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Indoor air —

Part 14: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Extraction, clean-up and analysis by high-resolution gas chromatography and mass spectrometry

Air intérieur —

Partie 14: Dosage des polychlorobiphényles (PCB) de type dioxine et des polychlorodibenzo-p-dioxines (PCDD)/polychlorodibenzofuranes (PCDF) totaux (en phase gazeuse et en phase particulaire) — Extraction, purification et analyse par chromatographie en phase gazeuse haute résolution et 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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Foreword

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

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 16000-14 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

ISO 16000 consists of the following parts, under the general title *Indoor air*:

— *Part 1: General aspects of sampling strategy*

— *Part 2: Sampling strategy for formaldehyde*

— *Part 3: Determination of formaldehyde and other carbonyl compounds — Active sampling method*

— *Part 4: Determination of formaldehyde — Diffusive sampling method*

— *Part 5: Sampling strategy for volatile organic compounds (VOCs)*

— *Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA® sorbent, thermal desorption and gas chromatography using MS/FID*

— *Part 7: Sampling strategy for determination of airborne asbestos fibre concentrations*

— *Part 8: Determination of local mean ages of air in buildings for characterizing ventilation conditions*

— *Part 9: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test chamber method*

— *Part 10: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test cell method*

— *Part 11: Determination of the emission of volatile organic compounds from building products and furnishing — Sampling, storage of samples and preparation of test specimens*

— *Part 12: Sampling strategy for polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polycyclic aromatic hydrocarbons (PAHs)*

— *Part 13: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDDs/PCDFs) — Collection on sorbent-backed filters*

— *Part 14: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Extraction, clean-up and analysis by high-resolution gas chromatography and mass spectrometry*

— *Part 15: Sampling strategy for nitrogen dioxide (NO_2)*

— *Part 16: Detection and enumeration of moulds — Sampling by filtration*

— *Part 17: Detection and enumeration of moulds — Culture-based method*

— *Part 18: Detection and enumeration of moulds — Sampling by impaction*

— *Part 23: Performance test for evaluating the reduction of formaldehyde concentrations by sorptive building materials*

— *Part 24: Performance test for evaluating the reduction of volatile organic compounds and carbonyl compounds without formaldehyde concentrations by sorptive building materials*

The following parts are under preparation:

— *Part 19: Sampling strategy for moulds*

— *Part 25: Determination of the emission of semi-volatile organic compounds by building products — Micro-chamber method*

— *Part 26: Measurement strategy for carbon dioxide (CO_2)*

— *Part 28: Sensory evaluation of emissions from building materials and products*

The following parts are planned:

— *Part 20: Detection and enumeration of moulds — Sampling from house dust*

— *Part 21: Detection and enumeration of moulds — Sampling from materials*

— *Part 22: Detection and enumeration of moulds — Molecular methods*

— *Part 27: Standard method for the quantitative analysis of asbestos fibres in settled dust*

Furthermore,

— ISO 12219-1[2] (under preparation), *Indoor air — Road vehicles — Part 1: Whole vehicle test chamber — Specification and method for the determination of volatile organic compounds in car interiors*,

— ISO 16017-1[4], *Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 1: Pumped sampling*, and

— ISO 16017-2[5], *Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 2: Diffusive sampling*

focus on volatile organic compound (VOC) measurements.

Introduction

ISO 16000 (all parts) specifies general requirements relating to the measurement of indoor air pollutants and the conditions to be observed before or during the sampling of individual pollutants or groups of pollutants as well as the measurement procedures themselves (see Foreword).

This part of ISO 16000 is applicable to the extraction, clean-up, and analysis from indoor air of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Sampling of PCBs, PCDDs/PCDFs are described in ISO 16000-13. Both ISO 16000-13 and ISO 16000-14 are parts of the complete PCB/PCDD/PCDF measurement procedure.

The sampling strategy to analyse PCBs, PCDDs/PCDFs and PAHs in indoor air is specified in ISO 16000-12.

Several PCBs and PCDDs/PCDFs are considered to be potential human carcinogens. There are 209 individual PCBs (congeners), 75 PCDDs and 135 PCDFs. The most toxic PCBs are those that are coplanar and structurally similar to PCDDs. The most toxic PCDD is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD).

PCBs are emitted into indoor air primarily from concrete sealers, certain paints or electrical capacitors, all of which have been banned in recent years. The principal sources of PCDDs/PCDFs in indoor air are impurities in wood preservatives containing pentachlorophenol (PCP) and emissions from fires involving chlorinated products. Tracked-in soil and emissions from nearby landfills and abandoned industrial sites may also contribute PCBs and PCDDs/PCDFs to the indoor environment.

Total PCB concentrations in urban outdoor air typically range from 10 pg/m^3 to several hundred picograms per cubic metre. PCDDs/PCDFs are usually found in urban outdoor air at extremely low concentrations; e.g. femtograms per cubic metre. PCBs and PCDDs/PCDFs may be distributed between the gas and particle-associated phases in ambient or indoor air, depending on the temperature, humidity, degree of chlorination, their concentration, and their capacity to associate with suspended particulate matter. These compounds, especially those of them having vapour pressures above 10^{-8} kPa, will tend to vaporise from particle filters during sampling. Consequently, a back-up vapour trap is included for efficient sampling. Separate analyses of the filter and vapour trap will not reflect the original atmospheric phase distributions at normal ambient temperatures because of volatilisation of compounds from the filter and should not be attempted.

This part of ISO 16000 is based on EN 1948-2[6], EN 1948-3[7], CEN/TS 1948-4[8], EPS I/RM/23[9], EPA SW 846 Method 8280B[10], VDI 2464-2[11], VDI 3498-1[12], VDI 3498-2[13] and References [14] to [16].

Indoor air —

Part 14: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Extraction, clean-up and analysis by high-resolution gas chromatography and mass spectrometry

WARNING — Persons using this part of ISO 16000 should be familiar with normal laboratory practice. This part of ISO 16000 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 health and safety practices and to ensure compliance with any national regulatory conditions.

1 Scope

This part of ISO 16000 specifies extraction, clean-up, and analysis procedures for the determination of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) collected from indoor air on particle filters backed by polyurethane foam (PUF). The method incorporates specific analyses by high resolution gas chromatography combined with high resolution mass spectrometry (HRGC/HRMS).

The method provides accurate quantitative data for tetra- to decachlorobiphenyls and tetra- to octachloro-dibenzo-*p*-dioxins/dibenzofurans (total concentrations for each isomeric series). It is capable of detecting 0,2 pg/m^3 or lower concentrations of most PCBs and PCDFs/PCDDs with air sampling volumes up to 360 m^3 or more in special cases. However, it may not be possible to detect all analytes at 0,2 pg/m^3 or lower, especially at lower sampling volumes.

Precision under normal conditions can be expected to be ± 25 % or better and uncertainty ± 50 % or better.

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 4793, *Laboratory sintered (fritted) filters — Porosity grading, classification and designation*

ISO 16000-13:2008, *Indoor air — Part 13: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Collection on sorbent-backed filters*

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
dioxin-like PCB
non- and mono-*ortho*-PCB having an affinity to the aryl hydrocarbon (Ah) receptor, showing similar toxicological effects as the 2,3,7,8-substituted PCDDs/PCDFs according to WHO

NOTE 1 See Reference [17].

NOTE 2 See Tables A.1 and A.2.

[ISO 16000-13:2008]

3.1.2
marker PCB
one of six PCBs

NOTE The six marker PCBs are: PCB-28, PCB-52, PCB-101, PCB-138, PCB-153, and PCB-180.

3.1.3
spiking
addition of $^{13}C_{12}$-labelled standards

[ISO 16000-13:2008]

3.1.4
statistical performance characteristic
measurement that quantifies the possible deviations of determined values resulting from the random part of the measuring process

EXAMPLE Repeatability (see ISO 9169[1])

3.1.5
field blank
unexposed but spiked sample of the sampling medium [e.g. filter, polurethane foam (PUF) trap, or complete sampling cartridge] that is carried to the field and through the complete analytical procedure, including the extraction, clean-up, and identification steps

NOTE The measurement value is needed to ensure that no significant contamination has occurred during all steps of the measurement process and to check that the operator can achieve a quantification level adapted to the task.

[ISO 16000-13:2008]

3.1.6
analytical blank
unexposed but spiked sample of a reagent or sampling medium that is carried through the complete analytical procedure including the extraction, clean-up, and identification steps

[ISO 16000-13:2008]

3.1.7
sampling standard
marker agent that is added to a sampling medium before sampling to determine the overall efficiency of the method

[ISO 16000-13:2008]

EXAMPLE $^{13}C_{12}$-labelled PCB, PCDD or PCDF.

3.1.8
extraction standard
marker agent added to a sampling medium before extraction and used for calculation of results

[ISO 16000-13:2008]

EXAMPLE $^{13}C_{12}$-labelled PCB, PCDD or PCDF.

3.1.9
recovery standard
marker agent added to the sample solution before injection into the GC

EXAMPLE $^{13}C_{12}$-labelled PCB, PCDD or PCDF.

3.1.10
congener
substance which belongs to the chemical group of PCB, PCDD or PCDF

NOTE Includes the 209 individual PCBs, 75 individual PCDDs, and 135 individual PCDFs.

[ISO 16000-13:2008]

3.1.11
isomer
PCB, PCDD or PCDF with identical elemental composition but different structure

[ISO 16000-13:2008]

EXAMPLE Among the chlorinated biphenyls, 1-chlorobiphenyl and 2-chlorobiphenyl. PCDDs and PCDFs also exhibit this phenomenon.

3.1.12
chromatographic profile
representation of the concentration levels of substances chromatographed

EXAMPLE Chromatographic profiles can be run for PCBs, PCDDs and PCDFs.

3.1.13
limit of detection
LOD
⟨chlorinated aromatic hydrocarbons in indoor air⟩ mean sample blank value plus three times the standard deviation, $3s$, of the blank

NOTE Adapted from Reference [18].

3.1.14
limit of quantification
mean sample blank value plus five, six or ten times the standard deviation of the blank

NOTE Adapted from Reference [18].

3.1.15
World Health Organization toxic equivalence factor
WHO-TEF
value assigned to the individual toxicity of a dibenzodioxin, dibenzofuran or dibenzodioxin-like PCB relative to the toxic effect of 2,3,7,8-TCDD

NOTE The WHO-TEF was first proposed by WHO in 1997. See Reference [17] and Annex B.

3.1.16
World Health Organization toxic equivalent
WHO-TEQ
product of the mass determined and the corresponding **WHO-TEF** (3.1.15)

NOTE 1 Subscripts are used to distinguish WHO-TEQ_{PCB}, and WHO-$TEQ_{PCDD/F}$ compound classes.

NOTE 2 See Annex A.

3.1.17
international toxic equivalence factor
I-TEF
value assigned to the toxicity of 2,3,7,8-chlorinated dibenzodioxins and dibenzofurans and certain dibenzodioxin-like PCBs

NOTE See Clause A.3.

3.2 Abbreviated terms

HpCB	heptachlorobiphenyl
HpCDD	heptachlorodibenzo-*p*-dioxin
HpCDF	heptachlorodibenzofuran
HRGC	high resolution gas chromatography
HRMS	high resolution mass spectrometry
HxCB	hexachlorobiphenyl
HxCDD	hexachlorodibenzo-*p*-dioxin
HxCDF	hexachlorodibenzofuran
I-TEF	international toxic equivalence factor
OCDD	octachlorodibenzo-*p*-dioxin
OCDF	octachlorodibenzofuran
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
PeCB	pentachlorobiphenyl
PeCDD	pentachlorodibenzo-*p*-dioxin
PeCDF	pentachlorodibenzofuran
PTFE	polytetrafluoroethylene
TCDD	tetrachlorodibenzo-*p*-dioxin
TCDF	tetrachlorodibenzofuran
TeCB	tetrachlorobiphenyl
WHO-TEF	World Health Organization toxic equivalence factor
WHO-TEQ	World Health Organization toxic equivalent

4 Principle

Separation, detection and quantification of PCBs/PCDDs/PCDFs collected from indoor air on particle filters backed up by polyurethane foam sampling media that have combined and extracted together is achieved by HRGC/HRMS using the isotope-dilution technique. Extraction procedures are normally based on Soxhlet extraction with toluene or an equivalent solvent. Sample clean-up is usually carried out by multi-column liquid chromatographic techniques based on specific adsorbents. The main purpose of the clean-up procedure is the removal of co-collected compounds and contaminants that may overburden the separation method, interfere with quantification or otherwise severely impact the performance of the identification and quantification steps. Furthermore, an enrichment of the analytes in the final sample extract is thereby achieved. The PCBs are separated from the PCDDs/PCDFs by desorption with different solvent volumes on an alumina column.

The GC should be equipped for temperature programming and all of the required accessories, such as gases and syringes, should be available. The GC injection port should be designed for capillary columns. Splitless injections, on-column injections, or moving needle injectors may be used. It is important to use the same technique and injection volume at all times. The HRMS system should be operated in the electron impact ionisation mode. The static resolving power of the instrument should be maintained at 10 000 or greater (10 % valley definition). The HRMS should be operated in the selected ion monitoring (SIM) mode with a total cycle time of $\leqslant$ 1 s.

The extract to be analysed for PCBs and the extract to be analysed for PCDDs/PCDFs contain extraction standards that are added before extraction and recovery standards added before GC quantification (see Tables 1 and 2). HRGC is used to separate the PCDD, PCDF and 12 dioxin-like PCB congeners from each other. HRMS enables differentiation between congeners with varying degrees of chlorine substitution and between native and labelled congeners.

$^{13}C_{12}$-labelled PCB/PCDD/PCDF standards are added at different stages of the overall method (before sampling, extraction and HRGC/HRMS-measurement). Spiking with $^{13}C_{12}$-labelled PCBs/PCDDs/PCDFs before sampling is necessary to determine the overall recovery rates of the PCB/PCDD/PCDF congeners. Losses during extraction and clean-up are detected and compensated by using these isotopically labelled surrogates as internal extraction standards for quantification, together with recovery standards that are added just before the HRGC/HRMS analysis.

5 Apparatus and materials

5.1 Apparatus

Usual laboratory equipment, and in particular the following.

5.1.1 Mass spectrometer (MS), whose absolute limit of detection for air measurements is at least 200 fg for 2,3,7,8-TCDD, signal/noise (S/N) ratio: 3:1, recent equipment achieves a ratio of $>$ 50:1. A high resolution gas chromatograph/high resolution mass spectrometer with resolution greater than or equal to 10 000 is required to achieve adequate sensitivity, selectivity and to allow the use of all the $^{13}C_{12}$-labelled standards.

5.1.2 Gas chromatograph (GC) direct coupling, injectors, e.g. on-column, splitless, programmable temperature vaporiser (PTV).

5.1.3 GC quartz capillary columns with polar separation phases, e.g. 90 % bis-cyanopropyl-10 % cyanopropylphenylpolysiloxane [1].

5.1.4 Injection syringes of appropriate sizes.

1) USP Type G8 phase, e.g. SP-2331, BPX 70, and CP-Sil 84, is a product identified by trade names. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of the product named.

5.1.5 Soxhlet extractor, of capacity 200 ml (43 mm × 123 mm) or larger, as needed, and appropriate condenser.

5.1.6 Boiling or **distillation flasks**, of capacities 50 ml, 250 ml, 500 ml, or as needed; round-bottom or pear shaped.

5.1.7 High power cooler.

5.1.8 Rotary evaporator and **flasks**, with vacuum monitoring.

5.1.9 Chromatography columns: a) 22 mm × 190 mm (i.d.) with a coarse glass sinter frit (porosity P 160) or other appropriate sizes; b) Pasteur pipette (7 mm × 150 mm) with silanised glass wool plug.

5.1.10 Concentrator [2] nitrogen blow-down apparatus; microprocessor-controlled, providing automated sample evaporation under mild thermal conditions, e.g. Kuderna-Danish type.

5.1.11 Threaded vial, clear, of capacity 1 ml with 0,25 ml micro insert.

5.1.12 Drying cabinet.

5.1.13 Oven, capable of being maintained at 300 °C.

5.2 Analytical reagents

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade (e.g. chromatographic or pesticide quality) and distilled water or water of equivalent purity.

5.2.1 Toluene, glass distilled, chromatographic or pesticide quality.

5.2.2 *n*-Hexane or ***n*-nonane**, glass distilled, chromatographic or pesticide quality.

5.2.3 Dichloromethane, glass distilled, chromatographic or pesticide quality.

5.2.4 Acetone, glass distilled, chromatographic or pesticide quality.

5.2.5 Diethyl ether.

5.2.6 *tert*-Butyl methyl ether.

5.2.7 Aluminium oxide, B Super I [3] for dioxin analysis.

5.2.8 Sodium sulfate, anhydrous.

5.2.9 Silica gel, 0,25 mm to 0,74 mm (63 mesh to 200 mesh).

5.2.10 Silica gel, KOH-coated. Mix 99 g of a KOH solution (1 mol/l) with 200 g of silica gel and homogenise, e.g. in a rotary evaporator (5.1.8).

5.2.11 Silica gel, H_2SO_4-coated. Mix 393 g of concentrated sulfuric acid and 500 g of silica gel and homogenise, e.g. in a rotary evaporator (5.1.8).

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2) Barkey Specimen Concentrator, N-evap® or TurboVap® are examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of these products.

3) Example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of this product.

5.2.12 Silica gel, $AgNO_3$-coated. Dissolve 25 g of $AgNO_3$ in 67,5 g of water in a 250 ml glass beaker. Place 225 g of silica gel in another 1 000 ml glass beaker. Pour the $AgNO_3$ solution slowly into the glass beaker containing the silica gel, and stir the mixture with a glass rod. Keep the mixture for half an hour in the dark, then transfer it to a dish and place in an oven having a nitrogen supply. Heat the oven from 70 °C to 125 °C over 5 h with nitrogen flushing. Maintain the temperature at 125 °C for 15 h with nitrogen flushing. Store all sorbents in airtight containers.

5.2.13 $^{13}C_{12}$-labelled standard (sampling standard), see Tables 1 and 2.

5.2.14 $^{13}C_{12}$-labelled standard (extraction standard), see Tables 1 and 2.

5.2.15 $^{13}C_{12}$-labelled standard (recovery standard), see Tables 1 and 2.

5.2.16 Unlabelled PCB/PCDD/PCDF standard for preparing the calibration curve; the selection is identical to the $^{13}C_{12}$-labelled standards compiled in Tables 1 and 2.

CAUTION — Shipping of PCDD/PCDF standards shall comply with national legal regulations. They shall be transported in special containers, which are commercially available. Handling should only be done by trained operators.

5.2.17 Keeper, solvent with a high boiling point that is added to the sampling standard solution and to the sample extract, e.g. *n*-tetradecane.

5.2.18 Lock-mass reference compound, e.g. perfluorokerosene (PFK).

5.3 Sampling materials

5.3.1 $^{13}C_{12}$-labelled standards. The masses of $^{13}C_{12}$-labelled sampling standards, in 100 µl of solvent, that should be added to each sample at a concentration level of approximately 100 fg of I-TEQ/m^3 for a sampling volume of approximately 180 m^3 are listed in Tables 1 and 2.

The extraction standards should be added to the various sampling media immediately after the samples are received in the laboratory. The $^{13}C_{12}$-labelled congeners are used for quantification because they behave exactly like the extracted native PCBs/PCDDs/PCDFs during the clean-up due to their nearly identical chemical and physical properties. The recovery standards (see Tables 1 and 2) are for determining the recovery rates. The masses specified in Tables 1 and 2 of standards to be used shall be adjusted appropriately if a considerably higher mass of native PCBs/PCDDs/PCDFs is expected in the sample. The use and the handling of the sampling standards are specified in ISO 16000-13.

5.3.2 PUF, open-cell, polyether type, density 22 mg/cm^3, cut into cylinders 76 mm long × 62 mm diameter, or other appropriate size depending on the specific sampling module used. The PUF cylinders should be slightly larger in diameter than the internal diameter of the sorbent cartridge so that the sampled air does not flow around it instead of through it.

5.3.3 Filter, micro-quartz or glass-fibre, binderless, acid-washed, with a filtration efficiency of 99,99 % mass fraction or better for particles below 0,5 µm, or other appropriate size filter depending on the specific sampling module used. This efficiency shall be certified by the filter supplier.

5.3.4 Forceps and **latex** or **neoprene gloves**, for handling the filter and PUF traps.

6 Analysis

6.1 General

The method specified in this part of ISO 16000 is based on the use of HRGC/HRMS together with the isotope dilution technique for separation, detection and quantification of PCBs/PCDDs/PCDFs. Chromatographic

separation and mass spectrometric detection permit identification of isomers and differentiation between congeners having a different number of chlorine substituents.

6.2 Sample extraction

Assemble the Soxhlet extractor (5.1.5) for pre-cleaning. Fill the boiling flask (5.1.6) with 300 ml or other appropriate volume of toluene (5.2.1) and reflux for 2 h. Allow the apparatus to cool, dismantle it, and discard the used solvent.

Using forceps (5.3.4), carefully fold the particle filter (5.3.3) and place it in the Soxhlet extractor. Place the PUF plug (5.3.2) on top of the filter to prevent its floatation. Before extraction, add 100 µl of the solution of the $^{13}C_{12}$-labelled extraction standards (see 5.3.1) to the top of the PUF trap. (The mass of each of the heptachlorinated and octachlorinated PCDD/PCDF standards should generally be at least twice the mass of the lower-chlorinated standards.)

If desired, the internal diameter of the extractor used may be smaller than the diameter of the PUF trap so that the PUF is compressed into the extractor, thereby reducing the amount of extraction solvent required.

Extract the PUF trap and filter in a Soxhlet extractor for 16 h to 24 h at a minimum of 3 cycles/h to 4 cycles/h with 300 ml of toluene (5.2.1).

Other solvents and solvent volumes may be used if first validated and documented by the user.

Concentrate the extract from the PUF trap and particle filter in a rotary evaporator (5.1.8) under controlled vacuum (45 °C bath temperature, 70 hPa) to approximately 20 ml.

Use of a concentrator (5.1.10) of the Kuderna-Danish type at 60 °C to 65 °C may be substituted, if desired.

A solvent recovery system may be required, especially if Kuderna-Danish concentration is employed.

Place the concentrated extract in clean, tightly sealed vials (5.1.11) and store in a freezer at 4 °C or below and for no longer than 30 days prior to analysis.

6.3 Clean-up

Clean-up methods employed shall prepare the sample extract in an appropriate manner for the subsequent quantitative analysis (an example is given in Annex B). Clean-up procedures are needed to concentrate the analytes and to remove interfering matrix components present in the raw extract.

The clean-up procedure results in two fractions containing either PCBs or PCDDs/PCDFs. This can be achieved by column chromatography, e.g. on activated magnesium silicate[4)] or alumina.

Proven clean-up procedures containing normally two or more of the following techniques shall be used. A detailed description of some of the procedures is given in Annex B. Other methods, such as an acid/base clean-up procedure followed by clean-up on microcolumns of silica gel, alumina, and activated carbon, can also be used, but shall be of proven equal performance to the following techniques:

a) gel permeation chromatography (GPC) — analytes in the molar mass range of 200 g/mol to 500 g/mol, which are of primary interest, can be isolated by GPC from larger molecules and polymers that might overload other clean-up methods;

b) multilayer column liquid chromatography with silica gel of different activity grades and surface characteristics — compounds with chemical properties different from PCBs/PCDDs/PCDFs can be removed by this process;

4) 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.

c) column adsorption chromatography using activated carbon — non-*ortho*-PCBs are separated from mono- and di-ortho-PCBs with activated carbon chromatography;

d) column liquid chromatography on alumina of different activity grade and acidity/basicity — interfering compounds with small differences in polarity or structure compared to PCBs can be removed in this manner.

The eluate of the sampling extraction procedure (approximately 20 ml) is added to chromatography column I (B.2.1). PCBs and PCDDs/PCDFs are eluted with 250 ml of *n*-hexane (5.2.2) and concentrated with a rotary evaporator (5.1.8). The concentrated extract of about 5 ml from chromatography column I is placed on the top of the sodium sulfate layer of chromatography column II described in Annex B. Elution is done with 60 ml of *n*-hexane (5.2.2), 90 ml of toluene (5.2.1) and 200 ml of *n*-hexane (5.2.2)/dichloromethane (5.2.3) (1+1 parts by volume). The first fraction is discarded, the second contains the PCBs and the third the PCDDs/PCDFs. The solvent of both fractions is evaporated to a volume of about 2 ml in a vacuum-controlled rotary evaporator, and further concentration is executed in a stream of nitrogen after addition of the recovery standards to a volume of 100 µl (see 6.4 and 6.5).

Separation of non-*ortho*-PCBs (77, 81, 126, 169) by means of a carbon column is described in Annex B. The final concentration of the cleaned extracts is described in 6.4 and the addition of the recovery standards in 6.5.

6.4 Final concentration of the sample extracts

To achieve sufficient detection limits, the cleaned sample fraction(s) are concentrated to a small volume before quantification.

Though dioxin-like PCBs and PCDDs/PCDFs have rather high boiling points, vapour phase transfer mechanisms and aerosol formation during solvent evaporation might lead to substantial losses when concentrating volumes below 10 ml. Depending on the method to be used for solvent volume reduction, take the following precautions:

a) rotary evaporators — losses can be substantial when reducing solvent volumes below 10 ml — countermeasures are the use of controlled vacuum conditions according to the vapour pressure and boiling point of the solvent, addition of a high-boiling solvent as a keeper as well as the use of specially shaped vessels (e.g. V-shaped);

b) counter gas flow evaporators — volumes should not be reduced to less than 1 ml;

c) nitrogen flow — an excessive flow of nitrogen which disturbs the solvent surface should be avoided — the vial shape has also some influence on possible losses: V-shaped vials or vial inserts shall be used for volume reductions below approximately 200 µl.

6.5 Addition of recovery standards

The very last step before injection into the GC (5.1.2) is the addition of the recovery standards (5.2.15) to measure the recovery rates of the extraction standards (5.2.14). For the determination of the PCDDs/PCDFs, add 25 µl of the standard solution containing 25 pg each of $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD (see Table 1) to the concentrated PCDD/PCDF extract. For the determination of the PCBs, add at least 10 µl of the standard solution, each containing 3 600 pg of $^{13}C_{12}$-2,3′,4′,5-TeCB (70), $^{13}C_{12}$-2,3,3′,5,5′-PeCB (111) and $^{13}C_{12}$-2,2′,3,3′,4,4′,5-HpCB (170) (see Table 2) to the concentrated PCB extract (see also Annex B). At the end the solution is concentrated as described in 6.4 c).

The recovery standards shall be added under conditions a) to c).

a) Add recovery standards at a minimum volume of 10 µl just prior to the injection. If the 12 dioxin-like PCBs are collected and concentrated in several fractions during clean-up procedure, add at least one of the four $^{13}C_{12}$-labelled congeners mentioned as recovery standards in Table 1 to each PCB-containing fraction. When selecting suitable congeners as recovery standards, ensure that both recovery standards and the corresponding extraction standards match with respect to retention time and mass range.

b) A slow evaporation to a volume of minimum 10 µl is acceptable.

c) Store samples with the recovery standard added which cannot be analysed for operational reasons (instrument failure) for as short a time as possible. Avoid any further uncontrolled solvent evaporation.

Table 1 — $^{13}C_{12}$-labelled 2,3,7,8-PCDD/PCDF congeners for addition to samples before sampling, extraction and GC injection to measure approximately 100 fg I-TEQ/m^3 and for a sampling volume of approximately 180 m^3

Congeners	Standard solution to be added before:		
	Sampling (5.2.13)	Extraction (5.2.14)	GC-injection recovery (5.2.15)
	Made up volume after addition of toluene (5.2.1) or *n*-nonane (5.2.2), µl		
	100	100	25
	Total mass, pg		
$^{13}C_{12}$-2,3,7,8-TCDF		25[a]	
$^{13}C_{12}$-1,2,3,4-TCDD			25
$^{13}C_{12}$-2,3,7,8-TCDD		25[a]	
$^{13}C_{12}$-1,2,3,7,8-PeCDF	25		
$^{13}C_{12}$-2,3,4,7,8-PeCDF		25[a]	
$^{13}C_{12}$-1,2,3,7,8-PeCDD		25[a]	
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF		25[a]	
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF		25	
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	25		
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF		25	
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD		25	
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD		25[a]	
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD			25
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF		50[a]	
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	50		
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD		50[a]	
$^{13}C_{12}$-OCDF		50	
$^{13}C_{12}$-OCDD		50	

[a] These standards are used to quantify the remaining congeners of the associated groups of chlorinated homologues for which no standard was added.

Table 2 — $^{13}C_{12}$-labelled PCB congeners to be added to the sample at different stages of the procedure for measurement of about 0,01 ng WHO-TEQ$_{PCB}$/m^3 assuming 180 m^3 of sampling volume

Congeners	Total mass to be added before:		
	Sampling (5.2.13)	**Extraction** (5.2.14)	**GC-injection recovery** (5.2.15)[a]
	Volume after dilution with toluene (5.2.1) or *n*-nonane (5.2.2), µl		
	100	100	⩾ 10
	Total mass, pg		
$^{13}C_{12}$-2,3,4,4′-TeCB (60)	3 600		
$^{13}C_{12}$-3,3′,4,5,5′-PeCB (127)[b]	3 600		
$^{13}C_{12}$-2,3,3′,4,5,5′-HxCB (159)	3 600		
$^{13}C_{12}$-3,4,4′,5-TeCB (81)		3 600	
$^{13}C_{12}$-3,3′,4,4′-TeCB (77)		3 600	
$^{13}C_{12}$-3,3′,4,4′,5-PeCB (126)		3 600	
$^{13}C_{12}$-3,3′,4,4′,5,5′-HxCB (169)		3 600	
$^{13}C_{12}$-2,3,3′,4,4′-PeCB (105)[b]		3 600	
$^{13}C_{12}$-2,3,4,4′,5-PeCB (114)		3 600	
$^{13}C_{12}$-2,3′,4,4′,5-PeCB (118)		3 600	
$^{13}C_{12}$-2′,3,4,4′,5-PeCB (123)		3 600	
$^{13}C_{12}$-2,3,3′,4,4′,5-HxCB (156)		3 600	
$^{13}C_{12}$-2,3,3′,4,4′,5′-HxCB (157)		3 600	
$^{13}C_{12}$-2,3′,4,4′,5,5′-HxCB (167)		3 600	
$^{13}C_{12}$-2,3,3′,4,4′,5,5′-HpCB (189)		3 600	
$^{13}C_{12}$-2,3′,4′,5-TeCB (70)			3 600
$^{13}C_{12}$-2,3,3′,5,5′-PeCB (111)			3 600
$^{13}C_{12}$-2,2′,3,3′,4,4′,5-HpCB (170)			3 600

[a] A selection of available $^{13}C_{12}$-labelled PCBs suitable as recovery standards is listed. At least one shall be added for each dioxin-like PCB-containing fraction.

[b] Attention should be paid to possible co-elution problems of PCB 127 and PCB 105 on certain commercially available columns.

6.6 GC conditions (example)

GC conditions for the separation of PCBs/PCDDs/PCDFs are specified in Table 3.

Table 3 — GC conditions for separation of PCBs/PCDDs/PCDFs

GC	Gas chromatograph with an on-column injector. Splitless injection technique, on-column injections, or moving needle injectors may be used. It is important to use the same technique and injection volume at all times.
Pre-column	Deactivated quartz capillary column; 2 m × 0,32 mm inner diameter.
GC separation capillary	90 % *bis*-Cyanopropyl-10 % cyanopropylphenylpolysiloxane (SP-2331) or 95 % dimethyl-5 % diphenylpolysiloxane (DB-5), 60 m × 0,25 mm inner diameter; 0,2 µm film thickness, directly coupled to MS via quartz capillary transfer column (0,5 m × 0,25 mm inner diameter). NOTE Other GC columns, such as 50 % cyanopropymethyl-50 % phenylmethyl-polysiloxane (DB-225), may be required for confirmation or specific separations. If the laboratory employs a column that has a different elution order than those specified here, the laboratory shall ensure that the isomers eluting closest to 2,3,7,8-TCDD are represented in the column performance solution.
Injection port temperature	130 °C
Inlet pressure	220 kPa He
Injection volume	1 µl to 3 µl in toluene or *n*-nonane
Temperature programme	80 °C (2 min) 20 °C/min to 225 °C 4 °C/min to 240 °C, 52 min 4 °C/min to 252 °C, 10 min
Transfer line temperature	280 °C

6.7 GC separation procedure

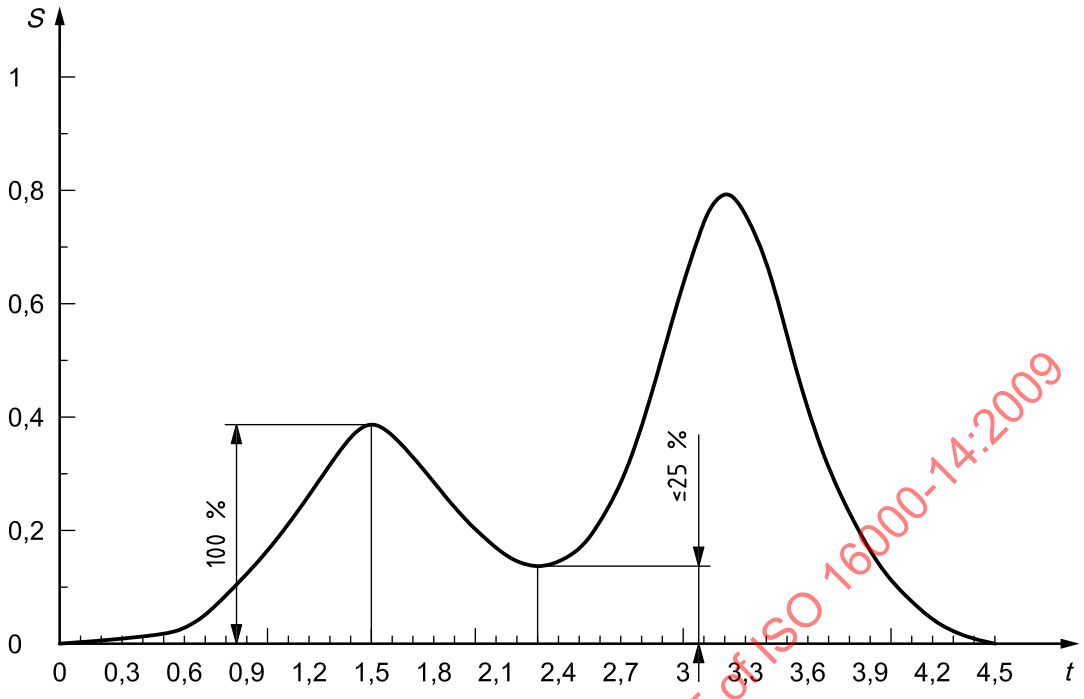
There is currently no GC column that is capable of separating all 2,3,7,8-chlorosubstituted PCDDs/PCDFs from all of the other chlorosubstituted congeners. Complete separation may only be achieved by multiple analysis of the sample on different GC columns of differing types (polarity). To avoid more labour- and cost-intensive laboratory methods, a result may be considered valid if the following conditions are fulfilled.

If a 2,3,7,8-PCDD/PCDF congener and a non-2,3,7,8-congener co-elute, quantify the total peak against the calibration factor for the 2,3,7,8-isomer. The TEQ value that results from this quantification and the TEF of the 2,3,7,8-isomer shall not exceed 5 % of the total TEQ of the sample. If there are multiple co-elutions, the sum of these individual contributions shall again not exceed 5 % of the total TEQ.

The GC column shall separate the 2,3,7,8-chlorosubstituted PCDD/PCDF congeners from all other interfering isomers with a "90 % valley" relative to the highest signal. On a polar separation capillary column, 2,3,7,8-TCDF shall be separated from all other interfering isomers with a "25 % valley" below the top of the minor peak, with respect to the height of this peak (see Figure 1). On a non-polar GC column, 2,3,7,8-TCDF and 1,2,3,7,8-PeCDD shall be separated from all other interfering isomers with a "30 % valley" below the top of the minor peak, with respect to the height of this peak.

Sample chromatograms (Figure C.1) clearly illustrate this problem and also reveal that while separation of most of the other congeners is easily achieved, the resolving powers of the current capillary GC columns are still insufficient to separate all congeners completely. All the chromatographic peaks have been assigned to the corresponding congeners (Reference [19]).

The retention time of a native 2,3,7,8-chlorosubstituted PCDD/PCDF congener shall not differ from that of its corresponding $^{13}C_{12}$-labelled congener in the spiked sample by more than $^{+3}_{0}$ s within a time window; deviations of $^{+3}_{-2}$ s are acceptable for the heptachloro- and octachlorocongeners.



Key

S signal, relative units

t time, min

Figure 1 — Illustration of the separation from another interfering isomer with a “25 % valley”

NOTE Normally the determination of single congeners is achieved by using a polar column, while groups of homologues may be determined with an non-polar column. A 95 % dimethyl-5 % diphenylpolysiloxane column may be used for both purposes under certain conditions.

Losses in sensitivity may result when polar GC columns are used for HpCDD/HpCDF and OCDD/OCDF. If, as a result, the evaluation of these congeners is no longer meaningful, these compounds can be quantified on a non-polar GC column.

Chromatographic resolution is verified using a test mixture of PCDDs/PCDFs specific to each example GC column show below.

90 % *bis*-Cyanopropyl-10 % cyanopropylphenyl polysiloxane test mixture:

2,3,7,8-TCDD

1,4,7,8-TCDD

1,2,3,7-TCDD

1,2,3,8-TCDD

95 % Dimethyl-5 % diphenyl polysiloxane test mixture:

1,2,3,7-TCDD/1,2,3,8-TCDD

2,3,7,8-TCDD

1,2,3,9-TCDD

50 % Cyanopropylmethyl-50 % phenylmethyl polysiloxane [5] test mixture:

2,3,4,7-TCDF

2,3,7,8-TCDF

1,2,3,9-TCDF

The concentrations of the TCDDs/TCDFs should be ~0,5 ng/µl in *n*-nonane (5.2.2) or toluene (5.2.1).

6.8 MS procedure

PCBs/PCDDs/PCDFs are identified and quantified by MS (multiple ion detection). HRGC/HRMS, with a resolution equal to or greater than 10 000 at *m/z* 292,982 5 (PFK), is required to achieve sufficient sensitivity and selectivity and to allow the use of all the $^{13}C_{12}$-labelled standards. Resolution in a range of 6 000 to 10 000 may be acceptable if the absence of interferences is documented. At least two ions of the molecular cluster of one of each degree of chlorination are measured to determine the individual congeners of PCBs, PCDDs or PCDFs, with the ion intensities being in a calculable ratio to one another. Combined with GC retention times, this approach provides further identification for specific compounds.

The isotope ratio measured shall not differ from the theoretical isotope ratio by more than ± 20 %.

The ions to be analysed are classified into a number of groups in such a way that the GC elution regions of the individual groups of chlorinated homologues do not overlap to the point of interference. With a sufficiently long dwell time per ion, high sensitivity and a sufficient number of scans to describe the GC signal (by individual measuring points) can be achieved for specific compounds (> 10 scans per peak).

To establish the time intervals for the individual groups of analytes, the retention times shall be determined for all congeners. This is best done with a standard fly ash extract, which usually contains all the native congeners. When a sample is analysed, the first and last eluting congener of each group shall be within the selected time window for this group. Selected ions of a lock-mass reference compound (5.2.18), e.g. PFK, are used in order to select the desired ions to be monitored precisely within the groups by setting an acceleration voltage. Adjust the level of the reference compound (PFK) metered inside the ion chamber during HRGC/HRMS analyses so that the amplitude of the most intense selected lock-mass ion signal is kept to a minimum. Under those conditions, sensitivity changes can be more effectively monitored. Using the peak matching unit and the PFK reference peak, verify that the exact *m/z* 392,976 1 (PFK) is within 3×10^{-6} of the required value. The MS resolving power should be documented by recording the peak profile of the high mass reference signal (*m/z* 392,976 1) obtained during the above peak matching calibration experiment by using the low mass PFK ion at *m/z* 292,982 5 as a reference. The minimum resolving power of 10 000 should be demonstrated on the high mass ion while it is transmitted at a lower accelerating voltage than the low mass reference ion, which is transmitted at full voltage and full sensitivity. There should be little, if any, loss in sensitivity on the high mass ion if the source parameters are properly tuned and optimised (see Annex D).

CAUTION — Excessive use of PFK or any reference substance causes high background signals and contamination of the ion source, resulting in an increase in downtime required for instrument maintenance.

During the analysis, the reference substance is added at a constant flow rate into the source in such a manner that an adequate signal for the lock mass is achieved at a given detector amplification without introducing interference (increasing noise) in the region of the targeted ions. The relative sensitivity of the PFK mass numbers (*m/z*) in an individual group should lie within ±15 % of the expected values. The mass resolution in a group should lie within ±10 % of the mean resolution in this group.

5) USP Type G7, G19 phase, e.g. DB-225, BP-225, and CP-Sil 43 CB are products that are identified by trade names. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of the products named.

MS conditions for analysing PCBs/PCDDs/PCDFs along with examples of mass fragmentograms and chromatograms are presented in Annex C. The masses of the ions recorded, including dwell times, delay times, and elution ranges for the groups (functions) for measurements on a double-focusing mass spectrometer (polar separation capillary), are given in Annex D.

7 Minimum requirements for extraction and clean-up

The following minimum requirements have to be fulfilled for the determination of PCB/PCDD/PCDF concentrations.

a) The recovery rates of the $^{13}C_{12}$-labelled tetra- to hexachlorinated PCDD/PCDF standards added before extraction shall be 50 % to 130 %, and those of the hepta- and octachlorinated standards shall be 40 % to 130 %. If the above ranges are exceeded, then provided the sum of the contributions to the total I-TEQ in the sample from all 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 hepta- and octachlorinated congeners.

b) The recovery rate for each of the individual congeners of the $^{13}C_{12}$-labelled dioxin-like PCB congeners added before extraction shall be at least 50 % and should not exceed 130 %. In exceptional cases, a recovery rate of 20 % to 150 % can be accepted for the field sample, if the contribution of an individual congener to the WHO-TEQ_{PCB} is less than 10 %.

c) To achieve sufficient detection limits, the cleaned sample fraction(s) shall be concentrated to a small volume before quantification, but not less than 10 µl. (When small volumes are used, solubility problems can arise for some congeners. In such cases, larger volumes are recommended.)

d) All collected particles and all adsorbents shall be extracted with toluene for 20 h in a Soxhlet extractor or comparable validated method.

8 Identification and quantification

8.1 Establishing the analytical function

The mass fragmentograms provide qualitative and quantitative information. Characteristic data for a congener are the molar mass, the isotope ratio, and the retention time. Qualitative assignment of the analytical signals (peaks, response) is made by comparing the retention times with those of the internal standard and with the correct isotope ratio at a given molar mass or isotope mass $M^+ + (M+2)^+$, $(M+4)^+$. The peak area is proportional to the mass of substance injected. Quantitative determination is performed by the method of internal standards, in which a known quantity of extraction standard is added to the sample before sample preparation (see 6.2). In the mass fragmentogram, the peak areas of the $^{13}C_{12}$-labelled extraction standard are related to the corresponding components to be determined. The mass of the native congener i, $^{12}C m_i$, is given by:

$$^{12}C\,m_i = \frac{^{13}C\,m_i \; ^{12}C\,A_i}{f_{rr\,i} \; ^{13}C\,A_i} \qquad (1)$$

where

$^{13}C m_i$ is the mass of the $^{13}C_{12}$-labelled standard (congener) i added to the sample;

$^{12}C A_i$ is the area of the peak of the native congener i;

$^{13}C A_i$ is the area of the peak of the $^{13}C_{12}$-labelled standard i added to the sample;

$f_{rr\,i}$ is the relative response factor of native congener i relative to the $^{13}C_{12}$-labelled congener i (see Clause 7).

The relative response factors are determined for all congeners used as standards (see Clause 7). The response of the individual isomers within one group of chlorinated homologues is different and deviations of greater than ±100 % are possible. For simplification, therefore, the convention that the relative response factor of a congener applies to all isomers for which there is no corresponding $^{13}C_{12}$-labelled standard is adopted.

For some native PCDD/PCDF congeners the corresponding $^{13}C_{12}$-labelled congeners are used as sampling or recovery standards and therefore cannot be used for calculation of the relative response factor. In this case, a congener with similar properties is used. The $^{13}C_{12}$-labelled PCDD/PCDF congeners to be used are given in Table 4.

Table 4 — Quantification scheme for PCDDs/PCDFs

Analyte	Extraction standard
2,3,7,8-TCDD	$^{13}C_{12}$-2,3,7,8-TCDD
1,2,3,7,8-PeCDD	$^{13}C_{12}$-1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDD	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,4,6,7,8-HpCDD	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
OCDD	$^{13}C_{12}$-OCDD
2,3,7,8-TCDF	$^{13}C_{12}$-2,3,7,8-TCDF
1,2,3,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF
2,3,4,7,8-PeCDF	$^{13}C_{12}$-2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDF	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF
1,2,3,4,6,7,8-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
OCDF	$^{13}C_{12}$-OCDF

For the determination of PCBs there are enough $^{13}C_{12}$-PCBs available so that for each PCB analyte the corresponding $^{13}C_{12}$-PCB is available for quantification.

8.2 Calibration and checking of GC/MS

Calibration is performed using five calibration solutions of different concentrations (example in Annex F) that contain defined amounts of all $^{13}C_{12}$-labelled standards (sampling, extraction and recovery standards) and the corresponding native PCB/PCDD/PCDF standards. The calibration curve is required for calculating the relative response factors of the analyte components. The usual calibration frequency depends on the stability of the instrument. Daily calibration checks using an injected control calibration standard have to be performed. In addition, complete calibration, including checking linearity using five calibration solutions, has to be performed after major changes, for example

a) when new or repaired instruments are used;

b) after changing the GC columns;

c) after cleaning the separation and detection systems;

d) if deviations from an injected control calibration standard exceed 20 %.

The relative response factor for congener i, f_{rri}, is defined and calculated as follows:

$$f_{\mathrm{rr}\,i} = \frac{^{12}\mathrm{C}\,A_i \;\; ^{13}\mathrm{C}\,m_i}{^{13}\mathrm{C}\,A_i \;\; ^{12}\mathrm{C}\,m_i} \tag{2}$$

where the variables on the right-hand side are as defined for Equation (1).

The calibration curve is a graphical plot of the response ratio $^{12}\mathrm{C}A_i/^{13}\mathrm{C}A_i$ on the ordinate against the mass ratio $^{12}\mathrm{C}m_i/^{13}\mathrm{C}m_i$ on the abscissa.

8.3 Checking the method and minimum requirement for method validation

It is not possible to calibrate the overall method, including sampling, since there are no calibration gases for PCBs/PCDDs/PCDFs. The analytical method has to be checked at regular intervals by a function control using suitable stock samples (e.g. fly ash). The stock samples have to be checked using certified solutions, and these test results shall be within the certified tolerance limits. Particular attention has to be paid to isomer-specific losses.

The entire analytical operation has to be checked regularly by determining blanks. A field blank (3.1.5) is a sample which is taken in an identical manner to the real sample, but without drawing air through the sampling apparatus. The measurement value for all chlorosubstituted PCB/PCDD/PCDF congeners of an analytical blank shall be equal to or less than the quantification limit (LOQ) of the method (see Clause 9). If the blank value is above the quantification limit the concentrations determined shall be below the lowest measured value of the sample series by a factor of 3. The extraction blank of all 2,3,7,8-chlorosubstituted congeners has to be determined by an analytical blank which covers the complete analytical method, including extraction, clean-up, and quantification:

— after major changes to the analytical method;

— after analysis of a sample with values which exceed the previous concentration level of the measurement solution by a factor of 10.

Due to the minimum requirement for method validation, the following shall be applied:

a) sampling:

   1) a filter efficiency of more than 99,5 % on a test aerosol with a mean particle diameter of 0,3 µm, at the maximum flow rate anticipated (or 99,9 % on a test aerosol of 0,6 µm mean diameter) — this efficiency shall be certified by the filter supplier,

   2) the breakthrough of the sampling train shall be less than 10 % for every single congener,

   3) the recovery rate of each of the sampling standards shall be greater than 50 %, calculated on the basis of the relevant extraction standard;

b) extraction: the extract of a repeated extraction procedure shall not contain more than 5 % of the amount of any individual native congener compared with the first extraction — for the second extraction the addition of $^{13}C_{12}$-labelled extraction standards is repeated;

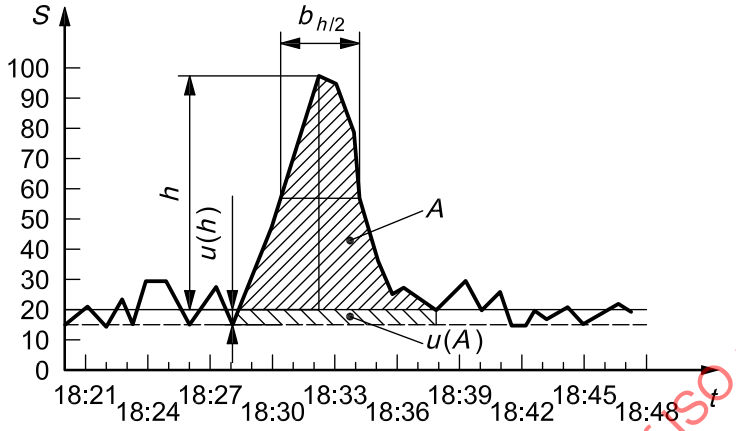
c) clean-up and separation: the final extract of a repeated clean-up procedure shall not contain less than 80 % of the amount of any individual native dioxin-like PCB compared with the first clean-up.

8.4 Quantification

Quantification is performed using the ion of the highest intensity (base peak) and is carried out according to Equation (1). It is also possible to quantify results using an additional second mass. The S/N ratio shall be at least 3:1 for native PCBs/PCDDs/PCDFs and shall be at least 20:1 for the $^{13}C_{12}$-labelled congeners. The isotope ratios of the measured sample shall agree with the theoretical isotope ratios to within ± 20 %.

Evaluation by peak height is used if the evaluation by peak area does not give a satisfactory result. This is the case at S/N ratios $\leqslant$ 20, i.e. in the vicinity of the detection limit.

Evaluation by peak height is more accurate than evaluation by peak area at low S/N ratios, since the position of the baseline is subject to relatively high scattering and the resultant error in calculation of area has a substantially greater effect than in calculation of height (Figure 2).



Key

A	area
$b_{h/2}$	peak width at half peak height
h	height
S	signal (relative units)
t	time
$u(A)$	uncertainty in the area
$u(h)$	uncertainty in the height

Figure 2 — Diagram of effect of S/N ratios on evaluation by peak height and peak area

An uncertainty in the baseline gives the relative uncertainty $u(h)/h$ for evaluation by peak height, but in contrast evaluation by peak area gives the relative uncertainty, $u(A)/A$

$$\frac{u(A)}{A} = \frac{u(h)\, b_{h/2}}{(h/2)\, b_{h/2}} = 2\frac{u(h)}{h} \tag{3}$$

where the variables are defined in the key to Figure 2.

In evaluation by peak area, the error is at least twice as high as in evaluation by peak height, provided that no tailing is present and that peak width of analyte and standard are equal.

In the quantitative determination of the PCB, PCDD, and PCDF homologue groups, two assumptions are made:

a) losses of all isomers of one degree of chlorination during sample preparation are identical;

b) the loss corresponds to the loss of the assigned $^{13}C_{12}$-labelled standard of the respective degree of chlorination, i.e. selective losses of individual isomers are excluded.

In addition, it is assumed that at the same concentration, the ion intensities in the mass fragmentograms are identical for all isomers of one degree of chlorination.

8.5 Minimum requirements for identification

8.5.1 A static resolution of at least 10 000 at 5 % valley shall be demonstrated. Resolution in the range of 5 000 to 10 000 shall only be accepted if the absence of interferences is documented and the sensitivity is reached.

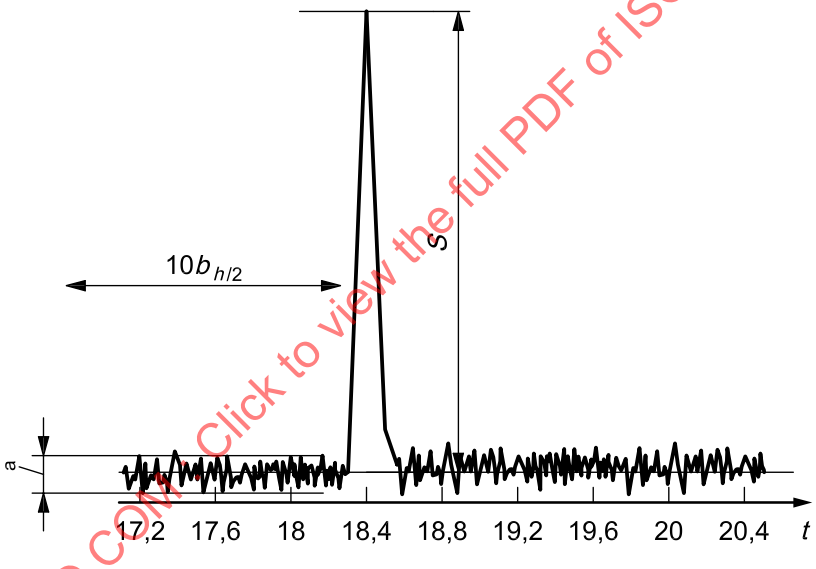
8.5.2 Identification shall be based on at least two ions of the molecular isotope cluster.

8.5.3 The isotope ratio between the ions monitored shall match the theoretical value to within ± 15 % (see Table 5).

8.5.4 The retention time of a native dioxin-like PCB congener shall be within a time window of + 3 s to 0 s based on the retention time of the corresponding $^{13}C_{12}$-labelled isomer in the sample.

8.5.5 The S/N ratio of the raw data as documented in Figure 3 shall be at least 3:1 for the signal used for identification.

8.5.6 The baseline noise shall be measured in front of the signal of the native congener within a signal-free window corresponding to 10 times the signal width at half height. Peak-to-peak values shall be taken.



Key

$b_{h/2}$ peak width at half peak height
S signal height
t time

a peak-to-peak noise, N

Figure 3 — Determination of the S/N ratio

8.6 Minimum requirements for quantification

In addition to the requirements for identification, the following points shall be fulfilled as quantification requirements a) to k).

a) The separation of all investigated dioxin-like PCB and PCDD/PCDF congeners relevant for the WHO-TEQ_{PCB} shall be demonstrated by using standard reference mixtures.

b) The peak shape of the gas chromatographic signal of a congener shall contain 10 or more sampling points (scanning units).

c) PCB-126 shall be separated from all other interfering congeners within a 25 % valley below the top of the minor peak with respect to the height of that peak.

d) The recovery rate of each individual $^{13}C_{12}$-labelled dioxin-like PCB and PCDD/PCDF of the extraction standards in each sample shall be within 40 % to 120 % — in exceptional cases, a recovery rate of 20 % to 150 % can be accepted for the field sample, if the contribution of an individual congener to the WHO-TEQ_{PCB} is less than 10 %.

e) The S/N ratio of the signal of the $^{13}C_{12}$-labelled congeners used for quantification shall be $>$ 20:1.

f) The measuring range shall be linear (at least over a concentration range of a factor of 100) — the standard deviation of the relative response factor shall not exceed $\pm$ 15 % and shall be based on a minimum of five measuring points over the whole range.

g) The limit of quantification (LOQ) at an S/N ratio for the individual congener i, LOQ_i, in picograms per cubic metre, shall not exceed the value given by Condition (4):

$$LOQ_i \leqslant \frac{0{,}5}{TEF_i} \tag{4}$$

where TEF_i is the toxic equivalent factor for compound i (according to WHO or NATO/CCMS; see Clause A.3).

The LOQ reported shall be no lower than three times the mean blank value derived from at least 10 individual blank value measurements.

h) Quantification is based on two isotope ions.

i) If quantification is only possible with a single ion due to a disturbed second trace or, in the case of PeCB, the second and third trace, this shall be reported.

j) The fragmentation products of Cl- and Cl_2-loss of higher chlorinated PCBs shall not overlap with the respective PCBs with one or two chlorine atoms less.

k) Interference with other compounds, especially PCDDs, shall be avoided by a suitable chromatographic column and/or temperature programme or an optimised clean-up.

9 Quality assurance criteria for the procedure blanks

9.1 Field blank requirements

The field blank is taken at the operator's site according to the following procedure (see also ISO 16000-13:2008, Clause 8):

a) no gas is drawn through the sampling train;

b) a leak check is performed.

A field blank procedure shall be performed before each measurement series.

The field blank shall not be deducted from the measured value.

All field blanks shall be reported with the corresponding measured values.

9.2 Analytical blank

The analytical blank value of all congeners shall be measured in a blank sample covering the complete analytical procedure, including extraction, clean-up, and quantification, when one of the following situations occurs.

a) After major changes in the extraction or clean-up procedure such as:

   1) use of new or repaired equipment;

   2) use of new batches of solvents or adsorbents.

b) After the analysis of samples with exceptionally high concentrations (e.g. those that exceed the average concentration by a factor of 10 or more).

   An analytical blank can be accepted when the following requirements are fulfilled.

c) The analytical blank value of all dioxin-like PCB and PCDD/PCDF congeners shall be lower than the lowest measured value in the samples.

d) For the two PCB congeners with the highest TEFs (PCB 126 and PCB 169) the analytical blank shall, by at least a factor of 10, be lower than the set LOQ of the method. For the other 10 PCB congeners the analytical blank shall, by at least a factor of 5, be lower than the LOQ (see 8.6).

If the analytical blank values exceed the values mentioned above, the laboratory specific LOQ has to be increased accordingly.

9.3 GC/MS blank

A GC/MS blank shall be measured on a regular basis in addition to the analytical blank. This GC/MS blank shall ensure that there is no contamination from either the measuring system itself or the sample ahead (e.g. syringe, previous sample, standard solution or septum).

For this purpose toluene (5.2.1) has to be injected, and the toluene chromatogram checked for all dioxin-like PCBs/PCDDs/PCDFs and for all $^{13}C_{12}$-standards. The relevant signals shall be below the detection limit, and at least 5 % lower than the corresponding signals of the next sample.

For GC/MS blank measurements, the injection volume shall be the same as for the samples.

The GC/MS blank shall be run at least once every 10 samples. If different concentrations are expected or different sample sources have to be analysed, a GC/MS blank shall be analysed in between.

10 Recovery

10.1 Sampling standards

To determine the recovery rates of the sampling standards, the following are applied to the glass fibre filter: 50 pg of $^{13}C_{12}$-1,2,3,7,8-PeCDF, 50 pg of $^{13}C_{12}$-1,2,3,7,8,9-HxCDF, 100 pg of $^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF. The reference standard for $^{13}C_{12}$-1,2,3,7,8-PeCDF is the extraction standard $^{13}C_{12}$-1,2,3,7,8-PeCDD, for $^{13}C_{12}$-1,2,3,7,8,9-HxCDF, it is the extraction standard $^{13}C_{12}$-2,3,4,6,7,8-HxCDF, and for $^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF it is the extraction standard $^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD (see Tables 1 and 5). For the PCBs, follow the appropriate procedure. The masses of the individual PCB sampling standards are given in Table 2 (see also Table 6).

The recovery of these sampling standards is an index for assessing the sampling. The recovery of the sampling standards shall be at least 50 % and is not used to correct the analytical results. The recovery rate of the sampling standard i, $R_{i,\mathrm{sa}}$, expressed as a percentage, is given by Equation (5):

$$R_{i,\mathrm{sa}} = \frac{100\, m_{i,\mathrm{ex}}}{f_{\mathrm{rr}\,i,\mathrm{sa}} \cdot m_{i,\mathrm{sa}}} \cdot \frac{A_{i,\mathrm{sa}}}{A_{i,\mathrm{ex}}} \qquad (5)$$

where

$A_{i,\mathrm{ex}}$ is the response of the extraction standard i corresponding to the sampling standard i;

$A_{i,\mathrm{sa}}$ is the response of the sampling standard i;

$f_{\mathrm{rr}i,\mathrm{sa}}$ is the relative response factor i of sampling standard i relative to the extraction standard i;

$m_{i,\mathrm{ex}}$ is the mass of the extraction standard i added to the sample;

$m_{i,\mathrm{sa}}$ is the mass of the individual sampling standard i added to the sample.

The variance of the recovery due to differing behaviour of extraction standard and sampling standard during sample preparation shall not exceed ± 20 %. Record any deviations.

Table 5 — Calculation scheme for the PCDD/PCDF recovery rates of the sampling standards

Sampling standard	Extraction standard
$^{13}C_{12}$-1,2,3,7,8-PeCDF	$^{13}C_{12}$-1,2,3,7,8-PeCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD

For the PCBs, take the relations listed in Table 6.

Table 6 — Calculation scheme for the PCB recovery rates of the sampling standards

Sampling standard	Extraction standard
$^{13}C_{12}$-2,3,4,4′-TeCB (PCB 60)	$^{13}C_{12}$-3,3′,4,4′-TeCB (PCB 77)
$^{13}C_{12}$-3,3′,4,5,5′-PeCB (PCB 127)	$^{13}C_{12}$-2,3′,4,4′,5-PeCB (PCB 118)
$^{13}C_{12}$-2,3,3′,4,5,5′-HxCB (PCB 159)	$^{13}C_{12}$-2,3,3′,4,4′,5-HxCB (PCB 156)

10.2 Extraction standards

The recovery rates of the $^{13}C_{12}$-labelled PCB and PCDD/PCDF standards are defined in Clause 7.

Recovery of the extraction standard i, $R_{i,\mathrm{ex}}$, expressed as a percentage, is calculated using Equation (6):

$$R_{i,\mathrm{ex}} = \frac{100\, m_{i,\mathrm{re}}}{f_{\mathrm{rr}i,\mathrm{ex}}\, m_{i,\mathrm{ex}}} \cdot \frac{A_{i,\mathrm{ex}}}{A_{i,\mathrm{re}}} \qquad (6)$$

where

$A_{i,\mathrm{ex}}$ is the response of the extraction standard i corresponding to the recovery standard i;

$A_{i,\mathrm{re}}$ is the response of the recovery standard i;

$f_{\mathrm{rr}i,\mathrm{ex}}$ is the relative response factor i of extraction standard i relative to the recovery standard i;

$m_{i,\mathrm{ex}}$ is the mass of the individual extraction standard i added to the sample;

$m_{i,\mathrm{re}}$ is the mass of the recovery standard i added to the sample.

For all tetra- and pentachlorinated extraction standards, the reference standard is the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD, and for all hexa- to octachlorinated extraction standards, the reference standard is the recovery standard $^{13}C_{12}$-1,2,3,7,8,9-HxCDD (see Tables 1 and 5).

11 Calculation and presentation of results

PCB, PCDD and PCDF indoor air concentrations shall be reported as mass concentrations, generally as the mass of the substance based on the volume in the state of the mean conditions under which sampling took place. The sampling conditions have to be recorded in order, if necessary, to convert the measurement results to standard conditions (101,325 kPa; 273,15 K).

For a toxicological assessment of the PCDD/PCDF concentrations in the matrices under test, the 2,3,7,8-toxic equivalents (TEQs) can be calculated according to WHO or NATO-CCMS. The TEQ value of a sample is calculated by multiplying the respective PCB/PCDD/PCDF congener concentration by the associated TEF (see Table A.3) and adding the resulting products.

Not only the concentrations of the individual congeners have to be reported, but also the TEQs in accordance with WHO, in femtograms per cubic metre (see Clause A.3 and Reference [17]).

For 2,3,7,8-congeners which are not detected, the detection limits have to be reported.

The mass concentration, in femtograms per cubic metre, of component i, ρ_i, based on the sampling volume determined, is calculated based on the mean sampling conditions using Equation (7):

$$\rho_i = \frac{^{12}m_i}{V_0} \tag{7}$$

where

$^{12}m_i$ is the mass, in femtograms, of the individual PCDD or PCDF congener i;

V_0 is the sampling volume, in cubic metres.

12 Safety measures

PCBs and PCDDs/PCDFs have been classified as carcinogens. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) are also extremely toxic (see Reference [20]). Care shall be exercised when working with these substances. The user shall be thoroughly familiar with the chemical and physical properties of targeted substances.

All PCBs and PCDDs/PCDFs shall be treated as carcinogens. Pure compounds shall be weighed in a glove box. Special precautions shall be taken to minimise the risk of human exposure, either through direct contact with contaminated materials, or through inhalation of contaminated air. Unused samples and standards are considered to be toxic waste and shall be properly disposed of according to regulations. Laboratory bench tops and equipment shall be regularly checked for contamination by analysing swab samples.

Some solvents specified in this part of ISO 16000 may present health hazards if breathed or absorbed through the skin. Toluene (5.2.1) is of particular concern. Special care should be exercised when using this solvent. All operations that require working with this solvent should be performed under a fume hood.

All work related to PCB/PCDD/PCDF analyses, including the preparation, handling, and storage of all samples and standards, should be conducted within a specially designed laboratory. This facility should include the following design features:

a) restricted access area;

b) sufficient ventilation;

c) negative pressure relative to surrounding areas;

d) all exhaust air ducting routed to a common, scrubbed outlet;

e) segregation, via doors and air pressure differentials, into low and high hazard areas;

f) an independent back-up air supply system designed to come into operation;

g) alternative power supply in the event of a commercial power failure;

h) capability to visually monitor ventilation system performance;

i) devices to monitor indoor air levels of organic vapours generated from solvent used;

j) a system of distinctive audio and visual alarms to alert lab personnel to potentially hazardous conditions.

13 Performance characteristics

13.1 Standard deviation of the overall method (sampling, preparation and analysis)

Using six ambient air samples taken in parallel, the standard deviations were calculated for the 2,3,7,8-chlorosubstituted PCDD/PCDF congeners, for the total of the isomer groups (tetra- to hepta-CDDs/CDFs), for the total PCDDs, for the total PCDFs, for the total PCDDs and PCDFs and for the toxic equivalents.

The standard deviation, s_{rel}, was calculated using the equation for random sample data from a finite population:

$$s_{rel} = \sqrt{\frac{\sum_{i=1}^{n}(X_i - \bar{X})^2}{(n-1)\,\bar{X}^2}} \qquad (8)$$

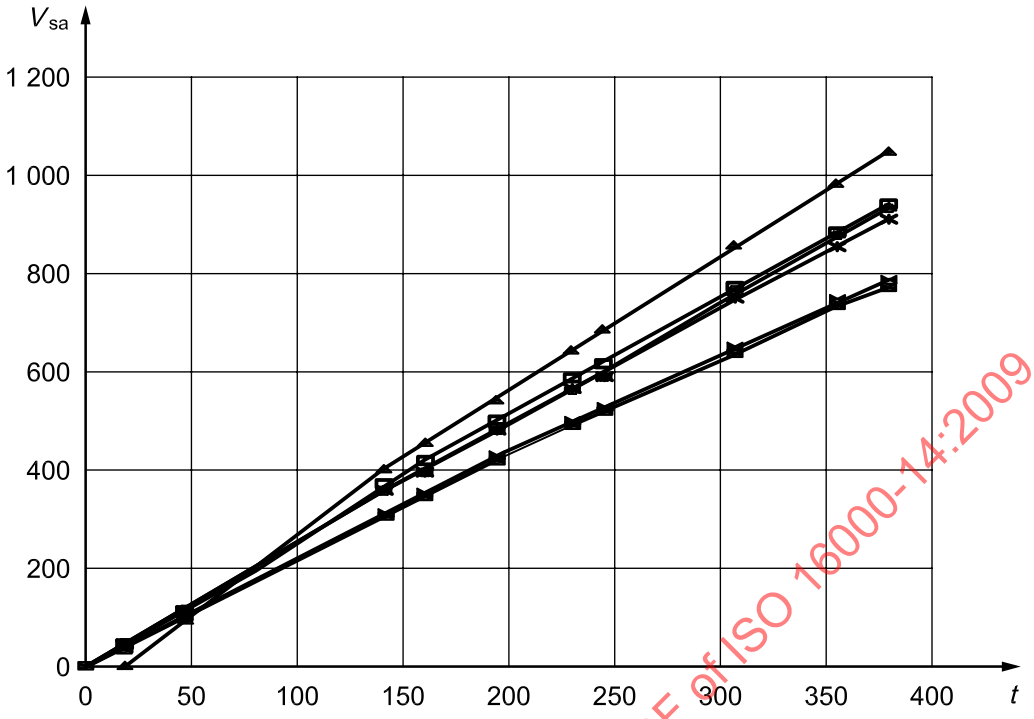
where

n is the number of measurements;

X_i is the ith measured value;

$\bar{X}$ is the mean value.

Figure 4 shows the sampling volumes as a function of measuring period of the six sampling devices operated in parallel.



Key

t sampling period

V_{sa} sampling volume

Figure 4 — Comparison between sampling volume and measuring period of the six sampling devices

13.2 Detection limits

The detection limits for the PCB/PCDD/PCDF congeners, based on the overall method for determining PCBs/PCDDs/PCDFs, depend on a number of factors (typical values are given in brackets):

a) sampling volume (50 m^3 to 1 000 m^3);

b) final volume of the analytical solution (10 µl to 50 µl);

c) injection volume (1 µl to 3 µl);

d) recovery of the $^{13}C_{12}$-labelled standards;

e) matrix effect on the S/N ratio;

f) effect of bleeding of the separation capillary on noise;

g) detection limit of the GC/MS system;

h) number of masses within a group in the mass-spectrometric determination;

i) position of the PCB/PCDD/PCDF congener mass within a group (see Table A.1).

Factors a) to c) can be varied within given limits (e.g. as stated). The optimum absolute sensitivity of the GC/MS system is instrument specific and, in conjunction with the other parameters, determines the attainable detection limits.

Table 7 gives an example of detection limits that can be achieved for given analytical parameters (see example) during long-term operation.

The other 2,3,7,8-substituted congeners, by definition, have similar detection limits. The detection limit is defined here as a S/N ratio 3:1, the baseline noise of the native trace being measured in a signal-free window equivalent to 10 times the signal width at half signal height before a signal of the corresponding standard trace.

Table 7 — Detection limits of PCDDs/PCDFs

PCDD	Detection limit fg/m^3	PCDF	Detection limit fg/m^3
2,3,7,8-TCDD	< 1,0	2,3,7,8-TCDF	< 1,0
1,2,3,7,8-PeCDD	< 1,5	1,2,3,7,8-PeCDF	< 1,0
1,2,3,6,7,8-HxCDD	< 2,0	1,2,3,4,7,8-HxCDF 2,3,4,6,7,8-HxCDF	< 1,5
1,2,3,4,6,7,8-HpCDD	< 3,0	1,2,3,4,6,7,8-HpCDF	< 3,0
OCDD	< 3,5	OCDF	< 3,0
Operating conditions			
Sampling volume	1 000 m^3	$^{13}C_{12}$-labelled standard recovery	88 %
Final volume of analyte solution	~ 10 µl	Gas-chromatographic separation on a 90 % *bis*-cyanopropyl-10 % cyanopropylphenylpolysiloxane capillary column	60 m × 0,25 mm inner diameter 0,2 µm film thickness
Injection volume	1 µl	Mass spectrometric determination	

The detection limits of all PCB congeners are ⩽ 1 pg/m^3 (taking 50 m^3 sampling volume as basis).

14 Interferences

Method interferences may be caused by contaminants in solvents, reagents, on glassware, and other sample processing hardware that result in discrete artefacts and/or elevated baselines in the detector profiles. Glassware shall be scrupulously cleaned (e.g. by acid washing, followed by heating to 450 °C in a muffle furnace, and rinsed with solvent immediately prior to use). All solvents and other materials shall be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks.

Interferences can be caused by components that have similar chemical and physical properties. Although sample preparation separates the most frequently occurring substances, some PCBs, polychlorinated naphthalenes and especially methoxychlorobiphenyls, methoxychloronapthalenes, methoxychlorodiphenyl ethers and chlorodiphenyl ethers, chlorobenzylphenyl ethers, bromobiphenyl ethers, mixed bromo/chlorodibenzodioxins and bromo/chlorodibenzofurans and others are only removed with difficulty.

If, by electron-impact ionisation, substances form ions that appear in the same mass window (function, m/z) as the PCB/PCDDs/PCDFs and, furthermore, have the same retention times as individual congeners, they interfere. At a mass spectrometric resolution of 7 000 to 10 000 (5 % valley), generally, in the case of indoor air measurements, no interference is observed.

Other interfering substances are those at high concentrations which lead to shifts in lock mass detection.

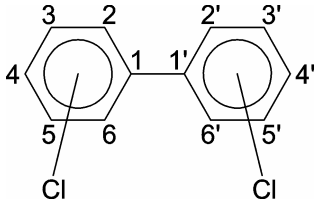
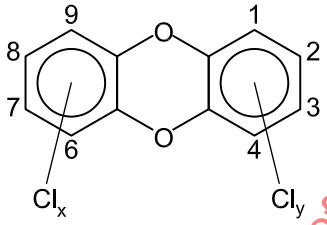
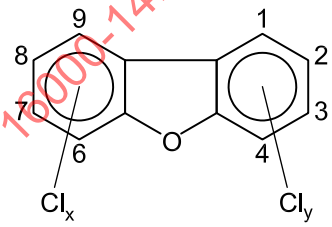
Interferences in chromatograms are given in Annex E.

Annex A
(informative)

Structure, toxicity and calculation of toxic equivalents

A.1 Generic structures of PCBs, PCDDs and PCDFs

Table A.1 — Structures of PCBs, PCDDs and PCDFs and number of possible isomers

No. chlorine atoms			
	PCB Polychlorinated biphenyls	**PCDD** Polychlorinated dibenzodioxins	**PCDF** Polychlorinated dibenzofurans
	No. PCB isomers	**No. PCDD isomers**	**No. PCDF isomers**
1	3	2	4
2	12	10	16
3	24	14	28
4	42	22	38
5	46	14	28
6	42	10	16
7	24	2	4
8	12	1	1
9	3	—	—
10	1	—	—
Totals	**209**	**75**	**135**

A.2 Structures of selected coplanar PCBs

Table A.2 — Structures of 12 selected coplanar polychlorinated biphenyls

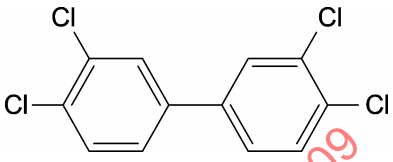
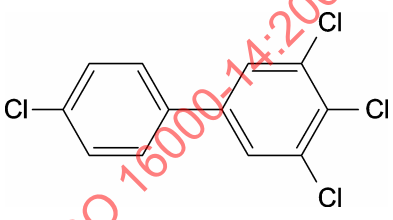
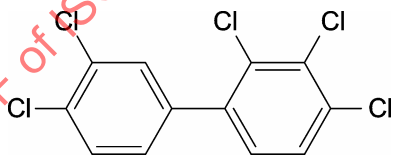
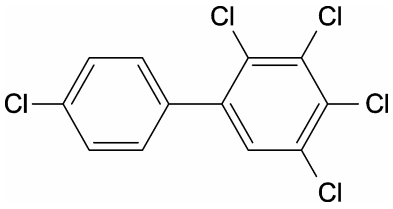
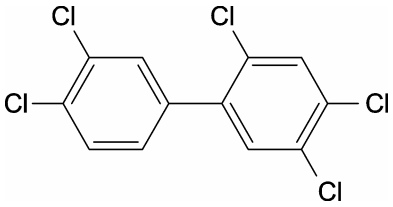
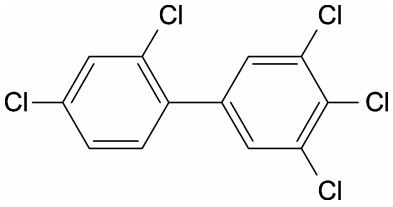
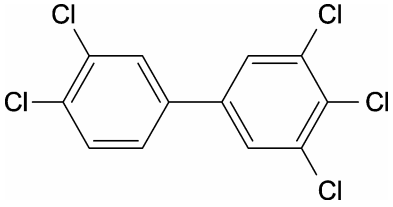
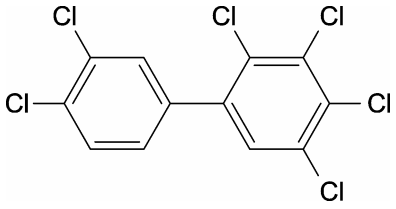
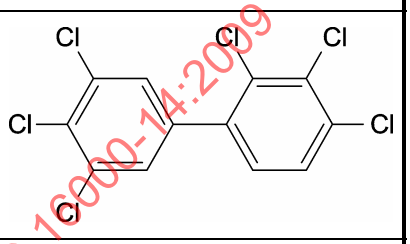
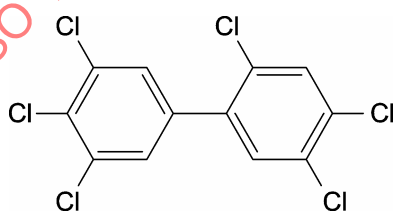
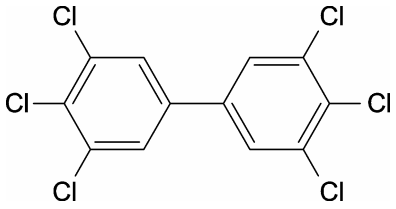
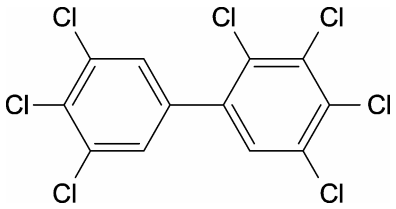
Chemical name IUPAC name	Empirical formula	Molar mass g/mol	Structural formula
3,3′,4,4′-Tetrachlorobiphenyl 3,3′,4,4′-TeCB PCB-77	$C_{12}H_6Cl_4$	291,9	
3,4,4′,5-Tetrachlorobiphenyl 3,4,4′,5-TeCB PCB-81	$C_{12}H_6Cl_4$	291,9	
2,3,3′,4,4′-Pentachlorobiphenyl 2,3,3′,4,4′-PeCB PCB-105	$C_{12}H_5Cl_5$	326,4	
2,3,4,4′,5-Pentachlorobiphenyl 2,3,4,4′,5-PeCB PCB-114	$C_{12}H_5Cl_5$	326,4	
2,3′,4,4′,5-Pentachlorobiphenyl 2,3′,4,4′,5-PeCB PCB-118	$C_{12}H_5Cl_5$	326,4	
2′,3,4,4′,5-Pentachlorobiphenyl 2′,3,4,4′,5-PeCB PCB-123	$C_{12}H_5Cl_5$	326,4	
3,3′,4,4′,5-Pentachlorobiphenyl 3,3′,4,4′,5-PeCB PCB-126	$C_{12}H_5Cl_5$	326,4	

Table A.2 (*continued*)

Chemical name IUPAC name	Empirical formula	Molar mass g/mol	Structural formula
2,3,3′,4,4′,5-Hexachlorobiphenyl 2,3,3′,4,4′,5-HxCB PCB-156	$C_{12}H_4Cl_6$	360,9	
2,3,3′,4,4′,5′-Hexachlorobiphenyl 2,3,3′,4,4′,5′-HxCB PCB-157	$C_{12}H_4Cl_6$	360,9	
2,3′,4,4′,5,5′-Hexachlorobiphenyl 2,3′,4,4′,5,5′-HxCB PCB-167	$C_{12}H_4Cl_6$	360,9	
3,3′,4,4′,5,5′-Hexachlorobiphenyl 3,3′,4,4′,5,5′-HxCB PCB-169	$C_{12}H_4Cl_6$	360,9	
2,3,3′,4,4′,5,5′-Heptachlorobiphenyl 2,3,3′,4,4′,5,5′-HpCB PCB-189	$C_{12}H_3Cl_7$	396,3	

A.3 Toxicity and calculation of the toxic equivalents for PCDDs/PCDFs and PCBs

In the environment, PCDDs/PCDFs practically never appear as single compounds but as a complex mixture associated with other structurally related (“dioxin-like”) compounds such as PCBs.

The TEQ system uses 2,3,7,8-TCDD as the standard to which the toxicity of the other compounds is related by means of a weighting. This relationship with the standard is based on the assumption that PCDDs/PCDFs and dioxin-like compounds act through the same mechanism of action. The toxic effects are assessed through subchronic toxicity studies and from certain biochemical properties such as Ah receptor binding capacity.

The toxic potential of a single congener is indicated through its TEF describing the individual toxicity relative to the toxic effect of 2,3,7,8-TCDD. For the TEQ calculation the amount or concentration of each relevant congener is multiplied with the corresponding TEF. When all congeners are given as “equivalents of 2,3,7,8-TCDD” they can simply be added up and the resulting TEQ represent the total toxicity of the mixture (see Table A.3).

There still remain uncertainties concerning the toxicity of PCDDs/PCDFs. Nevertheless, TEFs, first as I-TEF values assigned by NATO (Reference [21], see Table A.3) and more recently as WHO-TEFs (Reference [17]), have been established to standardise the toxicity first of 2,3,7,8-Cl substituted dibenzodioxins and dibenzofurans and latterly certain dioxin-like PCBs. For all other congeners which may be present in a sample, a TEQ value of zero is assigned.

The NATO scheme has been adopted internationally as a basis for the TEQ determination. During recent years the toxicity of PCDDs/PCDFs has been reported mainly in I-TEQ.

The most recent TEQ scheme, developed by WHO and the International Programme on Chemical Safety (IPCS), standardises the toxicity of 17 dibenzodioxin and dibenzofuran congeners and includes, for the first time, 12 dioxin-like PCBs (Reference [17]). It reflects the present knowledge of the toxic effects of PCDDs/PCDFs and dioxin-like PCBs (see Table A.3).

The WHO-TEQ approach is linked to a WHO recommendation concerning a tolerable daily intake (TDI) for humans of 1 pg to 4 pg WHO-TEQ per kilogram of body mass (including PCBs) which should not be exceeded. The TDI was recommended on the basis of critical effects (including developmental, reproductive, hormonal, immune system and neurobehavioural effects), dose-response relationships and quantitative risk extrapolation.

In the sense of an international harmonised risk assessment, which should be based on the most current knowledge, it appears to be reasonable to accept the WHO-TEQ system and to discuss the WHO-TDI as an assessment scale for future risk assessment at international level.

The calculation of the PCDD/PCDF TEQ results is normally performed using the NATO I-TEF (Reference [21]). The calculation of TEQ values for PCDDs/PCDFs and dioxin-like PCBs (coplanar and non-*ortho*-PCBs) is only possible using WHO-TEFs (WHO-TEQ_{PCB}). The calculation schemes are not mutually compatible (see Table A.3). Some countries mandate the calculation according to NATO/CCMS (I-TEQ) and others according to WHO (WHO-TEQ_{PCB}); consequently, it is essential to report both the results and the calculation scheme.

Table A.3 — WHO-TEFs (Reference [17]) and I-TEFs (Reference [21])

Congener	WHO-TEF	I-TEF
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	0,5
1,2,3,4,7,8-HxCDD	0,1	0,1
1,2,3,6,7,8-HxCDD	0,1	0,1
1,2,3,7,8,9-HxCDD	0,1	0,1
1,2,3,4,6,7,8-HpCDD	0,01	0,01
OCDD	0,000 3	0,001
2,3,7,8-TCDF	0,1	0,1
1,2,3,7,8-PeCDF	0,03	0,05
2,3,4,7,8-PeCDF	0,3	0,5
1,2,3,4,7,8-HxCDF	0,1	0,1
1,2,3,6,7,8-HxCDF	0,1	0,1
1,2,3,7,8,9-HxCDF	0,1	0,1
2,3,4,6,7,8-HxCDF	0,1	0,1
1,2,3,4,6,7,8-HpCDF	0,01	0,01
1,2,3,4,7,8,9-HpCDF	0,01	0,01
OCDF	0,000 3	0,001
Non-*ortho*-PCB		
3,4,4′,5-TeCB (81)	0,000 1	—
3,3′,4,4′-TeCB(77)	0,000 3	—
3,3′,4,4′,5-PeCB (126)	0,1	—
3,3′,4,4′,5,5′-HxCB (169)	0,03	—
Mono-*ortho*-PCB		
2,3,3′,4,4′-PeCB (105)	0,000 03	—
2,3,4,4′,5-PeCB (114)	0,000 03	—
2,3′,4,4′,5-PeCB (118)	0,000 03	—
2′,3,4,4′,5-PeCB (123)	0,000 03	—
2,3,3′,4,4′,5-HxCB (156)	0,000 03	—
2,3,3′,4,4′,5′-HxCB (157)	0,000 03	—
2,3′,4,4′,5,5′-HxCB (167)	0,000 03	—
2,3,3′,4,4′,5,5′-HpCB (189)	0,000 03	—

Annex B
(informative)

Example for the clean-up of PCBs/PCDDs/PCDFs and the separation of PCBs from PCDDs/PCDFs

B.1 General

This annex describes a clean-up method for a separate analysis of PCBs and PCDDs/PCDFs. The separation of PCBs from dibenzodioxins/dibenzofurans is carried out on an aluminium oxide column by different liquid phases and further separation of the PCB into a "non-*ortho*-fraction" (77, 81, 126, 169) and a fraction of PCB without "non-*ortho*-PCB". The clean-up follows the scheme below.

Sample extraction follows Clause 6. The $^{13}C_{12}$-labelled PCB congeners (see Table 2) are added to the sampling compartments as well as the $^{13}C_{12}$-labelled PCDD/PCDF congeners.

If this clean-up procedure is used, the calculation scheme of Table 6 cannot be used and the following alternative is recommended:

Table B.1 — Alternative calculation scheme for the recovery rates of the sampling standards

Sampling standard	Extraction standard
$^{13}C_{12}$-2,3,4,4′-TeCB (PCB 60)	$^{13}C_{12}$-2,3′,4,4′,5-PeCB (PCB 118)
$^{13}C_{12}$-3,3′,4,5,5′-PeCB (PCB 127)	$^{13}C_{12}$-3,3′,4,4′,5-PeCB (PCB 126)
$^{13}C_{12}$-2,3,3′,4,5,5′-HxCB (PCB 159)	$^{13}C_{12}$-2,3,3′,4,4′,5-HxCB (PCB 156)

B.2 Method

B.2.1 Chromatography column I

A column is filled with silica treated with different substances.

A glass column of 35 mm internal diameter, 300 mm length and a coarse glass sinter frit (porosity P 160 according to ISO 4793) is filled from bottom to top with 2 g of silica (5.2.9), 5 g of potassium hydroxide coated silica (5.2.10), 1 g of silica, 10 g of sulfuric acid coated silica (5.2.11), 1 g of silica, 5 g of $AgNO_3$ coated silica (5.2.12), 5 g of sodium sulfate (5.2.8).

The column is pre-washed with 120 ml of *n*-hexane (5.2.2). The sample extract of about 20 ml is placed on the sodium sulfate top layer. PCDDs/PCDFs and PCBs are eluted with 250 ml of *n*-hexane.

B.2.2 Chromatography column II

The column contains aluminium oxide B Super [3].

A glass column of 22 mm internal diameter, 250 mm length and a coarse glass sinter frit (porosity P 160 according to ISO 4793) is filled with *n*-hexane (5.2.2) followed by 25 g of aluminium oxide B Super I (5.2.7) and 10 g of sodium sulfate (5.2.8). The column is prewashed with 60 ml of *n*-hexane. The concentrated extract of about 5 ml from chromatography column I is placed on the top of the sodium sulfate layer.

Elution is done with 60 ml of *n*-hexane, 90 ml of toluene (5.2.1) and 200 ml of a mixture of *n*-hexane (5.2.2) and dichloromethane (5.2.3) (1+1 parts by volume). The first fraction is discarded, the second contains the PCBs and the third the PCDDs/PCDFs. The solvent of both fractions is evaporated to a volume of about 2 ml

in a vacuum-controlled rotary evaporator (5.1.8), and further concentration is executed in a stream of nitrogen to a volume of 100 µl. To avoid evaporation to dryness, it is advisable to add a high boiling solvent (e.g. *n*-tetradecane, 5.2.17) to the fraction (as a keeper) before concentrating to 50 µl to 100 µl. The volume of the keeper will depend on the final fraction volume.

NOTE Aluminium oxide B Super I is extremely moisture-sensitive. To avoid losses of activity and resultant selective losses, it is advisable to keep small batches of aluminium oxide in dry containers (e.g. desiccators or a glove box).

B.2.3 Additional clean-up I

An additional clean-up is recommended if a sample is very "dirty" or if the columns are to be preserved.

Chromatography with high performance liquid chromatography.

Column: ET 200/4 Nucleosil® 100-5 NO_2 [6].

Elution of the corresponding fraction (PCDDs/PCDFs or PCBs) is achieved with hexane/diethyl ether (95 + 5 parts by volume) and a flow of 0,5 ml/min. The fraction of 7 min to 15 min contains the PCDDs/PCDFs or the PCBs.

B.2.4 Additional clean-up II for separation of non-*ortho*-PCBs

B.2.4.1 General

The separation of non-*ortho*-PCBs (77, 81, 126, 169) on a carbon column is shown in Figure B.1.

B.2.4.2 Carbon column

It is advisable to separate the non-*ortho*-congeners, PCB 77, PCB 81, PCB 126, and PCB 169 from other PCB-congeners by an additional run through a column chromatograph with activated carbon (see B.2.4.3).

In some cases, the additional carbon clean-up causes problems with respect to the recovery.

B.2.4.3 Preparation of carbon column

Activated carbon [7] and Celite (0,02 mm-0,1 mm) [8] are mixed at 1 + 99 parts by volume (conditioning at 200 °C 20 h in a nitrogen stream). A glass column of 22 mm internal diameter, 250 mm length and a coarse glass sinter frit (porosity P 160 according to ISO 4793) is filled from bottom to top with 2 g of silica (5.2.9), 2 g of activated carbon/Celite (1 + 99), 2 g of silica and 10 g of Na_2SO_4 (5.2.8). The column is preliminarily rinsed with 100 ml of toluene (5.2.1) and 100 ml of *n*-hexane (5.2.2). The sample is placed on the Na_2SO_4 top layer and is eluted with 100 ml *n*-hexane/toluene (98+2 parts by volume). This fraction contains the whole PCB group except the non-*ortho*-substituted congeners PCB 77, 81, 126, 169. The second fraction of 75 ml of toluene contains the non-*ortho*-PCBs 77, 81, 126, 169. Both fractions are concentrated to a volume of approximately 2 ml in a vacuum-controlled rotary evaporator (5.1.8). Further concentration is executed with a gentle stream of nitrogen to a volume of 20 µl. In order to avoid evaporation to dryness, it is advisable to add a high boiling keeper (e.g. *n*-tetradecane, 5.2.17) to the fraction before evaporation to 50 µl to 100 µl. The volume of the keeper depends on the final fraction volume.

For addition of the recovery standard (5.2.15), see B.2.5 and Tables 1 and 2.

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6) Column ET 200/4 Nucleosil® 100-5 NO_2 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of this product.

7) Merck 1.02186.1000 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of this product.

8) Merck 1.02693.9010 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of this product.

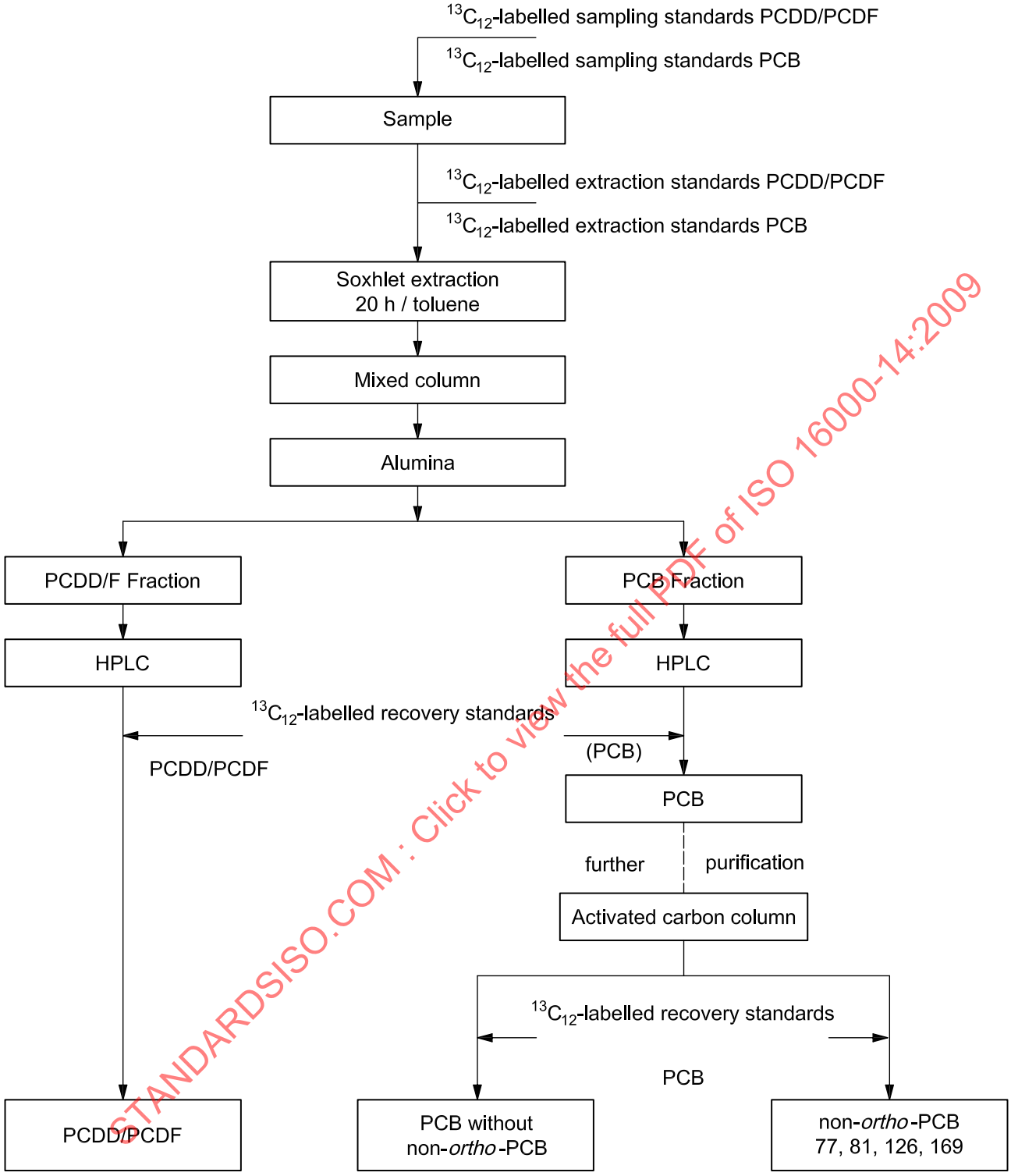


Figure B.1 — Schematic diagram of the separation of non-*ortho*-PCBs (77, 81, 126, 169) on a carbon column

B.2.5 Addition of the recovery standard

$^{13}C_{12}$-labelled PCDD/PCDF congeners (recovery standards, Table 1) are added to the dibenzodioxin/dibenzofuran fraction and $^{13}C_{12}$-labelled PCB congeners (recovery standards, Table 2) are added to the PCB fraction to control the recovery rate. The volumes of the sample extract are then reduced to final volumes of about 10 µl to 25 µl by a gentle stream of nitrogen. The samples (dibenzodioxin/dibenzofuran fraction and PCB fraction) are now ready for quantification.

B.2.6 GC/MS-analysis

GC/MS-analysis can be achieved by the conditions shown in Table C.1, depending on the column used and the injection technique.

Annex C
(informative)

GC/MS analysis

Table C.1 — Conditions of the GC separation

	PCDD/PCDF	PCB	Non-*ortho*-PCB (77, 81, 126, 169)
Injection	splitless 0,2 µl to 1 µl	on column 0,5 µl to1 µl	splitless 0,5 µl to 1 µl
Solvent	*n*-decane	*n*-decane	*n*-decane
Carrier gas	helium	helium	helium
Flow	constant flow 1,2 ml/min	constant flow 1,2 ml/min	constant flow 1,2 ml/min
Column			
Pre-column	deactivated fused silica - column intermediate polarity 5 m, inner diameter: 0,25 mm	deactivated fused silica - column non-polar 5 m, inner diameter: 0,32 mm	deactivated fused silica - column non-polar 5 m, inner diameter: 0,25 mm
Column	Supelco 2361, 60 m inner diameter: 0,25 mm film thickness: 0,1 µm	Hewlett Packard 5, 50 m inner diameter: 0,20 mm film thickness: 0,11 µm	Hewlett Packard 5, 60 m inner diameter: 0,25 mm film thickness: 0,25 µm
Temperature programme			
Initial temp. (°C)	140	145	140
Initial time (min)	1	1,5	1,5
Rate 1 (°C/min)	30	30	30
Final temp. 1 (°C)	220	180	190
Rate 2 (°C/min)	1,2	1,5	1,2
Final temp. 2 (°C)	260	230	240
Rate 3 (°C)		10	10
Final temp. 3 (°C)		300	300
Final time (min)	19	20	20

Figure C.1 shows examples of fly ash and the separation of PCDDs/PCDFs. Figure C.2 also presents examples of the HRGC/HRMS analysis of fly ash and the separation of dioxin-like mono-*ortho*-PCBs on a DB5 [9] column (length: 60 m; inner diameter: 0,25 mm; film thickness: 0,1 µm). Figure C.3 shows examples of the HRGC/HRMS analysis of fly ash and the separation of dioxin-like non-*ortho*-PCBs on the same column. The use of a HT8-PCB column [10] improves the separation of PCB 123 from PCB 118 and PCB 114.

9) Column DB5 is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of this product.

10) Column HT8-PCB is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 16000 and does not constitute an endorsement by ISO of this product.

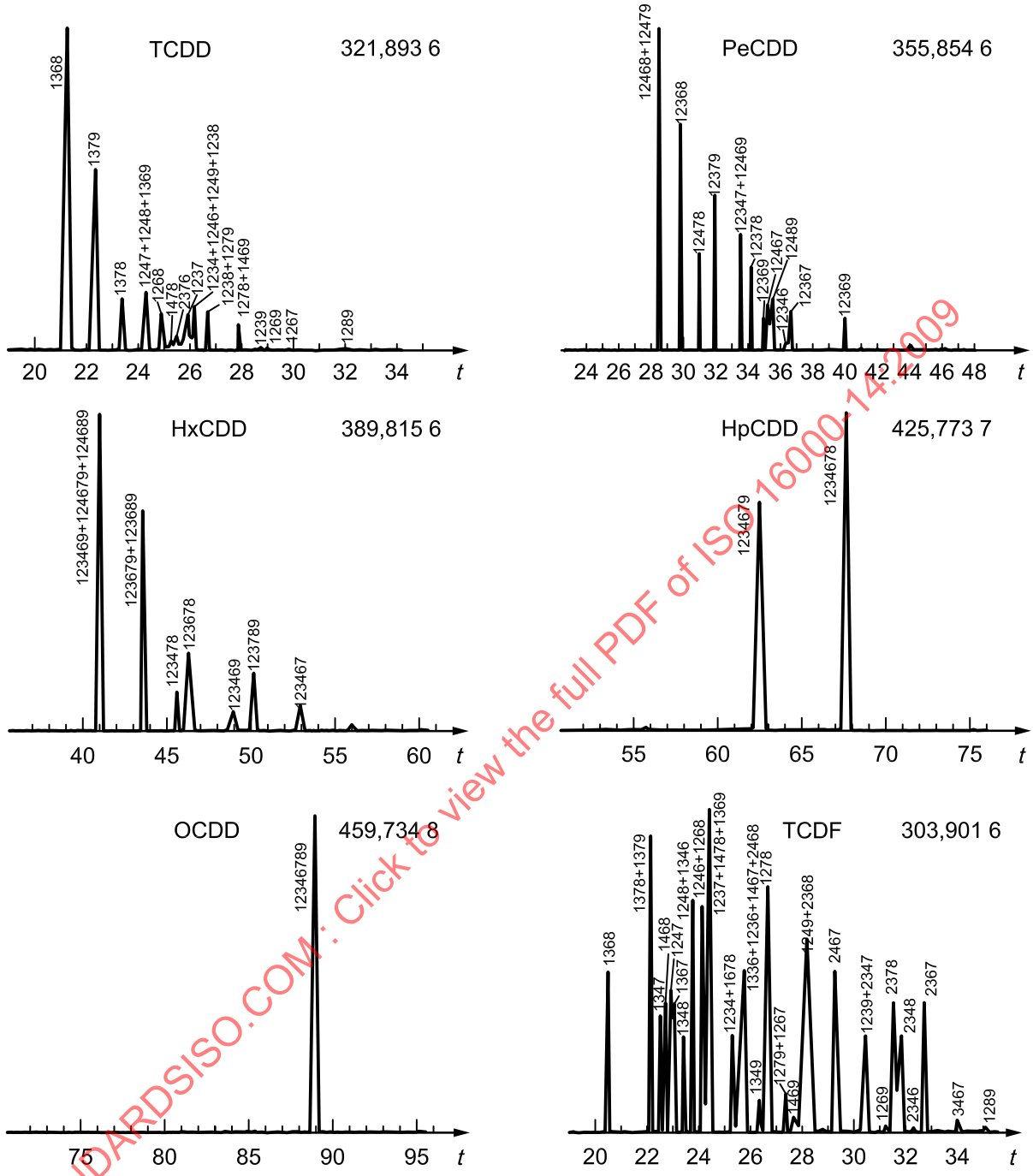
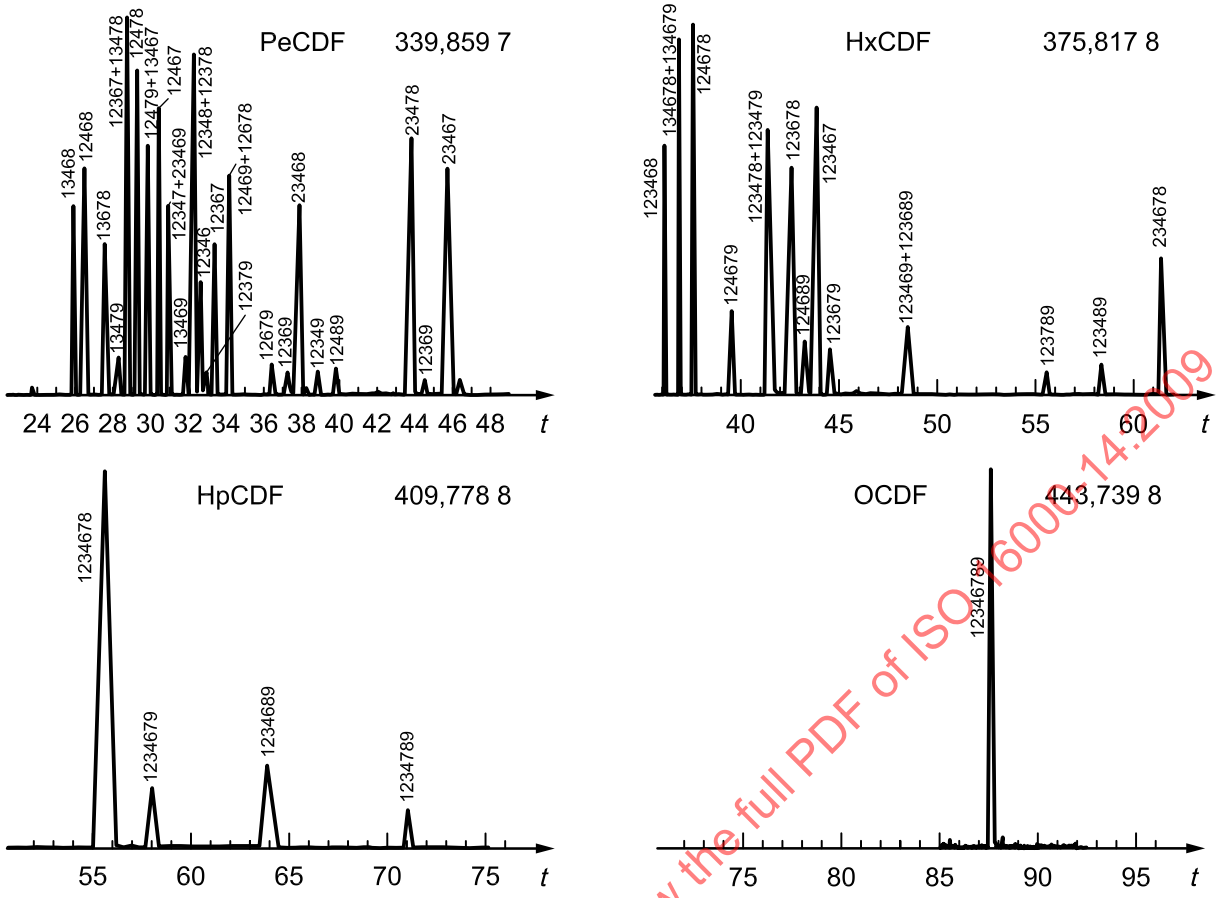


Figure C.1 (*continued*)

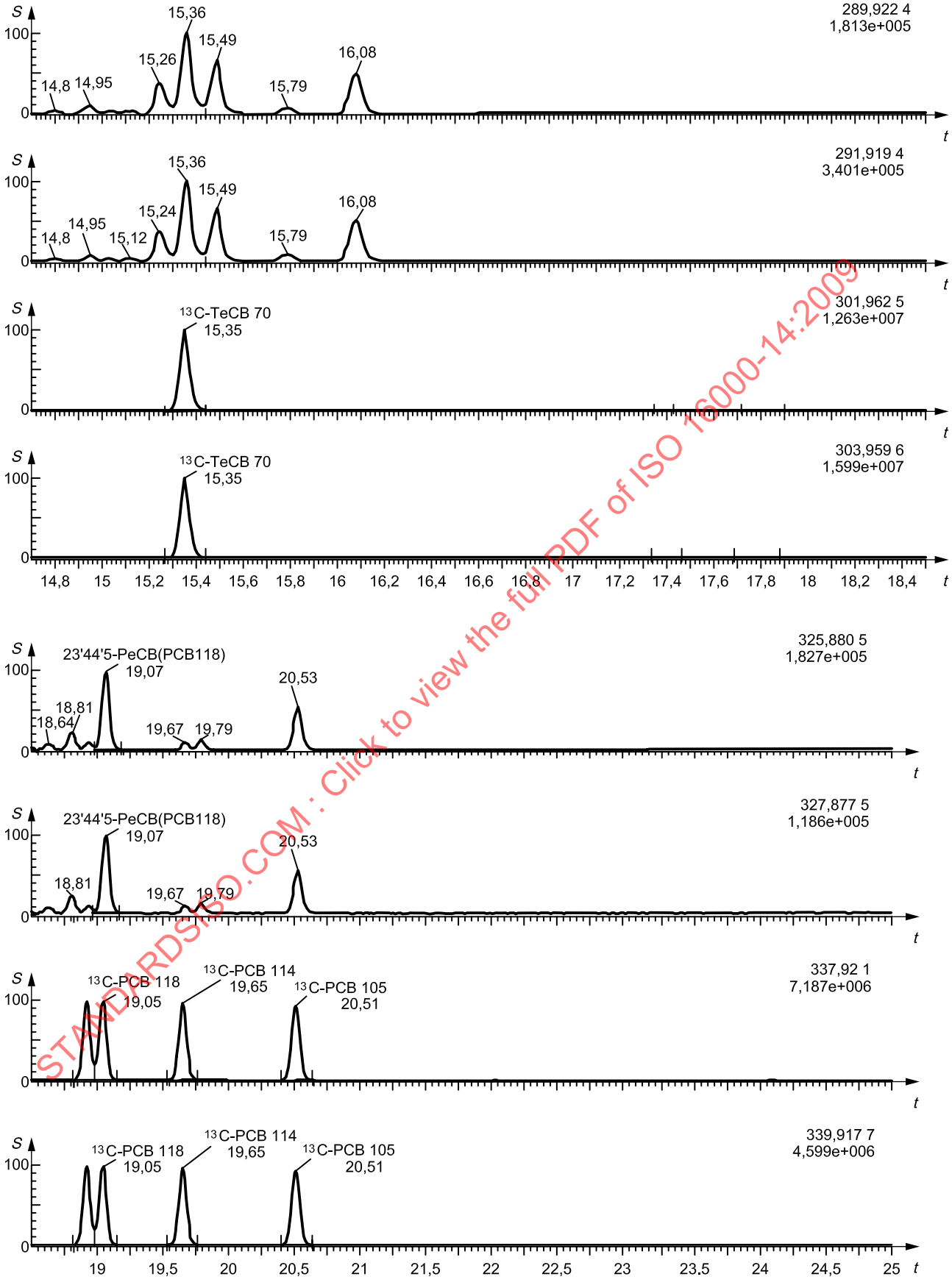


Key

t time, min

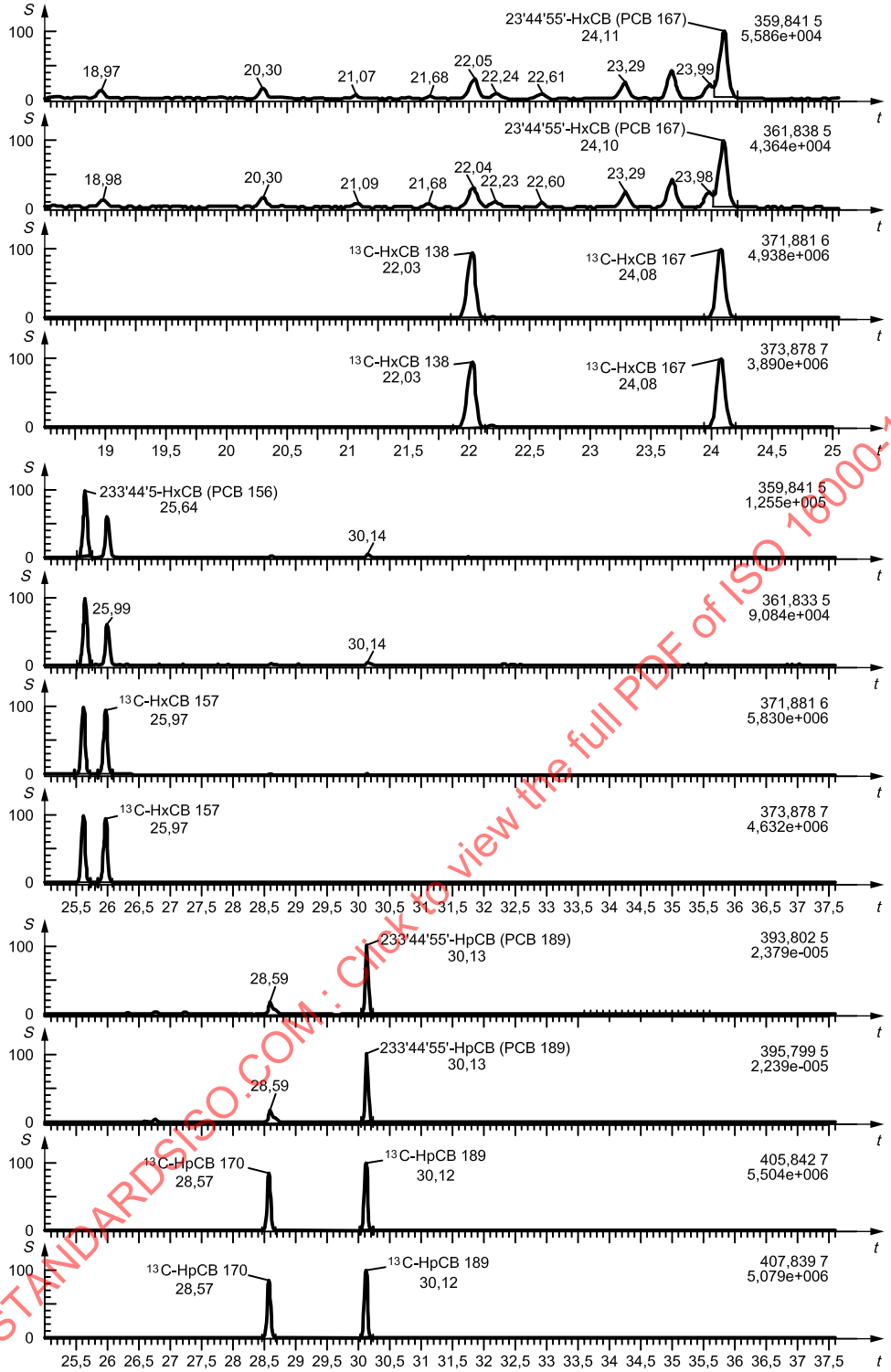
NOTE The number labels on the peaks correspond to the chlorine positions.

Figure C.1 — Example of HRGC/HRMS analysis of fly ash and the separation of the PCDDs and PCDFs



a)

Figure C.2 (*continued*)



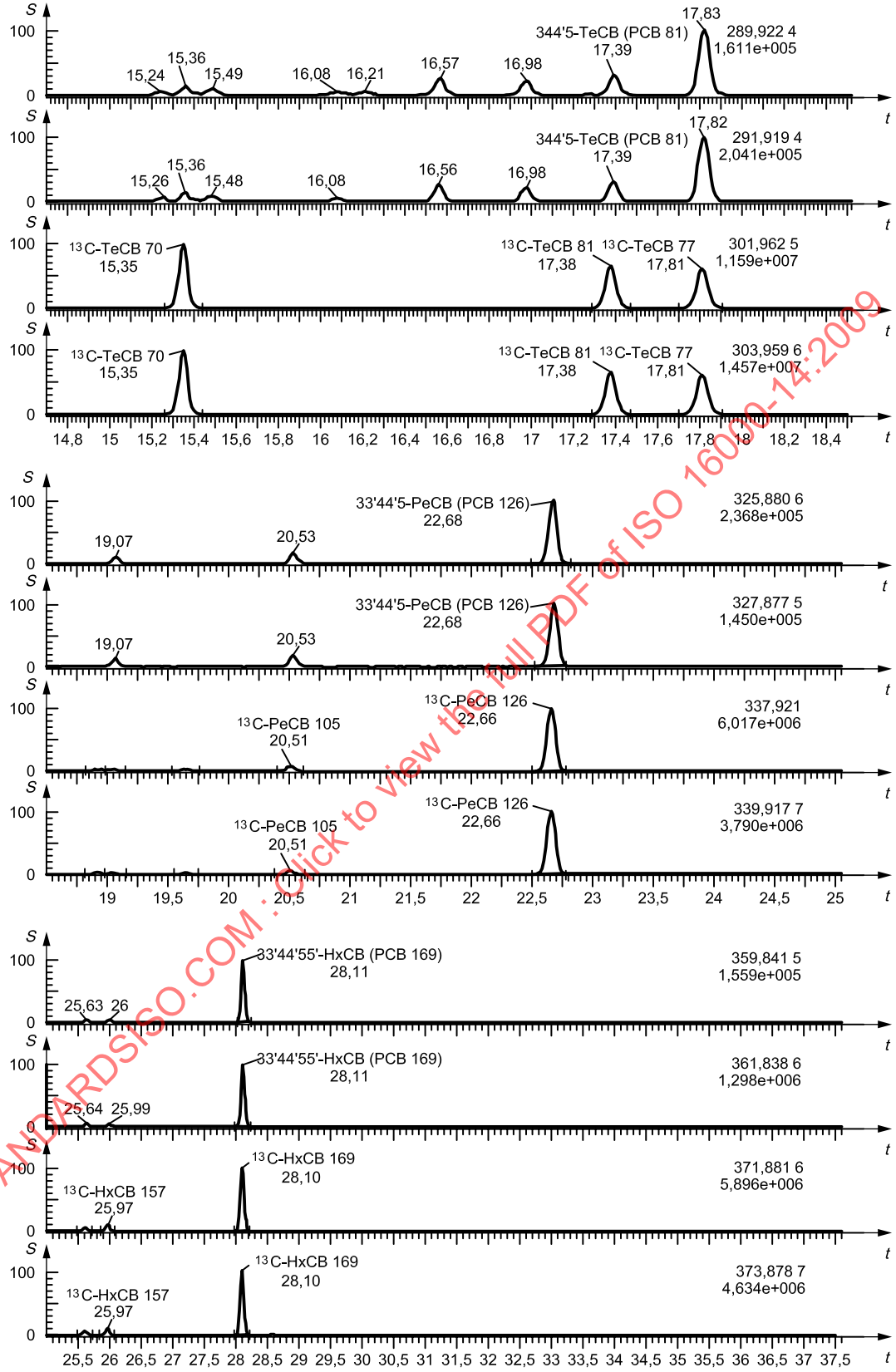
b)

Key

S intensity

t time, min

Figure C.2 — Examples of the HRGC/HRMS analysis of fly ash and the separation of dioxin-like mono-*ortho*-PCBs



Key

S intensity

t time, min

Figure C.3 — Examples of the HRGC/HRMS analysis of fly ash and the separation of dioxin-like non-*ortho*-PCBs

Annex D
(informative)

Masses of the ions for PCDDs/PCDFs and PCBs

Table D.1 shows the theoretical isotope ratio for all PCDDs/PCDFs with 4 to 8 chlorine substituents as an example for monitored PCDDs/PCDFs.

Table D.1 — Mass of ions for PCDD/PCDF

Substance	Ion	Natives	$^{13}C_{12}$ labelled	Relative abundance
TCDF	M	303,901 6	315,941 7	78
	$M+2$	305,898 6	317,938 7	100
TCDD	M	319,896 5	331,936 8	78
	$M+2$	321,893 6	333,933 9	100
PeCDF	M	337,862 7	349,902 9	63
	$M+2$	339,859 7	351,900 0	100
	$M+4$	341,856 8	353,897 0	64
PeCDD	M	353,857 6	365,897 8	63
	$M+2$	355,854 6	367,894 9	100
	$M+4$	357,851 8	369,892 0	64
HxCDF	$M+2$	373,820 7	385,861 0	100
	$M+4$	375,817 8	387,858 1	80
HxCDD	$M+2$	389,815 6	401,855 9	100
HpCDF	$M+2$	407,781 8	419,822 0	100
	$M+4$	409,778 8	421,819 1	80
HpCDD	$M+2$	423,776 7	435,816 9	100
	$M+4$	425,773 7	437,814 0	80
OCDF	$M+2$	441,742 8	453,783 1	88
	$M+4$	443,739 8	455,780 1	100
OCDD	$M+2$	457,737 7	469,778 0	88
	$M+4$	459,734 8	471,775 0	100

Table D.2 shows the theoretical isotope ratio for all PCBs with 4 to 7 chlorine substituents as an example for monitored PCBs.

Table D.2 — Mass of ions for PCBs

	Ion	Natives	$^{13}C_{12}$ labelled	Relative abundance
TeCB	M	289,922 4	301,962 6	78
	$M+2$	291,919 4	303,959 7	100
	$M+4$	293,916 5	305,956 7	48
PeCB	M	323,883 4	335,923 6	63
	$M+2$	325,880 4	337,920 7	100
	$M+4$	327,877 5	339,917 7	64
HxCB	$M+2$	359,841 5	371,881 7	100
	$M+4$	361,838 5	373,878 8	80
HpCB	$M+2$	393,802 5	405,842 8	100
	$M+4$	395,799 5	407,839 8	96