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Water quality — Determination of dioxin-like polychlorinated biphenyls — Method using gas chromatography/mass spectrometry

Qualité de l'eau — Dosage des biphényls polychlorés de type dioxine — Méthode par chromatographie en phase gazeuse/spectrométrie de masse

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Contents

Page

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 17858 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Introduction

When using this International Standard, it may be necessary in some cases to determine whether and to what extent particular problems will require the specification of minor additional conditions.

Water quality — Determination of dioxin-like polychlorinated biphenyls — Method using gas chromatography/mass spectrometry

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

Attention is drawn to any relevant national safety regulations. The non-*ortho* and mono-*ortho* PCBs are co-planar and are among the most toxic of chemicals. All work with dioxin-like PCBs requires therefore the utmost care; the national safety measures which correspond to those for toxic substances shall be strictly adhered to.

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

1 Scope

This International Standard specifies a method for the determination of dioxin-like tetra- to hepta-chlorinated biphenyls (PCBs) in waters and wastewaters (containing less than 1 % suspended solids) using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The method is optimized for dioxin-like PCBs, but can include other co-planar compounds such as polychlorinated dioxins and furans (PCDDs/PCDFs) and polychlorinated naphthalenes (PCNs). This method can be used to determine dioxin-like PCBs in other matrices (e.g. biota, sediments, air); however, additional clean-up steps and techniques can be required for samples with high organic loadings.

This method is applicable to the twelve non- and mono-*ortho* PCBs designated by the World Health Organization, as well as to other PCBs and co-planar compounds.

The detection limits and quantification levels in this method are dependent on the level of interferences as well as instrumental limitations. The minimum levels (ML) in Table 2 are the levels at which the dioxin-like PCBs can typically be determined with no interferences present.

This method is “performance based”. The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements, provided that all performance criteria in this method are met. The requirements for establishing method equivalency are given in 9.2.

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.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

3 Terms, definitions and abbreviated terms

3.1 Terms and definitions

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

3.1.1
analyte
dioxin-like polychlorinated biphenyl tested for by this method

See Table 1.

3.1.2
calibration standard
solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration

3.1.3
calibration verification standard
VER
midpoint calibration standard that is used to verify calibration

3.1.4
certified reference material
CRM
quality control sample used to determine accuracy and precision of method

3.1.5
congener
member of the same kind, class or group

EXAMPLE Any one of the 209 individual PCBs.

3.1.6
critical pair
pair of isomers that must be separated to a predefined degree (e.g. 25 % valley) to ensure chromatographic separation meets minimum quality criteria

3.1.7
dioxin-like isomer
PCB with identical chemical composition but different structure

3.1.8
homologue group
complete group of isomers

EXAMPLE Tetrachlorobiphenyls.

3.1.9
isotope dilution
method using labelled (usually $^{13}C_{12}$) internal standards to correct for losses during sample preparation and analysis

3.1.10
keeper solvent
high boiling point solvent added to the sampling standard solution

3.1.11
method blank
aliquot of reagent water that is treated exactly as a sample through the complete analytical procedure including extraction, clean-up, identification and quantification including all the relevant reagents and materials

3.1.12
operational performance characteristics
influence of the physical and chemical environment and maintenance problems, for example, mains voltage, temperature, supply of certain substances, set-up time, period of unattended operation

3.1.13
pattern
chromatographic fingerprint of any series of PCB isomers

3.1.14
profile
graphic representation of the sums of the isomer concentrations of the PCBs

3.1.15
spiking
addition of $^{13}C_{12}$-labelled PCB standards of which the recovery is calculated and used to correct values of native analytes of interest

3.1.16
statistical performance characteristics
quantification, for measured values, of the possible deviations resulting from the random part of the measuring process, e.g. repeatability or instability

3.1.17
toxic equivalent factor
TEF
relative toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

3.1.18
toxic equivalent quantity
TEQ
sum of toxic equivalents of each individual congener

3.2 Abbreviated terms

CRM	certified reference material
GC/MS	gas chromatography/mass spectrometry
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high-resolution gas chromatography
HRMS	high-resolution mass spectrometry
IPR	initial precision and recovery
LRMS	low-resolution mass spectrometry
MDL	method detection limit
ML	minimum level (see Table 2)
PAR	precision and recovery
PCB	polychlorinated biphenyl

PCDD/PCDF polychlorinated dibenzo-*p*-dioxin/dibenzofuran

PCN polychlorinated naphthalene

PFK perfluorokerosene

SIM selected ion monitoring

SPE solid-phase extraction

TEF toxic equivalent factor

TEQ toxic equivalent quantity

VER calibration verification standard

4 Principle

4.1 Spiking and extraction

Stable isotopically labelled analogues of dioxin-like PCBs (diluted in a suitable solvent such as acetone) are spiked into a 1 litre aqueous sample (a sample containing less than 1 % suspended solids). A minimum of one labelled standard per homologue group is used and the sample is extracted by one of three procedures noted in 4.1 a), 4.1 b) and 4.1 c). If the sample contains more than 1 % solid material, the solid portion can be analysed directly after filtration or drying and the aqueous portion can be discarded.

a) Samples containing no visible particles are extracted with dichloromethane [6.4 f)] in a separatory funnel or by solid-phase extraction. The extract is concentrated for clean-up.

b) Samples containing visible particles are vacuum filtered through a glass-fibre filter. The filter is extracted in a Soxhlet extractor using toluene and the filtrate is extracted with dichloromethane [6.4 f)] in a separatory funnel. The dichloromethane extract is concentrated and combined with the Soxhlet extract prior to clean-up.

c) The sample is vacuum filtered through a glass-fibre filter on top of a solid-phase extraction (SPE) disk. The filter and disk are eluted with suitable solvent mixtures or extracted in a Soxhlet or pressure filtration extractor, and the extract is concentrated for clean-up.

Other solvents and extraction techniques may be substituted, provided that all the performance criteria can be met.

4.2 Clean-up

After extraction, sample extracts are cleaned to remove interfering components. Sample clean-up procedures can include washes with acid and/or base, gel permeation, alumina, silica, Florisil[1)] and activated carbon chromatography. High-performance liquid chromatography (HPLC) can be used for further isolation of other specific co-planar compounds if required. Due to the large number of potential interfering compounds, sample extracts shall be fractioned or analysed on at least two distinct GC column phases to ensure unique identification and accurate quantification of each dioxin-like PCB congener.

4.3 Concentration

After clean-up, the extract(s) is concentrated to near dryness. Prior to injection, recovery standards are added to each extract, and an aliquot of the extract is injected into the gas chromatograph. The analytes are separated by GC and detected by a high-resolution mass spectrometer. Two exact masses are monitored for each analyte.

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

Resolution greater than or equal to 10 000 is recommended. High-resolution gas chromatography/high-resolution mass spectrometry at a resolution greater than or equal to 10 000 is at present required to achieve adequate sensitivity and selectivity, and to allow the use of all $^{13}C_{12}$-labelled standards. If the sample extract is being analysed for multi-component analyte groups (PCDD/Fs, PCBs, PCNs), a resolution of 10 000 is necessary. At resolutions less than 10 000, some $^{13}C_{12}$ PCDFs and PCBs interfere with native PCDDs of the same level of chlorination. Resolutions less than 10 000 can be used for specific analyte groups (PCBs, PCNs) where the matrix and potential interferences are well characterized.

4.4 Identification

An individual dioxin-like PCB is identified by comparing the GC retention time and ion abundance ratio of two exact masses monitored (see Table 7) with the corresponding retention time of an authentic internal standard and the theoretical or acquired ion-abundance ratio of the two exact masses. The isomers and congeners for which there are no labelled analogues are identified when retention times or relative retention times and ion-abundance ratios agree within predefined limits. Masses of those PCBs with a degree of chlorination higher than three (e.g. PentaCB 110 for TetraCB 77) shall be monitored to ensure there is no contribution to the mass of interest.

4.5 Quantification

Quantitative analysis is performed using selected ion monitoring (SIM) areas, in one of two ways.

a) For the dioxin-like PCBs for which labelled analogues have been added to the sample (4.1), the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.

b) For the dioxin-like PCBs for which labelled analogues are not added, the GC/MS system is calibrated for each compound using an isomer or congener with the most similar structure and the concentration of each compound is determined using the internal standard technique.

4.6 Analytical quality

The quality of the analysis is assured through reproducible calibration and testing of the extraction, clean-up, and GC/MS systems. Interferences, biases and limitations should be determined and identified for each target analyte through intercalibration (interlaboratory) studies, certified reference materials (CRM) and spiked matrix samples (SMS). A series of quality control (QC) samples (CRM, SMS) should be analysed with each set of samples and monitored through control charting or other quality review procedures.

5 Contamination and interferences

5.1 Where possible, monitor or clean reagents by extraction or solvent rinse.

Solvents, reagents, labware, and other sample processing hardware can yield artefacts and/or elevated baselines causing misinterpretation of chromatograms. (Example chromatograms showing typical retention times of native and labelled PCBs are given in Annex A.) Specific selection of reagents and purification of solvents by distillation in all-glass systems can be required. Many reagents, solvents and labware contain background levels of dioxin-like compounds, e.g. PCB118 and PCB105.

5.2 Clean labware such that the method blank requirements given in 9.5.3 are met. An example of a cleaning procedure is given below in a) to c).

a) Disassemble labware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, prior to detergent washing. Rinse labware with solvent and wash with a detergent solution as soon after use as is practical. Sonication of labware containing a detergent solution for approximately 30 s can aid in cleaning.

b) After detergent washing, rinse labware immediately with hot tap water. The tap water rinse shall be followed by an acetone rinse, then a dichloromethane [6.4 f)] rinse/soak. For known contaminated labware, use toluene as a final rinse/soak.

c) Soxhlet apparatus should be cycled with toluene for at least 20 cycles. Shake separatory funnels with dichloromethane [6.4 f)] and/or toluene for 2 min, drain, and then shake with pure dichloromethane [6.4 f)] for 2 min.

Proper cleaning of labware is extremely important because labware can contaminate the samples but can also remove the analytes of interest by surface adsorption if the surface is activated during the cleaning procedure. Glassware can be checked for contamination by analysing solvent rinses.

5.3 Demonstrate that all materials used in the analysis are free from interferences by running reference matrix method blanks initially and with each sample batch (samples started through the extraction process on a given 12-h shift, to a maximum of 20 samples); see 9.5, 15.5.

5.4 The reference matrix shall simulate, as closely as possible, the sample matrix under test. Ideally, the reference matrix shall not contain dioxin-like compounds in detectable amounts, but shall contain potential interferants in the concentrations expected to be found in the samples to be analysed.

Interferences co-extracted from samples can vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds, including PCBs of higher degrees of chlorination can be present at concentrations several orders of magnitude higher than the dioxin-like PCBs being analysed. The most frequently encountered interferences are dibenzo-*p*-dioxins, dibenzofurans, diphenyl ethers, methoxy biphenyls, hydroxydiphenyl ethers, benzylphenyl ethers, aromatic sulfur compounds, polynuclear aromatics, and pesticides. Because very low levels of dioxin-like PCBs are measured by this method, the elimination of interferences is essential. The example clean-ups given in Clause 13 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the dioxin-like PCBs at the levels shown in Table 2.

5.5 When a clean reference matrix that simulates the sample matrix under test is not available, use reagent water (6.7) or a matrix that most closely resembles the sample.

5.6 Number each piece of reusable labware or minimally identify each set of specific type of labware (e.g. Soxhlet extractors, round-bottom flasks) to associate that specific labware with the processing of a particular sample or set of samples. This will assist the laboratory in tracking possible sources of contamination for individual samples, identifying labware associated with highly contaminated samples that may require extra cleaning, and determining when labware shall be discarded.

6 Reagents and standards

Use only reagents of recognized analytical grade, unless otherwise specified.

6.1 **Water**, complying with grade 3 as defined in ISO 3696.

6.2 **pH adjustment and back-extraction reagents**.

6.2.1 **Potassium hydroxide solution**.

Dissolve 20 g of potassium hydroxide, KOH, in 100 ml of water.

6.2.2 **Sulfuric acid**, H_2SO_4, ρ = 1,84 g/ml.

6.2.3 **1 mol/l sulfuric acid**.

Dilute with care 56 ml of concentrated sulfuric acid (6.2.2) to 1 litre of water (6.1).

6.2.4 **Sodium chloride solution**.

Dissolve 5 g of sodium chloride, NaCl, in 100 ml of water.

6.2.5 **Sodium thiosulfate**, $Na_2S_2O_3$.

6.3 Solution drying and evaporation reagents.

6.3.1 Sodium sulfate, Na_2SO_4, granular, anhydrous.

Bake at 300 °C for at least 24 h, cool in a desiccator, and store in a precleaned glass bottle with a screw cap that prevents moisture from entering.

If, after heating, the sodium sulfate develops a noticeable greyish cast (due to the presence of carbon in the crystal matrix), discard that batch of reagent as it is not suitable for use. Rinse with about 20 ml of dichloromethane [6.4 f)] per gram of Na_2SO_4 or extract with dichloromethane [6.4 f)] if background contamination is detected.

6.3.2 Prepurified nitrogen, N_2 99,999 %.

6.4 Solvents for extraction and clean-up.

The extraction and clean-up solvents, distilled in glass, of pesticide quality and free of interferences, include the following:

a) **Acetone**, C_3H_6O.

b) **Toluene**, C_7H_8.

c) **Cyclohexane**, C_6H_{12}.

d) **Hexane**, C_6H_{14}.

e) **Methanol**, CH_3OH.

f) **Dichloromethane**, CH_2Cl_2.

g) **Diethyl ether**, $C_4H_{10}O$.

h) **Ethanol**, C_2H_6O.

i) **Nonane**, C_9H_{20}.

6.5 GPC calibration solution.

Dissolve 300 mg/ml of corn oil, 15 mg/ml of bis(2-ethylhexyl) phthalate, $C_{24}H_{38}O_4$, 1,4 mg/ml of pentachlorophenol, C_6Cl_5OH, 0,1 mg/ml of perylene, $C_{20}H_{12}$, and 0,5 mg/ml of sulfur, S, in dichloromethane [6.4 f)]. Store in glass and keep refrigerated. Prepare fresh monthly.

6.6 Adsorbents for sample clean-up.

6.6.1 Silica, 70 µm to 230 µm.

6.6.1.1 Activated silica, baked at 180 °C for a minimum of 1 h, cooled in a desiccator, and stored in a precleaned glass bottle with a screw cap that prevents moisture from entering. Prepare fresh every two weeks.

6.6.1.2 Acid silica, 30 % mass fraction.

Thoroughly mix 44,0 g of sulfuric acid (6.2.2) with 100 g of activated silica in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw cap. 22 % acid silica and 44 % acid silica are prepared in a similar manner by adding 29 g and 80 g of sulfuric acid, respectively, to 100 g of activated silica. Prepare fresh every two weeks.

6.6.1.3 Basic silica.

Thoroughly mix 30 g of 1 mol/l sodium hydroxide solution [c(NAOH) = 1 mol/l] with 100 g of activated silica in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw cap. Prepare fresh every two weeks.

6.6.1.4 Potassium silicate, 36 % mass fraction.

Dissolve 56 g of high purity potassium hydroxide (6.2.1) in 300 ml of methanol [6.4 e)] in a 750 ml to 1 000 ml flat-bottom flask. Add 100 g of silica (6.6.1) and a stirring bar, and stir on a hotplate at 60 °C to 70 °C for 1 h to 2 h. Decant the liquid and rinse the potassium silicate twice with 100 ml portions of methanol, followed by a single rinse with 100 ml of dichloromethane [6.4 f)]. Spread the potassium silicate on solvent-rinsed aluminium foil and dry for 2 h to 4 h in a hood. Activate overnight at 200 °C to 250 °C. Store in a bottle with a fluoropolymer-lined screw cap. Prepare fresh every two weeks.

6.6.2 Alumina.

6.6.2.1 One of two types of alumina, acid or basic, can be used in the clean-up of sample extracts, provided that the laboratory can meet the performance specifications for the recovery of labelled compounds described in 9.4. The same type of alumina shall be used for all samples, including those used to demonstrate initial precision and recovery (9.2).

a) **Acid alumina**, activate by heating to 130 °C for a minimum of 12 h.

b) **Basic alumina**, activate by heating to 600 °C for a minimum of 24 h.

6.6.2.2 Alternatively, activate by heating in a tube furnace at 650 °C to 700 °C under an airflow rate of approximately 400 ml/min. Do not heat over 700 °C, as this can lead to reduced capacity for retaining the analytes. Store at 130 °C in a covered flask. Use within 5 d of baking.

ICN Alumina Super I[2] or an equivalent may be used without activation.

6.6.3 Activated carbon.

6.6.3.1 Mixture of carbon and silica, for example, Carbopak C [3] (e.g. Supelco 1-0258, or equivalent) and Celite 545 [4] (e.g. Supelco 2-0199, or equivalent).

Thoroughly mix 9,0 g of Carbopak C and 41,0 g of Celite 545 to produce a mass fraction of 18 % of the mixture. Activate the mixture at 130 °C for at least 6 h. Store in a desiccator. Prepare fresh every two weeks.

NOTE Active carbon-impregnated silica[5] (Wako Pure Chemical Industries 019-11941) and active carbon-dispersed silica gel[6] (Kanto Chemical Co. 01875-43) are examples of alternative materials.

6.6.3.2 Carbon, e.g. Amoco PX21[7].

Thoroughly mix 0,30 g of PX21 with 5,7 g of activated silica (6.6.1.1) in a vial. Cap and shake the vial for at least 2 min or until mixture is homogeneous. Remove cap and place vial in oven at 300 °C for at least 60 h. Store in a desiccator.

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2) ICN Alumina Super I is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) Carbopak C is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4) Celite 545 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5) Active carbon-impregnated silica (019-11941) from Wako Pure Chemical Industries is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

6) Active carbon-dispersed silica gel (01875-43) from Kanto Chemical Company is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

7) Amoco PX21 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

6.6.4 Florisil, 70 µm to 250 µm.

Activate in an oven above 130 °C for a minimum of 24 h. Use as soon as possible after removal from oven. Activity of Florisil can be dependant on relative humidity. Prepare fresh for each use.

6.6.5 Silver nitrate/silica, (10 % mass fraction) for elimination of organosulfur and organohalogen compounds, made of silver nitrate ($AgNO_3$) of AR (Analytical Reagent) grade or equivalent and silica (6.6.1).

Dissolve 10 g of silver nitrate in 40 ml of water, add in portions 90 g of silica and shake until the mixture is homogeneous. Let stand for 30 min. Transfer the mixture to a drying oven preheated to 70 °C and heat from 70 °C to 125 °C over a 2 h period. Activate at 125 °C for at least 10 h. Store the mixture in a brown glass bottle. Prepare fresh for each use.

6.7 Reference matrices.

Reference matrices are matrices in which the dioxin-like PCBs and interfering compounds are not detected by this method, e.g. reagent water, bottled water purchased locally, HPLC grade water or water prepared by passage through activated carbon.

6.8 Standard solutions.

Purchase standard solutions as final working/calibration solutions or mixtures with certification indicating their purity, concentration, and authenticity. Alternatively, prepare solutions from materials of known purity and composition.

If the chemical purity is 98 % or greater, it is not necessary to correct the mass during the computation of the concentration of analytes in the standard.

When not being used, store standards in the dark in sealed ampoules or screw-capped vials with fluoropolymer-lined caps. Check the concentrations regularly so that solvent loss by evaporation can be detected. If solvent loss has occurred, replace the solution.

Standard preparation (6.8 to 6.16) and Tables 2, 3 and 4 give examples of a standard scheme that is acceptable. Other concentrations and spiking schemes may be used provided the performance criteria of the method can be met (see also 4.1).

Observe the safety precautions in the warning note. Check stock standard solutions for signs of degradation prior to the preparation of calibration or performance test standards.

NOTE Use certified reference standards and solutions to determine the accuracy of calibration standards wherever possible.

6.9 Precision and recovery (PAR) stock solution.

The PAR stock solution should contain the dioxin-like PCBs at the concentrations shown in Table 4. When diluted to the final concentration, the solution is referred to as the PAR standard solution (6.13). If possible, obtain this solution from an alternate supplier. This enables an ongoing verification and validation of the calibration (6.12) and labelled spiking solutions (6.10).

6.10 Labelled-compound spiking solution.

Prepare the labelled-compound spiking solution to contain the labelled dioxin-like compounds in nonane at the concentrations shown in Table 4.

Dilute a sufficient volume of the labelled-compound solution with acetone [6.4 a)] to prepare a diluted spiking solution.

Each sample requires 1,0 ml of the diluted solution, but no more solution should be prepared than can be used in 1 d.

6.11 Recovery standard(s).

Prepare the recovery standard solution to contain $^{13}C_{12}$-labelled compounds in nonane at the concentrations shown in Table 4 for the specific groups of compounds analysed.

6.12 Calibration standards.

Combine the solutions in 6.9 to 6.11 to produce at least five of the calibration solutions shown in Table 3 in nonane.

These solutions permit the relative response (labelled to native) and response factor to be measured as a function of concentration. A minimum of five solutions over the calibration range of the method should be used. Each concentration step should range between 3 times and 10 times the lower concentrated standard.

Use the mid-point standard for calibration verification (VER).

6.13 Precision and recovery (PAR) standard solution.

Use this standard solution for determination of initial and ongoing precision and recovery. For each sample matrix, dilute the required amount of the precision and recovery stock solution (6.9) to 2,0 ml with acetone [6.4 a)]. Use an amount that is representative of the levels being determined in the samples being analysed or at the regulatory limit that the samples under test are governed by.

6.14 GC retention time window defining solution and isomer specificity test standard.

Use this standard to define the beginning and ending retention time windows for the dioxin-like PCBs under test and to demonstrate isomer specificity of the GC columns employed for separation of the critical pair in the chromatographic analysis for each GC column used. The ability to isolate all congeners from interferences must be demonstrated (e.g. PCB 110 from PCB 77, 1,2,3,7,8-P_5CDD from PCB 169), either by use of separate GC columns or by the splitting of extracts using sample preparation procedures (e.g. carbon column chromatography to separate PCB 110 from PCB 77). This standard shall contain at least the compounds listed in Table 6.

6.15 Quality control (QC) check samples.

Obtain at least one QC check sample from a source independent of the calibration standards. Ideally, this check shall be a certified reference material or sample from an intercalibration study containing the dioxin-like PCBs in known concentrations in a sample matrix at levels similar to the matrix under test. Alternatively, a blank spiked with reference standards of dioxin-like PCBs from a source other than those used in the calibration solutions can be used as a QC check sample. This enables verification and validation of calibration solutions (6.12).

6.16 Stability of solutions

Analyse standard solutions used for quantitative purposes (6.9 to 6.13) periodically, and assay against reference standards (see Note in 6.8) before further use.

7 Apparatus and materials

7.1 Sampling equipment for discrete sampling

7.1.1 Sample bottles, made from amber glass, 1 l, with screw cap for liquid samples (waters, sludges and similar materials containing 1 % suspended solids or less).

If amber bottles are not available, protect samples from light.

Bottle caps can be lined with either fluoropolymer or metal foil.

Wash bottles in detergent water and rinse with solvent before use. Wash fluoropolymer liners in detergent water, rinse with reagent water and dry prior to use.

7.2 Equipment for sample preparation

7.2.1 Laboratory fume hood, of sufficient size to contain the sample preparation equipment listed below.

7.2.2 Glove box (optional).

7.2.3 Oven, capable of maintaining temperatures within 5 % of the target temperature and in the range of 100 °C to 600 °C.

7.2.4 Desiccator.

7.2.5 Balances, consisting of an analytical type capable of weighing 0,1 mg and a top-loading type capable of weighing 10 mg.

7.3 Extraction apparatus

7.3.1 pH meter, with combination glass electrode.

7.3.2 pH paper, wide range.

7.3.3 Graduated cylinder, 1 l capacity.

7.3.4 Liquid/liquid extraction separatory funnels, 250 ml, 500 ml, and 2 000 ml, with glass or fluoropolymer stopcocks.

7.3.5 Solid-phase extraction equipment, consisting of the following.

7.3.5.1 Filtration apparatus, 1 l, including glass funnel, glass frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing. For wastewater samples, the apparatus shall accept 90 mm or 144 mm extraction disks. For drinking water or other samples containing low suspended solids, smaller disks may be used.

7.3.5.2 Vacuum source, capable of maintaining 635 mm Hg, equipped with shut-off valve and vacuum gauge.

7.3.5.3 Glass-fibre filter, 1 micron pore size, to fit filtration apparatus in 7.3.5.1.

7.3.5.4 Solid-phase extraction disk, containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix, to fit filtration apparatus in 7.3.5.1. (Urethane foam has also been used where large volumes of water are extracted.)

7.3.5.5 Glass Petri dishes, appropriate to filter size.

7.3.6 Soxhlet extractor, for filters and SPE disks, consisting of the following.

7.3.6.1 Soxhlet, 50 mm ID, 200 ml capacity with a 500 ml round-bottom flask.

7.3.6.2 Thimble, 43 × 123 to fit Soxhlet.

7.3.6.3 Hemispherical heating mantle, to fit 500 ml round-bottom flask.

7.3.7 Beakers, 250 ml to 500 ml.

7.3.8 Spatulas, made of stainless steel.

7.3.9 Heated pressure filtration extractor, such as the Dionex Accelerated Solvent Extractor (ASE) for extraction of filters, SPE disks and other solid materials.

7.4 Filtration apparatus

7.4.1 Glass wool, extracted by Soxhlet for 3 h minimum or accelerated solvent extraction (ASE) using toluene.

7.4.2 Glass funnel, of 125 ml to 250 ml.

7.4.3 Glass-fibre filter paper, to fit glass funnel in 7.4.2.

7.4.4 Drying column, 15 mm to 20 mm ID quartz chromatographic column equipped with a coarse-glass frit or glass-wool plug.

7.4.5 Buchner funnel, with a minimum diameter of 40 mm.

7.4.6 Glass-fibre filter paper, capable of fitting the Buchner funnel in 7.4.5.

7.4.7 Filtration flasks, of 1,5 l to 2,0 l with side arm.

7.4.8 Pressure filtration apparatus.

7.5 Clean-up apparatus

7.5.1 Automated gel permeation chromatograph, consisting of the following.

7.5.1.1 Column, 600 mm to 700 mm long × 25 mm ID, packed with 70 g of SX-3 Bio-beads[8)].

7.5.1.2 Syringe, 10 ml, with Luer fitting.

7.5.1.3 Syringe filter holder, stainless steel, and glass-fibre or fluoropolymer filters.

7.5.1.4 UV detectors, 254 nm, preparative or semi-preparative flow cell.

7.5.2 High-performance liquid chromatograph equipment, consisting of the following.

7.5.2.1 Column oven and detector, operated at 0,02 AUFS (Adsorbance Units Full Scale) at 235 nm.

7.5.2.2 Injection valve, with 50 µl sample loop.

7.5.2.3 Column, two 6,2 mm × 250 mm columns in series, operated at 50 °C with 2,0 ml/min methanol isocratic effluent.

7.5.2.4 HPLC pump.

7.5.3 Disposable pipettes.

Either disposable Pasteur pipettes, 150 mm long × 5 mm ID or disposable serological pipettes, 10 ml (6 mm ID).

7.5.4 Glass chromatographic columns of the following sizes:

a) 150 mm to 280 mm long × 6 mm ID, with coarse-glass frit or glass-wool plug and 250 ml reservoir;

b) 280 mm long × 6 mm ID, with 300 ml reservoir and glass or fluoropolymer stopcock;

c) 300 mm long × 11 mm ID, with 300 ml reservoir and glass or fluoropolymer stopcock;

d) 200 mm long × 15 mm ID, with coarse-glass frit or glass-wool plug and 250 ml reservoir.

8) SX-3 Bio-beads is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product.

7.6 Concentration apparatus

7.6.1 Rotary evaporator, equipped with a variable temperature water bath and the following.

7.6.1.1 Vacuum source, for rotary evaporator equipped with shut-off valve at the evaporator and vacuum gauge.

7.6.1.2 Recirculating water pump and chiller, or other suitable vacuum source.

7.6.1.3 Round-bottom flasks, 100 ml and 500 ml or larger, with ground-glass fitting compatible with the rotary evaporator.

7.6.2 Kuderna-Danish (K-D) concentrator, consisting of the following.

7.6.2.1 Concentrator tube, 10 ml, graduated, with calibration verified. Use a ground-glass stopper (size 19/22 joint) to prevent evaporation of extracts.

7.6.2.2 Evaporation flask, 500 ml, attached to concentrator tube with springs.

7.6.2.3 Snyder column, three-ball macro and two-ball micro.

7.6.2.4 Boiling chips, of the following types.

7.6.2.4.1 Glass or silicon carbide, approximately 10/40 mesh, extracted with dichloromethane [6.4 f)] and baked at 450 °C for 1 h minimum.

7.6.2.4.2 Fluoropolymer (optional), extracted with methylene chloride.

7.6.2.5 Water bath, heated, with concentric ring cover, capable of maintaining a temperature within ± 2 °C, installed in a fume hood.

7.6.3 Nitrogen blowdown apparatus, equipped with a water bath controlled in the range of 30 °C to 60 °C, installed in a fume hood.

7.6.4 Sample vials, of the following types:

7.6.4.1 Amber glass, 2 ml to 5 ml, with fluoropolymer-lined screw cap.

7.6.4.2 Glass, 0,3 ml, conical, with fluoropolymer-lined screw or crimp cap.

7.7 Other equipment

7.7.1 Gas chromatograph, with a splitless or on-column injection port for capillary column, multi-level temperature programme with isothermal hold.

The chromatograph shall meet all of the performance specifications in 10.5.

7.7.2 GC column for dioxin-like PCBs, with isomer specificity for the 12 dioxin-like PCBs listed in Table 1.

A column or series of columns that is able to resolve the dioxin-like PCBs from any other PCB that does not have an identical TEF. Suggested columns include: 60 m × 0,25 mm ID; 0,25 μm 5 % phenyl, 94 % methyl, 1 % vinyl silicone bonded-phase fused-silica capillary column and 30 m × 0,25 mm ID; 0,25 μm SPB-Octyl fused-silica capillary column (see Table 5).

7.7.3 Mass spectrometer, 28 eV to 80 eV electron impact ionization, capable of repetitively selectively monitoring at least 12 exact masses at high resolution (> 10 000) during a period of approximately one second, and shall meet all of the performance specifications in Clause 14.

7.7.4 GC/MS interface.

The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column terminates near the inside edge of the ion source but does not intercept the electron or ion beams.

7.7.5 Data system, capable of collecting, recording, and storing MS data.

8 Sample collection, preservation, storage and holding times

8.1 Collect samples in amber glass containers as specified in ISO 5667-1 and ISO 5667-2.

8.2 Maintain aqueous samples in the dark $\leqslant$ 4 °C from the time of collection until receipt at the laboratory. If residual chlorine is present in aqueous samples, add 80 mg of sodium thiosulfate ($Na_2S_2O_3$) (6.2.5) per litre of water. If the sample pH is greater than 9, adjust to pH 7 to pH 9 with 1 mol/l sulfuric acid (6.2.3).

8.3 Store sample extracts in the dark until analysed.

NOTE 1 If stored in the dark at $\leqslant$ 10 °C, cleaned sample extracts can be stored indefinitely.

NOTE 2 There are no demonstrated maximum holding times associated with dioxin-like PCBs in aqueous sample matrices. If stored in the dark at $\leqslant$ 4 °C and preserved in accordance with 8.1 and 8.2 (if required), aqueous samples can be stored for up to one year.

9 Quality assurance (QA)/quality control (QC)

9.1 General

9.1.1 Each laboratory that uses this method shall operate a formal quality assurance programme. The minimum requirements of this programme consist of an initial demonstration of laboratory capability, analysis of samples spiked with labelled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance shall be compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1.2 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability shall be established as described in 9.2.

9.1.3 In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst shall be permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration and clean-up procedures, and changes in columns and detectors. Alternative determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, shall not be allowed. These techniques can be used for screening purposes if false negatives are not observed. If an analytical technique other than the techniques specified in this method is used, that technique shall have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

9.1.3.1 Each time a modification is made to this method, the analyst shall repeat the procedure in 9.2. If the detection limit of the method will be affected by the change, the laboratory shall demonstrate that the method detection limit (MDL) is lower than one-third of any regulatory compliance level in force or one-third the minimum level (ML) in this method, whichever is higher. If calibration will be affected by the change, the analyst shall recalibrate the instrument in accordance with Clause 10.

9.1.3.2 The laboratory shall maintain records of modifications made to this method.

9.1.4 Analyses of method blanks shall demonstrate freedom from contamination (5.3). The procedures and criteria for analysis of a method blank are described in 9.5 and 15.5.

9.1.5 The staff shall spike all samples with labelled compounds to monitor method performance.

NOTE 1 This is described in 9.3.

When results of these spikes indicate atypical method performance for samples, dilute, reclean or reanalyse the samples to bring method performance within acceptable limits.

NOTE 2 Procedures for dilution are given in 17.3.1.

9.1.6 The laboratory shall, on an ongoing basis, demonstrate through calibration verification that the analytical system is in control.

NOTE These procedures are described in 15.1 to 15.5.

9.1.7 The laboratory shall maintain records to define the quality of data that is generated.

NOTE Development of accuracy statements is described in 9.4.

9.2 Initial precision and recovery (IPR)

To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

a) Extract, concentrate and analyse at least four (eight preferred) aliquots of reagent water spiked with the diluted labelled-compound spiking solution (6.10) and the precision and recovery standard (6.13) according to the procedures in Clauses 11 to 18. For an alternative sample matrix, at least four aliquots (eight preferred) of the alternative reference matrix (6.7) are used. All sample-processing steps that are to be used for processing samples, including preparation (see Clause 11), extraction (see Clause 12), and clean-up (see Clause 13) shall be included in this test.

b) Using results of the samples prepared and analysed as in a) above, compute the average concentration ($\bar{c}$) of the extracts in percent, %, and the relative standard deviation of the concentration (s_{rel}) in percent, %, for each compound, by isotope dilution for dioxin-like PCBs with a labelled analogue, and by internal standard for those without a labelled analogue.

c) For each dioxin-like PCB, compare s_{rel} and $\bar{c}$ with the corresponding limits for initial precision and recovery: < 20 % for s_{rel} and ± 20 % of test concentration for $\bar{c}$. If s_{rel} and $\bar{c}$ for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples can begin. If, however, any individual s_{rel} exceeds the precision limit or any individual $\bar{c}$ falls outside the range for accuracy, system performance is unacceptable for that compound. Correct the problem and repeat the test. Conditions in Table 2 shall be met for minimum sensitivity.

9.3 Spiking

9.3.1 Spike all samples with the diluted labelled-compound spiking solution (6.10) to assess method performance on the sample matrix.

9.3.2 Analyse each sample in accordance with the procedures in Clauses 11 to 18.

9.3.3 Determine the percent recovery of the labelled compounds using the internal standard method (10.7).

9.3.4 If the surrogate standard recovery exceeds 25 % to 150 %, method performance shall be considered to be unacceptable for that compound in that sample. To overcome such difficulties, samples shall be diluted and/or reanalysed.

9.4 Recovery of labelled compounds assessment

9.4.1 Assess recovery of labelled compounds from samples and maintain records.

9.4.2 After the analysis of five samples of a given matrix type for which the labelled compounds pass the tests in 9.3, compute the average percent recovery ($\bar{F}_{rec}$) and the standard deviation of the percent recovery (s_{rec}) for the labelled compounds only. Express the assessment as a percent recovery interval from [$\bar{F}_{rec} - 2(s_{rec})$] to [$\bar{F}_{rec} + 2(s_{rec})$] for each matrix.

EXAMPLE If $\bar{F}_{rec}$ = 90 % and s_{rec} = 10 % for five analyses, the recovery interval is expressed as 70 % to 110 %.

9.4.3 Update the accuracy assessment for each labelled compound in each matrix on a regular basis (e.g. after each 5 to 10 new measurements).

9.5 Method blanks

9.5.1 Analyse reference matrix method blanks to demonstrate freedom from contamination (5.3).

9.5.2 Prepare, extract, clean up and concentrate a method blank with each sample batch (samples of the same matrix started through the extraction process on the same 12 h shift, to a maximum of 20 samples). The matrix for the method blank shall be similar to the sample matrix for the batch, e.g. an aliquot of reagent water blank (6.7). Analyse the blank immediately prior to analysis of the samples to demonstrate freedom from contamination.

9.5.3 If any dioxin-like PCBs are found in the blank at greater than the minimum level (Table 2) or greater than one-third of any regulatory compliance level in force, whichever is greater, or if any potentially interfering compound is found in the blank at the minimum level for each level of chlorination given in Table 2, halt analysis of samples until the blank associated with the sample batch shows no evidence of contamination at this level. Associate all samples with a method blank that does not significantly impact results before the results for those samples can be reported for regulatory compliance purposes. PCB 118 and PCB 105 are typically detected in blank materials. If results are greater than five times the blank, they can be reported without qualification. Flag results if they are less than five times the blank.

9.6 QC check sample

Analyse the QC check sample (6.15) regularly to assure the accuracy of calibration standards and the overall reliability of the analytical process.

The QC check sample should be analysed at least quarterly. This is not required if a QC check sample (e.g. CRM or blank spiked with external standards) is used with each set. If this method is used less frequently than once every four months, the QC check sample shall be run in duplicate to confirm original IPR results. If IPR limits cannot be met, the complete IPR procedure shall be repeated.

9.7 Method precision

The specifications contained in this method shall be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for calibration (Clause 10), calibration verification (15.3), and for initial precision and recovery (9.2) should be identical, so that the most precise results will be obtained.

NOTE 1 This subclause (9.7) is entitled “Method precision”, and as such is only concerned with the random variation of results given by the instrument. The same can be said for the calibration check and recovery standards. If standards from other sources are included, this can give a measure of systematic error, in addition to random error, which can be better measured elsewhere, i.e. with quality control standards.

NOTE 2 A GC/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of dioxin-like PCBs by this method.

Depending on specific programme requirements, field replicates can be collected to determine the precision of the sampling technique, and spiked samples can be required to determine the accuracy of the analysis when the internal standard method is used.

10 Calibration

10.1 Operating conditions

Establish the operating conditions necessary to meet the relative retention times for the dioxin-like PCBs in Table 2.

GC operating conditions are dependent on GC column type. Examples of suggested conditions taken from a method such as EPA 1668 [12] are shown in Table 5.

Optimize GC conditions for compound separation and sensitivity. Once optimized, use the same GC conditions for the analysis of all standards, blanks, IPR aliquots and samples.

10.2 Mass spectrometer (MS) resolution

Obtain a selected ion monitoring (SIM) of each analyte in Table 3 at the two quantification ions chosen (examples in Table 7) and at > 10 000 resolving power by injecting an authentic standard of the dioxin-like PCBs either singly or as part of a mixture in which there is no interference between closely eluted components.

Using a PFK molecular leak (or any other reference material), tune the instrument to meet the minimum required resolving power of 10 000 (10 % valley) at mass 292,982 5 or 330,979 2 (PFK) or any other reference signal in the analytical mass range. For each descriptor (examples in Table 7), monitor and record the resolution and exact masses of three to five reference peaks covering the mass range of the descriptor. The resolution shall be greater than or equal to 10 000.

NOTE Excessive perfluorokerosene (PFK) (or any other reference substance) can cause noise problems and contamination of the ion source necessitating increased frequency of source cleaning. After tuning, reduce PFK levels into the ion source to levels as low as possible.

10.3 Ion abundance ratios, minimum levels, signal-to-noise ratios, and absolute retention times

Choose an injection volume consistent with the capability of the HRGC/HRMS instrument and dimension of the GC column used. An injection volume of 1 µl is typically used for 0,25 mm ID columns. Smaller injection volumes (0,5 µl) are used for narrow bore (0,18 mm and 0,10 mm) ID columns. Injection volumes should be identical for standards and sample extracts.

Large volume injection may be used.

Inject an aliquot of the CS1 [or CS2 if a 5 point calibration set (e.g. CS2 to CS6) is used] calibration solution (Table 3) using the GC conditions from 10.1.

Measure the SIM areas for each analyte, and compute the ion abundance ratios at the exact masses specified in Table 7. Compare the computed ratio to the theoretical ratio given in Table 7.

A signal to noise ratio (S/N) of at least 5 shall be obtained for all analytes in the calibration solution CS1 (S/N of 10 in other calibration solutions).

Make sure that all analytes are within the QC limits in Table 7 for their respective ion abundance ratios. Minimum levels in Table 2 should be obtained for all analytes.

Otherwise, adjust the mass spectrometer and repeat this test until the limits specified are obtained. If the adjustment alters the resolution of the mass spectrometer, verify the resolution (10.2) prior to repetition of the test.

Additional masses may be monitored in each descriptor, and the masses may be divided among more than the four descriptors listed in Table 7, provided that the laboratory is able to monitor the masses of all the dioxin-like PCBs that can elute from the GC in a given retention time window.

Operate the mass spectrometer in a mass-drift correction mode, using PFK or any other suitable reference compound to provide lockmasses. Monitor each lockmass and make sure that it does not vary by more than ± 20 % throughout its respective retention time window.

NOTE The lockmass for each group of masses is shown in Table 7. Variations of the intensity of the lockmass by more than 20 % indicate the presence of co-eluting interferences that can significantly reduce the sensitivity of the mass spectrometer. Re-injection of another aliquot of the sample extract will usually not resolve the problem. Additional clean-up or dilution of the extract can be required to remove the interferences.

10.4 Retention time

Analyse the window defining mixtures (6.14) using the optimized temperature programme in 10.1 (see Table 5).

10.5 Isomer specificity

Analyse the isomer specificity test standards (6.14) using the procedure in Clause 14 and the optimized conditions for sample analysis (10.1).

Compute the percent valley between the GC peaks of each critical pair on their respective columns.

Verify that the height of the valley between the most closely eluted isomers is less than the maximum level selected (e.g. 25 %). If the valley exceeds the maximum level; adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (10.2 to 10.8).

10.6 Calibration by isotope dilution

Isotope dilution calibration is used for the dioxin like PCBs for which labelled compounds are added to samples prior to extraction. The reference compound for each dioxin-like PCB compound is shown in Table 2.

Prepare a calibration curve encompassing the concentration range for each compound to be determined. Plot the relative response, R_{rel}, (of the labelled to native compound) versus concentration in standard solutions or compute using a linear regression. Determine the relative response factor for each dioxin-like PCB according to the procedures described below. Employ at least five calibration points.

Determine the response of each dioxin-like PCB relative to its labelled analogue using the area responses of both the primary and secondary exact masses specified in Table 7, for each calibration standard, as follows:

$$R_{rel} = \frac{\left(A_{1n} + A_{2n}\right)\rho_L}{\left(A_{1L} + A_{2L}\right)\rho_n} \quad (1)$$

where

A_{1n} is the area of the primary mass for PCB;

A_{2n} is the area of the secondary mass for PCB;

A_{1L} is the area of the primary mass for the labelled compound;

A_{2L} is the area of the secondary mass for the labelled compound;

ρ_L is the concentration of the labelled compound in the calibration standard (Table 3), in micrograms per litre, µg/l;

ρ_n is the concentration of the native compound in the calibration standard (Table 3), in micrograms per litre, µg/l.

To calibrate the analytical system by isotope dilution, inject a volume of calibration standards CS1 to CS7 (6.12 and Table 3) identical to the volume chosen in 10.3, using the procedure in Clause 14 and the conditions in 10.1 and Table 2. Compute the relative response at each concentration.

If the relative response for any compound is constant (less than 20 % coefficient of variation) over the five-point calibration range, an averaged relative response can be used for that compound; otherwise, use the complete calibration curve for that compound over the five-point calibration range.

10.7 Calibration by internal standard

The internal standard method is applied to determination of other PCBs for which no labelled standards have been added to the sample (9.4).

Calibration requires the determination of response factors, F_R, defined by Equation (2):

$$F_R = \frac{(A_{1s} + A_{2s})\rho_{is}}{(A_{1is} + A_{2is})\rho_s} \quad (2)$$

where

A_{1s} is the area of the primary mass for the PCB;

A_{2s} is the area of the secondary mass for the PCB;

A_{1is} is the area of the primary mass for the internal standard;

A_{2is} is the area of the secondary mass for the internal standard;

ρ_{is} is the concentration of the internal standard (Table 3) in micrograms per litre, µg/l;

ρ_s is the concentration of the compound in the calibration standard (Table 3) in micrograms per litre, µg/l.

To calibrate the analytical system by internal standard, inject a volume of calibration standards CS1 to CS7 (6.12 and Table 3) identical to the volume chosen in 10.3, using the procedure in Clause 14 and the conditions in 10.1 and Table 2. Compute the response factor (F_R) at each concentration.

If the response factor (F_R) for any compound is constant (less than 20 % coefficient of variation) over the five-point calibration range, an averaged response factor can be used for that compound; otherwise, use the complete calibration curve for that compound over the five-point range.

10.8 Combined calibration

10.8.1 General

By using calibration solutions (6.12 and Table 3) containing the dioxin-like PCBs and labelled compounds and the recovery standards, use a single set of analyses to produce calibration curves for the isotope dilution and internal standard methods. Verify these curves each shift or analytical run (15.3) by analysing the calibration verification standard (VER – CS4, Table 3). Recalibration is required if any of the calibration verification criteria (15.3) cannot be met.

10.8.2 Data storage

Collect, record and store MS data on media.

Media should be retrievable and in a common format so that data can be restored and transferred between instruments.

10.8.3 Data acquisition

Collect the signal at each mass repetitively throughout the monitoring period and store on a mass storage device.

10.8.4 Response factors and multipoint calibrations

Use the data system to record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibration curves. Use computations of relative standard deviation (coefficient of variation) to test calibration linearity. Statistics on initial performance (9.2) should be computed and maintained, either on the instrument data system, on a separate computer system or in the form of a hard copy that is available for review.

11 Sample preparation

11.1 General

Sample preparation involves modifying the physical form of the sample so that the dioxin-like PCBs can be extracted quantitatively. In general, the samples shall be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Samples should be as homogeneous as possible in order to more accurately reflect the results in the sample.

For samples known or expected to contain high levels of the dioxin-like PCBs, use an appropriate sample size representative of the entire sample (17.3). Sample size, internal standard concentrations and spikes should be adjusted to bring the levels into the linear range of the method.

For all samples, process the blank and IPR aliquots through the same steps as the sample to check for contamination and losses in the preparation processes.

For samples that contain particles, determine percent suspended solids using the procedure in 11.2.

Prepare aqueous samples visibly absent of particles in accordance with 11.3 and extract directly using the separatory funnel or SPE techniques in 12.1 or 12.2, respectively.

Prepare aqueous samples containing visible particles and containing 1 % of suspended solids or less using the procedure in 11.3. After preparation, extract the sample directly using the SPE technique in 12.2 or filter in accordance with 11.3.3. After filtration, extract the particles and filter using the procedures detailed in 12.3 and extract the filtrate using the procedure described in 12.1.

NOTE Most dioxin-like PCBs are strongly bound to suspended particles and therefore the preparation of aqueous samples is dependent on the suspended solids content of the sample.

11.2 Determination of percent suspended solids

11.2.1 Desiccate and weigh a glass-fibre filter paper (7.3.5.3) to three significant figures.

11.2.2 Filter (10,0 ± 0,02) ml of well-mixed sample through the filter media.

11.2.3 Dry the filter for a minimum of 12 h at (110 ± 5) °C and cool in a desiccator for a minimum of 1 h.

11.2.4 Calculate the percent suspended solids, w_{sol}, as follows:

$$w_{sol} = \frac{(m_{ds} - m_f) \times 100}{10} \quad (3)$$

where

10 is the mass, in grams (g), of the filtered sample;

m_{ds} is mass, in grams (g), of the dried sample;

m_f is mass, in grams (g), of the filter.

11.3 Preparation of aqueous samples containing 1 % of suspended solids or less

11.3.1 General

Prepare aqueous samples visibly absent of particles as follows and extract directly using the separatory funnel or SPE techniques in 12.1 or 12.2, respectively. Prepare aqueous samples containing visible particles and 1 % of suspended solids or less using the procedure below and extract using either the SPE technique in 12.2 or further prepare using the filtration procedure in 11.3.3. After the filtration procedure, continue with extraction of the filter and particles (12.3) and extraction of the filtrate (12.1). If a SPE procedure is used, proceed with extraction of the filter and disk after the sample has passed though the disk.

11.3.2 Preparation of sample and QC aliquots

11.3.2.1 Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ± 1 g.

11.3.2.2 Spike 1,0 ml of the diluted labelled-compound spiking solution (6.10) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for at least 30 min, with occasional shaking.

11.3.2.3 For each sample or sample batch (to a maximum of 20 samples) to be extracted during the same 12-h shift, place one 1,0 l aliquot of reagent water in a clean sample bottle or flask.

11.3.2.4 Spike 1,0 ml of the diluted labelled-compound spiking solution (6.10) into the reagent water aliquot. Use this as the method blank.

11.3.2.5 If SPE is to be used, add 5 ml of methanol [6.4 e)] to the sample, cap and shake the sample to mix thoroughly, and proceed to 12.2 for extraction. If SPE is not to be used, and the sample is visibly absent of particles, proceed to 12.1 for extraction. If SPE is not to be used and the sample contains visible particles, proceed to 11.3.3 for filtration of particles.

11.3.3 Filtration of particles

11.3.3.1 Assemble a Buchner funnel (7.4.5) on top of a clean filtration flask. Apply vacuum to the flask, and pour the entire contents of the sample bottle through a glass-fibre filter (7.4.6) in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particles.

11.3.3.2 Rinse the sample bottle twice with approximately 5 ml portions of reagent water to transfer any remaining particles onto the filter.

11.3.3.3 Rinse any particles off the sides of the Buchner funnel with two 5 ml portions of reagent water.

11.3.3.4 Weigh the empty sample bottle to ± 1 g. Determine the mass of the sample by difference. Save the bottle for further use.

11.3.3.5 Extract the filtrate using the separatory funnel procedure in 12.1.

11.3.3.6 Extract the filter containing the particles using the Soxhlet procedure or ASE in 12.3.

12 Extraction and concentration

12.1 Separatory funnel extraction of filtrates and of aqueous samples that are visibly absent of particles

12.1.1 Pour the spiked sample (11.3.2.2) or filtrate (11.3.3.5) into a 2 l separatory funnel. Rinse the bottle or flask twice with 5 ml of reagent water and add these rinses to the separatory funnel.

12.1.2 Add 60 ml of dichloromethane [6.4 f)] to the empty sample bottle, seal, and shake for 60 s to rinse the inner surface.

NOTE 1 Other solvents can be used provided that all of the performance criteria of the method can be met.

Transfer the solvent to the separatory funnel, and extract the sample by shaking the funnel for 2 min with periodic venting. Allow the organic layer to separate from the aqueous phase for a minimum of 10 min. If an emulsion forms and is more than one third the volume of the solvent layer, employ mechanical techniques to complete the phase separation (see Note 2 below). Drain the dichloromethane extract through a solvent-rinsed glass funnel approximately one-half full of granular anhydrous sodium sulfate (6.3.1) supported on clean glass-fibre paper into a solvent-rinsed concentration device (12.5).

NOTE 2 The optimum technique depends upon the sample, but can include stirring, filtration through glass wool, use of phase separation paper, centrifugation, use of an ultrasonic bath with ice, addition of NaCl, or other physical methods. Alternatively, solid-phase or other extraction techniques can be used to prevent emulsion formation.

Any alternative technique is acceptable so long as the requirements in Clause 9 are met.

12.1.3 Extract the water sample two more times with 60 ml portions of dichloromethane [6.4 f)]. Drain each portion through the sodium sulfate into the concentrator. After the third extraction, rinse the separatory funnel with at least 20 ml of dichloromethane [6.4 f)], and drain this rinse through the sodium sulfate into the concentrator. Repeat this rinse at least twice. Set aside the funnel with sodium sulfate if the extract is to be combined with the extract from the particles.

Concentrate the extract using one of the macro-concentration procedures in 12.5.

If the extract is from a sample visibly free of particles (11.1), adjust the final volume of the concentrated extract to approximately 10 ml with hexane [6.4 d)], transfer to a 250 ml separatory funnel, and back-extract using the procedure in 12.4. If the extract is from the aqueous filtrate (11.3.3.5), set aside the concentration apparatus for addition of the Soxhlet extract from the particles [12.3.9 b)].

12.2 Solid-phase extraction (SPE) of samples containing less than 1 % suspended solids

12.2.1 Disk preparation

12.2.1.1 Place an SPE disk on the base of the filter holder and wet with toluene. While holding a glass-fibre filter above the SPE disk with tweezers, wet the filter with toluene and lay the filter on the SPE disk, making sure that air is not trapped between the filter and disk. Clamp the filter and SPE disk between the 1 l glass reservoir and the vacuum filtration flask.

12.2.1.2 Rinse the sides of the filtration flask with approximately 15 ml of toluene using a squeeze bottle or syringe. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approximately 1 min. Apply vacuum and draw all of the toluene through the filter/disk. Repeat the wash step with approximately 15 ml of acetone and allow the filter/disk to air dry.

12.2.1.3 Re-wet the filter/disk with approximately 15 ml of methanol, allowing the filter/disk to soak for approximately 1 min. Pull the methanol through the filter/disk using the vacuum, but retain a layer of methanol

approximately 1 mm thick on the filter. Do not allow the disk to get dry from this point until the end of the extraction.

12.2.1.4 Rinse the filter/disk with two 50 ml portions of reagent water by adding the water to the reservoir and pulling most through, leaving a layer of water on the surface of the filter.

12.2.2 Extraction

12.2.2.1 Pour the spiked sample (11.3.2.2), blank (11.3.2.4), or IPR aliquot into the reservoir and turn on the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in no less than 10 min.

NOTE For samples containing a high concentration of particles (suspended solids), such as pulp mill effluents, filtration times can be 8 h or longer.

12.2.2.2 Before the entire sample has been pulled through the filter/disk, rinse the sample bottle with approximately 50 ml of reagent water to remove any solids, and pour into the reservoir. Pull through the filter/disk. Use additional reagent water rinses until all visible solids are removed.

12.2.2.3 Before all of the sample and rinses have been pulled through the filter/disk, rinse the sides of the reservoir with two 5 ml portions of reagent water.

12.2.2.4 Allow the filter/disk to dry, then remove the filter and disk and place in a glass Petri dish (7.3.5.5). Extract the filter and disk in accordance with 12.3. Filters can also be extracted directly *in situ* using ethanol/toluene. A 30 %/70 % ethanol/toluene mixture is allowed to soak the particles and SPE disk for 30 min after which the disk is eluted with the solvent in the reservoir. This step is repeated two more times.

12.3 Soxhlet extraction of filters and/or disks

12.3.1 Charge a clean extraction thimble (7.3.6.2) with 5,0 g of activated silica (6.6.1.1).

Do not disturb the silica layer throughout the extraction process.

12.3.2 Place the thimble in a clean extractor. Place 30 ml to 40 ml of toluene in the receiver and 200 ml to 250 ml of toluene in the flask.

12.3.3 Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 drop to 2 drops of toluene will fall per second from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 h.

12.3.4 After pre-extraction, cool and disassemble the apparatus and allow the thimble/silica to dry, taking precautions to avoid contamination.

12.3.5 Load the filter and/or disk into the thimble.

12.3.6 Reassemble the pre-extracted Soxhlet apparatus, and add a fresh charge of toluene to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the silica bed until water removal lessens the restriction to toluene flow. Frequently check the apparatus for foaming during the first 2 h of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

12.3.7 Reflux the sample for at least 16 h or 100 cycles. Cool and disassemble the apparatus.

12.3.8 Remove the distilling flask and add any toluene in the receiver to the extract in the flask.

12.3.9 Concentrate the extract using one of the macro-concentration procedures in 12.5.

For extracts from the particles in an aqueous sample containing less than 1 % suspended solids (11.3.3.6):

a) concentrate the extract to approximately 5 ml using the rotary evaporator or heating mantle procedures in 12.5;

b) quantitatively transfer the extract through the sodium sulfate (6.3.1) into the apparatus that was set aside (12.1.3) and reconcentrate to the level of the toluene;

c) adjust to approximately 10 ml with hexane [6.4 d)], transfer to a 250 ml separatory funnel, and proceed with back-extraction (12.4).

For extracts from the SPE filter and disk (12.2.2.4), concentrate to approximately 10 ml using a rotary evaporator or heating mantle (12.5), transfer to a 250 ml separatory funnel, and proceed with back-extraction (12.4) if required.

12.4 Back-extraction with acid and base

12.4.1 Partition the extract against 50 ml of sulfuric acid (6.2.2). Shake for 2 min with periodic venting into a hood. Remove and discard the aqueous layer. Repeat the acid washing until no colour is visible in the aqueous layer, to a maximum of four washings.

12.4.2 Partition the extract against 50 ml of sodium chloride solution (6.2.4) in the same way as with acid. Discard the aqueous layer. Proceed with 12.4.5 if only acid washing is required or 12.4.3 if additional base washing is required.

12.4.3 Partition the extract against 50 ml of potassium hydroxide solution (6.2.1) in the same way as with acid. Repeat the base washing until no colour is visible in the aqueous layer, to a maximum of four washings.

NOTE Stronger potassium hydroxide solutions can be employed for back-extraction, provided that the laboratory meets the specifications for labelled-compound recovery and demonstrates acceptable performance using the procedure in 9.2.

12.4.4 Repeat the partitioning against sodium chloride solution and discard the aqueous layer.

12.4.5 Pour each extract through a drying column containing 7 cm to 10 cm of granular anhydrous sodium sulfate (6.3.1). Rinse the separatory funnel with 30 ml to 50 ml of solvent, and pour through the drying column. Collect each extract in a round-bottom flask. Re-concentrate the sample and QC aliquots in accordance with 12.5 and 12.6, and clean up the samples and QC aliquots in accordance with Clause 13.

12.5 Macro-concentration

12.5.1 General

Concentrate extracts in toluene using a rotary evaporator or a heating mantle. Concentrate extracts in dichloromethane [6.4 f)] or hexane [6.4 d)] using a rotary evaporator, heating mantle, or Kuderna-Danish apparatus.

12.5.2 Rotary evaporation

12.5.2.1 Assemble the rotary evaporator in accordance with the manufacturer's instructions, and warm the water bath to 45 °C. Between samples, rinse three 2 ml to 3 ml aliquots of solvent down the feed tube into a waste beaker.

12.5.2.2 Attach a round-bottom flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

12.5.2.3 Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete concentration in 15 min to 20 min. At the proper rate of concentration, the flow of solvent into the receiving flask will be steady, but no bumping or visible boiling of the extract will occur.

NOTE If the rate of concentration is too fast, analyte loss can occur.

12.5.2.4 When the liquid in the concentration flask has reached an apparent volume of approximately 2 ml, remove the flask from the water bath and stop the rotation. Slowly and carefully admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 ml of solvent.

12.5.2.5 Proceed to 12.5.5 for preparation for back-extraction or micro-concentration and solvent exchange.

12.5.3 Heating mantle

12.5.3.1 Add one or two clean boiling chips to a round-bottom flask, and attach a three-ball macro Snyder column. Pre-wet the column by adding approximately 1 ml of solvent through the top. Place the round-bottom flask in a heating mantle, and apply heat as required to complete the concentration in 15 min to 20 min.

NOTE At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.5.3.2 When the liquid has reached an apparent volume of approximately 10 ml, remove the round-bottom flask from the heating mantle and allow the solvent to drain and cool for at least 10 min. Remove the Snyder column and rinse the glass joint into the receiver with two 5 ml portions of solvent.

12.5.3.3 Proceed to 12.5.5 for preparation for back-extraction or micro-concentration and solvent exchange.

12.5.4 Kuderna-Danish (K-D)

Concentrate the extracts in separate 500 ml K-D flasks equipped with 10 ml concentrator tubes. The K-D technique is used for solvents such as dichloromethane [6.4 f)] and hexane [6.4 d)]. Toluene is difficult to concentrate using the K-D technique unless a water bath fed by a steam generator is used.

12.5.4.1 Add one to two clean boiling chips to the receiver. Attach a three-ball macro Snyder column. Pre-wet the column by adding approximately 1 ml of solvent through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

12.5.4.2 Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 min to 20 min.

NOTE At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.5.4.3 When the liquid has reached an apparent volume of 1 ml, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 min. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 ml to 2 ml of solvent. A 5 ml syringe is recommended for this operation.

12.5.4.4 Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two-ball micro Snyder column to the concentrator tube. Pre-wet the column by adding approximately 0,5 ml of solvent through the top. Place the apparatus in the hot water bath.

12.5.4.5 Adjust the vertical position and the water temperature as required to complete the concentration in 5 min to 10 min. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood.

12.5.4.6 When the liquid reaches an apparent volume of 0,5 ml, remove the apparatus from the water bath and allow to drain and cool for at least 10 min.

12.5.4.7 Proceed to 12.5.5 for preparation for back-extraction or micro-concentration and solvent exchange.

12.5.5 Preparation for back-extraction or micro-concentration and solvent exchange

12.5.5.1 For back-extraction (12.4), transfer the extract to a 250 ml separatory funnel. Rinse the concentration vessel with small portions of hexane [6.4 d)], adjust the hexane [6.4 d)] volume in the separatory funnel to 10 ml to 20 ml, and proceed to back-extraction (12.4).

12.5.5.2 For clean-up procedures other than back-extraction, transfer the extract to a blowdown vial using two to three rinses of solvent. Proceed with micro-concentration and solvent exchange (12.6).

12.6 Micro-concentration and solvent exchange

12.6.1 Extracts to be subjected to GPC or HPLC clean-up are exchanged into dichloromethane [6.4 f)]. Extracts to be cleaned up using silica, alumina, carbon, and/or Florisil are exchanged into hexane [6.4 d)].

12.6.2 Transfer the vial containing the sample extract to a nitrogen blowdown device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

NOTE A large vortex in the solvent can cause analyte loss.

12.6.3 Lower the vial into a 45 °C water bath and continue concentrating.

12.6.4 If the extract is to be concentrated for injection into the GC/MS or the solvent is to be exchanged for extract clean-up, follow the directions given in 12.6.5 to 12.6.8.

12.6.5 When the volume of the liquid is approximately 100 µl, add 2 ml to 3 ml of the desired solvent (dichloromethane [6.4 f)] for GPC and HPLC, or hexane [6.4 d)] for the other clean-ups) and continue concentration to approximately 100 µl. Repeat the addition of solvent and concentrate once more.

12.6.6 If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5,0 ml with dichloromethane [6.4 f)]. If the extract is to be cleaned up by HPLC, further concentrate the extract to 30 µl. Proceed with GPC or HPLC clean-up (13.2 or 13.6, respectively).

12.6.7 If the extract is to be cleaned up by column chromatography (alumina, silica, carbon, or Florisil), bring the final volume to 1,0 ml with hexane [6.4 d)]. Proceed with column clean-ups (13.3 to 13.5 and 13.7).

12.6.8 If the extract is to be concentrated for injection into the GC/MS (Clause 14), quantitatively transfer the extract to a conical vial of suitable volume for final concentration, rinsing the larger vial with hexane [6.4 d)] and adding the rinse to the conical vial. Reduce the volume to approximately 100 µl. Add 10 µl of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/MS analysis. If GC/MS analysis will not be performed on the same day, store the vial in the dark at a suitable temperature to avoid losses by evaporation.

13 Extract clean-up

Exact masses of packing materials and volumes or mixtures of elution solvents for each clean-up stage shall be determined and confirmed (see 9.2) before routine sample analysis can commence.

13.1 General

13.1.1 The analyst may use any of the example procedures below or any suitable alternative procedures. Before using a clean-up procedure, the analyst shall demonstrate that the requirements of 9.2 can be met using the clean-up procedure. In addition, the isomer pattern and congener profile of a well-characterized sample extract containing all PCBs (e.g. Aroclor 1248) shall not be altered by the applied clean-up procedure. Area response changes relative to PCB 118 shall not exceed 20 %.

13.1.2 Gel permeation chromatography (13.2) removes high molecular mass interferences that cause GC column performance to degrade; for this reason, it can be used for water extracts that are expected to contain high molecular mass organic compounds (e.g. polymeric materials, humic acids).

13.1.3 Acid, neutral, and basic silica (13.3), alumina (13.4), and Florisil (13.7) can be used to remove non-polar and polar interferences. Alumina and Florisil only shall be used to remove polychlorinated dibenzo-*p*-dioxins, dibenzofurans, *ortho*-substituted PCBs and diphenyl ethers.

13.1.4 Carbon (13.5) can be used to remove non-polar interferences and interfering *ortho*-substituted PCBs (e.g. PCB 110) from non-*ortho* PCBs (e.g. PCB 77 on 5 % phenyl columns).

13.1.5 HPLC (13.6) can be used to provide specificity for specific PCBs if required.

13.1.6 Silver nitrate/silica can be used to remove sulfur compounds and some organohalogen compounds.

13.2 Gel permeation chromatography (GPC)

13.2.1 Column packing

13.2.1.1 Place 70 g to 75 g of SX-3 Bio-beads in a 400 ml to 500 ml beaker.

13.2.1.2 Cover the beads with dichloromethane [6.4 f)] and allow to swell overnight (a minimum of 12 h).

13.2.1.3 Transfer the swelled beads to the column and pump dichloromethane [6.4 f)] through the column, from bottom to top, at 4,5 ml/min to 5,5 ml/min prior to connecting the column to the detector.

13.2.1.4 After purging the column with dichloromethane [6.4 f)] for 1 h to 2 h, adjust the column head pressure to 7 Pa to 10 Pa and purge for 4 h to 5 h to remove air. Maintain a head pressure of 7 Pa to 10 Pa. Connect the column to the detector.

13.2.2 Column calibration

13.2.2.1 Load 5 ml of the calibration solution (6.5) into the sample loop.

13.2.2.2 Inject the calibration solution and record the signal from the detector. The elution pattern will be corn oil, bis(2-ethyl hexyl) phthalate, pentachlorophenol, perylene, and sulfur (6.5).

13.2.2.3 Set the “dump time” to allow > 85 % removal of the corn oil and > 85 % collection of the phthalate.

13.2.2.4 Set the “collect time” to the peak minimum between perylene and sulfur.

13.2.2.5 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85 %. If calibration is not verified, the system shall be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

13.2.3 Extract clean-up

13.2.3.1 General

It is a requirement of GPC that the column shall not be overloaded. If the extract is known or expected to contain more than 0,5 g of high molecular mass material, split the extract into aliquots for GPC, and combine the aliquots after elution from the column.

NOTE The column specified in this method is designed to handle a maximum of 0,5 g of high molecular mass material in a 5 ml extract. The residue content of the extract can be obtained gravimetrically by evaporating the solvent from a 50 µl aliquot.

13.2.3.2 Filter the extract or load through the filter holder to remove the particles. Load the 5,0 ml extract into the column.

13.2.3.3 Elute the extract using the calibration data determined in 13.2.2. Collect the eluate in a clean 400 ml to 500 ml beaker.

13.2.3.4 Rinse the sample loading tube thoroughly with dichloromethane [6.4 f)] between extracts to prepare for the next sample.

13.2.3.5 If a particularly dirty extract is encountered, run a 5,0 ml dichloromethane [6.4 f)] blank through the system to check for carry-over.

13.2.3.6 Concentrate the eluate in accordance with 12.5 and 12.6 for further clean-up or injection into the GC/MS.

13.3 Silica clean-up

13.3.1 Place a glass-wool plug in a 15 mm ID chromatography column [7.5.4 d)]. Pack the column bottom to top with: 1 g of activated silica (6.6.1.1), 4 g of basic silica (6.6.1.3), 1 g of silica (6.6.1), 8 g of acid silica (6.6.1.2), 2 g of activated silica, and 4 g of granular anhydrous sodium sulfate (6.3.1). Tap the column to settle the adsorbents.

13.3.2 Pre-elute the column with 50 ml to 100 ml of hexane [6.4 d)]. Close the stopcock when the hexane is within 1 mm of the sodium sulfate. Discard the eluate. Check the column for channelling. If channelling is present, discard the column and prepare another.

13.3.3 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

13.3.4 Rinse the receiver twice with 1 ml portions of hexane [6.4 d)], and apply separately to the column. Elute the PCBs with 100 ml hexane [6.4 d)], and collect the eluate.

13.3.5 Concentrate the eluate in accordance with 12.5 and 12.6 for further clean-up or injection into the HPLC or GC/MS.

NOTE 1 For extracts of samples known to contain large quantities of other organic compounds, it can be advisable to increase the capacity of the silica column. This can be accomplished by increasing the strengths of the acid and basic silicas. The acid silica (6.6.1.2) can be increased in strength to as much as 44 % mass fraction (7,9 g of sulfuric acid added to 10 g of silica). The basic silica (6.6.1.3) can be increased in strength to as much as 33 % mass fraction (50 ml in NaOH added to 100 g silica), or the potassium silicate (6.6.1.4) can be used.

NOTE 2 The use of stronger acid silica (44 % mass fraction) can lead to charring of organic compounds in some extracts. The charred material can retain some of the analytes and lead to lower recoveries of PCBs. Increasing the strengths of the acid and basic silica can also require different volumes of hexane [6.4 d)] than those specified above to elute the analytes off the column.

After modification, verify the performance of the method by the procedure in 9.2.

13.4 Alumina clean-up

13.4.1 Place a glass-wool plug in a 15 mm ID chromatography column [7.5.4 d)].

13.4.2 If using acid alumina, pack the column by adding 6 g of acid alumina [6.6.2.1 a)]. With basic alumina, also use 6 g of basic alumina [6.6.2.1 b)]. Tap the column to settle the adsorbents.

13.4.3 Pre-elute the column with 50 ml to 100 ml of hexane [6.4 d)]. Close the stopcock when the hexane is within 1 mm of the alumina.

13.4.4 Discard the eluate. Check the column for channelling. If channelling is present, discard the column and prepare another.

13.4.5 Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the alumina.

13.4.6 Rinse the receiver twice with 1 ml portions of hexane [6.4 d)] and apply separately to the column. Elute the interfering compounds with 100 ml of hexane [6.4 d)] and discard the eluate.

13.4.7 The choice of eluting solvents will depend on the choice of alumina (acid or basic) made in 13.4.2; follow the instructions given below:

a) if using acid alumina, elute the dioxin-like PCBs from the column with 20 ml of dichloromethane [6.4 f)]: hexane [6.4 d)] (volume fraction 20:80) and collect the eluate;

b) if using basic alumina, elute the dioxin-like PCBs from the column with 20 ml of dichloromethane [6.4 f)]: hexane [6.4 d)] (volume fraction 50:50) and collect the eluate.

13.4.8 Concentrate the eluate in accordance with 12.5 and 12.6 for further clean-up or injection into the HPLC or GC/MS.

13.5 Carbon column

13.5.1 Cut both ends from a 10 ml disposable serological pipette to produce a 10 cm column. Fire-polish both ends and flare both ends if desired. Insert a glass-wool plug at one end, and pack the column with 0,55 g of carbon [Carbopak/Celite (6.6.3.1)] to form an adsorbent bed approximately 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

13.5.2 Pre-elute the column with 5 ml of toluene followed by 2 ml of dichloromethane [6.4 f)]: methanol: toluene (volume fraction 15:4:1), 1 ml of dichloromethane [6.4 f)]: cyclohexane [6.4 c)] (volume fraction 1:1), and 5 ml of hexane [6.4 d)]. If the flow rate of eluate exceeds 0,5 ml/min, discard the column.

13.5.3 When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1 ml portions of hexane [6.4 d)] and apply separately to the column. Apply 2 ml of hexane [6.4 d)] to complete the transfer.

13.5.4 Elute the interfering compounds with two 3 ml portions of hexane [6.4 d)], 2 ml of dichloromethane [6.4 f)]: cyclohexane [6.4 c)] (volume fraction 1:1), and 2 ml of dichloromethane [6.4 f)]: methanol: toluene (volume fraction 15:4:1). Discard the eluate.

13.5.5 Invert the column, and elute the desired dioxin-like PCBs with 20 ml of toluene. If carbon particles are present in the eluate, filter through glass-fibre filter paper.

13.5.6 Concentrate the eluate in accordance with 12.5 and 12.6 for further clean-up or injection into the HPLC or GC/MS.

NOTE Carbon chromatography can be optimized to separate the non-*ortho* from mono-*ortho* PCBs if desired.

13.6 High-performance liquid chromatography (HPLC)

13.6.1 Column calibration

13.6.1.1 Prepare a calibration standard containing the dioxin-like PCBs and/or other congeners of interest at a concentration of approximately 500 pg/µl in dichloromethane [6.4 f)].

13.6.1.2 Inject 30 µl of the calibration solution into the HPLC equipment (7.5.2) and record the signal from the detector. Collect the eluant for reuse. The elution order will be the tetra- through hepta-isomers.

13.6.1.3 Establish the collection time for the desired dioxin-like PCBs and for the other isomers of interest. Following calibration, flush the injection system with copious quantities of dichloromethane [6.4 f)], to ensure that residual PCBs are removed from the system.

13.6.1.4 Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the PCBs from the calibration standard (13.6.1.1) is 75 % to 125 % compared to the calibration (13.6.1.2). If calibration is not verified, recalibrate the system using the calibration solution, and re-extract and clean up the previous 20 samples using the calibrated system.

13.6.2 Extract clean-up

13.6.2.1 General

It is a requirement of HPLC that the column shall not be overloaded. If the extract cannot be concentrated to less than 30 µl, split it into fractions and combine the fractions after elution from the column.

NOTE The column specified in this method is designed to handle a maximum of 30 µl of extract.

13.6.2.2 Rinse the sides of the vial twice with 30 µl of dichloromethane [6.4 f)] and reduce to 30 µl with the evaporation apparatus (7.6.3), following the procedure in 12.6.

13.6.2.3 Inject the 30 µl extract into the HPLC. Elute the extract using the calibration data determined in 13.6.1. Collect the fraction(s) in a clean 20 ml concentrator tube containing 5 ml of hexane [6.4 d)]: acetone (volume fraction 1:1).

13.6.2.4 If an extract containing more than 500 ng/ml of total PCB is encountered, run a 30 µl dichloromethane [6.4 f)] blank through the system to check for carry-over.

13.6.2.5 Concentrate the eluate in accordance with 12.6 for injection into the GC/MS.

Alternatively 100 µl extracts can be cleaned using e.g. a COSMOSIL PYE[9)] column (5 µm particle size, 4,6 mm × 150 mm). Use a mobile phase of 100 % pentane at 0,7 ml/min. *Ortho*-substituted PCBs elute in Fraction 1 (1 min to 5,5 min) and non- and mono-*ortho* PCs elute in Fraction 2 (5,5 min to 16 min). Fraction 2 is taken to dryness under a gentle stream of nitrogen and redissolved with 20 µl of recovery standard.

13.7 Florisil clean-up

13.7.1 Place a glass-wool plug into a column (7.5.4). Pack with 1,5 g (approximately 2 ml) of Florisil (6.6.4) and top with approximately 1 ml of sodium sulfate (6.3.1) and a glass-wool plug.

13.7.2 Pre-elute the activated Florisil column (6.6.4) with 100 ml of hexane [6.4 d)] and discard the solvents.

13.7.3 When the solvent is within 1 mm of the packing, apply the sample extract {in hexane [6.4 d)]} to the column. Rinse the sample container twice with 1 ml portions of hexane [6.4 d)] and apply to the column.

13.7.4 Elute and collect the mono and di-*ortho* PCBs with approximately 165 ml of hexane [6.4 d)].

13.7.5 Elute and collect the non-*ortho* and mono-*ortho* PCBs with 100 ml of 6 % diethyl ether: hexane by volume [6.4 d)].

13.7.6 Concentrate the eluate in accordance with 12.5 to 12.6 for further clean-up or injection into HPLC or GC/MS.

9) COSMOSIL PYE is an example of a suitable product available commercially from e.g. Phenomenex USA. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the products.

13.8 Silver nitrate/silica column

13.8.1 Fill a chromatography column to about 5 mm with sodium sulfate and add 2 g of silver nitrate/silica (6.6.5). Top with a 5 mm layer of sodium sulfate.

13.8.2 Rinse the column with 50 ml of hexane [6.4 d)] and add the extract {approximately 3 ml in hexane [6.4 d)]} to the column. Rinse the extract container three times with hexane [6.4 d)] and add to the column ensuring that the column does not run dry.

13.8.3 Elute and collect the PCBs with 35 ml of hexane [6.4 d)].

13.8.4 Concentrate the eluate in accordance with 12.5 to 12.6 for further clean-up or for injection into the GC/MS.

NOTE The silver nitrate/silica packing can be placed at the bottom of the silica column (13.3) and run in tandem.

14 HRGC/HRMS analysis

14.1 Establish the operating conditions given in 10.1.

14.2 Add 10 µl of the appropriate recovery standard solution (6.11) to the sample. If an extract is to be reanalyzed and evaporation has occurred, do not add more recovery standard solution. Rather, bring the extract back to its previous volume (e.g. 19 µl) with solvent.

14.3 Inject a volume of the concentrated extract containing the recovery standard using on-column or splitless injection. The volume injected shall be identical to the volume used for calibration (see Clause 10). Start the GC column initial isothermal hold upon injection. Start MS data collection after the solvent peak elutes. Stop data collection not less than 30 s after the last target PCB has eluted. Return the column to the initial temperature for analysis of the next extract or standard.

NOTE Annex B provides a procedure for the use of high-resolution gas chromatography/low-resolution mass spectrometry (HRGC/LRMS) as a screening method.

15 System and laboratory performance

15.1 General

At the beginning of a batch of samples (up to 20), verify GC/MS system performance and calibration for all PCBs and labelled compounds. For these tests, use analysis of the CS3 or CS4 calibration verification (VER) standard (6.12 and Table 3) and the isomer specificity test standards (6.14) to verify all performance criteria. Perform adjustment and/or recalibration (see Clause 10) until all performance criteria are met. Only after all performance criteria are met can samples, blanks and IPRs be analysed.

15.2 MS resolution

A static resolving power of at least 10 000 (10 % valley definition) shall be demonstrated at the appropriate mass before any analysis is performed. Implement corrective actions whenever the resolving power does not meet the requirement.

15.3 Calibration verification

15.3.1 Inject the VER standard using the procedure in Clause 14.

15.3.2 The mass abundance ratios for all PCBs shall be within 20 % of the theoretical ratios shown in Table 7; otherwise, adjust the mass spectrometer until the mass abundance ratios fall within the limits specified, and repeat the verification test. If the adjustment alters the resolution of the mass spectrometer, verify the resolution (10.2) prior to repetition of the verification test.

15.3.3 The peaks representing each PCB and labelled compound in the low level standard (CS1 or CS2 – whichever is used) shall be present with S/N of at least 5 (10.3); otherwise, adjust the mass spectrometer and repeat the verification test.

15.3.4 Compute the concentration of each PCB compound by isotope dilution (17.1) for those compounds that have labelled analogues (Table 2). Compute the concentration of the labelled compounds by the internal standard method (17.2). These concentrations are computed based on the calibration data in Clause 10.

15.3.5 For each compound, confirm that the result of the VER analysis is within 20 % of the nominal concentration shown in Table 3. If all compounds are within this limit, calibration has been verified and analysis of standards and sample extracts shall proceed. If, however, any compound fails its respective limit, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (15.2) and verification (15.3) tests, or recalibrate (see Clause 10).

15.4 GC resolution

Inject the isomer specificity standards (6.14) on their respective columns.

15.5 Blank

Make sure that the results of the analysis of the blank meet the specifications in 9.5.3 before sample analyses proceed.

16 Qualitative determination

16.1 A dioxin-like PCB or labelled compound is identified as being present in a standard, blank or sample when all of the criteria in 16.2 to 16.5 are met. If the criteria are not met, the PCB has not been identified and the results shall not be reported for regulatory compliance purposes. If interferences preclude identification, extract a new aliquot of sample, further cleaned up, and analyse. If the interference cannot be removed, flag the data to indicate results are maximum concentrations.

16.2 The signals for the two exact masses in Table 7 shall be present and shall maximize within ± 2 s.

16.3 The signal to noise ratio (S/N) for the GC peak at each exact mass shall be greater than or equal to 3 for each PCB detected in a sample extract, and greater than or equal to 10 for all PCBs in the calibration standard (10.3 and 15.3.3).

16.4 The ratio of the integrated areas of the two exact masses specified in Table 7 shall be within 20 % of the theoretical ratio shown in Table 7, or within ± 10 % of the ratio in the midpoint (CS3 or CS4) calibration or calibration verification (VER), whichever is most recent.

16.5 The retention time of a native dioxin-like PCB shall be within a time window of ± 3 s based on the retention time of the corresponding $^{13}C_{12}$–labelled congener in the sample.

NOTE At present, there is no chromatographic column available that is able to separate all PCB congeners. Complete separation can be achieved by multi-analysis of the sample extract on different columns of different nature (polarity) and/or separation of PCBs by degree of *ortho*-substitution on carbon columns. However, in practice, the contribution of non-toxic congeners to the total TEQ amount from a single column analysis can be of the same order as the precision of the test (10 % to 20 %) if carbon column chromatography is used. Single column data can therefore be reported by this method if it can be documented that results are not significantly biased (> 20 %); however, in cases where a regulatory limit is exceeded by 20 % or less, perform a confirmatory analysis on a second column.

17 Quantitative determination

17.1 Isotope dilution quantification

17.1.1 By adding a known amount of a labelled compound to every sample prior to extraction, correction for recovery of the dioxin-like PCBs can be made because the dioxin-like PCBs and their labelled analogues

exhibit similar effects upon extraction, concentration, and gas chromatography. Use relative response (R_{rel}) values in conjunction with the initial calibration data described in 10.6 to determine concentrations directly, so long as labelled-compound spiking levels are constant, using Equation (4):

$$m_{ex} = \frac{(A_{1_n} + A_{2_n})m_l}{(A_{1_l} + A_{2_l})R_{rel}} \quad (4)$$

where

m_{ex} is the amount of the dioxin-like PCBs in the extract, in picograms (pg);

A_{1_n} is the area of the primary mass for the native compound;

A_{2_n} is the area of the secondary mass for the native compound;

A_{1_l} is the area of the primary mass for the labelled compound;

A_{2_l} is the area of the secondary mass for the labelled compound;

m_l is the amount of the labelled compound in the calibration standard (see Table 3), in picograms (pg);

R_{rel} is the relative response as defined in 10.6.

17.1.2 Quantify PCB 170 against labelled PCB 189. As a result, correct the concentration of PCB 170 for the recovery of the labelled PCB 189.

NOTE In instances where PCB 170 and PCB 189 behave differently during sample extraction, concentration and clean-up procedures, the accuracy of the PCB 170 results may be decreased. However, given the low toxicity of this compound relative to the other dioxin-like PCBs, the potential decrease in accuracy is not considered significant.

17.1.3 Because some $^{13}C_{12}$-labelled standards are used as recovery standards (i.e. not added before extraction of the sample), do not use them to quantify the corresponding native congener by strict isotope dilution procedures. Therefore, quantify those native congeners using the response of the labelled analogue of the same homologue series.

17.1.4 Quantify any peaks representing PCBs without corresponding labelled compounds using an average of the response factors from all of the labelled dioxin-like PCB-isomers at the same level of chlorination.

17.2 Internal standard quantification and labelled-compound recovery

17.2.1 Compute the concentrations of those native congeners referred to in 17.1.2 and 17.1.3 and the ^{13}C-labelled analogues in the extract using the response factors determined from the initial calibration data (10.6) and Equation (5):

$$m_{ex} = \frac{(A_{1_s} + A_{2_s})m_{is}}{(A_{1_{is}} + A_{2_{is}})F_R} \quad (5)$$

where

m_{ex} is the amount of the dioxin-like PCBs in the extract, in picograms (pg);

A_{1_s} is the area of the primary mass for the dioxin-like PCBs;

A_{2_s} is the areas of the secondary mass for the dioxin-like PCBs;

$A_{1_{is}}$ is the area of the primary mass for the internal standard;

$A_{2_{is}}$ is the area of the secondary mass for the internal standard;

m_{is} is the amount of the internal standard (Table 3) in picograms (pg);

F_R is the response factor as defined in 10.7.

17.2.2 Using the concentration in the extract determined above, compute the percent recovery, w, of the $^{13}C_{12}$-labelled compounds using Equation (6):

$$w = \frac{m_{ex}}{m_{spk}} \times 100 \quad (6)$$

where

m_{ex} is the amount found in picograms (pg), from 17.2.1 above;

m_{spk} is the amount spiked in picograms (pg).

17.3 Concentration in sample

17.3.1 Computation of the concentration of a dioxin-like PCB

Compute the concentration of a dioxin-like PCB in the aqueous phase of the sample using the concentration of the compound in the extract and the volume of water extracted (11.3) as follows:

$$\rho_{aq} = \frac{m_{ex}}{V_s} \quad (7)$$

where

ρ_{aq} is the concentration in the aqueous phase in picograms per litre (pg/l);

m_{ex} is the amount of the compound in the extract in picograms (pg);

V_s is the sample volume in litres (l).

17.3.2 Treatment of results exceeding calibration range

If the SIM area at either quantification mass for any compound exceeds the calibration range of the system, extract a smaller sample aliquot. Sample extracts can be diluted by a factor of 10 and reanalysed. If a dilution of greater than 50 × is required, the sample should be diluted and or internal standards/surrogates should be adjusted to compensate.

Dilute 100 ml, 10 ml, etc. of sample to 1 l with reagent water and re-prepare, extract, clean up, and analyse in accordance with Clauses 11 to 14.

If a smaller sample size will not be representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/µl in the extract, and analyse an aliquot of this diluted extract by the internal standard method.

17.4 Results and reporting

Report results to two significant figures for the dioxin-like PCBs and labelled compounds found in all standards, blanks, and samples. For standards (VER, IPR) and samples, report results at or above the minimum level (ML); see Table 2. Report results below the minimum level as not detected or as required by the regulatory authority. For blanks, report results that are above one-third of the ML. Calculate detection limits based on a signal to noise ratio of 3:1 as described in 16.3.

Report sample results in picograms per litre (pg/l) and TEQs as calculated in 17.5.

If the mass of a congener or congeners is below the detection limit then two TEQ concentrations should be reported per sample:

a) with the concentration of those congener(s) below the lower detection limit being taken as equal to the detection limit multiplied by 1, 1/2, or any value indicated in applicable regulations;

b) with the concentration of those congener(s) taken as zero.

Report results for dioxin-like PCBs in samples that have been diluted to the least dilute level at which the areas at the quantification masses are within the calibration range.

For dioxin-like PCBs having a labelled analogue, report results to the least dilute level at which the area at the quantification mass is within the calibration range and the labelled-compound recovery is within the normal range for the method.

NOTE Additionally, if requested, the total concentration of all isomers in an individual level of chlorination (i.e. total TetraCBs, total PentaCBs, etc.) can be reported by summing the concentrations of all isomers identified in that level of chlorination.

17.5 Toxic equivalents (TEQ)

17.5.1 In order to assess the toxicity of complex mixtures of PCDDs, PCDFs and dioxin-like PCBs, the concept of toxic equivalents was devised. Toxic equivalent factors (TEFs) are assigned to individual dioxins, furans and PCBs on the basis of how toxic they are in comparison with 2,3,7,8-TCDD, the most potent dioxin which has been assigned a value of 1,0. By comparison, animal and cell tests show that 2,3,7,8-TCDF and PCB 126 are approximately one-tenth as toxic as 2,3,7,8-TCDD. Consequently its toxic equivalent value is 0,1.

17.5.2 Toxic equivalent factors have been developed for those PCDDs, PCDFs and PCBs that contribute most to the toxicity of a complex mixture, which are those that have chlorine substitution in at least the 2,3,7 and 8 positions or PCBs that are co-planar (non-*ortho*-substituted) or mono-*ortho*-substituted. Multiplication of the concentration of a particular dioxin-like PCB by its toxic equivalent factor therefore gives a 2,3,7,8-TCDD toxic equivalent quantity (TEQ). The toxicity of any mixture, relative to 2,3,7,8-TCDD is therefore the sum of individual toxic equivalents.

17.5.3 Of the 210 PCDDs and PCDFs and 209 PCBs, 17 PCDDs/PCDFs and 12 PCBs contribute most to the toxicity of a complex mixture and are of most concern. This does not mean that the remaining 193 PCDDs and PCDFs and 197 PCBs are not toxic, but that they contribute comparatively little to the toxicity of a complex mixture.

NOTE Examples of toxic equivalent factors for dioxin-like PCBs are shown in Table 6.

17.5.4 To calculate a TEQ use Equation (8):

$$R_{\mathrm{TEQ}} = \sum_{i=1}^{12} R_{\mathrm{teq},i} = \sum_{i=1}^{12} \left(\rho_{\mathrm{aq},i} \times R_{\mathrm{tef},i} \right) \tag{8}$$

where

R_{TEQ} is the sum of the toxic equivalents for the individual compounds;

$R_{teq,i}$ is the concentration of congener "i", equal to its concentration times its toxic equivalent factor; see Table 6.

18 Analysis of complex samples

18.1 General

When the extract will not concentrate to 10 µl after all clean-up procedures have been exhausted, analyse a smaller aliquot of the sample (17.3) or diluted extract.

NOTE Some samples can contain high levels (> 10 µg/l) of the compounds of interest, interfering compounds, and/or polymeric materials. Some extracts will not concentrate to 10 µl; others can overload the GC column and/or mass spectrometer.

18.2 Recovery of labelled compounds

18.2.1 In most samples, recoveries of the labelled compounds will be similar to those from reagent water.

18.2.2 If the extraction standard recovery range exceeds 50 % to 130 % for tetra- to hexachlorinated congeners or 40 % to 130 % for heptachlorinated congeners, then, provided the sum of the contributions to the total TEQ in the sample from all of the congeners with recoveries not within these ranges does not exceed 10 %, the acceptable ranges shall be 30 % to 150 % for the tetra- to hexachlorinated congeners and 20 % to 150 % for the heptachlorinated congeners.

18.2.3 If the recovery of any of the labelled compounds is outside of these ranges, analyse a diluted sample (17.3.1).

18.2.4 If the recovery of any of the labelled compounds in the diluted sample is outside of the normal range, analyse the calibration verification standard (6.12) and verify the calibration (15.3).

18.2.5 If the calibration cannot be verified, perform a new calibration and reanalyse the original sample extract.

18.2.6 If the calibration is verified and the diluted sample does not meet the limits for labelled-compound recovery, the method does not apply to the sample being analysed and the result shall not be reported for regulatory compliance purposes. In this case, employ alternative extraction and clean-up procedures in this method to resolve the interference. If all clean-up procedures in this method have been employed and labelled-compound recovery remains outside of the normal range, extraction and/or clean-up procedures that are beyond the scope of this method shall be required to analyse these samples.

19 Pollution prevention

19.1 The solvents used in this method shall pose little threat to the environment when managed properly. The solvent evaporation techniques used in this method shall be amenable to solvent recovery, and it is recommended that the laboratory recover solvents wherever feasible.

19.2 Standards shall be prepared in volumes consistent with laboratory use to minimize disposal of standards.

20 Waste management

20.1 The laboratory shall comply with all national and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. The laboratory shall also comply with any sewage discharge permits and regulations.

20.2 Samples containing HCl to pH < 2 are hazardous and shall be neutralized before being poured down a drain or shall be handled as hazardous waste.

20.3 Low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves shall be burned in an appropriate incinerator. Gross quantities (milligrams) shall be packaged securely and disposed of through commercial or governmental channels that are capable of handling extremely toxic waste.

NOTE The dioxin-like PCBs decompose above 800 °C.

20.4 Liquid or soluble waste shall be dissolved in methanol or ethanol and irradiated with ultraviolet light with a wavelength shorter than 290 nm for several days. Liquid waste shall be analysed and the solutions shall be disposed of when the dioxin-like PCBs can no longer be detected.

21 Precision

Precision data from four samples is given in Annex C.

Table 1 — Dioxin-like PCBs determined by this method

Congener	CAS number	Labelled congener	CAS number
3,3',4,4'-Tetrachlorobiphenyl – PCB (77)	**32598-13-3**	$^{13}C_{12}$- 3,3',4,4'-tetraCB (77)	**105600-23-2**
3,4,4',5-Tetrachlorobiphenyl – PCB (81)	**70362-50-4**	$^{13}C_{12}$- 3,4,4',5-tetraCB (81)	**208461-24-9**
2,3,3',4,4'-Pentachlorobiphenyl – PCB (105)	**32598-14-4**	$^{13}C_{12}$- 2,3,3',4,4'-pentaCB (105)	**208263-62-1**
2,3,4,4',5-Pentachlorobiphenyl – PCB (114)	**74472-37-0**	$^{13}C_{12}$- 2,3,4,4',5-pentaCB (114)	**208263-63-2**
2,3',4,4',5-Pentachlorobiphenyl – PCB (118)	**31508-00-6**	$^{13}C_{12}$- 2,3',4,4',5-pentaCB (118)	**104130-40-7**
2',3,4,4',5-Pentachlorobiphenyl – PCB (123)	**65510-44-3**	$^{13}C_{12}$- 2',3,4,4',5-pentaCB (123)	**208263-64-3**
3,3',4,4',5-Pentachlorobiphenyl – PCB (126)	**57465-28-8**	$^{13}C_{12}$- 3,3',4,4',5-pentaCB (126)	**208263-65-4**
2,3,3',4,4',5-Hexachlorobiphenyl – PCB (156)	**38380-08-4**	$^{13}C_{12}$- 2,3,3',4,4',5-hexaCB (156)	**208263-68-7**
2,3,3',4,4',5'-Hexachlorobiphenyl – PCB (157)	**69782-90-7**	$^{13}C_{12}$- 2,3,3',4,4',5'-hexaCB (157)	**235416-30-5**
2,3',4,4',5,5'-Hexachlorobiphenyl – PCB (167)	**52663-72-6**	$^{13}C_{12}$- 2,3',4,4',5,5'-hexaCB (167)	**208263-69-8**
3,3',4,4',5,5'-Hexachlorobiphenyl – PCB (169)	**32774-16-6**	$^{13}C_{12}$- 3,3',4,4',5,5'-hexaCB (169)	**208263-70-1**
2,3,3',4,4',5,5'-Heptachlorobiphenyl – PCB (189)	**39635-31-9**	$^{13}C_{12}$-2,3,3',4,4',5,5'-heptaCB (189)	**208263-73-4**
2,2',3,3',4,4',5-Heptachlorobiphenyl – PCB (170)	**35065-30-6**		
2,2',3,4,4',5,5'-Heptachlorobiphenyl – PCB (180)	**35065-29-3**		

Table 2 — Suggested quantification relationships

Dioxin-like PCBs	Retention time and quantification reference	Relative retention time	Minimum level[a] Waters pg/l	Minimum level[a] Extract pg/µl
Compounds using $^{13}C_{12}$-tetraCB (70) as the recovery standard				
3,3',4,4'-TetraCB (77)	$^{13}C_{12}$- 3,3',4,4'-tetraCB (77)	0,999 to 1,002	10	0,5
3,4,4',5-TetraCB (81)	$^{13}C_{12}$- 3,4,4',5-tetraCB (81)	0,999 to 1,002	10	0,5
Compounds using $^{13}C_{12}$-pentaCB (138) as the recovery standard				
2,3,3',4,4'-PentaCB (105)	$^{13}C_{12}$- 2,3,3',4,4'-pentaCB (105)	0,999 to 1,002	50	2,5
2,3,4,4',5-PentaCB (114)	$^{13}C_{12}$- 2,3,4,4',5-pentaCB (114)	0,999 to 1,002	10	0,5
2,3',4,4',5-PentaCB (118)	$^{13}C_{12}$- 2,3',4,4',5-pentaCB (118)	0,999 to 1,002	50	2,5
2',3,4,4',5-PentaCB (123)	$^{13}C_{12}$- 2',3,4,4',5-pentaCB (123)	0,999 to 1,002	10	0,5
3,3',4,4',5-PentaCB (126)	$^{13}C_{12}$- 3,3',4,4',5-pentaCB (126)	0,999 to 1,002	10	0,5
Compounds using $^{13}C_{12}$-heptaCB (170) as the recovery standard				
2,3,3',4,4',5-HexaCB (156)	$^{13}C_{12}$- 2,3,3',4,4',5-hexaCB (156)	0,999 to 1,002	10	0,5
2,3,3',4,4',5'-HexaCB (157)	$^{13}C_{12}$- 2,3,3',4,4',5'-hexaCB (157)	0,999 to 1,002	10	0,5
2,3',4,4',5,5'-HexaCB (167)	$^{13}C_{12}$- 2,3',4,4',5,5'-hexaCB (167)	0,999 to 1,002	10	0,5
3,3',4,4',5,5'-HexaCB (169)	$^{13}C_{12}$- 3,3',4,4',5,5'-hexaCB (169)	0,999 to 1,002	10	0,5
2,3,3',4,4',5,5'-HeptaCB (189)	$^{13}C_{12}$- 2,3',4,4',5,5'-hexaCB (189)	0,999 to 1,002	10	0,5
Optional congeners				
2,2',3,3',4,4',5 -HeptaCB (170)			50	2,5
2,2',3,4,4',5,5'-HeptaCB (180)			50	2,5

NOTE Minimum levels and relative retention times are given for guidance only.

[a] The minimum level (ML) for each analyte is defined as the level for which the entire analytical system shall give a recognisable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specific sample masses/volumes and clean-up procedures have been used, i.e. based on 1 l of sample.

Table 3 — Suggested calibration standard concentrations

Congener	CS1 µg/l	CS2 µg/l	CS3 µg/l	CS4 µg/l	CS5 µg/l	CS6 µg/l	CS7 µg/l
3,3',4,4'-TetraCB (77)	0,1	0,5	2	10	40	200	800
3,4,4',5-TetraCB (81)	0,1	0,5	2	10	40	200	800
2,3,3',4,4'-PentaCB (105)	0,1	0,5	2	10	40	200	800
2,3,4,4',5-PentaCB (114)	0,1	0,5	2	10	40	200	800
2,3',4,4',5-PentaCB (118)	0,1	0,5	2	10	40	200	800
2',3,4,4',5-PentaCB (123)	0,1	0,5	2	10	40	200	800
3,3',4,4',5-PentaCB (126)	0,1	0,5	2	10	40	200	800
2,3,3',4,4',5-HexaCB (156)	0,1	0,5	2	10	40	200	800
2,3,3',4,4',5'-HexaCB (157)	0,1	0,5	2	10	40	200	800
2,3',4,4',5,5'-HexaCB (167)	0,1	0,5	2	10	40	200	800
3,3',4,4',5,5'-HexaCB (169)	0,1	0,5	2	10	40	200	800
2,3,3',4,4',5,5'-HeptaCB (189)	0,1	0,5	2	10	40	200	800
Optional congeners							
2,2',3,3,4,4',5 -HeptaCB (170)	0,1	0,5	2	10	40	200	800
2,2',3,4,4',5,5'-HeptaCB (180)	0,1	0,5	2	10	40	200	800
$^{13}C_{12}$- 3,3',4,4'-TetraCB (77)	50	50	50	50	50	50	50
$^{13}C_{12}$- 3,4,4',5-TetraCB (81)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3,3',4,4'-PentaCB (105)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3,4,4',5-PentaCB (114)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3',4,4',5-PentaCB (118)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2',3,4,4',5-PentaCB (123)	50	50	50	50	50	50	50
$^{13}C_{12}$- 3,3',4,4',5-PentaCB (126)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3,3',4,4',5-HexaCB (156)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3,3',4,4',5'-HexaCB (157)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3',4,4',5,5'-HexaCB (167)	50	50	50	50	50	50	50
$^{13}C_{12}$- 3,3',4,4',5,5'-HexaCB (169)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,3,3',4,4',5,5'-HeptaCB (189)	50	50	50	50	50	50	50
$^{13}C_{12}$-2,3',4',5-TetraCB (70)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,2',3,4,4',5,'-HexaCB (138)	50	50	50	50	50	50	50
$^{13}C_{12}$- 2,2',3,3,4,4',5 –HeptaCB (170)	50	50	50	50	50	50	50

Table 4 — Suggested concentration of dioxin-like PCBs in stock and spiking solutions

Dioxin-like PCBs	Labelled-compound stock solution ng/ml	PAR stock solution ng/ml	Recovery solution ng/ml
3,3',4,4'-TetraCB (77)	—	20	—
3,4,4',5-TetraCB (81)	—	20	—
2,3,3',4,4'-PentaCB (105)	—	20	—
2,3,4,4',5-PentaCB (114)	—	20	—
2,3',4,4',5-PentaCB (118)	—	20	—
2',3,4,4',5-PentaCB (123)	—	20	—
3,3',4,4',5-PentaCB (126)	—	20	—
2,3,3',4,4',5-HexaCB (156)	—	20	—
2,3,3',4,4',5'-HexaCB (157)	—	20	—
2,3',4,4',5,5'-HexaCB (167)	—	20	—
3,3',4,4',5,5'-HexaCB (169)	—	20	—
2,3,3',4,4',5,5'-HeptaCB (189)	—	20	—
Optional congeners			
2,2',3,3,4,4',5-HeptaCB (170)	—	20	—
2,2',3,4,4',5,5'-HeptaCB (180)	—	20	—
$^{13}C_{12}$- 3,3',4,4'-TetraCB (77)	100	—	—
$^{13}C_{12}$- 3,4,4',5-TetraCB (81)	100	—	—
$^{13}C_{12}$- 2,3,3',4,4'-PentaCB (105)	100	—	—
$^{13}C_{12}$- 2,3,4,4',5-PentaCB (114)	100	—	—
$^{13}C_{12}$- 2,3',4,4',5-PentaCB (118)	100	—	—
$^{13}C_{12}$- 2',3,4,4',5-PentaCB (123)	100	—	—
$^{13}C_{12}$- 3,3',4,4',5-PentaCB (126)	100	—	—
$^{13}C_{12}$- 2,3,3',4,4',5-HexaCB (156)	100	—	—
$^{13}C_{12}$- 2,3,3',4,4',5'-HexaCB (157)	100	—	—
$^{13}C_{12}$- 2,3',4,4',5,5'-HexaCB (167)	100	—	—
$^{13}C_{12}$- 3,3',4,4',5,5'-HexaCB (169)	100	—	—
$^{13}C_{12}$- 2,3,3',4,4',5,5'-HeptaCB (189)	100	—	—
$^{13}C_{12}$-2,3',4',5-TetraCB (70)	—	—	100
$^{13}C_{12}$- 2,2',3,4,4',5,'-HexaCB (138)	—	—	100
$^{13}C_{12}$- 2,2',3,3,4,4',5-HeptaCB (170)	—	—	100

Table 5 — Typical GC columns and temperature programmes

Column type	Analysis	Temperature programme	Injector
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	Non- and mono-*ortho* PCBs in Table 1	110 °C (1,0 min), 40 °C/min to 210 °C, 2 °C/min to 225 °C, 8 °C/min to 309 °C, hold 8 min	275 °C
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	Non- and mono-*ortho* PCBs in Table 1	150 °C (1,0 min), 12 °C/min to 200 °C, 3 °C/min to 235 °C, 8 °C/min to 310 °C	300 °C
DB-XLB 30 m, 0,25 mm ID, 0,25 µm film thickness	Non- and mono-*ortho* PCBs in Table 1	90 °C (3,0 min), 15 °C/min to 205 °C, 5 °C/min to 285 °C, hold 10 min	On-column
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	Non-*ortho* PCBs in Table 1	100 °C (1,0 min), 40 °C/min to 200 °C, 3 °C/min to 235 °C, 10 °C/min to 300 °C, hold 6 min	280 °C
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	Mono-*ortho* PCBs in Table 1	150 °C (1,0 min), 5 °C/min to 200 °C, 3 °C/min to 235 °C, 12 °C/min to 300 °C, hold 1 min	280 °C
DB-1 30 m, 0,25 mm ID, 0,25 µm film thickness	Non- and mono-*ortho* PCBs in Table 1	75 °C (0 min), 15 °C/min to 150 °C, 2,5 °C/min to 270 °C, hold 7 min	270 °C
SPB-Octyl 30 m, 0,25 mm ID, 0,25 µm film thickness	Non- and mono-*ortho* PCBs in Table 1		
RTX-5 60 m, 0,25 mm ID, 0,25 µm film thickness	Non-*ortho* PCBs in Table 1	90 °C (1 min), 15 °C/min to 180 °C, 5 °C/min to 290 °C, hold 10 min	On-column
NOTE Other columns typical for the analysis of PCBs are, for example, DB5-MS and HT-8[10)].			

10) DB5-MS and HT-8 are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

Table 6 — Examples of toxic equivalent factors

Compound	WHO/IPCS 1998 [a]
3,3',4,4'-Tetrachlorobiphenyl – PCB (77)	0,000 1
3,4,4',5-Tetrachlorobiphenyl – PCB (81)	0,000 1
2,3,3',4,4'-Pentachlorobiphenyl – PCB (105)	0,000 1
2,3,4,4',5-Pentachlorobiphenyl – PCB (114)	0,000 5
2,3',4,4',5-Pentachlorobiphenyl – PCB (118)	0,000 1
2',3,4,4',5-Pentachlorobiphenyl – PCB (123)	0,000 1
3,3',4,4',5-Pentachlorobiphenyl – PCB (126)	0,1
2,3,3',4,4',5-Hexachlorobiphenyl – PCB (156)	0,000 5
2,3,3',4,4',5'-Hexachlorobiphenyl – PCB (157)	0,000 5
2,3',4,4',5,5'-Hexachlorobiphenyl – PCB (167)	0,000 01
3,3',4,4',5,5'-Hexachlorobiphenyl – PCB (169)	0,01
2,3,3',4,4',5,5'-Heptachlorobiphenyl – PCB (189)	0,000 1
2,2',3,3',4,4',5-Heptachlorobiphenyl – PCB (170)	
2,2',3,4,4',5,5'-Heptachlorobiphenyl – PCB (180)	

[a] WHO International Programme on Chemical Safety.