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Soil quality — Determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high-resolution mass selective detection (GC/HRMS)

Qualité du sol — Détermination des dioxines et furanes comme biphényls polychlorés par chromatographie en phase gazeuse avec spectrométrie de masse à haute résolution (CG/SMHR)

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Foreword

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

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Introduction

Two groups of related chlorinated aromatic ethers are known as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). They consist of a total of 210 individual substances (congeners): 75 PCDDs and 135 PCDFs.

A group of chlorinated aromatic compounds similar to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) is known as polychlorinated biphenyls (PCBs) which consist of 209 individual substances.

PCDDs and PCDFs can form in the combustion of organic materials. They also occur as undesirable by-products in the manufacture or further processing of chlorinated organic chemicals. PCDDs/PCDFs enter the environment via these emission paths and through the use of contaminated materials. In fact, they are universally present at very small concentrations. The 2,3,7,8-substituted congeners are toxicologically significant. Toxicologically much less significant than the tetrachlorinated to octachlorinated dibenzo-p-dioxins/dibenzofurans are the 74 monochlorinated to trichlorinated dibenzo-p-dioxins/dibenzofurans.

PCBs have been produced over a period of approximately 50 y until the end of the 1990s for the purpose of different uses in open and closed systems, e.g. as electrical insulators or dielectric fluids in capacitors and transformers, as specialized hydraulic fluids, or as a plasticizer in sealing material. Worldwide, more than 1 million tons of PCBs were produced.

PCDD/Fs as well as PCBs are emitted during thermal processes such as waste incineration. In 1997, a group of experts of the World Health Organization (WHO) fixed toxicity equivalent factors (TEF) for PCDDs and 12 PCBs, known as dioxin-like PCBs (see Annex A). These 12 dioxin-like PCBs consist of four non-ortho PCBs and eight mono-ortho PCBs (no or only one chlorine atoms in 2-, 2'-, 6- and 6'-position), having a planar or mostly planar structure. Dioxin-like PCBs can contribute considerably to the total WHO-TEQ.

Only skilled operators who are trained in handling highly toxic compounds should apply the method described in this International Standard.

This International Standard is applicable for several types of matrices and validated for municipal sludge (see Annex B for the results of the validation).

Soil quality — Determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high-resolution mass selective detection (GC/HRMS)

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

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

1 Scope

This International Standard specifies a method for quantitative determination of 17 2,3,7,8-chlorine substituted dibenzo-p-dioxins and dibenzofurans and dioxin-like polychlorinated biphenyls in sludge, treated biowaste, and soil using liquid column chromatographic clean-up methods and GC/HRMS.

The analytes to be determined with this International Standard are listed in Table 1.

Table 1 — Analytes and their abbreviations

Substance	Abbreviation
Tetrachlorodibenzo-p-dioxin	TCDD
Pentachlorodibenzo-p-dioxin	PeCDD
Hexachlorodibenzo-p-dioxin	HxCDD
Heptachlorodibenzo-p-dioxin	HpCDD
Octachlorodibenzo-p-dioxin	OCDD
Tetrachlorodibenzofuran	TCDF
Pentachlorodibenzofuran	PeCDF
Hexachlorodibenzofuran	HxCDF
Heptachlorodibenzofuran	HpCDF
Octachlorodibenzofuran	OCDF
Polychlorinated biphenyl	PCB
Trichlorobiphenyl	TCB
Tetrachlorobiphenyl	TeCB
Pentachlorobiphenyl	PeCB
Hexachlorobiphenyl	HxCB
Heptachlorobiphenyl	HpCB
Decachlorobiphenyl	DecaCB

The limit of detection depends on the kind of sample, the congener, the equipment used, and the quality of chemicals used for extraction and clean-up. Under the conditions specified in this International Standard, limits of detection better than 1 ng/kg (expressed as dry matter) can be achieved.

This method is “performance based”. It is permitted to modify the method if all performance criteria given in this method are met.

NOTE In principle, this method can also be applied for sediments, mineral wastes, and for vegetation. It is the responsibility of the user of this International Standard to validate the application for these matrices. For measurement in complex matrices like fly ashes adsorbed on vegetation, it can be necessary to further improve the clean-up. This can also apply to sediments and mineral wastes.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

3 Abbreviated terms

PCB	polychlorinated biphenyls
PCDD/PCDF or PCDD/F	polychlorinated dibenzo-p-dioxins/dibenzofurans
I-TEF NATO/CCMS	international toxic equivalent factor, proposed by NATO-CCMS in 1988 (for a detailed description, see Annex A)
I-TEQ	international toxic equivalent, obtained by multiplying the mass determined with the corresponding I-TEF including PCDDs and PCDFs (for a detailed description, see Annex A). Should only be used for comparison with older data
WHO-TEF	toxic equivalent factor, proposed by WHO in 2005 (for detailed description, see Annex A)
WHO-TEQ	toxic equivalent, obtained by multiplying the mass determined with the corresponding WHO-TEF including PCDD, PCDF, and PCB (for detailed description, see Annex A). $WHO\text{-}TEQ_{PCB}$, $WHO\text{-}TEQ_{PCDD/F}$ should be used to distinguish different compound classes

4 Principle

This International Standard is based on the use of gas chromatography/mass spectrometry combined with the isotope dilution technique to enable the separation, detection, and quantification of PCDD/PCDF and dioxin-like PCB in sludge, biowaste, and soil. For the isotope dilution method, 17 labelled PCDD/F and 12 labelled PCB internal standards are used. The extracts for the GC-MS measurements contain one or two recovery standards. The gas chromatographic parameters offer information which enables the identification of congeners (position of chlorine substitutes) whereas the mass spectrometric parameters enable the differentiation between isomers with different numbers of chlorine substitutes and between dibenzo-p-dioxins, furans, and PCBs.

$^{13}C_{12}$-labelled PCDD/F and PCB congeners are added to the sample prior to extraction and GC/HRMS measurement. Losses during extraction and clean-up are detected and compensated by using these added congeners as internal standards for quantification together with recovery standards which are added just before the GC/HRMS analysis. For the determination of these substances, it is necessary to separate PCBs from PCDDs/PCDFs and vice versa.

The main purpose of the clean-up procedure of the raw sample extract is the removal of sample matrix components, which can overload the separation method, disturb the quantification, or otherwise

severely impact the performance of the identification and quantification method and the separation of PCDD/F from dioxin-like PCB. Furthermore, the enrichment of the analytes in the final sample extract is achieved. Extraction procedures are usually based on Soxhlet or equivalent extraction methods of dried, preferably freeze-dried, samples. Sample clean-up is usually carried out by multi-column liquid chromatographic techniques using different adsorbents. The determination of PCDD/Fs and PCBs is based on quantification by the isotope dilution technique using GC/HRMS.

5 Reagents

5.1 Chemicals

Solvents used for extraction and clean-up shall be of pesticide grade or equivalent quality and checked for blanks. Adsorbents like aluminium oxide, silica gel, diatomaceous earth, and others used for clean-up shall be of analytical grade quality or better and pre-cleaned and activated if necessary.

NOTE See Annex C for a specific list of solvents and chemicals.

5.2 Standards

— $^{13}C_{12}$-spiking solution for PCDD/F (internal standard);

— $^{13}C_{12}$-spiking solution for PCB (internal standard);

— calibration solutions PCDD/F;

— calibration solutions PCB;

— recovery standard PCDD/F;

— recovery standard PCB.

NOTE See Annex C for examples of concentration of the standard solutions.

6 Apparatus and materials

6.1 General

The apparatus and materials listed below are meant as minimum requirements for “conventional” sample treatment with Soxhlet extraction and column chromatographic clean-up. Additional apparatus and materials may be necessary due to different methods of sample extraction and clean-up methods.

6.2 Equipment for sample preparation

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

6.2.2 Desiccator.

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

6.3 Soxhlet extractor

6.3.1 Soxhlet, 50 mm internal diameter, 150 ml or 250 ml capacity with 500 ml round bottom flask.

6.3.2 Thimble, 43 mm × 123 mm, to fit Soxhlet.

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

6.4 Clean-up apparatus

6.4.1 Disposable pipettes, either disposable Pasteur pipettes, or disposable serological pipettes.

6.4.2 Glass chromatographic columns, of the following sizes:

— 150 mm length × 8 mm internal diameter, with coarse-glass frit or glass-wool plug, 250 ml reservoir, and glass or polytetrafluoroethylene (PTFE) stopcock;

— 200 mm length × 15 mm internal diameter, with coarse-glass frit or glass-wool plug, 250 ml reservoir, and glass or PTFE stopcock;

— 300 mm length × 25 mm internal diameter, with coarse-glass frit or glass-wool plug, 300 ml reservoir, and glass or PTFE stopcock.

6.4.3 Oven, capable of maintaining a constant temperature (±5 °C) in the range of 105 °C to 450 °C for baking and storage of adsorbents.

6.5 Concentration apparatus

6.5.1 Rotary evaporator, equipped with a variable temperature water bath and:

— a vacuum source for the rotary evaporator equipped with a shutoff valve at the evaporator and vacuum gauge;

— a recirculating water pump and chiller, providing cooling water of (9 ± 4) °C (use of tap water for cooling the evaporator wastes large volumes of water and can lead to inconsistent performance as water temperatures and pressures vary);

— round-bottom flask, 100 ml and 500 ml or larger, with ground-glass fitting compatible with the rotary evaporator.

6.5.2 Nitrogen blowdown apparatus, equipped with either a water bath controlled in the range of 30 °C to 60 °C or a heated stream of nitrogen, installed in a fume hood.

6.5.3 Kuderna-Danish[1)] **concentrator**.

6.5.4 Sample vials, of the following types:

— amber glass, nominated volume 2 ml to 5 ml, with PTFE-lined screw cap;

— glass, 0,3 ml, conical, with PTFE-lined screw or crimp cap.

6.6 Other equipment

6.6.1 Gas chromatograph, equipped with a splitless or on-column or temperature-programmed injection port for use with capillary columns, and an oven temperature programme which enables isothermal hold.

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

6.6.2 GC column for PCDDs/PCDFs and for isomer specificity for 2,3,7,8-TCDD (e.g. 60 m length × 0,32 mm internal diameter; 0,25 µm; 5 % phenyl, 94 % methyl, 1 % vinyl silicone bonded-phase fused-silica capillary column).

6.6.3 Mass spectrometer, 28 eV to 80 eV electron impact ionization, capable of repetitively selectively monitoring 12 exact masses minimum at high resolution (>10 000) during a period of approximately 1 s.

6.6.4 Data system, capable of collecting, recording, and storing mass spectrometric data.

7 Sample storage and sample pretreatment

7.1 Sample storage

Samples should be stored in suitable containers with an appropriate closure material such as polytetrafluoroethylene (PTFE). Samples to be frozen can be stored in aluminium containers pre-cleaned by heating to 450 °C for a minimum of 4 h or by rinsing with a non-chlorinated solvent.

Samples should be kept cold (<8 °C) and in the dark. The sample pretreatment should take place within 3 d of sampling. Alternatively, samples can be frozen (–18 °C) directly after sampling and kept frozen before sample pretreatment.

7.2 Sample pretreatment

Drying and homogenization should be carried out according to ISO 14507, if not otherwise specified. Store the ground material in a desiccator or a tightly closed glass container.

8 Extraction and clean-up

8.1 General

In this International Standard, the minimum requirements for extraction and clean-up to be met are described as well as examples of operation. The analyst can use any of the procedures given below and in Annex C or any suitable alternative procedures.

The determination of PCDDs/PCDFs is based on quantification by the isotope dilution technique using GC/HRMS. $^{13}C_{12}$-labelled 2,3,7,8-chlorine substituted PCDD/PCDFs congeners are added at different stages of the whole method. Losses during extraction and clean-up can be detected and compensated by using these added congeners as internal standards for quantification together with recovery standards which are added just before the GC/HRMS analysis. However, due to possible differences in the binding and adsorption characteristics between the native PCDDs/PCDFs and the $^{13}C_{12}$-labelled congeners, which are added during analysis, complete substantiation of the extraction efficiency and compensation of losses during clean-up is not ensured. Therefore, in addition, the applied methods shall be validated thoroughly. Examples of well-proven extraction and clean-up methods are given in Annex C.

The main purpose of the clean-up procedure of the raw sample extract is the removal of sample matrix components, which can overload the separation method, disturb the quantification, or otherwise severely impact the performance of the identification and quantification method and to separate dioxin-like PCB from PCDD/F. Furthermore, an enrichment of the analytes in the final sample extract is achieved. Extraction procedures are usually based on Soxhlet extraction of the <2 mm fraction of the dry and ground or sieved solid sample. Sample clean-up is usually carried out by multi-column liquid chromatographic techniques using different adsorbents.

In principle, any clean-up method can be used which recovers the analytes in sufficient quantities. Furthermore, the final sample extract shall not affect adversely the performance of the analytical system or the quantification step. However, all applied methods shall be tested thoroughly and shall pass a

set of method validation requirements before they can be employed. In addition, the verification of the method performance for each single sample shall be part of the applied quality assurance protocol.

8.2 Extraction

The sample amount used for extraction can vary from 5 g to 50 g depending on the expected level of contamination.

The internal standard consisting of $^{13}C_{12}$-labelled congeners listed in Table 2 shall be added directly into the sample before extraction.

The extraction procedure is carried out using Soxhlet extraction with toluene. The duration of extraction should be adjusted according to the kind and amount of sample used. The minimum requirement is 50 extraction cycles or approximately 12 h.

Other solvents or other methods like pressurized liquid extraction can also be used but shall be of proven equal performance.

Table 2 — $^{13}C_{12}$-labelled congeners included in the internal standard

$^{13}C_{12}$-spiking solution — internal standard	
PCDD/F congeners	**PCB congeners**
2,3,7,8-$^{13}C_{12}$-TCDD	$^{13}C_{12}$-PCB-77
1,2,3,7,8-$^{13}C_{12}$-PeCDD	$^{13}C_{12}$-PCB-81
1,2,3,4,7,8-$^{13}C_{12}$-HxCDD	$^{13}C_{12}$-PCB-126
1,2,3,6,7,8-$^{13}C_{12}$-HxCDD	$^{13}C_{12}$-PCB-169
1,2,3,7,8,9-$^{13}C_{12}$-HxCDD	
1,2,3,4,6,7,8-$^{13}C_{12}$-HpCDD	$^{13}C_{12}$-PCB-105
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-PCB-114
	$^{13}C_{12}$-PCB-118
2,3,7,8-$^{13}C_{12}$-TCDF	$^{13}C_{12}$-PCB-123
1,2,3,7,8-$^{13}C_{12}$-PeCDF	$^{13}C_{12}$-PCB-156
2,3,4,7,8-$^{13}C_{12}$-PeCDF	$^{13}C_{12}$-PCB-157
1,2,3,4,7,8-$^{13}C_{12}$-HxCDF	$^{13}C_{12}$-PCB-167
1,2,3,6,7,8-$^{13}C_{12}$-HxCDF	$^{13}C_{12}$-PCB-189
2,3,4,6,7,8-$^{13}C_{12}$-HxCDF	
1,2,3,7,8,9-$^{13}C_{12}$-HxCDF	
1,2,3,4,6,7,8-$^{13}C_{12}$-HpCDF	
1,2,3,4,7,8,9-$^{13}C_{12}$-HpCDF	
$^{13}C_{12}$-OCDF	

8.3 Clean-up

8.3.1 General

Clean-up methods shall prepare the sample extract in an appropriate manner for the subsequent quantitative determination. Clean-up procedures shall concentrate PCDD/Fs and dioxin-like PCBs in the extracts and remove interfering matrix components present in the raw extract.

Proven clean-up procedures shall be used including usually two or more of the following techniques which can be combined in different orders. A detailed description of some of the procedures is given in Annex C.

Other methods can also be used but shall be of proven equal performance as the techniques described below.

8.3.2 Gel permeation chromatography

The interesting molecular weight range for PCDD/Fs and dioxin-like PCBs of 200 g/mol to 500 g/mol can be isolated from larger molecules and polymers which might overload other clean-up methods. This method can also be used for the removal of sulfur.

8.3.3 Multilayer column

Multilayer column liquid chromatography using silica with different activity grades and surface modifications. Compounds with different chemical properties than PCDD/Fs and dioxin-like PCBs can be removed.

8.3.4 Sulfuric acid treatment

A direct treatment of the sample extract with sulfuric acid is possible but is not recommended due to risk of accident. If applied, this shall be carried out very carefully to avoid losses of PCDD/Fs and dioxin-like PCBs on the formed carboniferous surfaces.

8.3.5 Activated carbon column

Column adsorption chromatography using activated carbon can be used to separate planar PCDD/F and coplanar PCB molecules from mono-ortho PCB and other interfering non-planar molecules.

8.3.6 Aluminium oxide column

Column liquid chromatography on aluminium oxide of different activity grade and acidity/basicity. Interfering compounds with small differences in polarity or structure compared to PCDD/Fs and dioxin-like PCBs can be removed.

Additionally, aluminium oxide columns can be used to separate PCDD/Fs from dioxin-like PCBs.

8.3.7 Removal of sulfur

The removal of sulfur can be achieved by refluxing the extract with powdered copper or by gel permeation chromatography.

8.4 Final concentration of cleaned sample extract

To achieve sufficient detection limits, the cleaned sample extract shall be concentrated to a volume in the order of 25 µl to 100 µl before quantification. The final solvent shall be nonane, toluene, or another solvent with a high boiling point.

Though PCDD/Fs have rather high boiling points (>320 °C), 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, the following precautions shall be taken into consideration:

a) Rotary evaporators

Losses might be substantial when reducing solvent volumes below 10 ml. Counter measures include 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