. contamination of the chromatographic system). If no or negligible interfering substances are present, no clean-up is necessary. Depending on the substances to be removed, Table 5 shall be used. If polar compounds shall be removed take special care on the recoveries of the low chlorinated PCBs.

Table 5 — Clean-up methods

Method	Clean-up	For removal of	Suitable for	Remarks
Clean-up A	Aluminium oxide	Polar compounds		Difficult to adjust water content and keep it constant
Clean-up B	Silica	Polar compounds		Attention: some charges of silica can contain low concentrations of PCBs
Clean-up C	Gel permeation	High molecular compounds, lipids	MS	
Clean-up D	Florisil®	Polar compounds		Analysis of pesticides is possible after this clean-up
Clean-up E	H_2SO_4/Silica NaOH	Polar compounds, PAH, lipids		Especially suitable for lipid containing samples
Clean-up F	Benzenesulfonic acid/sulfuric acid	Polar compounds, (poly)aromatics, bases, hetero compounds, oil		Especially suitable for samples containing high concentration of mineral oil
Clean-up G	DMF/hexane	Aliphatic hydrocarbons, lipids, oil	MS	
Clean-up H	H_2SO_4 (conc.)	Lipids		
Clean-up I	TBA	Sulfur	ECD	
Clean-up J	Cu	Sulfur	ECD	
Clean-up K	$AgNO_3$/Silica	Sulfur + polar compounds	ECD	Also applicable for MS

Before application of the clean-up to real samples the laboratory shall ensure that recoveries after use of the clean-up for a standard are at least 80 % for all relevant congeners (including internal standards).

Other clean-up procedures may also be used, provided they remove the interfering peaks in the chromatogram and recoveries after use of the clean-up are at least 80 % for all relevant congeners (including internal standards).

The extract obtained in 10.3 or in a previous clean-up step shall be quantitatively transferred to the clean-up system; alternatively, an aliquot may be used.

10.4.2 Clean-up A – Aluminium oxide

Prepare an adsorption column by placing a small plug of quartz wool (8.1.9) in the chromatography tube (8.1.11) and packing it dry with 2,0 g ± 0,1 g of aluminium oxide (7.3.1.1).

Apply the extract to the dry packed adsorption column. Rinse the test tube twice with 1 ml of petroleum ether (7.2.3) and transfer the rinsings to the column with the same pipette as soon as the liquid level reaches the upper side of the column packing. Elute with approximately 20 ml of petroleum ether. Collect the entire eluate.

Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

If a new batch of aluminium oxide is used, the solvent volume to eluate the specified PCB congeners completely from the column shall be determined using a proper PCB standard solution.

NOTE Commercially available disposable aluminium oxide cartridges may be used as an alternative if found suitable. A column is suitable if the performance of the method is in agreement with 8.2.2.

10.4.3 Clean-up B – Silica gel

Put glass wool (8.1.9) and 10 g silica gel (7.3.2.2) into the chromatographic tube (8.1.11). Then add a 1 cm layer of sodium sulfate (7.2.4) and condition with 20 ml petroleum ether (7.2.3). Apply the extract to the column when the level of the solvent mixture is drained to approximately 0,5 cm above the column packing.

Elution is performed using a total of 10 ml petroleum ether (7.2.3). Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.4 Clean-up C – Gel permeation chromatography

The extract is carefully reduced under a gentle nitrogen flow. The residue is immediately dissolved in 5 ml solvent mixture [(ethyl acetate (7.3.3.2) and cyclohexane (7.3.3.3) (1+1)]. The dissolved residue is put into the GPC column.

The solvent mixture for GPC is used for elution.

The GPC system-settings should be:

— Flow rate: 5 ml/min;

— Volume of the sample loop: 5 ml;

— First fraction: 120 ml (24 min);

— PCB elution: 155 ml (31 min);

— Last fraction: 20 ml (4 min).

The elution volumes of the first fraction, eluate and last fraction shall be considered recommended values and shall be regularly verified by means of the multi-component PCB-standard solution.

Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3)

NOTE During use of the gel permeation column a small shift in volume to be collected may occur. This is visible in a decrease of recoveries of the internal standards. If this occurs, readjustment of the sampled volume may be necessary.

10.4.5 Clean-up D – Florisil®

Add into a chromatographic tube (8.1.11) 5 mm sodium sulfate (7.2.4), 1,5 g Florisil (7.3.4.1), and again 5 mm sodium sulfate. To fix the mixture, place glass wool (8.1.9) on the top. Rinse the column with approximately 50 ml isooctane (7.3.4.2). Apply the extract to the column. Rinse the extraction tube/vessel for two times with 1 ml isooctane/toluene (95/5) (7.3.4.3) and give it onto the column. Afterwards elute with 7 ml isooctane /toluene. One drop of keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.6 Clean-up E – silica H_2SO_4/silica NaOH

The combined silica H_2SO_4/silica NaOH phase is effective in the removal of polar compounds, polycyclic aromatic compounds and triglycerides.

Prepare an adsorption column by pouring consecutively 1 g silica NaOH (7.3.5.4), 5 g silica H_2SO_4 (7.3.5.2) and 2 g sodium sulfate (7.2.4) in a clean chromatography column (8.1.11). Add a sufficient amount of *n*-hexane (7.3.5.5) and elute until the top of the *n*-hexane phase reaches the top of the sodium sulfate layer. Apply the extract to the top of the sodium sulfate layer and make it penetrate into the sodium sulfate layer. Elute with about 60 ml of *n*-hexane and collect the entire *n*-hexane fraction. One drop of keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.7 Clean-up F – Benzenesulfonic acid/sulfuric acid

Benzenesulfonic acid/sulfuric acid pretreatment is effective if the sample contains large amounts of oil.

Condition the silica cartridges by eluting three times with 2 ml portions of *n*-hexane. Discard the eluate and vacuum dry the columns. Apply 500 µl of the extract to the column and let slowly seep into the column. After 30 s, add 2 × 1 ml *n*-hexane like solvent (7.3.5.5) to the column and wait once again for 30 s. Elute the PCB from the column with 3 × 0,5 ml of *n*-hexane like solvent (7.3.5.5). Collect the entire eluate. Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.8 Clean-up G – DMF/*n*-hexane partitioning for aliphatic hydrocarbons removal

Extracts of samples containing a high amount of aliphatic compounds (e.g. oil) need additional clean-up by dimethylformamide/hexane partitioning.

This additional clean-up step shall only be applied in case of GC-MS and not for GC-ECD. Indeed in the later case, the extracts are more diluted and interference by aliphatic hydrocarbons are not expected in the ECD signal.

Transfer the extract to a separating funnel of 100 ml and extract the PCBs with 25 ml of DMF (7.3.7.1). Repeat twice. Transfer the combined DMF extracts to a separatory funnel of 500 ml, add 100 ml of water (7.2.5) and extract the PCBs with 50 ml of *n*-hexane (7.3.5.5). Repeat once. One drop of keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.9 Clean-up H – concentrated sulfuric acid

This treatment is recommended if sulfoniable compounds are present. Face shields, gloves and protective clothing shall be worn.

Transfer the extract to a convenient stoppered glass vial. Dilute the extract to 20 ml with petroleum ether (7.2.3). Pour in 5 ml of concentrated sulfuric acid (7.3.8.1) and shake vigorously at intervals for 5 min. Allow to

separate completely (about 15 min). Take the upper layer, rinse the remaining sulfuric acid with petroleum ether. Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.10 Clean-up I – TBA sulfite reagent

Add 2 ml of TBA sulfite reagent (7.3.9.1) to 1 ml of concentrated extract and shake for 1 min. Add 10 ml of water (7.2.5) and shake again for 1 min. Separate the organic phase from the water with a Pasteur pipette and add a few crystals of anhydrous sodium sulfate (7.2.4) to remove the remaining traces of water.

10.4.11 Clean-up J – Clean-up using pyrogenic copper to remove elemental sulfur and some other organic sulfur compounds

Add 1 ml of the extract (in petroleum ether) to a centrifuge tube. Add 100 mg pyrogenic copper powder (prepared according to procedure given in 7.3.10). Centrifuge the tube more than 5 min at approximately 3 500 rpm (ensure that there is no visible turbidity). Remove the extract and if necessary, clean-up further using column chromatography.

10.4.12 Clean up K – $AgNO_3$/silica

Add into a chromatographic tube (8.1.11) sodium sulfate (7.2.4) e.g. 5 mm high, 2 g of the $AgNO_3$/silica mixture (7.3.11.2) and again 5 mm high sodium sulfate (7.2.4). Rinse the column with approximately 50 ml *n*-hexane (7.3.5.5). Apply the extract to the filled column. Rinse the extraction vessel for three times with 2 ml *n*-hexane and give it onto the column, when the meniscus of the extract riches the surface of the sodium sulfate (7.2.4). Add in the same manner 40 ml of hexane onto the column. Keeper substance (7.2.7) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

If the eluate is still coloured after the clean-up, the procedure should be repeated.

10.5 Addition of the injection standard

Add an appropriate amount of the secondary injection standard (7.6.3) to the extract obtained after clean-up (this amount shall be in line with the concentration of the calibration standard). Record the final volume *V*.

10.6 Gas chromatographic analysis (GC)

10.6.1 General

Both MS and ECD detectors are allowed, but in special cases only one gives the proper results. In general MS is recommended. In the following cases ECD-detection may be preferred:

— Presence of mineral oil. Removal of mineral oil may be difficult, because the polarity of these compounds can be comparable to PCBs. An ECD-detector is not sensitive for mineral oil and no clean-up or a less effective clean-up is possible.

— Using an ECD-detector, the pattern of the PCBs is more easily recognised.

— An ECD-detector can be used for a first screening to select the samples having PCB-concentrations higher than the minimum reporting value. For samples with PCB-concentrations lower than this value further identification is not necessary.

For both detection techniques the internal standard method is used for quantification.

10.6.2 Setting the gas chromatograph

Set the gas chromatograph (8.2) in such a way that sufficient separation of the PCBs is achieved (see 5.2). Optimise the gas chromatograph starting from the following conditions:

— Separation column: Capillary column (see 8.2.2);

— Oven temperature program: 60 °C, 2 min;
30 °C/min to 120 °C;
5 °C/min to 300 °C;
300 °C, 15 min;

— Injector temperature: 260 °C;

— Splitless injection: 1 µl, keep the split 1,8 min closed;

— Carrier gas: Helium 0,8 ml/min to 1 ml/min.

Annex B gives the elution order of the target PCBs as can be expected on two different columns.

10.7 Mass spectrometry (MS)

10.7.1 Mass spectrometric conditions

Tune the mass spectrometer in accordance with the manufacturer's instructions. Chromatograms are recorded in full scan or selected ion monitoring/recording mode (SIM/SIR). The ions to be selected are given in Table 6. For each native congener, two ions making part of the chlorine isotope cluster of the molecular ion and one specific fragment ion are chosen.

Table 6 — Diagnostic ions for PCBs to be used with MS detection

Compound	**Diagnostic ion 1** *m*/*z*	**Diagnostic ion 2** *m*/*z*	**Diagnostic ion 3** *m*/*z*
PCB28	256 (100)	258 (74)	186 (82)
$^{13}C_{12}$-PCB28	268	270	
PCB52	292 (100)	294 (49)	220 (95)
$^{13}C_{12}$-PCB52	304	306	
PCB101	326 (100)	328 (65)	256 (62)
$^{13}C_{12}$-PCB101	338	340	
PCB118	326 (100)	328 (62)	254 (57)
$^{13}C_{12}$-PCB118	338	340	
PCB138	360 (100)	358 (42)	290 (106)
$^{13}C_{12}$-PCB138	372	374	
PCB153	360 (100)	362 (92)	290 (73)
$^{13}C_{12}$-PCB153	372	374	
PCB180	394 (100)	396 (96)	324 (84)
$^{13}C_{12}$-PCB180	406	408	
NOTE Brackets values are abundance values which are normalized to the diagnostic ion 1. Values for diagnostic ions 2 and 3 may depend on the MS-system and its actual condition. Presented values should be considered indications.			

10.7.2 Calibration of the method using an internal standard

10.7.2.1 General

This is an independent method for the determination of the mass concentrations and is not influenced by injection errors, the volume of water present in the sample or matrix effects in the sample, provided that recovery of the compounds to be analysed is about equal to that of the internal standard.

Add a specific mass of the internal standard and injection standard (7.5.3) to dilutions of the mixed calibration solution (7.5.2). The mass concentration of both standards shall be the same for all calibration solutions and comparable with the concentration of both standards in the final extract. Run the GC-MS analysis with the calibration solutions, prepared as described in 7.5.2. Calculate the relative response ratio for the native PCB and the $^{13}C_{12}$-PCB after obtaining a calibration curve by plotting the ratio of the mass concentrations against the ratio of the peak areas (or peak heights) using Formula (2):

$$\frac{A_{\mathrm{n}}}{A_{\mathrm{C13}}} = s \cdot \frac{\rho_{\mathrm{n}}}{\rho_{\mathrm{C13}}} + b \qquad (2)$$

where

A_{n} is the measured response of the native PCB, e.g. peak area;

A_{C13} is the measured response of the $^{13}C_{12}$-labelled PCB internal standard, e.g. peak area;

s is the slope of the calibration function;

ρ_{n} is the mass concentration of the native PCB in the calibration solution, expressed in micrograms per litre (µg/l);

ρ_{C13} is the mass concentration of the $^{13}C_{12}$-labelled PCB internal standard in the calibration solution, expressed in micrograms per litre (µg/l);

b is the intercept of the calibration curve with the ordinate.

Two types of calibration are distinguished: the initial calibration (10.7.2.2) and the daily calibration (validity check of the initial calibration); the last one is called calibration verification (10.7.2.3).

Non-linear calibration methods may be applied.

10.7.2.2 Initial calibration

The initial calibration serves to establish the linear working range of the calibration curve. This calibration is performed when the method is used for the first time and after maintenance and/or repair of the equipment.

Take a gas chromatogram of a series of at least five standard solutions with equidistant concentrations, including the solvent blank. Identify the peaks, using MS or the gas chromatograms of the individual compounds. Prepare a calibration graph for each compound.

Check for linearity according to ISO 8466-1.

It is allowed to use non-linear calibration using all five standards. In that case, the same five standards shall be used for recalibration and not the selection of two described below (see 10.7.2.3).

10.7.2.3 Calibration verification

The calibration verification checks the validity of the linear working range of the initial calibration curve and shall be performed before each series of samples.

For every batch of samples, inject at least two calibration standards with concentrations of (20 ± 10) % and (80 ± 10) % of the established linear range and calculate the straight line from these measurements. If the straight line falls within ± 10 % of the reference values of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line shall be established according to 10.7.2.2.

10.7.3 Measurement

Measure the gas chromatograms of the extracts obtained according to 10.5. With the aid of the absolute retention times, identify the peaks to be used to calculate the relative retention times. Use the internal standard or injection standard as close as possible to the PCB-peak to be quantified. For the other relevant peaks in the gas chromatograms, determine the relative retention times.

If the concentration is above the level for proper identification or quantification, a diluted extract shall be injected for proper identification or quantification of the relevant PCBs or re-extract the sample using a lower amount of sample.

If as a result of dilution, the internal standard is outside the linear range, Formula (4) in 10.7.5 does not give the proper quantification and the deviation from linearity shall be taken into account.

10.7.4 Identification

Apply EN ISO 22892 for the identification of the PCBs. In EN ISO 22892, the chromatographic criteria and MS-criteria are described, necessary for proper identification. Use the diagnostic ions as given in Table 6.

10.7.5 Check on method performance

Because this European Standard allows using different modules, comparing the measured response of the internal standards and injection standards in both the injected performance standard solution and the injected sample is a check on the performance of the total procedure.

Use for this analysis

— the same final volume,

— the same definite volume of internal standard, and

— the same definite volume of injection standard,

as used for the samples.

This is the performance standard.

The performance standard may be one of the calibration standards, provided that the ratio of the volumes (internal standard/injection standard) used is the same.

Calculate for each internal standard the ratio between sample and performance standard solution using the closest injection standard with Formula (3):

$$U = \frac{A_1(S)}{A_2(S)} \times \frac{A_2(ps)}{A_1(ps)} \times 100 \quad (3)$$

where

U is the recovery rate, expressed in percent (%);

A_1 is the measured response of the $^{13}C_{12}$-labelled PCB internal standard, e.g. peak area;

A_2 is the measured response of the $^{13}C_{12}$-labelled PCB injection standard, e.g. peak area;

ps is the performance standard;

S is the sample.

The average ratio in the sample shall be at least 70 % and do not exceed 110 % of the ratio in the standard. The ratio for an individual PCB should be at least 60 %. If these values are not achieved, the analyses shall be repeated using modules more suitable for the sample.

If multiple clean-up is necessary, lower ratios can be found, because with each clean-up step losses are accepted by this European Standard. Lower ratios are acceptable if this can be explained by the accepted losses in each clean-up step. The minimum ratio shall be 50 %.

If the two-step procedure for addition of the internal standard has been used, calculate the extraction ratio between the non-labelled PCB added to the sample and the labelled PCB to the extract using Formula (4):

$$E = \frac{A_{1,\text{mean}}(S) \times f}{A_3(S)} \times \frac{A_3(ps)}{A_{1,\text{mean}}(ps)} \times 100 \quad (4)$$

where

E is the extraction recovery rate in percent (%);

$A_{1,\text{mean}}$ is the average measured response of the $^{13}C_{12}$-labelled PCB internal standard, e.g. peak area;

A_3 is the measured response of the non-labelled PCB internal standard, e.g. peak area;

f is the fraction of the original extract used for clean-up;

ps is the performance standard;

S is the sample.

The extraction recovery of the non-labelled standard shall be at least 75 %.

The values calculated for the concentrations of native congeners in the sample are only considered to be acceptable if the recoveries of the internal standards are within the limits described before. In other cases the values should be reported as indicative.

10.7.6 Calculation

Calculate the mass content of the individual PCB from the multipoint calibration of the total method by using Formula (5). In general diagnostic ion 1 can be used for this calculation.

$$w_\text{n} = \frac{(A_\text{n} / A_{\text{C13}}) - b}{s \cdot m \cdot d_\text{s}} \cdot \rho_{\text{C13}} \cdot f_\text{e} \cdot f_\text{t} \cdot V \quad (5)$$

where

w_n is the content of the individual PCB found in the sample, expressed in milligrams per kilogram (mg/kg) on the basis of the dry matter;

A_{C13} is the measured response of the $^{13}C_{12}$-labelled PCB internal standard in the sample extract;

A_n is the measured response of the native PCB in the sample extract;

ρ_{C13} is the mass of the $^{13}C_{12}$-labelled PCB internal standard added to the sample, expressed in micrograms per litre (µg/l);

m is the mass of the test sample used for extraction, expressed in grams (g);

d_s is the dry matter fraction in the field moist sample, determined according to EN 15934, expressed in percent (%);

f_e is the ratio of the total organic solvent volume used for extraction to that of the aliquot used for the analysis; f = 1 if the whole extract is used;

f_t is the addition factor;

V is the volume of the final solution, expressed in millilitres (ml);

s is the slope of the recalibration function;

b is the intercept of the recalibration curve with the ordinate.

The result shall be expressed in milligrams per kilogram (mg/kg) dry matter and rounded to two significant figures.

10.8 Electron capture detection (ECD)

10.8.1 General

Using ECD, the same procedure as for MS can be followed, except the points described below for all specific steps in the measurement. For ECD, the same order in paragraphs has been used as in 10.7 (MS-detection). Only differences are described.

10.8.2 ECD conditions

The ECD shall be operated at temperatures of 300 °C to 350 °C. Use the manufacturer's recommended settings to give the best conditions for linearity of the detector response.

10.8.3 Calibration of the method using internal standards

Using an ECD, internal and injection standards are not $^{13}C_{12}$-PCBs but standards described in 7.5.3.3. Replace $^{13}C_{12}$-labelled PCB by used internal standard in Formula (2).

Formula (2) shall be used replacing A_{C13} by $A_{IS,i}$ and ρ_{C13} by $\rho_{IS,i}$ with

$A_{IS,i}$ is the measured response of internal standard i, e.g. peak area;

$\rho_{IS,i}$ is the mass concentration of internal standard i in the calibration solution, in micrograms per litre (µg/l).

10.8.4 Measurement

Refer to 10.7.3.

10.8.5 Identification

Check the presence of any assigned compound by repeating the gas chromatographic analysis from 10.8.1, using GC-MS (see above) or using a column with a moderate polar phase (8.2.2) in combination with ECD. According to EN ISO 22892, three identification points shall be obtained. Measuring only with ECD, at least

check the results with another column and recognition of the PCB-pattern are necessary. The results using the second column should be within 10 %. If both are correct, the three identification points (EN ISO 22892) for identification are obtained. If one is missing, only indication can be reported.

10.8.6 Check on ECD method performance

Mistakes are probable when a peak of an interfering compound appears at the same position in the chromatogram as that of the internal standard. Therefore the following procedure is used to check if interfering compounds are present.

The presence or absence of interfering compounds is determined from the measured responses of the injection standards. When no interfering compounds are present in the extract, the ratio between the responses of the injection standards in the extracts is equal to that ratio in the standard solutions. The quotient of these ratios is called the relative response ratio, RRR. When no interfering compounds are present in the extract the value of RRR is in principle 1,00. In this European Standard, it is assumed that no interfering compounds are present in the extract when RRR = 1,00 $\pm$ 0,05.

When the value of RRR deviates from 1,00 $\pm$ 0,05, it is assumed that the response of one of the injection standards is influenced by an interfering compound present in the extract. In this case the performance of the method is calculated using the undisturbed injection standard.

Verify the correctness of the response of the injection standards as follows:

Calculate the relative response ratio RRR for the PCB injection standards by using Formula (6):

$$RRR = \frac{R_{e,198}}{R_{e,2}} \times \frac{R_{s,2}}{R_{s,209}} \quad (6)$$

where

RRR is the relative response ratio;

$R_{e,198}$ is the response of PCB198 in the extract;

$R_{e,2}$ is the response of the selected second internal standard in the extract;

$R_{s,209}$ is the response of PCB209 in the working standard solution;

$R_{s,2}$ is the response of the selected second recovery standard in the working standard solution.

NOTE PCB198 or PCB209 are recommended as injection standards for ECD-detection because of fewer interferences, other internal standards may be used.

The theoretical value of the relative response ratio RRR is 1,00. If RRR = 1,00 $\pm$ 0,05, regard the injection standards as correctly quantified and enter the value 1,00 for RRR in Formula (6). If RRR < 0,95 or RRR > 1,05, the gas chromatogram shall be checked for correct quantification of both injection standards. Take particular note of the peak shapes and peak widths. If the quantification has been correctly carried out, use both standards if RRR = 1,00 $\pm$ 0,05. Use only the injection standard PCB198 if RRR < 1,05 and use only PCB209 if RRR > 1,05 .

Calculate the ratio between sample and performance standard solution for each internal standard using the closest injection standard according to Formula (3) (see 10.7.5).

The average recovery ratio in the sample shall be at least 70 % of the ratio in the standard. The ratio for an individual PCB should be at least 60 %. If these values are not achieved, the analyses shall be repeated using modules more suitable for the sample. If one of the ratios is higher and exceeds 100 %, there is probably an

interfering compound present in the sample, which has the same retention time. This internal standard should not be used for further calculation.

If multiple clean-up is necessary, lower ratios can be found, because with each clean-up step losses are accepted by this standard. Lower ratios are acceptable if this can be explained by the accepted losses in each clean-up step. The minimum ratio shall be 50 %.

10.8.7 Calculation

Internal and injection standards are not $^{13}C_{12}$-labelled-PCB standards but standards as described in 7.5.3. Replace $^{13}C_{12}$-labelled PCB internal standard by the applied internal standards (7.5.3.) in Formula (5).

11 Performance characteristics

The method is "performance based". It is permitted to modify the method to overcome interferences not specified in this European Standard, provided that the performance criteria are met. Internal standards shall be used to check the pretreatment, extraction and clean-up procedures. Recoveries of these standards should be 70 % to 110 %. If the recovery is lower or outside the limits (i.e. 70 % to 110 %) the method shall be modified using other modules described in this European Standard.

Some samples may require multiple clean-up in case of lower recoveries.

12 Precision

The performance characteristics of the method data have been evaluated (see Annex A).

13 Test report

The test report shall contain at least the following information:

a) a reference to this European Standard (EN 16167);

b) complete identification of the sample;

c) the extraction module, clean-up module and detection module used for the analysis;

d) the results of the determination according to 10.7 (GC-MS) and 10.8 (GC-ECD);

e) any details not specified in this European Standard or which are optional, as well as any factor which may have affected the results.

Annex A
(informative)

Repeatability and reproducibility data

A.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison for the determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in sludge and treated biowaste was carried out by 10 to 13 European laboratories on three materials Detailed information can be found in the final report on the Interlaboratory comparison study mentioned in [6].

Table A.1 lists the types of materials tested.

Table A.1 — Material tested and parameters analysed in the interlaboratory comparison for the determination of PCB by GC-MS and GC-ECD in sludge and treated biowaste

Grain size	Sample	Material tested	Parameters
Sludge (< 0,5 mm)	Sludge 1	Mix of municipal waste water treatment plant sludges from North Rhine Westphalia, Germany	PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180
Fine grained (< 2,0 mm)	Compost 1	Fresh compost from Vienna, Austria	PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180

The validation has been carried out for soil also but no results are presented because the concentrations in the test materials were lower than the limit of applicability.

A.2 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table A.2).

Table A.2 — Results of the interlaboratory comparison studies of the determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD

Matrix	l	n	n_o	$\bar{\bar{x}}$ µg/kg	s_R µg/kg	$C_{V,R}$ %	s_r µg/kg	$C_{V,r}$ %	BD
PCB 28									
Sludge 1	14	47	1	50,13	18,49	37	3,19	6,4	3
Compost 1	6	15	2	0,79	0,09	12	0,06	7,9	14
PCB 52									
Sludge 1	13	42	2	53,72	21,02	39	3,74	7,0	4
Compost 1	5	18	0	0,78	0,26	33	0,05	6,2	17
PCB 101									
Sludge 1	13	49	0	41,21	16,86	41	2,56	6,2	4
Compost 1	12	40	1	3,04	1,18	39	0,29	9,5	1
PCB 118									
Sludge 1	13	47	0	27,02	10,36	38	1,84	6,8	5
Compost 1	8	26	1	2,22	1,08	49	0,15	6,9	6
PCB 138									
Sludge 1	12	48	0	50,38	17,21	34	3,81	7,6	4
Compost 1	12	39	2	6,62	2,38	36	0,58	8,7	0
PCB 153									
Sludge 1	13	47	1	49,64	18,48	37	3,64	7,3	2
Compost 1	12	39	2	6,97	2,54	36	0,58	8,3	0
PCB 180									
Sludge 1	12	48	0	40,63	12,94	32	3,65	9,0	4
Compost 1	12	43	1	5,81	2,12	37	0,57	9,7	0
PCB sum									
Sludge 1	18	70	1	205	84	41	16	7,7	2
Compost 1	12	48	2	26,1	10,3	39	4,7	18,1	0

Explanation of symbols

l	number of participating laboratories
n	number of analytical results
n_o	number of rejected laboratories
$\bar{\bar{x}}$	total mean of analytical results (without outliers)
s_R	reproducibility standard deviation
$C_{V,R}$	coefficient of variation of reproducibility
s_r	repeatability standard deviation
$C_{V,r}$	coefficient of variation of repeatability
BD	number of measurements below detection limit

Annex B
(informative)

Examples for retention times of PCBs

Table B.1 shows examples for retentions times of the target PCBs determined on different columns.

Table B.1 — Examples for retention times of PCBs for two different capillary columns

Component	Retention time min	
	A[a]	**B**[b]
PCB28	33,32	32,98
PCB52	34,85	34,54
PCB101	38,71	38,27
PCB118	41,89	41,61
PCB138	45,00	44,54
PCB153	43,18	42,49
PCB180	50,41	49,47

[a] 50 m CP-Sil 8[4)]; internal diameter 0,22 mm; film layer 0,12 µm

[b] 50 m CP-Sil 19[4)]; internal diameter 0,22 mm; film layer 0,12 µm

NOTE Depending upon the column used the co-elution of the stated PCBs with other congeners is possible. For the co-elution information please consult the column specification or column procedures.

4) CP-Sil 8, CP-Sil 19 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. Equivalent products may be used if they can be shown to lead to the same results.

Bibliography

[1] EN 12766-1, *Petroleum products and used oils — Determination of PCBs and related products — Part 1: Separation and determination of selected PCB congeners by gas chromatography (GC) using an electron capture detector (ECD)*

[2] EN 15308:2008, *Characterization of waste — Determination of selected polychlorinated biphenyls (PCB) in solid waste by using capillary gas chromatography with electron capture or mass spectrometric detection*

[3] EN 61619, *Insulating liquids — Contamination by polychlorinated biphenyls (PCBs) — Method of determination by capillary column gas chromatography (IEC 61619)*

[4] EN ISO 6468, *Water quality — Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes — Gas chromatographic method after liquid-liquid extraction (ISO 6468)*

[5] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[6] Horizontal Project Interlaboratory Comparison, www.horizontal.ecn.nl

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