INTERNATIONAL STANDARD

ISO 16000-14

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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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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.

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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.

