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Soils, sludges and treated bio-waste — Determination of polychlorinated biphenyls — Method by GC-MS and GC-ECD

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Foreword

This document (BT/TF151 WI CSS99016) has been prepared by Technical Committee CEN/TC BT "", the secretariat of which is held by .

This document is a working document.

Project Horizontal is commissioned to develop standards in the field of sludge, soil and treated biowaste that are common to several matrices, are consistent with regard to EU regulation, and which lead to equivalent results as far as is technically feasible – so-called Horizontal Standards. These draft standards have been prepared by experts involved in ISO and CEN technical committees in the field of soil, sludge and biowaste. The standards will be applicable only to matrices for which they have been validated in accordance with ISO 5725-5. In the course of the work of the project Horizontal, three development levels of draft are produced, combined with consultation of relevant CEN bodies.The final draft standards produced by project Horizontal will be further processed by BT/TF151. 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.

This standard is applicable and validated for several types of matrices. The table below indicates which ones

Material	Validated	Document
Soil	X	Report on Horizontal Validation Study, (JRC, 2007)
Sludge	X	[Report on Horizontal Validation Study, (JRC, 2007)
Treated bio-waste	X	Report on Horizontal Validation Study, (JRC, 2007)
Soil improvers	Not validated yet	
Waste	validated by CEN 292 in 2007	

Introduction

This document is developed in the framework of the 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 standard does not contain one single possible way of working. Several choices are possible, in particular relating to clean-up. Detection with both MS-detection and ECD-detection is possible. Two different extraction procedures are described and 11 clean-up procedures. The use of internal and injection standards is described in order to have an internal check on choice of the extraction and clean-up procedure. The method is as far as possible in agreement with the method described for PAHs.

After an evaluation study, in which e.g. the ruggedness of the method was studies, a European wide validation of the draft standard has taken place. The results of the desk studies as well as the evaluation and validation studies have been subject to discussions with all parties concerned in CEN. The standard is part of a modular horizontal approach in which the standard belongs to the analytical step.

Until now test methods determining properties of materials were often prepared in Technical Committees (TCs) working on specific products or specific sectors. In those test methods often steps as sampling, extraction, release or other processing, analyses, etc were included. In this approach it was necessary to develop, edit and validate similar procedural steps over and over again for every material or product. Consequently this has resulted in duplication of work. To avoid such duplication of work for parts of a testing procedure references to parts of test methods from other TCs were introduced. However the following problems are often encountered while using references in this way: 1) The referenced parts are often not edited in a way that they could easily be referred to, 2) the referenced parts are often not validated for the other type of material and 3) the updates of such test standards on products might lead to inadequate references.

In the growing amount of product and sector oriented test methods it was recognised that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that can be used for all materials and products or for all materials and products with certain specifications.

Based on this hypothesis a horizontal modular approach is being investigated and developed in the project 'Horizontal'. 'Horizontal' means that the methods can be used for a wide range of materials and products with certain properties. 'Modular' means that a test standard developed in this approach concerns a specific step in assessing a property and not the whole ” chain of measurement” (from sampling to analyses). A beneficial feature of this approach is that “modules” can be replaced by better ones without jeopardizing the standard “chain”.

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the test procedure.

The modules that relates to this standard are specified in section XX Normative references.

An overview of modules and the manner, in which modules are selected will be worked out later, at which time proper reference in this standard will be provided.

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 treated biowaste using GC/MS and GC/ECD (See also annex A) The content of this European Standard is identical to EN 15308 and is therefore applicable to waste. Validation for waste is carried out by CEN 292 and not in the framework of HORIZONTAL.

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 European 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 European Standard, provided that all performance criteria mentioned in this European Standard are met.

NOTE 1The method may be applied to the analysis of other PCB congeners not specified in the scope, but its suitability should be proven by proper in-house validation experiments.

NOTE 2 This European Standard is not developed for the analysis of PCB 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 referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CSS99031 Sludge, treated biowaste, and soils in the landscape – Sampling – Framework for the preparation and application of a sampling plan

CSS99058 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 1: Guidance on selection and application of criteria for sampling under various conditions

CSS99057 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 2: Guidance on sampling techniques

CSS99032 Sludge, treated biowaste, and soils in the landscape – Sampling - Part 3: Guidance on sub-sampling in the field

CSS99059 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 4: Guidance on procedures for sample packaging, storage, preservation, transport and delivery

CSS99060 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 5: Guidance on the process of defining the sampling plan

CSS99035 Soil, sludge and treated biowaste – Pre-treatment for organic characterisation

CSS99022 Soil, sludge and treated biowaste – Determination of dry matter – Gravimetric method

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

ISO/DIS 16720[1)] *Soil quality - Pretreatment of samples by freeze drying for subsequent analysis*

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 European Standard, the following terms and definitions apply.

3.1 Polychlorinated biphenyl (PCB)
biphenyl substituted by one to ten chlorine atoms

3.2 Congener
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
polychlorinated biphenyls (PCBs)

3,4 Calibration standard
A solution prepared from a secondary standard and/or stock solutions and use 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 resolution to ensure chromatographic separation meets minimum quality criteria

3.8 Limit of detection
lowest content that can be measured with reasonable statistical certainty

Remarks: It was deeply discussed within the experts who had attended the TF151/TG3 Meetings and it was resolved that the definition of limit of detection is to be given according to the CEN rules, if there is any. Otherwise a definition is to be given, which is to be stated in all the Horizontal standards.

1) Under preparation

3.9 Resolution
Difference in retention times of the two peaks a and b, which constitute the critical pair, divided by the arithmetic mean of the peak widths at base of a and b, calculated as:

$$\frac{2\,\Delta t}{Y_a + Y_b}$$

where Δt is the difference in retention times of the two peaks a and b (sec), which constitute the critical pair and Y_a and Y_b are the peak widths at base of a and b (sec), respectively, as indicated in fig. x

4 Principle

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

After pretreatment according methods referred to in 8.2, 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 between 70 % and 110%. If the recovery is lower the method shall be modified using other modules described in this European Standard.

Note Some samples may require multiple clean-up in that case lower recoveries are accepted (see also 9.7.5)

5 Interferences and hazards

5.1 Interferences

5.1.1 Interference with sampling and extraction

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

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

5.1.2 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 does 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. The critical pair PCB28 and PCB31 is used for selection of the capillary column (see . 7.2.2) If molecular mass differences are present, quantification can be made by mass selective detection. If not or using ECD, the specific PCB is reported as the sum of all PCBs present in the peak. Typically the concentrations of the co-eluting congeners compared to those of the target congeners are low. When incomplete resolution is encountered, peak integration shall be checked and, when necessary, corrected. .

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

Presence of considerable amounts of mineral oil in the sample may interfere with the quantification of PCB in GC-MS. In presence of mineral oil GC-ECD may be preferred or mineral oil can be removed using clean-up procedure G (see 9.4) using DMSO/n-hexane.

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

5.2 Hazards

PCBs are highly toxic and shall 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 shall be disposed of in a manner approved for disposal of toxic wastes.

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

Anyone dealing with waste and sludge analysis shall be aware of the typical risks of that kind of material irrespective of the parameter to be determined. Waste and sludge samples may contain hazardous (e. g. toxic, reactive, flammable, infectious) substances, which can be liable to biological and/or chemical reaction. Consequently it is recommended that these samples should be handled with special care. The gases, which may be produced by microbiological or chemical activity, are potentially flammable and pressurise sealed bottles. Bursting bottles are likely to result in hazardous shrapnel, dust and/or aerosol. National regulations shall be followed with respect to all hazards associated with this method.

6 Reagents

6.1 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 9.1. If the blank value is unreasonably high, i.e. more than half of the lowest reported value, find the cause through a step by step examination of the whole procedure.

6.1.1 n-heptane

6.2 Reagents for extraction procedures

6.2.1 Acetone, C_2H_4O

6.2.2 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 30 and 68 are allowed to use.

Tempory remark: prEN 15308 (CEN292) accepts n-heptane in that case the boiling range should be 30-98

6.2.3 Anhydrous sodium sulphate, Na_2SO_4

The anhydrous sodium sulfate shall be kept carefully sealed.

6.2.4 Sodium chloride, anhydrous, NaCl

6.2.5 Distilled water or water of equivalent quality, H_2O

6.2.6 Keeper substance

High boiling compound i.e. octane, nonane

6.3 Reagents for clean-up procedures

6.3.1 Clean-up A – Aluminium oxide

6.3.1.1 Aluminium oxide

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

6.3.1.2 Deactivated aluminium oxide

Deactivated with 10 % water.

To 90 g of aluminium oxide (6.3.1.1) 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, use it for maximum two weeks after testing.

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

6.3.2 Clean up B - Silica gel 60 for column chromatography,

6.3.2.1 Silica gel 60, particle size 63 µm to 200 µm,

6.3.2.2 Silica gel 60, water content: mass fraction w(H2O) = 10 %.

Silica gel 60 (.6.3.2.1), 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 two weeks.

6.3.3 Clean up C - Gel permeation chromatography

6.3.3.1 Bio beads®[2] SX-3

6.3.3.2 Ethyl acetate, $C_4H_8O_2$

6.3.3.3 Cyclohexane, C_6H_{12}

Preparation of GPC, for example: Put 50 g Bio-Beads® S-X3(6.3.3.1) into a 500ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane (7.3.3.2) and ethyl acetate (6.3.3.2) 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 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.

2) Bio-Beds is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

6.3.4 Clean-up D – Florisil®[3)]

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

6.3.4.1 Iso-octane C_8H_{18}

6.3.4.2 Iso-octane/Toluene 95/5

6.3.5 Clean up E silica H2SO4/silica NaOH

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

6.3.5.2 Silica treated with sulphuric acid. Mix 56 g silica (6.3.5.1) and 44 g sulphuric acid (6.3.8.1)

6.3.5.3 Silica treated with NaOH. Mix 33 g silica (6.3.5.1) and 17 g sodium hydroxide (NaOH) (1 mol/l)

6.3.5.4 n-Hexane, C_6H_{14}

6.3.6 Clean-up F Benzenesulfonic acid/sulfuric acid

6.3.6.1 3 ml silica gel column, of adsorbant mass 500 mg, particle size 40 µm

6.3.6.2 3 ml benzenesulfonic acid column, of adsorbant mass 500 mg, particle size 40 µm

6.3.7 Clean-up G – DMF/hexane partitioning

6.3.7.1 Dimethylformamide(DMF), C_3H_7NO

6.3.8 Clean-up H – Concentrated sulphuric acid, H_2SO_4

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

6.3.9 Clean-up I – TBA sulphite reagent

6.3.9.1 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.

6.3.10 Clean-up J- pyrogenic copper

WARNING — Pyrogenic copper is spontaneously inflammable. Suitable precautions shallbe taken.

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

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

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

6.3.10.3 zinc granules, Zn, particle size 0,3 mm to 1,4 mm

6.3.10.4 anionic detergent aqueous solution (e.g. 35 % *m*/*V* n-dodecane-1-sulfonic acid sodium salt $(CH_3(CH_2)_{11}SO_3Na)$[4)]

6.3.10.5 deoxygenated water

Dissolve 45 g copper(II) sulfate pentahydrate (6.3.10.1) in 480 ml water containing 20 ml hydrochloric acid (6.3.10.2) in a 1 000 ml beaker.

Take 15 g of zinc granules size (6.3.10.3) add 25 ml water and one drop of anionic detergent solution (6.3.10.4) in another 1 000 ml beaker.

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

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

Stirring is continued until the hydrogen generation almost ceases. Then the precipitated copper is allowed to settle. The supernatant water is carefully removed and the product washed with deoxygenated water (6.3.10.5) three times, to eliminate residual salts.

Then the water is carefully replaced with 250 ml acetone (6.2.1) (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 (6.3.5.4), to ensure elimination of the acetone.

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

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

6.3.11 Clean-up K Silica – Silvernitate Clean up

6.3.11.1 Silvernitrate, $AgNO_3$

6.3.11.2 $AgNO_3$/Silica adsorbant

Solve 10 g of $AgNO_3$ (6.3.11.1) in 40 ml water and add this mixture in portions to 90 g of silica (6.3.5.1). Shake the mixture until it is homogenous and leave it for 30 min. Put the mixture into a drying oven at (70 ± 5) °C. Within 5 h regular increase the temperature from 70°C to 125°C. Activate the mixture for 15 h at 125°C. Store the mixture in brown glass bottles.

6.4 Gas Chromatographic Analysis

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

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4) other commercial available detergents may also be suitable

6.5 Standards

6.5.1 General

Choose the internal standards (3.5) 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

6.5.2 Calibration standards

The calibration standard (3.4) should contain at least the following compounds:

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

6.5.3 Internal and injection Standards

The PCB congeners to be considered as internal and injection standards (3.6) 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, often an aliquot of the extract is 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 containing up to 2,5 % of PCB155

NOTE 2 For internal standard PCB30, PCB143 and PCB207 are advised

NOTE 3 For ECD-detection injection standard PCB198 or PCB209 is recommended because of little interferences.

6.5.3.1 Labelled PCB congeners

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

6.5.3.2 Non- labelled PCB congeners

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

6.5.3.3 PCB congeners for resolution check

PCB28	2,4,4'-trichlorobiphenyl	(CAS number: 7012-37-5)
PCB 31	2,4',5-trichlorobiphenyl	(CAS number: 16862-07-4)

6.6 Preparation of standard solutions

6.6.1 Preparation of calibration standard solutions of PCBs

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in n-heptane (6.1.1) by weighing approx. 10 mg of each of the standards (6.5.2) 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 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.

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

NOTE 2 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 one year, provided that evaporation of solvent is negligible.

NOTE 3 Components present in mixed standard solutions should be completely separated by the gas chromatographic columns used.

6.6.2 Preparation of internal standard solution

Prepare a concentrated primary internal solution, containing at least three different components (6.5.3), of about 0,4 mg/ml in n-heptane (6.1.1) 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 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 solution, 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.

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

6.6.3 Preparation of injection standard solution

Prepare a concentrated primary injection solution, containing at least two different components (6.5.3), of about 0,4 mg/ml in n-heptane (6.1.1) 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 with measurable peak area or peak surface in the chromatogram (at least 10 times the detection limit)

7 Apparatus

7.1 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.

7.1.1 Sample bottles

Glass, stainless steel or aluminium sample bottles with glass stopper or screw top and polytetrafluoroethene seal (PTFE) of appropriate volume.

NOTE Glass is not appropriate for sludges.

7.1.2 Shaking device

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

7.1.3 Water bath, adjustableup to 100 °C

7.1.4 Separating funnels of appropriate volume

7.1.5 Conical flasks of appropriate volume

7.1.6 Soxhlet extraction apparatus

Consisting of: round bottom flask,, Soxhlet extractors and Soxhlet thimbles e.g. 27*100mm, vertical condensers e.g. 300mm, water-bath as heating apparatus

7.1.7 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 9.7.5or 9.8.5.

7.1.8 Boiling chips, glass or porcelain beads

7.1.9 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.

7.1.10 Calibrated test tubes

With a capacity of 10 ml to 15 ml and ground glass stopper.

7.1.11 Chromatography tubes

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

See figure B.2.

7.2 Gas chromatograph

7.2.1 General

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.

7.2.2 Capillary columns

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 (see3.9) at least 0,5) for integrating the PCB28 peak. In general column length should be 25 m to 60 m. Internal diameter 0,18 mm to 0,32 mm and film thickness 0,1 µm to 0.5 µm.

Using ECD-detection, a second column coated with a moderate polar phase, e. g. CP-Sil 19, OV 1701 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 B.

8 Sampling and preservation of samples

8.1 Sampling

Sampling shall be carried out in accordance with sampling standards CSS99031-32 and 99057-60.

In principle, the samples shall be analyzed as soon as possible after sampling. This applies in particular to the examination of microbiologically active solids. Samples shall be stored on a cool (< 10 °C) dark place. Samples can be stored for one week at a temperature between 0 °C and < 10 °C and for a year at temperatures < -18 °C. Dried samples can be stored at room temperature in a dark place.

8.2 Sample preservation and pretreatment

Samples shall be pretreated according the mentioned International Standards using the following scheme (Figure 2) 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.

(Bulk) sample

↓

Homogenization and subdividing (ISO CD 23909)

↓

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/plas-tic 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

Figure 2 Sampling and pre-treatment scheme

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 according to CSS99022, 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. one month).

9 Procedure

9.1 Blank

Perform a blank determination following paragraphs for 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 (50 % of the lowest reported value).

9.2 Extraction

9.2.1 General

Depending on the test sample (origin, moisture content), choose a suitable extraction method (Table 1). Method 1 or 3 are recommended 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. With wet samples these methods shall be applied in order to eliminate the presence of water. If solving of the PCBs is the most important step (waste and organic rich materials) and the sample is dry, method 2 using Soxhlet is recommended. For sludges it has been shown that soxhlet is applicable. In presence of plastic, use of acetone shall be avoided, because use acetone leads to a high amount of co-extractives. However, a general rule cannot be given, because samples may contain all; aggregates, organic matter and (plastic) waste..

This European Standard allows other extraction procedures as ultrasonic extraction, microwave or pressurised extraction providing:

– The laboratory can show that the extraction is shown to be equivalent to one of the procedures 1; 2 or 3 as described in this European Standard, or

– The sample requires another approach as shown by the laboratory and the results of the procedure are in agreement with the performance criteria as described in 9.7.5 or 9.8.5.

NOTE For application of this European Standard for some wastes, addition of acetone with soxhlet extraction has been shown to be effective .

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 g – 20 g of soil; 2 g - 10 g of sewage sludge, 5 g - 20 g of compost or 2 g – 20 g of (bio)waste. The amount of sample shall be weighed with an accuracy of at east 1%.

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, suspended 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	

NOTE Annex C lists the applicability of the extraction procedure for other organic contaminants

9.2.2 Extraction 1; samples using acetone/petroleum ether and agitation

Take 2 g - 20 g test sample and place it in a sample bottle (8.1.1). Add a definite volumel of the secondary internal standard solution (6.5.2). Add 50 ml of acetone (6.2.1) to the test sample and extract by shaking thoroughly to break up aggregates for 30 min. Then add 50 ml of petroleum ether (6.2.2) and shake again thoroughly during at least 12 h. Use a horizontal shaking device (7.1.2) 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 (6.2.2) and decant again. Collect the extracts in a separating funnel (7.1.4) and remove the acetone by shaking twice with 400 ml of water (6.2.5). Dry the extract over anhydrous sodium sulphate (6.2.3) Rinse the sodium sulphate with petroleum ether (6.2.2) and add the rinsing to the extract.

NOTE 1 If the laboratory can prove sufficient extraction in a shorter time than 12 h, this is acceptable.

NOTE 2 Tap water has shown to be applicable for removal of the acetone in several laboratories, 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 definte amount 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 is 0,1 µg/ml. In order to 'wet' the complete sample, a minimum amount of 100 µl of internal standard is recommended.

NOTE3 In matrice with a high organic matter content (i.e. some sludges) longer extraction procedures can be necessary, Extraction procedure 2 (9.2.3) may be preferred for these samples

9.2.3 Extraction 2; dry samples using Soxhlet / Pressurised liquid extraction

Take 2 g -20 g dry test sample and place it in the extraction thimble (7.1.6). Add the definite amount of the secondary internal standard solution (6.5.3) and approximately 70 ml of the extraction solvent (6.2.2) to the extraction vessel. 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.

9.2.4 Extraction 3; of wet samples acetone/petroleum ether/Sodium chloride and agitation

Take an amount of sample equivalent to 2 g - 20 g of the dry sample and put it into a 1 l sample bottle (7.1.1). Add the definite amount of the secondary internal standard solution (6.5.3). If the sample is dry, add 50 ml water. For moist samples, the water quantity to be added is calculated as follows:

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

m_w is the water to be added in grams (g)

m_E is the quantity of the sifted soil sample in grams (g)

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

Add 40 g sodium chloride (6.2.4), 100 ml acetone (6.2.1) and 50 ml petroleum ether (6.2.2) to the moistened preparations, close the sample bottle and shake it by means of the shaking device (7.1.2) 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 (6.2.5). Dry the extract over anhydrous sodium sulphate (6.2.3) and transfer the dried extract to the concentrator (7.1.7). Rinse the sodium sulphate with petroleum ether (6.2.2) and add the rinsing to the extract.

9.3 Concentration

Add a boiling chip (7.1.8) to the extract and concentrate the extract to approx. 10 ml by evaporation using a cocentrator 7.1.7. Transfer the concentrated extract to a calibrated test tube (7.1.10) and concentrate to 1 ml using a gentle stream of nitrogen or another inert gas at room temperature. If clean up H is used, concentration is not necessary. Note 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 an amount of X µl of the secondary internal standard solution containing C_{13}-congeners.

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 (6.2.6).

9.4 Clean up of the extract

9.4.1 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 2 shall be used. If polar compounds shall be removed take special care on the recoveries of the low chlorinated PCB's. Reference to validation reports is made in annex D.

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, 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			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,	DMF/hexane	Alipahatic hydrocarbons, lipids, oil	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 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 are also allowed, providing 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 9.3 or in a previous clean-up step shall be quantitatively transferred to the clean-up system; alternatively, an aliquot may be used.

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

9.4.2 Clean-up A – Aluminium oxide

Prepare an adsorption column by placing a small plug of quartz wool (7.1.9) in the chromatography tube (7.1.11) and packing it dry with 2,0 0 g ± 0,1 g of aluminium oxide (6.3.1.1).

Apply the extract to the dry packed adsorption column; rinse the test tube twice with 1 ml of petroleum ether (6.2.2) 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. 20 ml of petroleum ether. Collect the entire eluate.

One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3).

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 Commercial available disposable aluminium cartridges may be used as an alternative if found suitable. A column is suitable if the performance of the method is in agreement with 9.7.5 or9.8.5.

9.4.3 Clean-up B silica gel

Put glass wool (7.1.9) and 10 g silica gel (6.3.2.2) into the chromatographic tube (6.1.11). Then add a 1 cm layer of sodium sulfate (6.2.3) and condition with 20 ml petroleum ether (6.2.2). Apply the extract to the column when the level of the solvent mixture is drained to approximately 0,5 cm above the column packing.

Elution is performed using a total of 10 ml petroleum ether (6.2.2). One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3).

9.4.4 Clean-up C – Gelpermeation

The extract is carefully reduced under a gentle nitrogen flow. The residue is immediately dissolved in 5 ml solvent mixture (ethyl acetate (6.3.3.2) and cyclohexane (6.3.3.3) 1+1). 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 shall be regularly verified by means of the multi-component PCB-standard solution.

One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3)

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

9.4.5 Clean-up D- Florisil

Add into a chromatographic tube (7.1.11) 5 mm sodium sulfate (6.2.3), 1.5.g Florisil (6.3.4.1), and again 5mm high sodium sulfate. To fix the mixture, place glass wool (7.1.9) on the top. Rinse the column with approx. 50 ml isooctane (6.3.4.2). Apply the extract to the column. Rinse the extraction tube/vessel for two times with 1ml isooctane / toluene (95/5) (6.3.4.3) and give it onto the column. Afterwards elute with 7 ml isooctane /toluene.

One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3).

9.4.6 Clean up E silica H_2SO_4/silica NaOH

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

Prepare an adsorption column by pouring consecutively 1 g silica NaOH (6.3.5.3), 5 g silica H_2SO_4 (6.3.5.2) and 2 g sodium sulphate (6.2.3) in a clean chromatography column (7.1.11). Add a sufficient amount of n-hexane (6.3.5.4) 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 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 (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see 9.33).

9.4.7 Clean-up F Benzenesulfonic acid/sulfuric acid

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

Conditioning 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 x 1 ml n-hexane like solvent (6.3.5.4) 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 (6.3.5.4). Collect the entire eluate. One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3).

9.4.8 Clean-up G DMF/n-hexane partitioning for aliphatic hydrocarbons removal

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

This additional clean-up step shall only 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 to a separating funnel of 100 ml and extract the PCBs with 25 ml of DMF (6.3.6.1). Repeat twice. Transfer the combined DMF extracts to a separatory funnel of 500 ml, add 100 ml of water (6.2.5) and extract the PCBs with 50 ml of n-hexane (6.3.5.4). Repeat once. One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3).

9.4.9 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 (6.2.2). Pour in 5 ml of concentrated sulfuric acid (6.3.8.1) and shake vigorously at intervals for 5 min. Allow to separate completely (about 15 min.). Take the upper layer, rinse the remaining sulphuric acid with petroleum-ether. One drop of keeper substance (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see 9.33).

9.4.10 Clean-up I – TBA-sulfite reagent

Add 2 ml of TBA sulfite reagent (6.3.9.1) to 1 ml of concentrated extract and shake for 1 min. Add 10 ml of water (6.2.5) 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 (6.2.3) to remove the remaining traces of water.

9.4.11 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 in6.3.10). 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.

9.4.12 Clean up K $AgNO_3$/Silica

Add into a chromatographic tube (7.1.11) sodium sulfate (6.2.3) e.g. 5mm high , 2g of the $AgNO_3$/silica mixture (6.3.11.2) and again 5mm high Na_2SO_4 . Rinse the column with approx. 50ml n-hexane (6.3.5.4). Apply the extract to 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 (6.2.6) is added to the eluate, and then the eluate is reduced to the desired volume (see9.3).

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

9.5 Addition of the injection standard

Add an appropriate amount of the secondary injection standard (6.6.3) to the extract obtained after clean-up (this amount shall be in line with the concentration of the calibration standard). Note the final volume *V.*

9.6 Gas chromatographic analysis

9.6.1 General

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

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

9.6.2 Setting the gas chromatograph

Set the gas chromatograph (7.2) 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 (see7.2.1),

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

Injector temperature: 260 °C

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

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

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

9.7 Mass spectrometric (MS) conditions

The mass spectrometer is tuned 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 3. 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 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			
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	

(..) 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.

NOTE: Typical examples of chromatograms with instrumental conditions are provided in Annex B.

9.7.1 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 (6.5.3) to dilutions of the mixed calibration solution (6.5.2). 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 (6.5.2). 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 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 $^{13}C_{12}$-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 nanograms per litre (µg/ml) ;

ρ_{C13} is the mass concentration of the $^{13}C_{12}$-labelled PCB internal standard in the calibration solution in nanograms per millilitre (µg/ml) ;

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

Two types of calibration are distinguished: the initial calibration (9.7.3.1) and the daily calibration (validity check of the initial calibration); the last one is called recalibration (9.7.3.2).

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.

9.7.1.1 Initial calibration

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.

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.

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

9.7.1.2 Recalibration.

For every batch of samples, inject at least two calibration standards with concentrations of (20 $\pm$ 10) % and (80 $\pm$ 10) % of the established linear range and calculate the straight line from these measurements. If the straight line falls within the ± 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 9.7.1.1.

9.7.2 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.

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 PCB's or re-extraxt 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 9.7.6 does not give the proper quantification and the deviation from linearity shall be taken into account

9.7.3 Identification

For identification of the PCBs apply ISO 22892. In this International Standard, the chromatographic criteria and MS-criteria are described, necessary for proper identification. Use the diagnostic ions as given in table 3.

9.7.4 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.

Use for this analysis (1) the same final volume (2) the same definite volume of internal standard and (3) the same definite volume of injection standard, as used for the samples. This is the performance standard.

NOTE 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. In general diagnostic ion 1 can be used for this calculation.

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

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

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

p.s. is performance standard

The average ratio in the sample shall be at least 70 % and do not exceed 110 % of the ratio in the standard. The ratio for an individual PCB should be at least 60 %. If not 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 standard. Lower ratios are acceptable if this can be explained by the accepted losses in each clean-up step. The minum 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.

$$Extraction\ recovery\ ratio = \frac{A_{1\text{-average}}(sample) * f}{A_3(sample)} \cdot \frac{A_3(p.s.)}{A_{1\text{-average}}(p.s.)}.100\% \quad (3a)$$

$A_{1\text{-average}}$ is the average measured response of the C_{13}-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 de fraction of the original extract used for clean-up

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.

9.7.5 Calculation

Calculate the mass content of the individual PCB from the multipoint calibration of the total method by using equation (4). In general diagnostic ion 1 can be used for this calculation.

$$\omega_{\mathrm{n}} = \frac{(A_{\mathrm{n}} / A_{\mathrm{C13}}) - b}{s \cdot m \cdot d_{\mathrm{s}}} \cdot \rho_{C13} \cdot f_e \cdot f_t \cdot V \qquad (4)$$

where:

ω_{n} is the content of the individual PCB found in the sample in milligrams per kilogram (mg/kg) on the basis of the dry substance;

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

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

ρ_{C13} is the mass of the $^{13}C_{12}$-labelled PCB internal standard added to the sample in nanograms per gram (µg) ;

m is the mass of the soil test sample used for extraction in grams (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 percent (%) ;

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

f_t is the addition factor in accordance with ISO 14507;

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

S is the slope of the recalibration function;

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

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

9.8 ECD-detection

Using ECD-detection, the same procedure as for MS can be followed, except the points described below for all specific steps in the measurement. For ECD-detection, the same order in paragraphs has been used as in 9.7 (MS-detection). Only differences are described.

9.8.1 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.

9.8.2 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 $^{13}C_{12}$-labelled PCB by used internal standard.

9.8.3 Measurement

No differences.

9.8.4 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 22892, three identification points shall be obtained. Measuring only with ECD, at least check the results with another column and recognition of the PCB-pattern are necessary. The results using the second column should be within 10 % If both are correct, the three identification points (ISO 22892) for identification are obtained. If one is missing, only indication can be reported.

9.8.5 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 occurs more often with ECD-detection. Therefore the following procedure is used to check if interfering compounds are present

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

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

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

Calculate the relative response ratio RRR for the PCB injection standards, by using 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;

$R_{\mathrm{e},198}$ is the response of PCB198 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 PCB209 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 ± 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 both standards if RRR = 1.00 ± 0.05. Use only the injection standard PCB198 if RRR < 1.05 and use only PCB209 if RRR > 1,05

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

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

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 shall 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. 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).

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

9.8.6 Calculation

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

10 Precision data

The performance characteristics of the method (Annex E) data have been evaluated. Table 4 gives the resulting typical values for repeatability and reproducibility limits as their observed ranges. The typical value is derived from the data in Table E.2 in Annex E by taking the median value and rounding the numbers.

Table 4 — Typical values and observed ranges of the repeatability and reproducibility limits

The reproducibility limit provides a determination of the differences (positive and negative) that can be found (with a 95 % statistical confidence) between a single test result obtained by a laboratory using its own facilities and another test result obtained by another laboratory using its own facilities, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard and the two laboratory samples are obtained from the same primary field sample and prepared under identical procedures. Conversely, the repeatability limit refers to measurements obtained from the same laboratory, all other conditions being identical. The reproducibility limit and the repeatability limit do not cover sampling but cover all activities carried out on the laboratory sample including its preparation from the primary field sample.

Results of the validation of the Determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste	Typical value %	Observed range %
Repeatability limit, r Compost and Sludge	20	*17 - 25*
Reproducibility limit, R Compost and Sludge	92	84 - 112
Repeatability limit, r Soil	25	20 – 31
Reproducibility limit, R Soil	98	84 - 112

NOTE 1. The above results refer to the difference that may be found between two test results performed on two laboratory samples obtained under the same conditions. In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the above typical reproducibility values and observed reproducibility ranges should be divided by √2 to obtain the corresponding typical dispersion limit and its observed range. In the example of PCB 138 in Sludge 1 the result and its dispersion limit is 50.4 ± 34.4 (2 * sR = 68.3 % of 50.4). This means that with a 95 % statistical confidence, the values reasonably attributable to the measured parameter are larger than 16 ug/kg and lower than 85 ug/kg.

NOTE 2 The repeatability limit (r) and the reproducibility limit (R) as given in Table E.2 (Annex E) and in this table are indicative values of the attainable precision if the determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD is performed in accordance with this standard [CSS99016].

NOTE 3 A limited number of materials and parameters were tested. Consequently, for other materials and parameters, performance characteristics may fall outside the limits as derived from the validation of the the Determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste.

NOTE 4 In particular for relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Table E.2 (Annex E) and this table.

11 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 European Standard ;

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

d) 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 mg/kg d.m.; 0.36 mg/kg d.m.; 0.0069 mg/kg d.m.).

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

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

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[5]
  - 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

[5] More important for PAHs

Annex B
(informative)

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

Table C.1

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

Chromatograms, to be added.

Annex C Aplicability of extraction procedures for organic contaminants (informative)

	Extraction procedure 1	Extraction procedure 2	Extraction procedure 3
PCB	X	X	X
PAH	X		X

Table Aplicacability of extraction procedures mentioned in this standard for other organic contaminants. X = aplicable

Annex D Validation

(Normative)

This method has been validated for the samples described in chapter 11. The modules in this European Standard 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 recovery 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 Certified Reference Standards (that fit for purpose and/or their results in proficiency testing.
- If the method in use is not applicable for a specific sample (low recoveries of recovery 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 2007) are ……..

Report on Horizontal Validation Study, (JRC, 2007)

(INFORMATIVE)

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 E
(informative)

Repeatability and reproducibility data

E.1 Performance characteristics

E.1.1 Objective of the interlaboratory comparison

In a European wide interlaboratory comparison study according to ISO 5725-2, the performance characteristics of the standard “Determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste” were established.

E.1.2 Materials used in the interlaboratory comparison study

The interlaboratory comparison of determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste was carried out with 10 - 13 European laboratories on 3 materials. The materials selected for the interlaboratory comparison were chosen to represent soil, sludge and biowaste as broad as possible, because the standard will find general application across different types of soil and soil related materials. (detailed information can be found in the final report on the Interlaboratory comparison study mentioned in the Bibliography).

In the interlaboratory comparison study the following starting points were used:

The laboratory samples were all taken from one large batch of the different materials according to the normal practice. The normal size reduction and the normal repeated mixing were carried out as needed to obtain representative laboratory samples from the large batch sample (ref JRC).

> Note : the samples provided for the validation should not be confused with reference samples provided for certification purposes, as the performance results obtained have to be directly applicable to daily practice (less rigorous sample preparation than for a reference material).

The experimental plan was designed by project HORIZONTAL on the basis of each laboratory being given two laboratory samples of each material to be tested. This is in accordance with ISO 5725-2.

The materials examined cover all the grain size classes to which the the determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste applies: very fine grained materials (like sludge: 0 µm to about 125 µm) and fine-grained materials (soil and compost: 0 mm to 4 mm).

Table E.1 provides a list of the types of materials chosen for testing and the selected components.

Table E.AD.1 — Material types tested and components analysed in the interlaboratory comparison of the method for the determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste.

Grain size class	Sample code	Material type tested	Parameters/congeners
Sludge (<0.5 mm) Fine grained	Sludge 1	Sewage sludge 1 Mix 1 of municipal WWTP sludges from North Rhine Westphalia, Germany	PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180
(< 2 mm)	Compost 1	Compost 1 Fresh compost from Vienna, Austria	PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180
	Soil 3	Soil 3 A sludge amended soil from Barcelona, Spain	PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180

E.1.3 Interlaboratory comparison results

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

The repeatability is determined as an interval around a measurement result (i.e. "repeatability limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another, both test results being obtained under the following conditions: The tests are performed in accordance with all the requirements of the present standard by the same laboratory using its own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The repeatability limit was calculated using the relationship : $r_{test} = f \cdot \sqrt{2} \cdot s_{r,test}$ with the critical range factor f = 2.

For instance, the repeatability limit around a measurement result of 10.0ug PCB 138 /kg is ± 2.44 ug PCB 138/kg (i.e ± 24 % of 10)

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). However it may be necessary to use a larger value for f in situation as described in clause 10.

The reproducibility, like repeatability is also determined as an interval around a measurement result (i.e. "reproducibility limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another test result obtained by another laboratory, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard by two different laboratories using their own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The reproducibility limit was calculated using the relationship: $R = f \cdot \sqrt{2} \cdot s_R$ with the critical range factor f = 2.

For instance, the reproducibility limit around a measurement result 10.0 ug PCB 138/kg is ± 9.56 ug PCB 138/kg (i.e ± 96 % of 10)

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the dispersion limit is equal to $k \cdot s_R$ with the usual value k = 2, resulting in a dispersion limit lower than the reproducibility limit (i.e. a ratio of $\sqrt{2}$). However it may be necessary to use a larger value $f \cdot \sqrt{2}$ (or k) in situation as described in clause 10 .

In case of relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Tables E.2 (this means that the value chosen for the critical range factor f is larger than 2 as well as for the coverage factor k for dispersion). This is because the extreme results may have been obtained in accordance with the present standard and/or be caused by the variability within, or in between, the laboratory samples.

Table E.AD.2 — Results of the interlaboratory comparison studies of the determination of polychlorinated biphenyls (PCB) by GC-MS and GC-ECD in soil, sludge and treated biowaste. All concentrations in ug/kg.

Matrix	Parameter	Mean	sr	sR	r	R	p	Outliers	Used number of data	Number of data reported below detection	Total no of data
Sludge 1	PCB 28	50.13	6.4%	37%	8.93	51.77	13	1	47	3	54
Compost 1	PCB 28	0.79	7.9%	12%	0.17	0.26	4	2	15	14	37
Soil 3	PCB 28	*0.44*		*98%*			3		10	28	38
Sludge 1	PCB 52	53.72	7.0%	39%	10.48	58.85	11	2	42	4	54
Compost 1	PCB 52	0.78	6.2%	33%	0.14	0.73	5	0	18	17	35
Soil 3	PCB 52	*1.29*		*114%*			3		12	26	38
Sludge 1	PCB 101	41.21	6.2%	41%	7.17	47.21	13	0	49	4	53
Compost 1	PCB 101	3.04	9.5%	39%	0.81	3.31	11	1	40	1	45
Soil 3	PCB 101	0.33	6.7%	24%	0.06	0.23	4	0	13	21	34
Sludge 1	PCB 118	27.02	6.8%	38%	5.14	29.01	13	0	47	5	52
Compost 1	PCB 118	2.22	6.9%	49%	0.43	3.03	7	1	26	6	36
Soil 3	PCB 118	*0.80*		*104%*			3		12	24	36
Sludge 1	PCB 138	50.38	7.6%	34%	10.68	48.18	12	0	48	4	52
Compost 1	PCB 138	6.62	8.7%	36%	1.62	6.68	10	2	39	0	45
Soil 3	PCB 138	0.36	11.9%	30%	0.12	0.30	4	1	16	20	40
Sludge 1	PCB 153	49.64	7.3%	37%	10.18	51.73	12	1	47	2	53
Compost 1	PCB 153	6.97	8.3%	36%	1.63	7.11	10	2	39	0	47
Soil 3	PCB 153	0.57	10.6%	25%	0.17	0.40	5	0	18	13	31
Sludge 1	PCB 180	40.63	9.0%	32%	10.21	36.24	12	0	48	4	52
Compost 1	PCB 180	5.81	9.7%	37%	1.58	5.95	11	1	43	0	47
Soil 3	PCB 180	0.56	7.7%	44%	0.12	0.70	6	0	20	18	38

Matrix	Parameter	Mean	sr	sR	r	R	p	Outliers	Used number of data	Number of data reported below detection
Sludge	PCBsum	205.29	7.7%	41%	44.1	236	18	0	70	
Compost 1	PCBsum	26.11	18.1%	39%	13.2	28.9	12	0	48	
Soil 3	PCBsum	1.92	10.7%	43%	0.58	2.3	7	0	26	

Abbreviations: sr Repeatability standard deviation; SR Reproducibility standard deviation; r Repeatability limit

(comparing two measurements); R Reproducibility limit (comparing two measurements); p Number of labs.

Note 1. In judging the results it is important to consider the concentration levels, at which measurements have been carried out. The choice was made to avoid spiking of samples. This implies that particularly in soil and compost low concentrations have been observed for some congeners and results below detection for other congeners. If measurement results are well below a possible critical level (regulation), between lab variabilities of up to 70 % may prove fit for purpose.

Note 2. In the framework of characterisation of waste the method described in this document is equal to the protocol describing the quantification of PCB in waste (prEN 15308). This standard has been validated. Based on the results, which complement the present standard very well, a broader horizontal standard could be developed covering the matrices covered in this standard and waste.

Annex E Bibliography

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

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

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

[4] JRC, 2007. Report on Horizontal Validation Study.