INTERNATIONAL STANDARD

ISO 13876

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Soil quality — Determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)

Qualité du sol — Détermination des polychlorobiphényles (PCB) par chromatographie en phase gazeuse avec détection sélective de masse (GC-MS) et chromatographie en phase gazeuse avec détection par capture d'électrons (GC-ECD)

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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

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, and 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).

The European Standard EN 16167:2012 on which this International Standard is based, 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 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 and 11 clean-up procedures are described. 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 ISO 13859). It has been tested for ruggedness.

This International 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 International Standard is applicable and validated

Matrix	Materials used for validation
Sludge	Municipal sewage sludge
Biowaste	Compost

Soil quality — Determination of polychlorinated biphenyls (PCB) by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)

WARNING — Persons using this International Standard should be familiar with usual laboratory practice. This International 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 International Standard be carried out by suitably trained staff.

1 Scope

This International 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 International 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] 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 International Standard, a limit of application of 1 µg/kg (expressed as dry matter) can be achieved.

Sludge and treated biowaste can 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 International 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.

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

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 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

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

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

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

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 209 individual PCBs

[SOURCE: EN 15308:2008, 3.2]

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

3.3
critical pair
pair of congeners that is 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 International Standard, different procedures for different steps (modules) are allowed. Which modules should be used depends on the sample. A recommendation is given in this International Standard. Performance criteria are described and it is the responsibility of the laboratories applying this International Standard to show that these criteria are met. Use of spiking standards (internal standards) allows an overall check on the efficiency of a specific combination of modules for a specific sample. However, 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 analysed 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) (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 can 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 can interfere with the determination. These interferences can lead to incompletely resolved signals and, depending on their magnitude, can 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 (8.2.1). 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 can interfere with the quantification of PCB in GC-MS. In presence of mineral oil, GC-ECD can 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 can 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. It is strongly advised that standard solutions are prepared centrally in suitably equipped laboratories or are purchased from suppliers specialized 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 recognized 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 of 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) is 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 for least 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 of 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 for 2 h at 600 °C, particle size of 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 of 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 of silica (7.3.5.1) and 44 g of 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 of silica (7.3.5.1) and 17 g of 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 of 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 International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same result.

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 International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

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

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 of 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 can also be suitable.

7.3.10.5 Deoxygenated water.

7.3.10.6 Pyrogenic copper.

Dissolve 45 g of copper(II)-sulfate pentahydrate (7.3.10.1) in 480 ml of water containing 20 ml of 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 of 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 while 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 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 of acetone (7.2.1) (while 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 of 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 at 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 of 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, regularly 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 are 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 DC 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)
PCB31	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, can 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 be 0,18 mm to 0,32 mm, and film thickness be 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.

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 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 ISO 14507, 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.

3) CP-Sil 19, OV 1701 are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products. Equivalent product may be used if they can be shown to lead to the same results.

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

Matrix	Drying technique			
	Air drying	Freeze drying (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 (see 10.2.2) or 3 (see 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 (see 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 can contain all aggregates, organic matter, and (plastic) waste.

Other extraction procedures, e.g. ultrasonic extraction, microwave, or high-pressure liquid extraction can 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 International 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 International Standard for some types of waste, the addition of acetone with Soxhlet extraction has been shown to be effective.

Extraction procedures described in this International 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, pressurized 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 for 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) can 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.

Pressurized liquid extraction can 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 of water. For moist samples, the water quantity to be added is calculated using Formula (1):

$$m_w = 50 - \frac{m_E \cdot w_{H_2O}}{100} \quad (1)$$

where

m_w is the mass of water to be added, in grams (g);

m_E is the mass of the sifted sample, in grams (g);

w_{H_2O} is the water content of the sample, determined according to ISO 11465, in percent (%).

Add 40 g of sodium chloride (7.2.6), 100 ml of acetone (7.2.1), and 50 ml of 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

Table 5 *(continued)*

Method	Clean-up	For removal of	Suitable for	Remarks
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 can 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 can 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 can 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 of 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 of 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 can occur. This is visible in a decrease of recoveries of the internal standards. If this occurs, readjustment of the sampled volume can be necessary.

10.4.5 Clean-up D — Florisil®

Add into a chromatographic tube (8.1.11) 5 mm of sodium sulfate (7.2.4), 1,5 g of Florisil (7.3.4.1), and again, 5 mm of sodium sulfate. To fix the mixture, place glass wool (8.1.9) on the top. Rinse the column with approximately 50 ml of isooctane (7.3.4.2). Apply the extract to the column. Rinse the extraction tube/vessel for two times with 1 ml of isooctane/toluene (95/5) (7.3.4.3) and give it onto the column. Afterwards, elute with 7 ml of 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 of silica NaOH (7.3.5.4), 5 g of silica H_2SO_4 (7.3.5.2), and 2 g of 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 latter case, the extracts are more diluted and interference by aliphatic hydrocarbons is 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 for 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 sulphate (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 of *n*-hexane (7.3.5.5). Apply the extract to the filled column. Rinse the extraction vessel for three times with 2 ml of *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 can be preferred.

— Presence of mineral oil: Removal of mineral oil can 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 recognized.

— 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). Optimize the gas chromatograph starting from the following conditions:

— separation column: Capillary column (8.2.2);

— oven temperature program: 60 °C, 2 min;
30 °C/min to 120 °C/min;
5 °C/min to 300 °C/min;
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 can 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):

$$\frac{A_n}{A_{C13}} = s \cdot \frac{\rho_n}{\rho_{C13}} + b \quad (2)$$

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, 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, 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).

Nonlinear calibration methods can 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 nonlinear 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 ISO 22892 for the identification of the PCBs. In 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 International 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 can 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, in percentage (%);

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 does 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 International 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 percentage (%);

$A_{1,\text{mean}}$ 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.