HORIZONTAL

Date: 2005-25-11

6th draft

Ad-hoc group PAH/PCB

Soils, sludges and treated bio-waste – Organic constituents – Polychlorinated biphenyls (PCB) by GC-MS and GC-ECD

Document type: International Standard
Document subtype:
Document stage: draft november 2005
Document language:

C:\Documents and Settings\Harms009\Local Settings\Temporary Internet Files\OLK21\PCB-Berlin-nov05.doc STD Version 2.0

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Foreword

The European project HORIZONTAL is focussed on the standardisation of test procedures in environmental samples. Several studies have been started to elaborate the possibility of horizontal standardisation on specific subjects. One of the subjects is the horizontal standardisation of Polychlorinated Biphenyls (PCBs) as described in this standard. PCB's are subject of standardisation in CEN 292 (waste), CEN 308 (sludge) and ISO TC190 (soil). This PCB-standard has been developed in co-operation with experts from CEN 308, CEN 292 and ISO/TC 190.

Introduction

Taken into account the different matrices and possible interfering compounds, this standard do 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 spiking 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.

Soils, sludges and treated bio-waste – Organic constituents – Polychlorinated biphenyls (PCB) by GC-MS and GC-ECD

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 soil, sludge, sediments, suspended solids and waste using GC/MS and GC/ECD (See also annex A)

The limit of detection is dependent 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 standard, limits of detection of 1 µg/kg (expressed as dry matter) may be achieved.

Waste, sludge and soil 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 standard contains decision tables based on the properties of the sample and the extraction and clean up procedure to be used. This method is 'performance based'. It is permitted to modify the method to overcome interferences not foreseen in this standard, provided that all performance criteria in this method are met.

Note 1: With this method also other PCBs-congeners can be analysed provided suitability is proven.

Note 2: This standard is not developed for the analysis of PCB in in liquids. For insulating liquids, petroleum products, used oils and aqueous samples is referred to EN 61619, EN 12766-1 and EN ISO 6468, respectively.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this European Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this European Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO/DIS 10381-1 *Soil quality – Sampling – Part 1: Guidance on the design of sampling programmes.*

ISO/DIS 10381-2 *Soil quality – Sampling – Part 2: Guidance on sampling techniques.*

ISO 11465:1993 *Soil quality – Determination of dry matter and water content on mass basis – Gravimetric method.*

ISO 14507 *Soil quality – Guidance for sample pretreatment for the determination of organic contaminants in soil.*

ISO/DIS 16720 *Soil quality - Pretreatment of samples by freeze drying for subsequent analysis*

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)*

prEN 14346 *Characterization of waste — Calculation of dry matter by determination of dry residue and water content*

prEN 15002 *Characterization of waste — Preparation of test portions from the laboratory sample*

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

EN ISO 6468 *Water quality — Determination of certain organochlorine insecticides, polychlorinated biphenyls*

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

3.2 Congener

a member of the same kind, class or group of chemicals – e. g. anyone of the 209 individual PCB

NOTE The IUPAC congener numbers are for easy identification; they do not represent the order of chromatographic elution.

3.3 Analyte

In the context of this international standard, these are polychlorinated biphenyls (PCBs)

3.4 Calibration standard

A solution prepared from primary and/or secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration

3.5 Internal standard

$^{13}C_{12}$-labelled PCB standards or a PCB not mentioned in the scope and unlike to be present in the sample. They are added to the sample to be extracted. They are used to quantify the PCBs to be measured. Recoveries of these standards are also calculated and used to check the performance of the procedure.

3.6 Injection standard

A $^{13}C_{12}$-labelled PCB or a PCB not mentioned in the scope and not added as internal standard and unlike to be present in the sample, is added to the extract before injection into the GC, to monitor variability of the instrument response and to calculate the recovery of the internal standards

3.7 Critical pair

a pair of congeners that must be separated to a predefined degree (e. g. 50 % valley) to ensure chromatographic separation meets minimum quality criteria

4 Principle

Due to the horizontal character of this standard, different procedures for different steps (modules) are allowed. Which modules should be used depends on the sample a recommendation is given in this standard. Performance criteria are described and it is the responsibility of the laboratories applying this 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 gives the information upon the extensive extraction efficiency of the native PCB bonded to the matrix.

After pretreatment according 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 an immobile phase of low polarity. Detection occurs with mass spectrometry (MS) or an electron capture detector (ECD).

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. Internal standards are to be used to have a check on the pretreatment, extraction and clean-up procedures. The average recovery of these standards should be at least 70. If the recovery is lower the method has to be modified using other modules described in this standard.

Temporally remark: the recovery of 70% may change after the validation study.

5 Interferences and hazards

5.1 Interferences

5.1.1 Interference with sampling and extraction

Use sampling containers of materials (preferably of steel 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.

5.1.2 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 will not allow an interpretation of the result. Unsymmetrical peaks and peaks being broader than the corresponding peaks of the reference substance suggest interferences.

Chromatographic separation between the following pairs can be critical. Due to their molecular mass differences, quantification can be made by mass selective detection. When incomplete resolution is encountered, peak integration shall be checked and, when necessary, corrected.

- PCB 52- PCB 73
- PCB 101 - PCB 89 / PCB 90
- PCB 118 - PCB 106
- PCB 138 - PCB 164 / PCB 163

Samples containing simultaneous PCB-and Tetrachlorobiphenymethan (TCBT)-mixtures the determination of the PCB with GC-ECD might be disturbed.

6 Safety remarks

PCBs are highly toxic and must be handled with extreme care. Contact of solid materials, solvent extracts and solutions of standard PCB with the body must 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 must be disposed of in a manner approved for disposal of toxic wastes.

In handling hexane precautions have to taken because of the neurotoxic properties of hexane.

7 Reagents

7.1 General

All reagents shall be of recognised analytical grade or better. The purity of the reagents used shall be checked by running a blank determination as described in 10.1. If the blank value is unreasonably high, i.e. more than 10 % of the lowest value of interest, find the cause through a step by step examination of the whole procedure.

7.1.1 n-heptane

7.2 Reagents for extraction procedures

7.2.1 Acetone

7.2.2 Petroleum ether

Boiling range 40 °C to 60 °C

Note Instead of the above petroleum ether, all hexane-like solvents with a boiling range between 34 and 68 °C are allowed to use

7.2.3 Anhydrous sodium sulfate

Heated for at least 6 h to 550 °C ± 20 °C, cooled to about 200 °C in the furnace and then to ambient temperature in a desiccator containing magnesium perchlorate or a suitable alternative. The anhydrous sodium sulfate shall be kept carefully sealed.

7.2.4 Sodium chloride, anhydrous

7.2.5 Distilled water or water of equivalent quality

7.2.6 Keeper substance

High boiling compound i.e. octane, nonane

7.3 Reagents for clean-up procedures

7.3.1 Clean-up A – Aluminium oxide

7.3.1.1 Aluminium oxide

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

7.3.1.2 Deactivated aluminium oxide

Deactivated with 10 % water.

To 90 g of aluminium oxide (7.3.1.1) add 10 g of water. Shake until all lumps have disappeared. Allow the aluminium oxide to condition before use for some 16 h, sealed from the air.

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

7.3.2 Clean up B - 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(H2O) = 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 to cool in a desiccator and add 10 % water (*w/w*) in a flask. Shake for 5 min intensively by hand until all lumps have disappeared and then for 2 h in a shaking machine. Store the desactivated silica gel in the absence of air, use it for maximum of one week.

7.3.3 Clean up C - Gel permeation chromatography

7.3.3.1 Bio beads SX-3

7.3.3.2 Ethyl acetate

7.3.3.3 Cyclohexane

Preparation of GPC, for example: Put 50 g Bio-Beads® S-X3(7.3.3.1) into a 500ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane (7.3.3.2) 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 hours; drain the slurry into the chromatography tube for GPC; after approximately 3 days, push in the plungers of the column so that a filling level of approx. 35 cm is obtained; to further compress the gel, pump approx. 2 l 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 approx. 33 cm.

7.3.4 Clean-up D – Florisil

7.3.4.1 Florisil baked 2 hours at 600°C). Florisil® is a commercial denomination of a silicate of magnesium compounds grain size: 100-200mesh

7.3.4.2 Iso-octane

7.3.4.3 Iso-octane/Toluene 95/5

7.3.5 Clean up E silica H2SO4/silica NaOH

7.3.5.1 Silica, particle size 70 µm to 230 µm, baked at 180 ºC for a minimum of 1 hour, and stored in a precleaned glass bottle with screwcap that prevents moisture from entering

7.3.5.2 Silica treated with sulphuric acid. Mix 56 g silica (7.3.5.1) and 44 g sulphuric acid (7.3.8.1)

7.3.5.3 Silica treated with NaOH. Mix 33 g silica (7.3.5.1) and 17 g sodium hydroxide (NaOH) (1 mol/l)

7.3.5.4 n-Hexane

7.3.6 Clean-up F Benzenesulfonic acid/sulfuric acid

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

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

7.3.7 Clean-up G – DMF or DMSO/cyclohexane

7.3.7.1 Dimethylsulfoxide(DMSO)

7.3.7.2 Dimethylformamide(DMF)

7.3.7.3 Cyclohexane

Temporally remark. DMF and DMSO are both used. Decision will be made after the validation study

7.3.8 Clean-up H – Concentrated sulphuric acid

7.3.8.1 Sulphuric acid, of purity 96% (*m/m*) to 98% (*m/m*)

7.3.9 Clean-up I – TBA sulphite reagent

7.3.9.1 Tetrabutylammonium reagent (TBA sulphite reagent)

Saturate a solution of tetrabutylammonium hydrogen sulphate in a mixture of equal volumina of water and 2-propanol, $\underline{c}((C_4H_9)_4NHSO_4)$ = 0.1 mol/l, with sodium sulphite.

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

7.3.10 Clean-up J- pyrogenic copper

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

7.3.10.1 copper(II)-sulphate pentahydrate, $CuSO_4 \cdot 5\ H_2O$

7.3.10.2 hydrochloric acid, HCl, *c* = 2 mol/l

7.3.10.3 zinc granules, particle size 0.3 mm to 1.4 mm

7.3.10.4 anionic detergent aqueous solution (e.g. 35 % *m*/*V* n-dodecane-1-sulfonic acid sodium salt $(CH_3(CH_2)_{11}SO_3Na)$[1)]

7.3.10.5 deoxygenated water

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 1000 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 1000 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 (7.3.5.4), to ensure elimination of the acetone.

Carefully transfer the copper with hexane into an Erlenmeyer flask and store under hexane. The flask must 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 month. The clean up efficiency will then decline. The copper will change colour as the clean-up efficiency decreases.

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1) other commercial available detergents may also be suitable.

7.3.11 Clean-up K Silica – Silvernitate Clean up

7.3.11.1 Silvernitrate, $AgNO_3$

7.3.11.2 $AgNO_3$/Silica adsorbant

Solve 10g of $AgNO_3$ (7.3.10.1) in 40 ml water and add this mixture in portions to 90g Silica (7.3.5.1). Shake the mixture until it is homogenous and leave it for 30 minutes. Put the mixture into a drying oven at 70 ± 5°C. Within 5 hours regular increase the temperature from 70°C to 125°C. Activate the mixture for 15 hours at 125°C. Store the mixture in brown glass bottles.

7.4 Gas Chromatic Analysis

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

7.5 Standards

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

7.5.1 Calibration standards

The calibration standard should contain at least the following compounds:

PCB-28	2,4,4'-trichlorobiphenyl	(CAS number: 7012-37-5)
PCB-52	2,2',5,5'-tetrachlorobiphenyl	(CAS number: 35693-99-3)
PCB-101	2,2',4,5,5'-pentachlorobiphenyl	(CAS number: 37680-37-2)
PCB-118	2,3',4,4',5-pentachlorobiphenyl	(CAS number: 31508-00-6)
PCB-138	2,2',3,4,4',5'-hexachlorobiphenyl	(CAS number: 35056-28-2)
PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl	(CAS number: 35065-27-1)
PCB-180	2,2',3,4,4',5,5'-heptachlorobiphenyl	(CAS number: 35065-29-3)

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

7.5.2 Internal and injection Standards

7.5.2.1 MS-detection

The labelled PCB congeners to be considered as internal standards are listed below. Use a mixture containing at least PCB 28, PCB 52, PCB 101 PCB 138, PCB 153, PCB 180. Internal standards shall be used for calculation of the relative retention times

- $13C_{12}$-2,4,4'-trichlorobiphenyl (PCB 28)
- $13C_{12}$-2,2',5,5'-tetrachlorobiphenyl (PCB 52)

- $13C_{12}$-2,2‘,4,5,5‘-pentachlorobiphenyl (PCB 101) (CAS number 37680-73-2)
- $13C_{12}$-2,3‘,4,4‘,5-pentachlorobiphenyl (PCB 118)
- $13C_{12}$-2,2‘,3,4,4‘,5‘-hexachlorobiphenyl (PCB 138) (CAS number 35065-28-2)
- $13C_{12}$-2,2‘,4,4‘,5,5‘-hexachlorobiphenyl (PCB 153)
- $13C_{12}$-2,2‘,3,4,4‘,5,5‘-heptachlorobiphenyl (PCB 180)

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

7.5.2.2 ECD-detection

Also for ECD-detection internal and injection standards shall be used.

At least three of the following compounds shall be used as internal and at least 1 as an injection standard. The standards shall not interfere with the analytes

PCB-29	2,4,5-trichlorobiphenyl	(CAS number: 15862-07-4)
PCB-30	2,4,6-trichlorobiphenyl	(CAS number: 35693-92-6)
PCB-143	2,2',3,4,5,6'-hexachlorobiphenyl	(CAS number: 68194-15-0)
PCB-155	2,2',4,4',6,6'-hexachlorobiphenyl	(CAS number: 33979-03-2)
PCB-198	2,2',3,3',4,5,5’,6,-octachlorobiphenyl	(CAS number: 68194-17-2)
PCB-207	2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl	(CAS number: 52663-79-3)
PCB-209.	2,2',3,3',4,4',5,5’,6,6'-decachlorobiphenyl	(CAS number: 2051-24-3)

Note Some PCB mixtures containing up to 2.5% of PCB-155

Note For internal standard PCB 30, PCB 143 and PCB 207 are advised

Note For injection standard PCB-198 or PCB-209 is recommended because of less interferences.

7.5.2.3 Column performance

PCB-28	2,4,4'-trichlorobiphenyl	(CAS number: 7012-37-5)
PCB-31	2,4',5-trichlorobiphenyl	(CAS number: 16862-07-4)

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 (7.1.1) by weighing approx. 10 mg of each of the standards (7.5.1) to the nearest 0.1 mg and dissolving them in 25 ml of n-heptane.

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

NOTE 1 Because of the dangerous nature of the substances to be used, commercially available - preferably certified - standard solutions are preferred to be used. Avoid skin contact.

NOTE 2 The working standard solutions shall be in the same solvent like the extract

NOTE 3 Store the primary and diluted standard solutions in a dark place at a temperature of less than 4 °C. The solutions

are stable for at least 1 year, provided that evaporation of solvent is negligible.

NOTE 4 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 solution, containing at least two (or all) different components (7.5.2), of about 0.4 mg/ml in n-heptane (7.1.1) by weighing approx. 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 which such a concentration that the added amount gives a peak area or peak surface of the same order of magnitude of the peaks of the injected calibration solutions.

Temporally remark: as part of the validation the number of internal standards will be investigated

7.6.3 Preparation of injection standard solution

Prepare a concentrated primary injection solution, containing at least two different components (7.5.2), of about 0.4 mg/ml in n-heptane (7.1.1) by weighing approx. 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 area or peak surface of the same order of magnitude of the peaks of the injected calibration solutions.

8 Apparatus

8.1 Extraction and clean-up procedures

Customary laboratory glassware.

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

8.1.1 Glass sample bottles

With glass stopper or screw top and polytetrafluorethene seal (PTFE) of appropriate volume

8.1.2 Shaking device

With horizontal movement (200 to 300 strokes a minute).

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.100ml, Soxhlet extractors and Soxhlet thimbles e.g. 27*100mm, vertical condensers e.g. 300mm, water-bath as heating apparatus

8.1.7 Concentrator

As per Kuderna Danish (see figure B.1).

Other evaporators, e.g. a rotary evaporator, may be used if found to be suitable. An evaporator is suitable if the performance of the method is in agreement with 10.7.5 or 10.8.5.

8.1.8 Boiling chips

Glass or porcelain beads.

8.1.9 Quartz wool or silanized glass wool

NOTE 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 capacity of 10 to 15 ml and ground glass stopper.

8.1.11 Chromatography tubes

Chromatography column of glass, 5mm inside diameter, length e.g. 600mm

See figure B.2.

8.2 Gas chromatograph

Equipped with a capillary column, Mass Spectrometric Detection (MS) or electron capture detector (ECD) based on ^{63}Ni.

NOTE 1 Working with an encapsulated radioactive source as present in an ECD requires a licence according to the appropriate national regulations.

NOTE 2 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.

8.2.1 Capillary columns

Comprising a 5% phenyl-methyl silicone stationary phase coated onto fused silica capillary column or an equivalent chemically bonded phase column. The dimensions should be sufficient to separate the critical pairs mentioned below. In general column length should be ca 50 m. Internal diameter ca 0.25 mm and film thickness ca 0.2 µm.

The chromatographic peaks of PCB 28 and PCB 31 shall be resolved sufficiently (resolution at least 0,5) for integrating the PCB 28 peak.

Using ECD-detection, a column coated with a moderate polar phase, e. g. CP-Sil 19, OV 1701 etc., shall be used to confirm the result obtained. Confirmation analysis using a second column is not necessary in case the analytical result is much below any regulatory level.

NOTE The retention times for the PCB on different capillary appear in annex C.

9 Sampling and preservation of samples

9.1 Sampling

Obtain representative samples in accordance with ISO 10381-1 (soil) using sampling apparatus in accordance with ISO 10381-2. ,,,,,, for waste and ..... for sludge

Samples shall be stored on a cool (< 10 °C) dark place. Samples can be stored for one week at a temperature between 0 and < 10 °C and for a year at temperatures < -18 °C.

9.2 Sample preservation and pretreatment

Samples shall be pretreated according the mentioned International Standards using the following scheme 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.

Following scheme will be removed if the Horizontal pre-treatment standard is available

(Bulk) sample

↓

Homogenization and subdividing (Method for bulk samples ,ISO)

↓

Laboratory sample

↓

If necessary, increasing solid matter (centrifugation[a], filtration[b])

↓

Drying

Sludge[c]	Sediment	Suspended solids	Soil (f.e. clay, sandy)	Waste (compost,Bio-waste, mixed waste	Waste (shredder/plastic materials
Freeze drying (ISO/DIS 16720)	Freeze drying (ISO/DIS 16720) No drying[d]	Freeze drying (ISO/DIS 16720)	Na_2SO_4 Air drying Freeze drying (ISO/DIS 16720) No drying	Na_2SO_4 Air drying Freeze drying (ISO/DIS 16720) No drying prEN 15002)	Air drying, (prEN15002)

↓

Grinding (ISO 14507)/sieving (homogenisation, subdividing)

↓

Test sample

(Dry or high solid matter content Water content < 50%)

a) Possible with several samples, but not practicable with material having approximately the same density of water (i.e. sludges)

b) Possible, but no good handling with several samples, blockages, water content to high, Extraction of target compound with filter paper

c) Na_2SO_4 can be used for preservation of the hygroscopic dried sludge

d) If water content enables direct extraction according 10.2.2

NOTE If it is possible to take a representative wet sample, the following procedure can be used to make a dry sample. Mix in a mortar ca 10 g of sample, weighed to the nearest 0.01 g, with one or more equivalents of Na_2SO_4, weighed to the nearest 0.01 g, so that a homogenous dry mass is obtained. Use this whole sample for further analysis.

Store the samples in a dark place at a temperature below 10 °C, if possible in a refrigerator. Determine the content of dry matter in the sample in according to ISO 11465 or prEN 14346.

NOTE It is permissible for dried samples, if kept sealed, to be stored for a longer period at room temperature (approx. 1 month).

10 Procedure

10.1 Blank

Perform a blank determination following paragraphs for the used procedure (selected extraction and clean-up) 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 freedom from contamination.

10.2 Extraction

10.2.1 General

Depending on the test sample, origin, moisture content, choose a suitable extraction method. Method 1 or 3 shall be applied if it is important to break up aggregates in the sample to reach the PCBs. This is especially important with soil samples containing clay particles. Also with wet samples these methods shall be applied. If solving of the PCBs is the most important step and the sample is dry, method 2 using Soxhlet shall be applied.

Extraction procedures described in this European standard are able 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: 10 - 20g of soil; 2 - 10 g of sewage sludge, 5 - 20 g of compost or 2 – 20 g of waste.

Table 1 Extraction methods to be used with different matrices

	Sample contains	Extractant	Equipment	Procedure	remarks
Dry test sample	Soil-like materials, sludge, sediments, biowaste, compost	Acetone/petroleum ether	agitation	Extraction 1	
Dry test sample	Plastic waste Sludge, biowaste, compost, suspendes solids, sediment	Petroleumether	Soxhlet, Pressurised Liquid Extration	Extraction 2	
Wet test sample	Soil-like material/sediments biowaste compost	Acetone/petroleum ether	agitation	Extraction 1	Limitations for the amount of water in the sample are given.
Wet test sample	Soil-like material/sediments biowaste compost	Acetone/petroleum ether/NaCl	agitation	Extraction 3	

10.2.2 Extraction 1 samples using acetone/petroleum ether

Take 2 g - 20 g test sample and place it in a sample bottle (8.1.1). Add X µl 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. Then add 50 ml of petroleum ether (7.2..2) and shake again thoroughly during at least 12 hours. Use a horizontal shaking device (8.1.2) and have the solvent movement in the sample bottle as long as possible (horizontal position). After settling, decant the supernatant. Wash the solid phase with 50 ml of petroleum ether (7.2.2) and decant again. Collect the extracts in a separating funnel of 1 litre capacity (8.1.4) and remove the acetone by shaking twice with 400 ml of water (7.2.5). Dry the extract over anhydrous sodium sulphate (7.2.3) and transfer the dried extract to the concentrator (8.1.7). Rinse the sodium sulphate with petroleum ether (7.2.2) and add the rinsing to the extract.

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

NOTE 2 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.

NOTE 3 Other extraction techniques, like ultrasonic extraction, microwave or pressurised extraction may be suitable. However if using other extraction techniques the comparability to the method described in this standard has to be proven.

NOTE 4 The amount X of the internal standard added in all extraction procedures 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 will be 0,1 µg/ml

10.2.3 Extraction 2 dry samples using Soxhlet

Take 2 g -20 g dry test sample and place it the extraction thimble (8.1.6). Add X µl of the secondary internal standard solution (7.5.2) and approximately 70 ml of the extraction solvent (7.2.2) to the sample. Extract the sample with the soxhlet extraction apparatus. The duration of the extraction should be calculated with a minimum of 100 extraction cycles.

Note If the sample is hydroscopic and is not dried just before analysis, add $NaSO_4$ to the test sample to get a free flowing material.

10.2.4 Extraction 3 of wet samples acetone/petroleum ether/Sodium chloride

Take an amount of sample equivalent to 2 g -20 g of the dry sample and put it into a 1 l sample bottle (8.1.1). Add X µl of the secondary internal standard solution (7.5.2). If the sample is dry, add 50 ml water. For moist samples, the water quantity to be added is calculated as follows:

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

m_{w} is the water to be added in grams

m_{E} is the quantity of the sifted soil sample in grams

$m_{\mathrm{H_2O}}$ is the water content of the sample according to ISO 11465 or prEN 14346 in percent mass (%)

Add 40 g sodium chloride (7.2.4), 100 ml acetone (7.2.1) and 50 ml petroleum ether (7.2.2) to the moistened preparations, close the sample bottle and shake it by means of the shaking device (8.1.2) for at least 12 h.

The organic phase is to be separated, if necessary, using a centrifuge with sealable centrifuge cups. Collect the extract in a separating funnel of 1 litre capacity and remove the acetone by shaking twice with 400 ml of water (7.2.5). Dry the extract over anhydrous sodium sulphate (7.2.3) and transfer the dried extract to the concentrator (8.1.7). Rinse with petroleum ether (7.2.2) and add the rinsing to the extract.

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

10.3 Concentration

Add a boiling chip (8.1.8) to the extract and concentrate the extract to approx. 10 ml by evaporation. Transfer the concentrated extract to a calibrated test tube (8.1.10) and concentrate to 1 ml using a gentle stream of nitrogen at room temperature. If clean up H is used, concentration is not necessary. Note the final volume of the extract.

NOTE Too high temperatures and a too high flow of nitrogen may result in loss of the more volatile PCB.

NOTE 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 (7.2.6).

10.4 Clean up of the extract

Clean-up has to 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 the following decision table has to be used. If polar compounds have to be removed take special care on the recoveries of the low chlorinated PCB's. Reference to validation reports is made in annex

A.

Table 2. Possible clean-up methods

	Clean-up	Designed for removal of	Special for	Validated	Remarks
Clean-up A	Aluminium oxide	Polar compounds		soil	Difficult to adjust water content and keep it constant
Clean-up B	Silica	Polar compounds		soil	Attention; some charges of silica can contain low concentrations of PCBs
Clean-up C	gelpermeation	High molecular compounds	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, lipides			Special for lipid containing samples
Clean-up F	Benzenesulfonic acid/sulfuric acid	Polar compounds, (poly)aromatics, bases, hetero compounds, oil		Waste?	Special suitable for samples containing high concentration of mineral oil
Clean-up G,	DMSO/hexane	Alipahatic hydrocarbons	MS		
Clean-up H	H_2SO_4 (conc)	lipids			
Clean-up I	TBA	sulphur	ECD	sludge/sediment	
Clean-up J	Cu	sulphur	ECD	sludge/sediment	
Clean-up K	$AgNO_3$/Silica	Sulphur + polar compounds	ECD	sludge/sediment/soil	Also applicable for MS

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

Temporally remark: Methods will be transferred to informative annex if no validation data are available of become available during the validation study

10.4.1 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 (7.3.2.1).

With a pipette transfer the extract to the dry packed adsorption column; rinse the test tube twice with 1 ml of petroleum ether (7.2.2) 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 approx. 10 ml of petroleum ether.

One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

NOTE 1 Before use, the elution pattern of each series of aluminium oxide columns and the necessary elution volume should be verified with the aid of a standard solution of PCBs.

NOTE 2 Commercial available disposable columns may be used as an alternative if found suitable. A column is suitable if the performance of the method is in agreement with 10.7.5 or 10.8.5.

10.4.2 Clean-up B silica gel

Put glass wool (8.1.9) and 10 g silica gel (7.3.4.2) into the chromatographic tube (8.1.11). Then add a 1 cm layer of sodium sulfate (7.2.3) and condition with 20 ml petroleum ether (7.2.2.). The sample is put into the column when the level of the solvent mixture is drained to approximately 0.5 cm above the column packing. The reduced extract

or the eluate obtained in the previous clean-up step is to be quantitatively transferred to the column; alternatively, an aliquot may be used.

Elution is performed using a total of 100 ml petroleum ether (7.2.2). One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

10.4.3 Clean-up C – Gelpermeation

The extract obtained 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) for gel permeation chromatography (6.2.3). The dissolved residue is put into the GPC column.

The solvent mixture for GPC is used for elution.

Settings of the GPC system (recommended values):

Flow rate:	5	$ml \cdot min^{-1}$
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 have to be regularly verified by means of the multi-component PCB-standard solution.

One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

Note During use of the gelpermeation column a small shift in volume to be collected may occur. This will be visible in a decrease of recoveries of the internal standards. If this occurs readjustment of the sampled volume may be necessary

10.4.4 Clean-up D- Florisil

Add into a chromatographic tube (8.1.11) 5 mm sodium sulfate (7.2.3), 1.5.g Florisil (7.3.4.1), and again 5mm high sodium sulfate. To fix the mixture, place glass wool (8.1.9) on the top. Rinse the column with approx. 50 ml isooctane (7.3.4.2). Give the evaporated extract received from the extraction quantitatively with a pipette onto the column. Rinse the extraction tube/vessel for two times with 1ml 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 (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

10.4.5 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 (7.3.5.3), 5 g silica H_2SO_4 (7.3.5.2) and 2 g sodium sulphate (7.2.3) in a clean chromatography column (8.1.11). Add a sufficient amount of n-hexane (7.3.5.4) and elute until the top of the n-hexane phase reaches the top of the sodium sulfate layer. Place with the aid of a Pasteur pipette the extract on top of the Na_2SO_4 layer and make it penetrate into the Na_2SO_4 layer. Elute with ca 60 ml of n-hexane and collect the entire n-hexane fraction. One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

10.4.6 Clean-up F Benzenesulfonic acid/sulfuric acid

Benzenesulfonic acid/sulfuric acid pre-treatment is effective if the sample contains large amounts of oil.

Directly prior to the clean-up procedure put 0.5 g of silica H_2SO_4 (7.3.5.2) onto the frit of the benzenesulfonic acid column. Place the combined benzenesulfonic acid/sulfuric acid column on the silca gel column (7.3.5.1). condition the two columns three times with 2 ml n-hexane (7.3.5.4), use vacuum assisted drying.

Transfer 250 µl of the extract onto the upper column and flush with 0.5 ml n-hexane. The extract shall be distributed

evenly over the packing of the upper column. After at least 30 seconds, elute the upper column twice with 1 ml n-hexane. Remove the upper column.

Elute the lower column twice with 0.5 ml n-hexane. One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

10.4.7 Clean-up G DMSO/n-hexane partitioning for aliphatic hydrocarbons removal

Extracts of samples containing a high amount of aliphatic compounds need additional clean up by dimethylsulfoxide/hexane partitioning.

This additional clean-up step has only to be applied in case of GC/MSD 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 of to a separating funnel of 100 ml and extract the PCBs with 25 ml of DMSO (7.3.6.1). Repeat twice. Transfer the combined DMSO extracts to a separatory funnel of 500 ml, add 100 ml of water (6.1.5) and extract the PCBs with 50 ml of n-hexane (7.3.5.4). Repeat once. One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

10.4.8 Clean-up H concentrated Sulphuric 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 (7.2.2). Pour in 5 ml of concentrated sulfuric acid (7.3.8.1) and shake vigorously at intervals for 5 minutes. Allow to separate completely (about 15 min.). Take the upper layer, rinse the remaining sulphuric acid with petroleum-ether. One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

10.4.9 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 (7.2.3) to remove the remaining traces of water.

10.4.10 Clean-up J- Clean-up using pyrogenic copper to remove elemental sulphur and some other organic sulphur 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 to above 5 min at approx. 3500 rpm (ensure that there is no visible turbidity). Remove the extract and if necessary, clean-up further using column chromatography.

10.4.11 Clean up K $AgNO_3$/Silica

Add into a chromatographic tube (8.1.11) sodium sulfate (7.2.3) e.g. 5mm high , 2g of the $AgNO_3$/silica mixture (7.3.11.2) and again 5mm high Na_2SO_4 . Rinse the column with approx. 50ml n-hexane (7.3.5.4). Give the concentrated extract quantitatively with a pipette onto the filled column. Rinse the extraction vessel for three times with 2ml n-hexane and give it onto the column, when the meniscus of the extract riches the surface of the Na_2SO_4. Add in the same manner 40 ml of hexane onto the column. One drop of keeper substance (7.2.6) is added to the eluate, and then the eluate is reduced to approximately 0.5 ml by means of the concentrator (8.1.7).

Note If the eluate is still coloured after the clean up, the proceeding should be repeated.

10.5 Addition of the injection standard

Add Y.µl of the secondary injection standard (7.6.3) to the extract obtained after clean-up (must be in line with the calibration standard) Note the final volume *V*

10.6 Gas chromatographic analysis

10.6.1 General

Both MS and ECD detectors are allowed, but in special cases only one will give 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 easier 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 5.1.2). Optimise the gas chromatograph starting from the following conditions:

GC-conditions:

Separation column:	Capillary column, non to medium polar stationary phase, film thickness 0,25 µm, length 30 m, internal diameter 0,25 mm
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

10.7 MS detection

10.7.1 Mass spectrometric (MS) conditions

MS Interface Temperature:	295 °C
Filament On	6 min

10.7.2 Identification

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

Use the diagnostic ions as given in table 3. For identification of the PCBs apply ISO 22892. In general diagnostic ion 1 can be used for quantification.

Table 3. Diagnostc 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*)
PCBs			
PCB 28	256 (100)	258 (74)	186 (82)
$^{13}C_{12}$-PCB 28	268	270	
PCB 52	292 (100)	294 (49)	220 (95)
$^{13}C_{12}$-PCB 52	304	306	
PCB101	326 (100)	328 (65)	256 (62)
$^{13}C_{12}$-PCB101	338	340	
PCB 118	326 (100)	328 (62)	254 (57)
$^{13}C_{12}$-PCB 118	338	340	
PCB 138	360(100)	358 (42)	290 (106)
$^{13}C_{12}$-PCB 138	372	374	
PCB 153	360 (100)	362 (92)	290 (73)
$^{13}C_{12}$-PCB 153	372	374	
PCB 180	394 (100)	396 (96)	324 (84)
$^{13}C_{12}$-PCB 180	406	408	

(..) 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 have to be considered as indication.

10.7.3 Calibration of the method using internal standard

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 standard.

Add a specific mass of the internal standard and injection standard (7.5.2) to dilutions of the mixed calibration solution (7.5.1). The mass concentration of both standards must 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 (7.5.1). 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 equation (2):

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

where:

A_n is the measured response of the native PCB e.g. peak area;

A_{C13} is the measured response of the $^{13}C_{12}$-labeled 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 µg/ml;

ρ_{C13} is the mass concentration of the $^{13}C_{12}$-labeled PCB internal standard in the calibration solution in µg/ml;

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

Two types of calibration are distinguished: the initial calibration (10.7.3.1) and the daily calibration (validity check of the initial calibration); the last one is called recalibration (10.7.3.2).

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.

The recalibration checks the validity of the linear working range of the initial calibration curve and is performed before each series of samples.

NOTE Non linear calibration methods are allowed.

10.7.3.1 Initial calibration

Take a gas chromatogram of a series of at least 5 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.

Determine the deviations between the measured values and the initial calibration line. When the deviation for the highest concentration is less than 5 %, assume linearity exists for the whole range. When this deviation is more than 5 %, decrease the range by deleting the value for the highest concentration.

Choose as a working standard the calibration solution with the concentration closest to the middle of the linear range. When the range of the samples is lower than the linear range found, it is permissible for a working standard with a lower concentration to be chosen, corresponding to the middle of the sample range.

NOTE It is allowed to use non-linear calibration using all 5 standards. In that case, the same 5 standards have to be used for recalibration and not the selection of 2 described below

10.7.3.2 Recalibration.

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 the 95 % confidence limits of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line has to be established according to 9.8.1.1.

10.7.4 Measurement

Measure the gas chromatograms of the extracts obtained under 9.5. 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. Assign the name of a compound if the relative retention time differs from the relative retention time obtained under 10.7.3.2 by less than 0.2 %.

If the concentration is above the level for proper identification or quantification, a diluted extract has to be injected for proper identification or quantification of the relevant PCB's.

NOTE If as a result of dilution, the internal standard is outside the linear range, formula 4 in 10.7.6 will not give the proper quantification and the deviation from linearity has to be taken into account

10.7.5 Check on method performance

Because this 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.

Add to the volume used for analysis X µl of internal standard and Y µl of injection standard. This is the performance standard.

Note The performance standard can be one of the calibration standards, provided that the ratio of the volumes (X/Y) used is the same.

Calculate for each internal standard the ratio between sample and performance standard solution using the closest injection standard.

$$recovery\ ratio = \frac{A_1(sample)}{A_2(sample)} \cdot \frac{A_2(p.s.)}{A_1(p.s.)} .100\% \quad (3)$$

A_1 is the measured response of the ^{13}C-labeled PCB internal standard e.g. peak area

A_2 is the measured response of the ^{13}C-labeled PCB injection standard e.g. peak area

p.s. is performance standard

The average ratio in the sample must be at least 70% of the ratio in the standard. The ratio for an individual PCB should be at least 60%. If not the analyses shall be repeated using modules more suitable for the sample.

10.7.6 Calculation

Calculate the mass ratio of the total individual PCB using equation (4):

$$\omega_n = \frac{(A_n / A_{C13}) - b}{s \cdot m \cdot d_s} \cdot \rho_{C13} \cdot f_e . f_t \cdot V \quad (4)$$

where:

ω_n is the content of the individual PCB found in the sample in mg/kg on the basis of the dry substance;

A_{C13} is the measured response of the $^{13}C_{12}$-labeled 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 $^{13}C_{12}$-labeled PCB internal standard added to the sample in µg;

m is the mass of the soil test sample used for extraction in g;

w_s is the content of the dry substance in the field moist sample in mass fraction, determined according to ISO 11465 or prEN14346 in %;

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 in accordance with ISO 14507;

V is the volume of the final solution in ml;

S is the slope of the calibration function;

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

The result shall be expressed in mg/kg dry material and rounded to two significant figures.

10.8 ECD-detection

Using ECD-detection, the same procedure as for MS can be followed, except the points described below

10.8.1 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.

10.8.2 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 (8.2.1) in combination with ECD. According to ISO 22982, 3 identification points have to 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 3 identification points (ISO 22982) for identification are obtained. If one is missing, only indication can be reported.

10.8.3 Calibration of the method using internal standards

Internal and injection standards are not $^{13}C_{12}$-PCBs but standards described in 7.5.2.2. Replace in equation 2 $^{13}C_{12}$-labeled PCB by used internal standard.

10.8.4 Measurement

No differences.

10.8.5 Check on 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. Compared to MS-detection, this will more often occur with ECD-detection. Therefore two injection standards are added to the extract to determine whether interfering compounds are present or absent. An injection standard can only be used if its retention time on the gas chromatography column(s) does not interfere with the retention time of one of the analytes.

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 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 the equation (5):

$$\mathrm{RRR} = \frac{R_{\mathrm{e},198}}{R_{\mathrm{e},2}} \times \frac{R_{\mathrm{s},2}}{R_{\mathrm{s},209}} \qquad (5)$$

where:

RRR is the relative response ratio;

$R_{\mathrm{e},143}$ is the response of PCB-198 in the extract;

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

$R_{\mathrm{s},207}$ is the response of PCB-209 in the working standard solution;

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

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 the formula below. 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 only the injection standard PCB 143 if RRR < 1.05, use only PCB-209 if RRR > 1,05

$$recovery\ ratio = \frac{A_1(sample)}{A_2(sample)} \cdot \frac{A_2(p.s.)}{A_1(p.s.)} .100\% \quad (6)$$

A_1 is the measured response of the PCB internal standard e.g. peak area

A_2 is the measured response of the PCB injection standard e.g. peak area

p.s. is performance standard

The average recovery ratio in the sample must be at least 7o% of the ratio in the standard. The ratio for an individual PCB should be at least 60%. If not the analyses shall be repeated using modules more suitable for the sample. If one of the ratios is higher and exceeds 100 % it is probably that an interfering compound is present in the sample having the same retention time. This internal standard should not be used for further calculation (see also 9.9.4.

10.1.6 Calculation

Internal and injection standards are not 13-labeled-PCBs but standards described in 7.5.2.2. Replace in equation 4 $^{13}C_{12}$ PCB by used internal standards.

11 Validation

This method has been validated for the samples and modules described in annex A. These modules are suitable for most samples described in the scope. Because a large amount of different modules and combination of modules can be used applying this method, which are not yet validated, additional preconditions are set.

- Follow the recommendations for selection of the different modules
- Recovery of the internal standard as described in 10.7.5 and 10.8.5 is an essential precondition performing this analysis
- The laboratory has to prove their proficiency according the combination of modules they are using with at least 3 Certified Reference Standards that fit for purpose.
- If the method in use is not applicable for a specific sample (low recoveries of internal standards), Validation of the new combination of modules is not necessary for this incidental case. Validation with at least 3 CRMs is necessary if this new combination is frequently used by the laboratory.

Note Suitable CRMs (State of the Art 2005) are ……..

Temporary remark: In CEN Environmental TC's the validation is subject of discussion.

12 Test report

The test report shall contain at least the following data:

a) the information required to identify the sample;

b) a reference to this international standard;

c) he extraction module, clean-up module and detection module used

d) the columns used and the gas chromatographic conditions;

e) the contents of individual PCB in mg/kg on the basis of dry matter, rounded off in such a way that no more than two significant values are obtained (for instance 12; 5.5; 0.36; 0.082; 0.0069 mg/kg d.m.)

f) 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 Description on materials for which the method is validated and also materials for which experience is present and future validation should be carried out

For the analysis of PCBs, the following relevant sample types are distinguished

- **Sludge**
  - Sewage sludge
  - Industrial sludge
- **Sediment, suspended solids**
- **Waste**
  - Soil-like waste
  - Building materials containing tar particles, creosote wood, surface treated materials
  - Bitumen[2]
  - Mixed waste (containing different phases)
- **Soil improvers**
  - Compost (stabilized)
  - Biowaste (not stabilized) containing organic matter of natural origin
- **Soil**

All mineral soils and organic rich soils

2 More important for PAHs

Annex B Validation

In this annex reference is made to standards and validation reports in which parts of this Horizontal standard were validated.

Extraction procedure

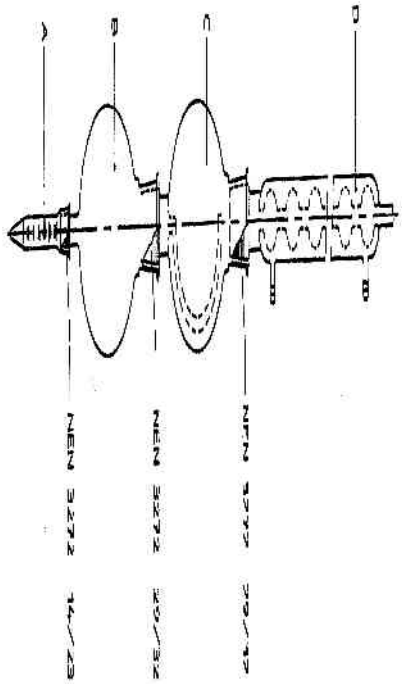
	Extraction	**matrix**	
Extraction 1		dry soil	ISO 10382
		Wet soil/sediment	ISO 10382
Extraction 2		sludge	DIN 38414-20
Extraction 3			

Clean-up procedure

	Clean-up	**matrix**	
Clean-up A	Aluminium oxide	soil	ISO 10382
		sediment	ISO 10382
Clean-up C	Silica		
Clean-up D	Florisil		
Clean-up E	H_2SO_4/Silica NaOH		
Clean-up F	Benzenesulfonic acid/sulfuric acid		
Clean-up G	gelpermeation		
Clean-up H	H_2SO_4 (conc)		
Clean-up I	TBA		
Clean-up J	Cu		
Clean-up K	$AgNO_3$/Silica	sludge	DIN38414-20
	AG/NO_3/Aluminium oxide	sediment	Waterschap Brabantse Delta
Clean-up L,	DMSO/hexane		

Annex C
(informative)

Figures



A = Test tube (15 ml)

B = Destillation bottle (500 ml)

C = Receiving bottle (500 ml)

D = Bol condensor

Figure B.1 = Drawing of the concentrator as per Kuderna Danish

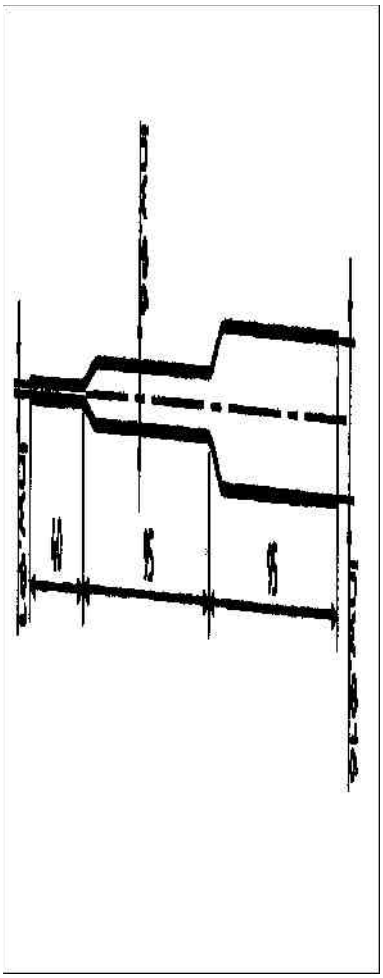


Figure B.2 Chromatographic tub. Sizes are given in mm

Annex D
(informative)

Table of retention times of polychlorinated biphenyls for two different capillary columns

Table C.1

Component	Retention time [min]	
PCB-28	33.32	32.98
PCB-52	34.85	34.54
PCB-101	38.71	38.27
PCB-118	41.89	41.61
PCB-138	45.00	44.54
PCB-153	43.18	42.49
PCB-180	50.41	49.47
[1)] A: 50 m CP-Sil 8; radius 0,22 mm; filmlayer 0,12 μm [2)] B: 50 m CP-Sil 19; radius 0,22 mm; filmlayer 0,12 μm		

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