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General methods of tests for pigments and extenders —

Part 28: Determination of total content of polychlorinated biphenyls (PCB) by dissolution, cleanup and GC-MS

Méthodes générales d'essai des pigments et matières de charge —

Partie 28: Détermination de la teneur totale en biphényles polychlorés dans les pigments organiques par dissolution, purification et CG-SM

Reference number
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Foreword

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

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This document was prepared by Technical Committee ISO/TC 256, *Pigments, dyestuffs and extenders*.

A list of all parts in the ISO 787 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at https://www.iso.org/members.html.

Introduction

A number of methods to quantify PCBs in “environmental samples” or oil residues prove inadequate for pigments due to being merely extractive on the particle surface without taking into account occlusions of contaminants in the crystal lattice of pigments (see References [1] to [3]).

Occurrence and formation principles are referred to in References [5], [6] and [8].

General methods of tests for pigments and extenders —

Part 28: Determination of total content of polychlorinated biphenyls (PCB) by dissolution, cleanup and GC-MS

1 Scope

This document specifies a method for determining the total content of polychlorinated biphenyls (PCBs), checking for all 209 possible congeners in pigment materials.

This document is applicable to a working range from1 mg/kg to 150 mg/kg. The lower quantitation limit of this method is 1 mg/kg per congener. Results below 1 mg/kg are considered to be qualitative only.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO 15528, *Paints, varnishes and raw materials for paints and varnishes — Sampling*

ISO 18451-1, *Pigments, dyestuffs and extenders — Terminology — Part 1: General terms*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 18451-1 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
polychlorinated biphenyls
PCB
209 congeners, from mono- through deca-chlorinated biphenyls, which may be subdivided into homologue groups comprising PCB congeners with the same degree of chlorination, i.e. same gross formula

Note 1 to entry: The general CAS-Number for polychlorinated biphenyls is 1336-36-3. For a comprehensive congener list with CAS-Numbers, see Annex A or Reference [5].

3.2
internal reference material
mixture of defined quantities of ^{13}C-Isotope-labelled PCBs added directly into the freshly weighed pigment sample

Note 1 to entry: No subsequent additions of internal reference materials are permitted. Surrogate standards reference materials can be added to assess recovery rates only, but these cannot be deemed an internal reference materials, nor can these be used for quantitation.

Note 2 to entry: Mass spectrometry assumes a vast linear range of detector sensitivity, thus a calibration with a single quantity will usually suffice.

Note 3 to entry: In case of major deviations between analytes and reference material concentrations, the quantitation might need to be confirmed by a second analysis (from the beginning) using an adequately adapted sample weight.

4 Principle

The principles have been developed from References [1] to [4], [6] and [7].

The use of solid surface extraction methods risks underestimating the actual content by orders of magnitude. Such errors are often further enhanced when the PCBs quantitation is carried out by using external or internal reference materials which do not undergo the same losses as the analytes that are occluded in the crystal lattice of the pigment.

5 Sampling

Take a representative sample of the product to be tested, as described in ISO 15528.

Samples shall be of dry powder consistency. Volatiles should be less than 1 % (mass fraction), determined with a sample portion separated from the sample portions used for PCB analyses.

Samples shall be kept in the dark and in capped glass bottles or vials.

Samples suspected of having high impurities content shall be handled in dedicated glassware and kept separately from other laboratory equipment; sample mass may be reduced and internal reference materials amounts doubled in order to cope with the possible elevated levels of interferences by impurities. A screening pre-run is recommended to avoid detector overload.

Bromine detection or presence of partially brominated samples (e.g. C.I. Pigment Red 168, C.I. Pigment Green 36) require caution due to occurrence of "mixed" halogenated aromatic compounds. Analysis can proceed if results are checked for non-interference (see 9.5).

6 Procedure

6.1 General

This method requires a strict sequential application of the following steps:

— sample weighing;

— addition of internal reference material and thorough mixing;

— dissolution in concentrated sulfuric acid from 92 % (mass fraction) to 95 % (mass fraction);

— sonicate the mixture until fully dissolved and a homogenous dark solution is obtained in the sulfuric acid phase. The temperature shall be kept lower than 50 °C at all times, e.g. by circulating or continuously exchanging the water in the sonication bath.

NOTE 1 Using this concentration range avoids major disintegration and rearrangement of mono- and bichlorinated biphenyls.

NOTE 2 Sufficient dissolution is indicated by absence of the "tyndall-effect" (i.e. no scattering of a preferably blue light beam by the solution).

Upon dissolution in concentrated sulphuric acid, pigment molecules are protonated and may be considered in a simplified way as being pulled out of their crystal lattice one by one in drawer-like fashion, until all the solid matter has dissolved, without cleavage occurring at the molecular (chromophor) level.

Any possible inadvertent destruction of analytes is likely to occur also to the internal reference materials at a related, nearly equal rate, thus compensating for any destructive effects, at the expense of a slightly lower signal-to-noise ratio (see Reference [5]). There maybe a slightly increased rate of destruction for low chlorinated biphenyls.

This may be offset by a preceding leaching step as described in the Note to 9.1.

6.2 Clean-up to eliminate interfering species

Interfering species such as aryl sulfuric acids, carboxylic acids, esters, polymeric surfactants, heterocyclic compounds are eliminated by a cleanup performed by sequential wet column-chromatography.

6.3 Separation and quantification

(Optional) separation from PCDD/PCDF, (which may be later eluated and analysed), as well as from other interfering substances on an alumina column.

Final separation and quantitation by gas chromatography (GC) using MS detection mode (see 8.18). The application of a 60 m column (see 8.3) will ensure that most PCB congeners are separated or recognizable at different retention times.

Exceptionally a 30 m column may be used for screening. The instrument should comprise automatic flow and pressure control, programmable temperature controls and auto sampling equipment (see 8.2).

MS detector should preferably be operated in SIM-mode.

NOTE SIM is the most suitable mode for quantitation. SCAN is best for qualitative identification of the signals, but suffers from reduced sensitivity.

Quantitation is achieved by using the ratio of peak areas of analytes (identified as analyte by three or more specific mass traces as “qualifiers”) versus the corresponding internal standards areas, to cope with overlapping ranges of chlorination groups and residual interfering peaks.

There are no intentional chemical reactions except for the dissolution step, which incurs protonation merely to cleave the crystal lattice, as all other steps are merely physical ones.

Chemical reaction leading to decay of analytes are considered to occur with both the internal reference material and the analytes alike, assuming full compensation of the resultant error. It is therefore essential to use internal reference materials matching the congeners distribution profile of the sample to a relatively high degree. Other approaches to assess artefacts in detail (see example in Reference [1]) shall be mentioned in the test report.

7 Reagents

7.1 Safety precautions

In case of spillage, apply 30 VA ultraviolet lamp for irradiation of spillage areas (254 nm wavelength emitted power maximum).

Staff is required to be qualified for and familiar with handling of hazardous substances in the laboratory.

All material, solvents, reagents, and standards shall be handled with appropriate care. Laboratory facilities, equipment and personnel shall comply with the latest legal and safety standards on PCB handling as may be locally applicable.

Upon accidental skin contact with PCB, triply wipe skin area with polyethylene glycol-wetted cloth (to be disposed of) and see emergency medical services immediately.

Major droplet spillages may result in shutdown of the entire laboratory facilities. Protect eyes when applying UV-lamp irradiation after spillages for decomposing contaminants.

7.2 Reagents, solvents and absorbents

Only reagents and solvents of acknowledged and certified analytical grade for pesticide analyses or comparable grades shall be used.

Solvents shall be used as delivered. New column adsorbents shall be purged by shaking in and decanting off dichloromethane (or a similarly suitable solvent), followed by drying under suitable cover.

7.2.1 Sulphuric acid, 92 % to 95 % (mass fraction).

7.2.2 Phosphoric acid, p.a., 85 % (mass fraction).

7.2.3 Diatomaceous earth.

7.2.4 Silica gel, 63 mesh to 200 mesh.

7.2.5 Aluminium oxide (alumina) (Basic, Activity: Super 1).

7.2.6 Silver nitrate, p.a., ultrapure.

7.2.7 Cesium hydroxide, hemihydrate.

7.2.8 Sodium sulfate, p.a., granulated.

NOTE Sodium sulfate (anhydrous) is used to eliminate residual aqueous and alcoholic matter in the hexane phase, as this can have detrimental effects on the GC column materials.

7.2.9 Porous styrene divinylbenzene beads for size exclusion chromatography.

7.2.10 *n*-Hexane.

7.2.11 Dichloromethane.

7.2.12 Toluene.

7.2.13 *n*-Nonane.

7.2.14 Ethanol.

7.2.15 Ethyl acetate.

7.2.16 Cyclohexane.

Original reference solutions shall be diluted by a factor 5 or 2 (MBP-MXP), using *n*-nonane, octane or similar high-boiling, non-aromatic solvents. Dilution should be carried out filling up vial content to 2 or 5 times its mass by adding the solvent. The error due to slightly different densities of reference concentrate and pure solvent is <0,5 %, hence negligible.

7.3 Internal reference materials for PCBs, 13C12-labelled

— PCB cong. ## 3, 15, 28, 52, 118, 153, 180, 194, 208, 209: LGC/Cambridge #CIL-EC-4189[1)]

— PCB cong. ## 3, 8, 28, 52, 101, 118, 138, 153, 180, 194, 206, 209: Wellington-CAMPRO # MBP-MXP[1)]

— PCB cong. ## 3, 8, 28, 52, 101, 114, 118, 138, 153, 180, 194, 206, 209: LGC/Cambridge #CIL-EC 5411[1)]

These internal reference materials or similar are recommended with respect to the requirement of a "reporting threshold" value of 2 mg/kg for any resolvable PCB peak, covering in principle all 209 congeners.

Optional: To assess low-chlorinated biphenyls (Mono-CB-s, Di-CB-s) in detail, the user may counter-check these by using a spiking series for the particular homologue groups.

7.4 Cleaning/disposal agents

All glassware shall be purged with a suitable solvent prior to use and then washed intensively with a high-alkaline detergent or soap with readily soluble anionic and phosphate-containing surfactants in order to exclude any source of cross contamination. Joint grease shall be excluded from the laboratory. New glassware shall be purged by shaking in and decanting off dichloromethane (or a similarly suitable solvent), followed by drying under suitable cover.

Decontamination soap, brushes for all glassware, discharge bins (separate waste streams); polyethylene glycol pharmaceutical grade as first aid in case of skin contamination.

8 Apparatus

8.1 Glassware, equipped with B (NS) 29 ground joints only as standard and inverse joint geometry.

8.2 Column (for the clean-up procedure), 30 cm long, 14 mm internal diameter, B (NS) 29/32 shells, 250 ml top reservoir as integral part and PTFE stop cock at the bottom of the column.

8.3 Capillary column (for the gas chromatographic determination) of 60 meters length, internal diameter 0,25 mm coated with 0,25 µm thick film of 5 % phenylmethylsiloxane equivalent to a phenyl arylene polymer with the following characteristics:

— non-polar with very low bleed characteristics, ideal for GC-MS;

— excellent inertness for active compounds;

— improved signal-to-noise ratio for better sensitivity and mass spectral integrity;

— bonded and cross-linked;

— solvent rinsable to separate most of the 209 PCB congeners.

NOTE "Fast GC" permitted for particular congeners to be sought (i.e. a selection of the spectrum of 209 congeners for special purposes, to be mentioned in the final report).

8.4 Round-bottomed flasks, B (NS) 29, 1 000 ml, 500 ml and 250 ml.

8.5 Flat-bottomed flasks, 100 ml.

8.6 Cylinders, calibrated, 100 ml and 500 ml.

1) Examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

8.7 Conical flasks, with ground joint B (NS) 29 and suitable glass stoppers, 500 ml.

8.8 Brown glass bottles, B-(NS) — 1 l, equipped with stoppers.

8.9 Magnetic stirrers.

8.10 Rotary evaporator, with automatic vacuum control; foam-and-flash evaporation stopper flask recommended.

8.11 Ultrasonic bath, ≥ 250 W.

8.12 Top-load balance, wind-shielded; accuracy to the nearest 0,001 g or better.

8.13 Pneumatic dispensers, 20 µl to 200 µl, 100 µl to 1 000 µl, and suitable tips.

8.14 Separation funnels, 500 ml.

8.15 GC-MS system, complying with the following requirements: GC-MS interfaces constructed of all glass or glass-lined materials are necessary. Glass can be deactivated by silanizing with dichloro dimethyl silane. Inserting a fused silica column (8.3) directly into the MS source is recommended. Care must be taken not to expose the end of the column to the electron beam, see Reference [9].

8.16 Reference material, matching 1 ml GC vials.

8.17 Air conditioning, continuously operating to avoid humidity errors with alumina or sulphuric acid during benchwork.

8.18 Mass spectrometric detector (MSD).

Unless indicated otherwise, this method merely refers to the electron-impact ionizing (EI) quadrupole-MS, low resolution MS (QPMS; LRMS), used as a detection and quantitation instrument following separation of analytes on the GC column.

Selective Ion Monitoring (SIM) mode of MSD to be used, with 3 qualifier mass traces to securely identify a peak as a "positive" by their area ratios.

NOTE In addition, ion trap or CI methods can be applied to yield equal or better results.

8.19 Column (optional), 50 cm long, 20 mm internal diameter, B (NS) 29/32 shells, 250 ml top reservoir and PTFE stop cock at the bottom of the column.

8.20 Column (optional), 30 cm long, 10 mm internal diameter, B (NS) 29/32 shells, 250 ml top reservoir and PTFE stop cock at the bottom of the column.

8.21 "Ludwig" extractors (optional), 500 ml, with suitable heating and cooler accessories.

9 Procedure

9.1 Preparation of test sample

Weigh to two decimal places 0,7 g to 1,5 g pigment sample into a 500 ml conical flask and add 0,5 g to 1,0 g ethanol to wet the pigment dropwise until it is completely wetted to form a stiff homogeneously creeping paste (avoid exceeding 1,5 g ethanol).

Add 100 µl $^{13}C_{12}$-labelled reference solution in *n*-nonane (50 ng to 200 ng/congener preferred) into the flask.

Add anhydrous sodium sulfate using approximately the same mass as the sample; Optional: Add 1 g to 2 g of phosphoric acid to buffer further against over-protonation, and/or to confine interference by aluminium or iron–III species.

Shake laterally and sonicate for 10 min.

Carefully add 80 g of 92 % to 95 % sulphuric acid stepwise under repeated shaking. The temperature of the mixture shall be kept at <50 °C to avoid chemical reactions on analytes and standards.

Sonicate the mixture until fully dissolved and a homogenous dark solution is obtained in the sulfuric acid phase. The temperature shall be kept at <50 °C at all times.

NOTE Inadvertent destruction, cleavage or rearrangement of mono- or di-chlorinated analytes can be minimized by first contacting the weighed sample, without reference materials, with small amounts of *n*-hexane + dichloromethane (1 + 1), drying the sample after centrifugation with decantation of supernatant organic extract, to be kept separately, then proceeding to spike with internal reference materials and to dissolve the residual pigment sample in conc. sulphuric acid, uniting organic phases after the liquid-liquid extraction of the sulphuric acid phase prior to clean-up. In this case, it seems equally appropriate to add mass-labelled reference materials for mono-and DiCB-s, monitoring isomerization of individual reference compounds and loss/recovery carefully during the GC-MS phase.

9.2 Liquid-liquid extraction

9.2.1 Separation funnel

Extract the sulphuric acid solution twice with 200 ml *n*-hexane (repeating if necessary with an additional 200 ml extraction).

In case of samples suspected of high impurities content, a mixture of 50 ml *n*-hexane and up to 200 ml dichloromethane shall be used instead of the second n-hexane extraction. Care with separation shall be taken due to increased specific mass of the organic phase.

Sonicate for 5 min and then rapidly stir it for 90 min at room temperature avoiding direct sunlight.

Transfer the mixture to a separating funnel and collect the organic phase in a 1 000 ml round-bottomed flask (hereafter round-bottomed flask).

Repeat the extraction/separation steps once more (more if deemed necessary).

Combine all the organic phases in the round-bottomed flask, i.e. of both an optional initial solvent extraction (see 9.1, NOTE) and the liquid-liquid-extraction of the sulphuric acid solution.

In case residual "greasy" sulphonation products are present at the bottom of the organic solvent phase, separate them by:

a) decantation; then

b) carefully adding 50 % aqueous KOH solution to the greasy residue (approximately 2 ml) up to a pH value of 7 to 8;

3) shaking out the aqueous phase twice with 100 ml *n*-hexane. In order to control the change of temperature due to the heat of neutralization, it is recommended to fill some *n*-hexane into the flask prior to the KOH solution: the solvent boiling will indicate in good time that the flask requires cooling;

4) after pH 8 has been reached, transfer the solution into the separation funnel, and wash the flask with 10 ml of *n*-hexane, which is also added into the separation funnel.

Combine all the clean organic phases in round-bottomed flask and dry them by adding 0,5 g of anhydrous sodium sulfate.

After adding a maximum of 0,5 ml to 1 ml of *n*-nonane as "keeper", remove the solvents carefully by rotary evaporation, reducing the extract to a volume of approximately 1 ml to 2 ml.

9.2.2 Ludwig extraction

Alternatively, the extraction can be carried out with a Ludwig extractor.

The solution is transferred into a Ludwig extractor and extracted with 500 ml *n*-hexane for about 90 min under reflux.

After addition of a maximum of 0,5 ml to1 ml of *n*-nonane as "keeper" the *n*-hexane extract is reduced in volume by rotary evaporation, to an approximate volume of ≤1 ml.

9.3 Clean-up

9.3.1 General

The clean-up procedure is carried out using sequential wet column chromatography. The specific columns used may be adapted according to the expected impurity profile of the sample. However, a multilayer column specified in c) below is obligatory and care needs to be exercised regarding the sequence in which they are run.

The following column type and sequence is given as guidance:

a) (Optional) Acidified diatomaceous earth column prepared according to 9.3.2.1 and run according to 9.3.2.2. may be used to retain excessive content of Lewis-basic nitrogen heterocycles, esters, ethers, etc.;

b) (Optional) Porous styrene divinylbenzene beads column may be used according to 9.3.3.1 and run according to 9.3.3.2 as size exclusion chromatography/gel permeation chromatography (GPC) to separate high molecular weight species (e.g. rosin, polymers);

c) (Obligatory) Multilayer column prepared as described in 9.3.4.1 and run as described in 9.3.4.2. Impregnated silica layers (separated by a shallow layer of non-impregnated silica gel) in this column may filter out following organic impurities to reduce the matrix effects, leading to a better signal-to-noise ratio as described in 9.3.4.1, and run as described in 9.3.4.2:

   — Silica gel impregnated with sulphuric acid: Retains residual amines, cleaves alkyl ethers;

   — Silica gel impregnated with cesium hydroxide: Eliminates acidic species by formation of insoluble salts;

   — Silica gel impregnated with silver nitrate: Oxidises alcohols and retain low-valent Sulfur compounds;

d) (Optional) Alumina B column may be used according to 9.3.5.1 and run according to 9.3.5.2 to additionally separate PCBs from larger amounts of "dioxins" (PCDD/PCDF).

9.3.2 Diatomaceous earth-sulfuric acid column (optional)

9.3.2.1 Preparation of the column

Geometry: length: 30 cm, int. width: 10 mm, top reservoir: 250 ml.

Fill the empty column with *n*-hexane; subsequently fill up to 1/3 of the top reservoir flask with *n*-hexane degassed by ultrasonic bath.

Pack the column as follows:

— 5 g Silica gel (see 7.2.4) (bottom layer);

— 30 g to 33 g Diatomaceous earth/H_2SO_4 (see 7.2.3) (1 + 1 — mix, compacted);

— 5 g to 6 g Silica gel (see 7.2.4);

— 5 g to 7 g Anhydrous sodium sulfate, granular (top layer) (see 7.2.8).

Let *n*-hexane run off until top of sodium sulfate is reached then pre-condition with 200 ml hexane/dichloromethane (4:1) and let run off to the top of sodium sulfate layer again. Discard the eluate.

9.3.2.2 Running the column

Add resulting *n*-nonane solution from step 9.2 to the top of sodium sulfate layer, let run in, then rinse the flask three times with *n*-hexane/dichloromethane (4 + 1).

Elute the column with 250 ml *n*-hexane/dichloromethane (4 + 1) at 3 ml/min to 5 ml/min as mobile phase.

Collect the eluate in a 500 ml round-bottomed flask.

Evaporate the eluate solution to 2 ml under addition of a max. of 1 ml to 2 ml of *n*-nonane as before and reduce to near dryness by nitrogen flushing. The residue will have an oily appearance.

9.3.3 Gel permeation (size exclusion) chromatography/porous styrene divinylbenzene beads-column (optional)

9.3.3.1 Preparation of the column

Geometry: length: 50 cm, int. width: 20 mm, top reservoir: 250 ml.

Weigh in 100 g of porous styrene divinylbenzene beads in a 1 l round bottom flask.

Add 700 ml ethyl acetate + cyclohexane (1 + 1).

Let it stand for 2,5 h (swelling).

Transfer the mixture into the column.

NOTE The stationary phase will reduce in volume with rinsing of the mobile phase.

Further rinse the column with 1,5 l of ethyl acetate + cyclohexane (1 + 1).

9.3.3.2 Running the column

Add resulting *n*-nonane solution from step 9.2 (or depending on the preceding optional column step the reduced volume of the *n*-nonane solution) to the top of column, let run in.

Elute with 90 ml ethyl acetate + cyclohexane (1 + 1) at 3 ml/min to 5 ml/min.

Discard the eluate.

Elute with 100 ml ethyl acetate + cyclohexane (1 + 1) at 3 ml/min to 5 ml/min.

Collect the eluate in a 250 ml round bottom flask.

Evaporate the eluate solution to 2 ml under addition of a maximum of 1 ml to 2 ml of *n*-nonane as before and reduce to near dryness by nitrogen flushing. The residue will have an oily appearance.

9.3.4 Multilayer columns (obligatory)

9.3.4.1 Preparation of a large-volume multilayer column

No bubbles, no adhesion of components on the wall are permitted (rotary shaking, gentle knocking).

Geometry: length: 30 cm, int. width: 14 mm, top reservoir: 250 ml.

Fill the empty, dry column with *n*-hexane to 1/3 of the top reservoir.

Fill the column consecutively according to Table 1.

Table 1 — Filling of the column

Material	Added weight
Silica gel, pure (bottom layer)	0,4 g to 0,6 g
Impregnated silica gel with $AgNO_3$	2,25 g to 2,3 g
Silica gel, pure	1,5 g
Impregnated silica gel with CsOH	3,75 g to 3,8 g
Silica gel, pure	1,1 g
Impregnated silica gel with H_2SO_4	7,5 g to 7,7 g
Silica gel, pure	4,5 g
Sodium sulfate, anhydrous (top layer)	3,0 g to 3,2 g

Let the solvent run off to near the surface of the top layer. Rinse once with 20 ml *n*-hexane (7.2.10).

NOTE Alternatively, a two step wet column chromatography (see 9.3.4.3) with modified silica (first) and alumina (second) can be used or a commercially available alternative with a similar specification.

9.3.4.2 Running the large-volume multilayer column

If red colour breaks through $AgNO_3$-silica gel layer, repeat CsOH and $AgNO_3$-silica gel layers in separate column.

Add resulting *n*-nonane solution from step 9.2 (or depending on preceding optional column steps the reduced volume of the *n*-nonane solution) to the top of sodium sulfate layer, let run in.

Rinse the flask three times with n-hexane.

Elute with 120 ml *n*-hexane.

Collect the eluate in a 500 ml round-bottomed flask.

Evaporate the eluate solution to 2 ml under addition of a max of 1 ml to 2 ml of *n*-nonane as before and reduce to near dryness by nitrogen flushing. The residue will have an oily appearance.

9.3.4.3 Two-step wet column

9.3.4.3.1 Preparation of the column

Geometry: length: 20 cm, int. width: 15 mm, top reservoir: 250 ml and a PTFE stop cock at the bottom of the column.

First column is packed with modified silica (1 g silica gel, 4 g basic silica gel, 1 g silica gel, 8 g acid silica gel, 2 g silica gel and 4 g granular anhydrous sodium sulfate).

Second column (same geometry) is packed with alumina (6 g).

9.3.4.3.2 Running the column

Pre-elute the column with 10 ml *n*-hexane.

Apply the concentrated sample extract or concentrated eluate from preceding step to the column.

Elute with 10 ml hexane (fraction 1).

Elute with 2 ml of a mix of 2 % dichloromethane in *n*-hexane (fraction 2).

Elute with 12 ml of a mix of 20 % dichloromethane in *n*-hexane (fraction 3).

Concentrate the eluate of fraction 3.

9.3.5 Alumina column (optional)

9.3.5.1 Preparation of the column

Geometry: length: 30 cm, intended width: 10 mm, top reservoir: 250 ml.

Relative air humidity shall not exceed an equivalent of 60 % at 26 °C, with suitable efficient air conditioning equipment recommended. Otherwise stop.

Rinse the empty and dry column twice with toluene and then fill the 1/3 of the column height with toluene.

Add 0,3 g to 0,5 g silica gel/alumina (3:1) as bottom isolating layer (especially important if the column is left standing overnight).

Rapidly weigh in approximately 12,5 g to 13 g anhydrous alumina (see 7.2.5).

Add it to the toluene-filled column, yielding slowly-settling slurry. Do not adjust amount of alumina as deactivation by air humidity will result in grave losses of analyte.

Rinse all alumina down the column with small quantities of toluene ("shooting" with the dropper). Electrostatic pushing by outside rubbing may also be applied, and confirms "activated" condition.

After collection of most alumina on the main column body, add a further isolating layer of 0,3 g + 0,3 g silica gel on top (see above; will also collect residual alumina from top reservoir). Wait for settling, and then add 3 g granular anhydrous sodium sulfate to the top of the column as a protecting top layer.

Let toluene run off until the top of the sodium sulfate layer.

9.3.5.2 Running the column

Load the concentrated eluate from the previous step onto the column. Rinse the flask twice (2 times 3 ml toluene), and add the solvent to the column.

Elute using the following sequence, collecting all eluates into a calibrated cylinder:

— 36 ml to 41 ml toluene;

— 2 times 2 ml *n*-hexane + dichloromethane (98 + 2);

— elute further with *n*-hexane + dichloromethane (98 + 2) until a volume of 120 ml is reached in the calibrated cylinder.

9.4 Preparation of GC solution and GC procedure

Transfer collected eluate of the final column quantitatively to a round-bottomed flask.

After addition of a maximum of 1 ml of n-nonane as “keeper” the n-hexane extract is reduced in volume by rotary evaporation, to an approximate volume of 0,5 ml, matching a 1 ml standard GC vial.

Transfer into the GC vial. The measuring solution is obtained by rinsing the flask with 2 times 25 µl *n*-nonane and adding the volume to the GC vial. Cap the vial.

Dioxins are still on the column and can be eluted with *n*-hexane + dichloromethane (1 + 1) for further analyses.

Specification of column as given in 8.3 (60 m length, 0,25 × 0,25; QP-MSD).

PFTBA (perfluorotributylamine) tuning of MS should be performed twice monthly or according to instrument performance monitoring requirements.

For the sample injection, it is recommended to:

— rinse the syringe at least once with the sample before injection, pumping 5 times with the injection sample;

— inject 0,6 µl to 1,5 µl into the injection port by means of a calibrated syringe or auto sampling device (exact quantity depends on apparatus and conditions);

— purge the syringe six times with pure solvent after injection.

The conditions for determination are dependent on the specific apparatus. The GC parameters shall be chosen to give best resolution:

— carrier gas (helium) flow shall be maintained between 0,8 ml/min and 1,3 ml/min;

— the temperature program shall be chosen as experienced to give the best resolution at lowest retention times of the very last congener, decachlorobiphenyl (at no more than 50 min in case of a 60 m-column), and separate monochlorobiphenyls from the injection solvent (at no less than 10 min). In a typical setting, the GC oven is programmed to warm up from 120 °C to 330 °C with approximately a 15 °C/min temperature ramp;

— the splitless injection system should be set up and operated to minimize crossover and general contamination and maximize reproducibility of injection. The temperature is recommended to remain below 270 °C;

— it is essential that column and injector be “blown out” at maximum temperature after each run for 10 min to 15 min.

9.5 Calculation and quantitation procedure

NOTE This procedure involves a simplification in that the analyte congeners are assumed to suffer the same loss rate and to undergo similar fragmentations as the respective reference materials added. Thus, their ratio versus the reference congeners can achieve sufficiently accurate, reliable results for judgement, the error not exceeding the normal ±20 % deviation. This will save cost and minimize hazardous waste volume. More detailed information can be found via Reference [5].

In case of higher PCB (i.e. above 150 mg/kg) content a reanalysis shall be done with a correspondingly lower sample mass.

Each chlorination degree (homologue group) should be quantified using a corresponding mass-labelled reference congener. Quantitation is carried out via isotope dilution method and is calculated using Formula (1):

$$C = \frac{R_n}{R_{ls}} \times \frac{W_l}{W_s} \quad (1)$$

where

C is the concentration of native PCB congener in the sample, in µg/g = mg/kg;

W_l is the mass, in microgram, of $^{13}C_{12}$-labelled PCB internal reference material added to sample;

W_s is the sample mass, in grams;

R_{ls} is the area of $^{13}C_{12}$-labelled PCB internal reference material in the sample;

R_n is the area of native PCB congener in the sample.

It is not part of this method to assess the system or parts of the procedure for recoveries, to permit rapid screening work. Congener reference material are taken as "fully recovered" for ratio calculations.

However, such recovery-assessing procedures are compatible with this method which may be modified to pursue the following approach.

Compute the percentage of recovery using Formula (2):

$$R_e = \frac{R_{ls}}{R_{is}} \times \frac{R_{lr}}{R_{ir}} \times 100 \quad (2)$$

where

R_e is the recovery of $^{13}C_{12}$-labelled PCB (if measured individually);

R_{ir} is the response of injection reference material (if any) in the reference sample;

R_{is} is the response of injection reference material in the sample;

R_{ls} is the area of $^{13}C_{12}$-labelled PCB internal reference material in the sample;

R_{lr} is the response of $^{13}C_{12}$-labelled reference material in the reference sample.

Integration of the M^+-peaks in GC-Quadrupole-MS-Detection [Electron Impact (EI) ionization] should be carried out "manually". Small peaks shall be strictly discarded if looking positive but lacking even an approximate qualifier isotopic ratio as predetermined for the analyte. Positive identification criteria may be considered 3 qualifier masses in a range of ±20 % of theoretical abundance statistics (High-resolution MS might permit different identification procedures).

Fragment masses (e.g. $[M\text{-}Cl]^+$) shall be checked for by retention time coincidence; even if isotopic ratios match, only the highest chlorination degree measured at this retention time counts as a "true" peak, the lighter ones being disregarded as "fragments".

Each chlorination degree (homologue group) may be quantified using only one reference congener, exception being the HexaCB-range, where suitable retention times shall be assigned to two respective reference material.

In case of bromine detection or presence of partially brominated samples, the absence of the characteristic "79 and 81" bromine peaks under any analyte peak shall be ensured, an option being the inclusion of these masses to the SIM/IonSet pattern for control purposes to prove absence of bromine.

10 Test report

The initial measuring printout shall provide a full description of the GC-MS method. The data report shall at least comprise:

a) all details necessary for complete identification of the product tested (e.g. manufacturer, trade name, batch number, sample ID, etc.);

b) a reference to this document, i.e. ISO 787-28:2019;

c) the sample mass in grams (2 decimals accuracy);

d) for all congener groups (including deca) the content in mg/kg;

e) the total PCB as summary from congener groups contents in mg/kg;

f) the apparatus used;

g) the conditions including time of dissolution in sulfuric acid and of solvent application to liquid-liquid-extraction;

h) the clean-up procedure in brief;

i) any deviations from the method;

j) reference materials used (type, quantity);

k) the date of testing.

Annex A
(informative)

Nomenclature of PCBs

Table A.1 — Nomenclature of PCBs

BZ-No(see note)	Compound	CAS No
Biphenyl (not a PCB)		92–52–4
Mono-CB		27323–18–8
1	2	2051–60–7
2	3	2051–61–8
3	4	2051–62–9
Di-CB		25512–42–9
4	2,2′	13029–08–8
5	2,3	16605–91–7
6	2,3′	25569–80–6
7	2,4	33284–50–3
8	2,4′	34883–43–7
9	2,5	34883–39–1
10	2,6	33146–45–1
11	3,3′	2050–67–1
12	3,4	2974–92–7
13	3,4′	2974–90–5
14	3,5	34883–41–5
15	4,4′	2050–68–2
Tri-CB		25323–68–6
16	2,2′,3	38444–78–9
17	2,2′,4	37680–66–3
18	2,2′,5	37680–65–2
19	2,2′,6	38444–73–4
20	2,3,3′	38444–84–7
21	2,3,4	55702–46–0
22	2,3,4′	38444–85–8
23	2,3,5	55720–44–0
24	2,3,6	58702–45–9
25	2,3′,4	55712–37–3
26	2,3′,5	38444–81–4
27	2,3′,6	38444–76–7

NOTE 1 Ballschmiter and Zell[9] proposed a system where a number, (called “BZ number”), is attributed to each individual congener. This number correlates the structural arrangement of the PCB congener and ascending order of number of chlorine substitutions within each sequential homologue. Slight changes have been made to the original numbering system since its introduction. A review can be found in the IARC monograph 107[11].

NOTE 2 Data for Table A.1 have been taken fro EPA publication “Table of PCB Species by Congener Number” htpps://www.epa.gov/sites/production/files/2015-09/documents/congenertable.pdf which uses the BZ numbering system published by Ballschmiter et al. 1992[12].

Table A.1 *(continued)*

BZ-No(see note)	Compound	CAS No
28	2,4,4′	7012–37–5
29	2,4,5	15862–07–4
30	2,4,6	35693–92–6
31	2,4′,5	16606–02–3
32	2,4′,6	38444–77–8
33	2,3′,4′	38444–86–9
34	2,3′,5′	37680–68–5
35	3,3′,4	37680–69–6
36	3,3′,5	38444–87–0
37	3,4,4′	38444–90–5
38	3,4,5	53555–66–1
39	3,4′,5	38444–88–1
Tetra-CB		26914–33–0
40	2,2′,3,3′	38444–93–8
41	2,2′,3,4	52663–59–9
42	2,2′,3,4′	36559–22–5
43	2,2′,3,5	70362–46–8
44	2,2′,3,5′	41464–39–5
45	2,2′3,6	70362–45–7
46	2,2′,3,6′	41464–47–5
47	2,2′,4,4′	2437–79–8
48	2,2′4,5	70362–47–9
49	2,2′4,5′	41464–40–8
50	2,2′4,6	62796–65–0
51	2,2′4,6′	65194–04–7
52	2,2′5,5′	35693–99–3
53	2,2′5,6′	41464–41–9
54	2,2′,6,6′	15968–05–5
55	2,3,3′,4	74338–24–2
56	2,3,3′,4′	41464–43–1
57	2,3,3′,5	70424–67–8
58	2,3,3′,5′	41464–49–7
59	2,3,3′,6	74472–33–6
60	2,3,4,4′	33025–41–1
61	2,3,4,5	33284–53–6
62	2,3,4,6	54230–22–7
63	2,3,4′,5	74472–34–7
64	2,3,4′,6	52663–58–8

NOTE 1 Ballschmiter and Zell[9] proposed a system where a number, (called “BZ number”), is attributed to each individual congener. This number correlates the structural arrangement of the PCB congener and ascending order of number of chlorine substitutions within each sequential homologue. Slight changes have been made to the original numbering system since its introduction. A review can be found in the IARC monograph 107[11].

NOTE 2 Data for Table A.1 have been taken fro EPA publication “Table of PCB Species by Congener Number” htpps://www.epa.gov/sites/production/files/2015-09/documents/congenertable.pdf which uses the BZ numbering system published by Ballschmiter et al. 1992[12].