INTERNATIONAL STANDARD

ISO 13914

First edition
2013-12-01

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)

Reference number
ISO 13914:2013(E)

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

Published in Switzerland

Contents

Page

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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

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 around 200 µl.

d) Kuderna Danish

To avoid initial losses, prewet the column with about 1 ml of solvent. Boiling chips should be added and the vertical position of the apparatus should be adjusted. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. Adjust the water bath temperature accordingly. When reaching an extract volume of 1 ml, remove the evaporation flask, replace the snyder column with a smaller one, and continue the evaporation.

8.5 Addition of recovery standard

The very last step before quantification is the addition of the recovery standards for calculation of the recovery rates of the internal standards.

Recovery standards shall be added just prior to the quantification procedure. Samples with the recovery standard added which could not be analysed due to operational reasons (instrument failure) should be stored as briefly as possible and any further uncontrolled solvent evaporation shall be avoided.

Recovery standards shall be added after the final volume reduction. Any further direct volume reduction shall be avoided. A slow evaporation at room temperature from the open sample vial to a volume of about 25 µl is acceptable.

9 GC/HRMS analysis

9.1 General

GC-MS analyses of PCDD/Fs and dioxin-like PCBs shall be carried out on a high-resolution GC-MS instrument equipped with a high-resolution gas chromatograph, an autosampler, a high-resolution mass spectrometer, and a data system for instrument control, data acquisition, and processing.

9.2 Gas chromatographic analysis

Gas chromatographic separation shall be carried out in such a way that sufficient separation of all PCDD/F and dioxin-like PCB congeners is achieved and the quality criteria specified in 9.4 and 9.5 are met.

For PCDD/F, there is no capillary column available at present that allows the separation of all 2,3,7,8-substituted congeners from all other non-2,3,7,8-substituted congeners. Complete separation can only be achieved by analysing a sample on different capillary columns of different polarity.

For dioxin-like PCB analysis, similar problems exist for the separation of all coplanar and mono-ortho congeners. There is no column available at present which is able to separate all 12 dioxin-like PCB congeners from all other non-dioxin-like PCB congeners.

9.3 Mass spectrometric detection

A high-resolution mass spectrometer at a minimum resolution of 10 000 is used for the detection of PCDD/F and dioxin-like PCB. This allows the use of $^{13}C_{12}$-labelled congeners as internal standards for all 17 PCDD/F congeners and 12 dioxin-like PCB congeners of interest.

The mass spectrometer is used in the MID mode (multiple ion detection). The GC column is directly coupled to the mass spectrometer. The ion source temperature should be between 250 °C to 270 °C depending on the type of instrument. To achieve appropriate sensitivity, the detection capability should be at least 200 fg for 2,3,7,8-TCDD.

For identification and quantification, the masses given in Table 3 and Table 4 shall be recorded in MID mode. For each PCDD/F or PCB congener of interest, at least two ions of the molecular isotope cluster shall be recorded for both the native and the added $^{13}C_{12}$-labelled congeners.

In addition, masses for quality control of the mass calibration shall be measured depending on the type of instrument, e.g. lock mass, calibration mass, lock mass check.

The time slots for the MID windows shall be defined by a calibration standard in a way that all congeners of interest elute within the related MID window. In case the sum of the concentrations of isomer groups are needed, the retention time window for all isomers of an isomer group shall be defined by measuring a standard mixture containing the first and last eluting isomers of each isomer group corresponding to the used GC column. As an alternative, a fly ash extract or any other solution containing all native PCDD/F congeners can be used.

Table 3 — Masses for the detection and quantification of PCDD/F

Substance	Dibenzofurans		Dibenzo-p-dioxins	
	^{12}C	^{13}C	^{12}C	^{13}C
Tetra-CDD/F	303,901 6	315,941 9	319,896 5	331,936 8
	305,898 7	317,938 9	321,893 7	333,933 9
Penta-CDD/F	339,859 8	351,900 0	355,854 7	367,894 9
	341,856 9	353,897 0	357,851 8	369,891 9
Hexa-CDD/F	373,820 8	385,861 0	389,815 7	401,855 9
	375,817 9	387,858 0	391,812 8	403,852 9
Hepta-CDD/F	407,781 8	419,822 0	423,776 7	435,816 9
	409,778 9	421,819 0	425,773 8	437,814 0
Octa-CDD/F	441,742 8	453,783 0	457,737 7	469,777 9
	443,739 9	455,780 1	459,734 8	471,775 0

Table 4 — Masses for the detection and quantification of PCB

Homologue groups	^{12}C	^{13}C
Trichloro-PCB	255,961 3	268,001 6
	257,958 4	269,998 6
Tetrachloro-PCB	289,922 3	301,962 6
	291,919 4	303,959 7
Pentachloro-PCB	325,880 4	337,920 7
	327,877 5	339,917 7
Hexachloro-PCB	359,841 5	371,881 7
	361,838 5	373,878 8
Heptachloro-PCB	393,802 5	405,842 7
	395,799 5	407,839 8
Octachloro-PCB	427,763 5	439,803 8
	429,760 6	441,800 8
Nonachloro-PCB	461,724 5	473,764 8
	463,721 6	475,761 8
Decachloro-PCB	497,682 6	509,722 9
	499,679 7	511,719 9

9.4 Minimum requirements for identification of PCDF/PCDD and PCB

9.4.1 The isotope ratio between the two ions of the molecular isotope cluster which are recorded shall match the theoretical value within ±15 % (see Table 5).

Table 5 — Limits of isotope ratios

Substance	Isotope ratio lower limit	Isotope ratio theoretical value	Isotope ratio upper limit
TCDD/F	0,65	0,77 (M/M+2)	0,88
PeCDD/F	0,55	0,64 (M+4/M+2)	0,75
HxCDD/F	0,69	0,81 (M+4/M+2)	0,94
HpCDD/F	0,83	0,96 (M+4/M+2)	1,13
OCDD/F	0,74	0,89 (M+2/M+4)	1,009

9.4.2 The retention time of a native 2,3,7,8-chlorine substituted isomer (Cl_4- to Cl_6-congeners) shall be within a time window of +3 s to –3 s based on the retention time of the corresponding $^{13}C_{12}$-labelled isomer in the sample. For the identification of low concentrations ($S/N < 10$), a time window of ±10 s is acceptable. Alternatively, relative retention times based on the recovery standard (e.g. $^{13}C_{12}$-1,2,3,4-TCDF) can be calculated. The difference shall not be more than 0,3 % compared with the calibration standard.

9.4.3 The signal-to-noise ratio of the raw data shall be at least 3:1 for three consecutive scans for the signal used for identification. The base line 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 are taken.

9.5 Minimum requirements for quantification of PCDF/PCDD and PCB

9.5.1 For PCDD/F analysis, there is no chromatographic column available at present that is able to separate all 2,3,7,8-chlorine substituted congeners from all other non-2,3,7,8-chlorine substituted congeners. Complete separation can only be achieved by multi-analysis of the sample on different columns of different natures (polarity).

Single column data can therefore be reported by this method. However, in cases where a regulatory limit is exceeded or congener-specific data are needed, a confirmatory analysis should be performed on a second column.

For dioxin-like PCB analysis, similar problems exist for the separation of all coplanar and mono-ortho congeners. There is no column available at present which is able to separate all 12 dioxin-like PCB congeners from all other non-dioxin-like PCB congeners. The use of one relatively non-polar column (e.g. DB-5) is the common technique. The separation of congener PCB-123 is the crucial point of the gas chromatographic separation. But due to the minor contribution to the overall TEQ, this leads to an inessential increase of the uncertainty of the method.

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

9.5.3 2,3,7,8-TCDD shall be separated from all other interfering isomers within a 25 % valley below the top of the minor peak with respect to the height of that peak.

9.5.4 The recovery rate of each individual 2,3,7,8-chlorine substituted PCDD/PCDF of the internal standards in each sample shall be within:

— 50 % to 130 % for the tetra- to hexa-chlorinated congeners or

— 40 % to 130 % for the hepta- and octachlorinated congeners.

NOTE If the above ranges are exceeded for one or more congeners, then the ranges given below are acceptable for congeners with recoveries not within these ranges if the sum of the concentrations of those congeners contribute less than 10 % to the total TEQ in the sample.

— 30 % to 150 % for the tetra- to hexa-chlorinated congeners or

— 20 % to 150 % for the hepta- and octa-chlorinated congeners.

9.5.5 The signal-to-noise ratio of the signal of the $^{13}C_{12}$-labelled congeners used for quantification shall be >20:1.

9.5.6 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 15 % and shall be based on a minimum of five measuring points over the whole range.

9.5.7 An analytical blank shall be analysed as defined in 9.6. The blank values of all congeners of interest shall be equal or less than the detection limit of the method. Alternatively, the levels found shall be at least a factor of 10 below the lowest measured concentrations in the series of samples.

9.6 Calibration of the GC/HRMS system

9.6.1 General

The calibration is carried out with at least five calibration solutions. These solutions contain all native congeners of interest in different precisely defined amounts and all $^{13}C_{12}$-labelled standards (internal and recovery standards) in the same concentrations as expected in the spiked sample solutions assuming 100 % recovery. The calibration range should encompass the concentrations of the sample.

9.6.2 Calibration for 2,3,7,8-congeners

The calibration curve is used to calculate the relative response factors for each congener of interest. The relative response factors are used together with the $^{13}C_{12}$-labelled congeners added to the sample to quantify the mass of the native congeners of interest by the isotope dilution method.

Calibration frequency depends on the stability of the instrument. Daily calibration checks shall be run. In addition, a full calibration shall be repeated after major changes such as:

a) use of new or repaired equipment;

b) replacement of GC columns;

c) after cleaning of the separation and detection systems;

d) if the deviation of an injected calibration standard exceeds 20 %.

The relative response factor for congener *i* is defined and calculated as given in Formula (1):

$$rrf_i = \frac{A_i\left[^{12}C\right]}{A_i\left[^{13}C\right]} \cdot \frac{c_i\left[^{13}C\right]}{c_i\left[^{12}C\right]} \quad (1)$$

where

rrf_i is the relative response factor of native congener *i* relative to $^{13}C_{12}$-labelled congener *i*;

$A_i[^{12}C]$ is the area of native congener *i*;

$A_i[^{13}C]$ is the area of $^{13}C_{12}$-labelled congener *i;*

$c_i[^{12}C]$ is the concentration of native congener *i* in the calibration solution;

c_i $[^{13}C]$ is the concentration of $^{13}C_{12}$-labelled congener *i* in the calibration solution.

9.6.3 Calibration for sum of homologue groups

The calibration of the mass spectrometer is done in the same way and with the same calibration solutions as for single congeners. The relative response factors for each homologue group is calculated by the addition of all peak areas of all native congeners of the same homologue group which are included in the calibration solution relative to one $^{13}C_{12}$-labelled congener. Table 6 shows the relations between native congeners and $^{13}C_{12}$-labelled congeners.

Table 6 — Relation for calibration of homologue groups

Substance	Calibration of PCDD homologues		Calibration of PCDF homologues	
	Native isomer	^{13}C isomer	Native isomer	^{13}C isomer
Tetrachloro homologues	2,3,7,8	2,3,7,8	2,3,7,8	2,3,7,8
Pentachloro homologues	1,2,3,7,8	1,2,3,7,8	1,2,3,7,8 2,3,4,7,8	1,2,3,7,8
Hexachloro homologues	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9	1,2,3,7,8,9	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9 2,3,4,6,7,8	2,3,4,6,7,8
Heptachloro homologues	1,2,3,4,6,7,8	1,2,3,4,6,7,8	1,2,3,4,6,7,8 1,2,3,4,7,8,9	1,2,3,4,6,7,8

9.7 Quantification of GC/HRMS results

9.7.1 Quantification of concentrations of 2,3,7,8-congeners

The concentration of congener *i* in the sample is calculated using Formula (2):

$$c_i[^{12}C] = \frac{A_i[^{12}C]}{A_i[^{13}C]} \cdot \frac{c_i[^{13}C]}{rrf_i} \quad (2)$$

where

rrf_i is the relative response factor of native congener *i* relative to $^{13}C_{12}$-labelled congener *i*;

$A_i[^{12}C]$ is the area of native congener *i*;

$A_i[^{13}C]$ is the area of $^{13}C_{12}$-labelled congener *i;*

$c_i[^{12}C]$ is the concentration of native congener *i* in the sample;

$c_i[^{13}C]$ is the concentration of $^{13}C_{12}$-labelled congener *i* in the sample.

The concentrations of all congeners of interest in the samples shall be within the linear range of the method. High concentrations of native congeners will cause overlapping in the mass window between high isotopic ions (i.e. M+12, M+14) of the native congeners with the lower isotopic ions (i.e. M, M+2) of the $^{13}C_{12}$-labelled standards especially for higher chlorinated congeners. This will result in a significant deviation from linearity beyond a mass ratio of 100. An overestimation of the recovery rate and an underestimation of the amount of the native congener caused by this should be avoided. Samples exceeding the mass ratio by more than 100 shall be repeated with smaller amounts of sample.

9.7.2 Quantification of recovery rates of $^{13}C_{12}$-labelled standards

The recovery rates of the internal standards are quantified against the recovery standard using Formula (3):

$$R_i = \frac{A_{i[E]}}{A_{[R]}} \cdot \frac{c_{[R]}}{rrf_i} \cdot \frac{100}{c_{i[E]}} \quad (3)$$

where

R_i is the recovery rate of the internal standard, in percent;

rrf_i is the relative response factor of internal standard *i* relative to the $^{13}C_{12}$-labelled recovery standard;

$A_{[R]}$ is the area of the recovery standard;

$A_{i[E]}$ is the area of internal standard *i;*

$c_{[R]}$ is the concentration of the recovery standard;

$c_{i[E]}$ is the concentration of internal standard *i*.

9.7.3 Quantification of the sum of homologue groups

The sum of concentrations of all congeners of a homologue group in the sample is calculated as given in Formula (4):

$$C_h[^{12}C] = \frac{\sum A_i[^{12}C]}{A_i[^{13}C]} \cdot \frac{c_i[^{13}C]}{rrf_i} \quad (4)$$

where

rrf_i is the relative response factor of native congener i relative to $^{13}C_{12}$-labelled congener i;

$\Sigma A_i[^{12}C]$ is the sum of areas of all native congeners of a homologue group;

$A_i[^{13}C]$ is the area of $^{13}C_{12}$-labelled congener i;

$C_h[^{12}C]$ is the sum of concentrations of all native congeners of a homologue group in the sample;

$c_i[^{13}C]$ is the concentration of $^{13}C_{12}$-labelled congener i in the sample.

9.7.4 Calculation of the toxic equivalent

The total TEQ concentration of PCDD/F is calculated using Formula (5) by the addition of the concentrations of the 17 individual 2,3,7,8-chlorine substituted PCDD/Fs multiplied by the appropriate TEF (see Annex A).

The total TEQ concentration of dioxin-like PCB is calculated using Formula (5) by the addition of the concentrations of the 12 individual coplanar and mono-ortho PCB congeners multiplied by the appropriate TEF (see Annex C).

$$TEQ = \sum \left(c_i[^{12}C] \cdot TEF_i \right) \quad (5)$$

where

TEQ is the sum of the concentrations of all individual congeners of interest multiplied by the appropriate toxic equivalency factor;

$c_i[^{12}C]$ is the concentration of native congener i in the sample;

TEF_i is the toxic equivalency factor of congener i.

9.7.5 Calculation of the limit of detection and the limit of quantification

9.7.5.1 Calculation of the limit of detection

If no analytical blank can be detected, the limit of detection (X_{LD}) is calculated by quantifying the virtual smallest possible peak defined by the minimum requirements for identification and quantification (see 9.4.3). Otherwise the mean analytical blank value adding three times the standard deviation of the analytical blank is defined as X_{LD}.

NOTE For PCDD/F, usually no analytical blanks are detected if glassware and other laboratory equipment are cleaned properly and chemicals of high quality are used. For PCB, it is not possible to eliminate analytical blanks completely due to their worldwide extensive use over a long period of time in different applications and the resulting ubiquitous background levels. Therefore, solvents and adsorbents as well as indoor air can be contaminated with detectable concentrations which leads to ubiquitous blank values.

9.7.5.2 Calculation of the limit of quantification

If no analytical blank can be detected, the limit of quantification (LOQ) is calculated by quantifying the virtual smallest possible peak as described in 9.4.3 but using a signal-to-noise ratio of 6 or 10 instead of 3, depending on the accepted uncertainty of the results.

Otherwise, the LOQ is defined as the mean analytical blank value plus five to 10 times the standard deviation of the analytical blank value. The factor of five to 10 depends on the accepted uncertainty of the results.

10 Precision

The performance characteristics of the method data have been evaluated (see Annex B).

11 Test report

The test report shall contain at least the following information:

a) a reference to this International Standard (ISO 13914);

b) complete identification of the sample;

c) a pretreatment report;

d) a short description of the method used for extraction and sample clean-up;

e) the analytical results containing the levels of the individual PCDD/F and PCB congeners;

f) the recoveries of the individual internal standards;

g) any details not specified in this International Standard or which are optional, as well as any factor which may have affected the results.

Annex A
(informative)

Toxic equivalent factors

The dioxins and furans with chlorine atoms at the 2, 3, 7, and 8 positions are considered the most toxic. Of these, 2,3,7,8-chlorodibenzo-p-dioxin (TCDD) has by far the highest toxicity, is the most studied and best known. Animal studies have shown that 2,3,7,8-TCDD can be lethal in very small concentrations. In the row of known toxins, it is one of the most toxic substances. Different PCDD/F congeners have many of the same biological effects but with different strengths.

In the environment, PCDD/Fs practically never appear as single compounds but always 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 weighted as toxic equivalents (TEQs). This normalization is based on the assumption that PCDD/Fs 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 toxic equivalence factor (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 represents the total toxicity of the mixture.

For PCDD/Fs, two different TEF concepts are currently in use. The I-TEF concept was created by NATO-CCMS in 1988 and the WHO-TEF concept was published in 1998 by WHO. For dioxin-like PCBs, only the WHO-TEF concept includes toxic equivalency factors. The TEF values for both schemes are given in Table A.1.

Table A.1 — TEF values for 2,3,7,8 PCDD/F congeners and dioxin-like PCB congeners according to I-TEF and WHO-TEF concepts

Congener	WHO 2005 TEF	I-TEF
	WHO_{Humans}	NATO-CCMS
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 1	0,001
2,3,7,8-TCDF	0,1	0,1
1,2,3,7,8-PeCDF	0,05	0,05
2,3,4,7,8-PeCDF	0,5	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

Table A.1 *(continued)*

Congener	WHO 2005 TEF	I-TEF
	WHO_{Humans}	NATO-CCMS
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 1	0,001
3,4,4',5-TCB (81)	0,000 1	—
3,3',4,4'-TCB (77)	0,000 1	—
3,3',4,4',5-PeCB (126)	0,1	—
3,3',4,4',5,5'-HxCB (169)	0,01	—
2,3,3',4,4'-PeCB (105)	0,000 1	—
2,3,4,4',5-PeCB (114)	0,000 5	—
2,3',4,4',5-PeCB (118)	0,000 1	—
2',3,4,4',5-PeCB (123)	0,000 1	—
2,3,3',4,4',5-HxCB (156)	0,000 5	—
2,3,3',4,4',5'-HxCB (157)	0,000 5	—
2,3',4,4',5,5'-HxCB (167)	0,000 01	—
2,3,3',4,4',5,5'-HpCB (189)	0,000 1	—

Annex B
(informative)

Repeatability and reproducibility data

B.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison of dioxins, furans, and dioxin-like polychlorinated biphenyls by gas chromatography with high-resolution mass spectrometry (GC-HRMS) in sludge, treated biowaste, and soil was carried out with four to six European laboratories on three materials. Detailed information can be found in the final report on the interlaboratory comparison study mentioned in Reference [6].

Table B.1 provides a list of the materials tested and the selected components.

Table B.1 — Materials tested and components analysed in the interlaboratory comparison of the method for the determination of dioxins, furans, and dioxin-like polychlorinated biphenyls by gas chromatography with high-resolution mass spectrometry (GC-HRMS) in sludge, treated biowaste, and soil

Grain size	Sample	Material tested	Components/congeners analysed
Sludge (<0,5 mm)	Sludge 1	Mix of municipal waste water treatment plant sludges from North Rhine Westphalia, Germany	1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD; OCDD; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF; 1,2,3,6,7,8-HxCDF; 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF; OCDF; PCB-77; PCB-81; PCB-105; PCB-114; PCB-118; PCB-126; PCB-156; PCB-157
Fine grained (<2,0 mm)	Compost 1	Fresh compost from Vienna, Austria	1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD; OCDD; 2,3,4,6,7,8-HxCDF; OCDF; PCB-77; PCB-105; PCB-118; PCB-156; PCB-157; PCB-189
	Soil 3	Sludge-amended soil from Barcelona, Spain	1,2,3,4,7,8-HxCDD; 1,2,3,4,6,7,8-HpCDD; OCDD; 2,3,4,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; OCDF; PCB-105; PCB-118

B.2 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability standard deviation (s_r), and the reproducibility standard deviation (s_R) were obtained (Table B.2). The results are presented for information, the data set being not exhaustive enough for validation.

Table B.2 — Results of the interlaboratory comparison studies of the determination of dioxins, furans, and dioxin-like polychlorinated biphenyls by gas chromatography with high-resolution mass spectrometry (GC-HRMS) in sludge, treated biowaste, and soil

Matrix	l	n	n_o	$\bar{\bar{x}}$ ng/kg	s_R ng/kg	$C_{V,R}$ %	s_r ng/kg	$C_{V,r}$ %	BD
DL PCB-77									
Sludge 1	5	19	0	6,666	0,777	11,7	0,334	5,0	0
Compost 1	4	16	0	0,132	0,015	11,7	0,009	7,0	0
DL PCB-81									
Sludge 1	5	19	0	0,524	0,252	48,1	0,055	10,5	0
DL PCB-105									
Sludge 1	5	16	1	19,184	2,829	14,7	1,357	7,1	0
Compost 1	4	16	0	0,683	0,132	19,4	0,063	9,2	0
Soil 3	4	15	0	0,096	0,012	12,3	0,005	5,5	4
DL PCB-114									
Sludge 1	5	16	1	1,369	0,395	28,8	0,067	4,9	0
DL PCB-118									
Sludge 1	5	16	1	32,585	3,899	12,0	0,845	2,6	0
Compost 1	4	16	0	1,945	0,303	15,6	0,190	9,7	0
Soil 3	5	19	0	0,218	0,043	19,5	0,016	7,5	0
DL PCB-126									
Sludge 1	5	19	0	0,250	0,083	33,0	0,029	11,4	0
DL PCB-156									
Sludge 1	5	15	1	7,310	0,745	10,2	0,259	3,5	1
Compost 1	4	16	0	0,882	0,254	28,7	0,241	27,3	0
DL PCB-157									
Sludge 1	5	16	1	0,864	0,167	19,3	0,057	6,5	0
Compost 1	4	16	0	0,102	0,022	21,7	0,018	17,2	0
DL PCB-189									
Compost 1	4	16	0	0,177	0,066	37,4	0,066	37,4	0
Total DL PCB									
Sludge 1	5	15	1	75,6	10,2	13,5	1,5	1,9	
Compost 1	4	16	0	4,66	1,13	24,3	0,83	17,7	
Soil 3	5	19	0	0,37	0,07	18,9	0,04	11,9	
1,2,3,4,6,7,8-HpCDD									
Sludge 1	6	24	0	185	37	20,0	22	11,7	4
Compost 1	5	20	0	176	72	40,7	14	7,8	0
Soil 3	6	15	2	6,54	1,23	18,8	0,53	8,1	0
1,2,3,4,7,8-HxCDD									
Sludge 1	5	15	1	3,50	0,49	14,1	0,24	6,9	5

Table B.2 — *(continued)*

Matrix	l	n	n_o	$\bar{\bar{x}}$ ng/kg	s_R ng/kg	$C_{V,R}$ %	s_r ng/kg	$C_{V,r}$ %	BD
1,2,3,7,8,9-HxCDD									
Sludge 1	5	16	1	6,427	3,132	48,7	0,927	14,4	4
Compost 1	4	14	0	2,086	0,627	30,1	0,206	9,9	6
1,2,3,7,8-PeCDD									
Sludge 1	4	16	0	2,285	1,235	54,0	0,413	18,1	8
OCDD									
Sludge 1	6	24	0	1479	337	22,8	205	13,8	0
Compost 1	5	20	0	1031	442	42,9	115	11,2	0
Soil 3	6	19	1	56,9	14,0	24,7	2,9	5,1	0
OCDF									
Sludge 1	6	24	0	197	43	22,0	27	13,9	0
Compost 1	5	16	1	26,4	7,3	27,6	6,4	24,2	0
Soil 3	6	19	1	42,3	9,1	21,4	3,9	9,3	0
1,2,3,4,6,7,8-HpCDF									
Sludge 1	6	24	0	95,4	18,3	19,2	8,6	9,0	0
Soil 3	6	23	0	12,4	2,7	21,6	2,5	20,0	0
1,2,3,4,7,8,9-HpCDF									
Sludge 1	5	20	0	7,4	2,6	34,7	1,8	23,6	4
1,2,3,6,7,8-HxCDF									
Sludge 1	5	20	0	7,4	1,6	21,3	0,8	10,9	4
1,2,3,7,8-PeCDF									
Sludge 1	4	15	0	5,2	1,2	23,8	0,4	8,3	9
2,3,4,7,8-PeCDF									
Sludge 1	5	20	0	9,8	3,3	33,3	3,1	31,9	4
2,3,7,8-TCDF									
Sludge 1	5	16	1	10,0	1,4	14,4	0,7	6,6	8

l number of laboratories
n number of analytical results
n_o number of rejected laboratories
$\bar{\bar{x}}$ total mean of analytical results (without outliers)
s_R reproducibility standard deviation
$C_{V,R}$ coefficient of variation of reproducibility
s_r repeatability standard deviation
$C_{V,r}$ coefficient of variation of repeatability
BD number of measurements below detection limit

Annex C
(informative)

Examples of extraction and clean-up methods

C.1 Example A

C.1.1 General

This method is applicable for the determination of PCDD/F and dioxin-like PCB in dry solid samples with particle size of <2 mm.

Sample volumes used for analysis shall be adapted in such a way that the expected amount of analyte lies between the detection limit and the upper end of the calibration range. Samples exceeding the upper limit of the calibration range shall be repeated with smaller amounts of sample.

The described method is also applicable for the determination of PCDD/Fs or PCBs solely. In this case, clean-up steps can be reduced accordingly.

C.1.2 Chemicals

C.1.2.1 Acetone, C_3H_6O.

C.1.2.2 Benzene, C_6H_6.

C.1.2.3 Celite™ 545.[2)]

C.1.2.4 Dichloromethane, CH_2Cl_2.

C.1.2.5 Ethanol, C_2H_5OH, absolute, analytical grade.

C.1.2.6 Extraction thimbles, pure cellulose.

C.1.2.7 Glass balls, 5 mm.

C.1.2.8 n-Hexane.

C.1.2.9 Basic aluminium oxide, Al_2O_3.

C.1.2.10 Silica gel, particle size 0,063 mm to 0,200 mm, active.

C.1.2.11 Sodium chloride, NaCl, analytical grade.

C.1.2.12 Sodium sulfate, $NaSO_4$, analytical grade.

C.1.2.13 Sodium hydroxide, NaOH, 1 mol/l.

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

C.1.2.14 Sulfuric acid, H_2SO_4, analytical grade, 95 % to 97 %.

C.1.2.15 Sea sand, analytical grade.

C.1.2.16 Toluene, $C_6H_5CH_3$.

C.1.3 Procedure

C.1.3.1 Spiking of the sample

Weigh an exact amount of 10 g to 25 g (±0,1 g) of the freeze-dried and ground sludge or compost sample into an Erlenmeyer flask with a ground neck.

The sample will be spiked with 100 µl of $^{13}C_{12}$-solution "sewage sludge" and 100 µl of $^{13}C_{12}$-solution "WHO" (PCB). The compositions of these spiking solutions are listed in Tables C.1 and C.2.

After spiking, close the flask and agitate the sample for 1 h using a mechanical shaker.

Table C.1 — Spiking solution "sewage sludge"

$^{13}C_{12}$-spiking solution "sewage sludge"	pg/100 µl
2,3,7,8-$^{13}C_{12}$-TCDD	20
1,2,3,7,8-$^{13}C_{12}$-PeCDD	40
1,2,3,4,7,8-$^{13}C_{12}$-HxCDD	40
1,2,3,6,7,8-$^{13}C_{12}$-HxCDD	140
1,2,3,7,8,9-$^{13}C_{12}$-HxCDD	80
1,2,3,4,6,7,8-$^{13}C_{12}$-HpCDD	2 500
$^{13}C_{12}$-OCDD	8 500
2,3,7,8-$^{13}C_{12}$-TCDF	60
1,2,3,7,8-$^{13}C_{12}$-PeCDF	40
2,3,4,7,8-$^{13}C_{12}$-PeCDF	40
1,2,3,4,7,8-$^{13}C_{12}$-HxCDF	40
1,2,3,6,7,8-$^{13}C_{12}$-HxCDF	40
2,3,4,6,7,8-$^{13}C_{12}$-HxCDF	80
1,2,3,7,8,9-$^{13}C_{12}$-HxCDF	20
1,2,3,4,6,7,8-$^{13}C_{12}$-HpCDF	500
1,2,3,4,7,8,9-$^{13}C_{12}$-HpCDF	40
$^{13}C_{12}$-OCDF	800

Table C.2 — Spiking solution "WHO"

$^{13}C_{12}$-spiking solution "WHO"	pg/100 µl
$^{13}C_{12}$-PCB – 77	500
$^{13}C_{12}$-PCB – 81	500
$^{13}C_{12}$-PCB – 126	500
$^{13}C_{12}$-PCB – 169	500
$^{13}C_{12}$-PCB – 105	1 000
$^{13}C_{12}$-PCB – 114	1 000
$^{13}C_{12}$-PCB – 118	1 000
$^{13}C_{12}$-PCB – 123	1 000
$^{13}C_{12}$-PCB – 156	1 000
$^{13}C_{12}$-PCB – 157	1 000
$^{13}C_{12}$-PCB – 167	1 000
$^{13}C_{12}$-PCB – 189	1 000

C.1.3.2 Extraction

Depending on the sample volume, use 150 ml or 250 ml Soxhlet devices for extraction.

The right size of core is needed - consisting of cellulose (33 mm × 130 mm for 150 ml adaptor and 33 mm × 205 mm for 250 ml adaptor).

The core will be set in a right-dimensioned beaker.

The spiked and homogenized sample of sludge or compost is filled into the core and some flask-resisting particles will be flushed with a small amount of toluene (C.1.2.16) and also put into the core. Flushing the flask will be repeated three times.

Afterwards the core will be closed with some cellulose drapery and fibreglass and put into the glass adaptor. Some resisting toluene in the beaker will also be flushed and filled into the glass adaptor.

Now the glass adaptor is set on the right 500 ml round-bottomed flask filled with some sea sand (C.1.2.15) and the whole equipment is set in a heater rounded by an isolation cover.

Now the adaptor is filled twice with toluene (C.1.2.16) until siphoning.

After complete run off of the toluene, the extraction is started. Extraction time is approximately 12 h or at least 50 extraction cycles. After cooling of the apparatus, the remaining toluene in the glass adaptor is added to the extract into the round-bottom flask. The extract is concentrated using a rotary evaporator to approximately 5 ml.

C.1.3.3 Clean-up

C.1.3.3.1 Schematics of clean-up procedure

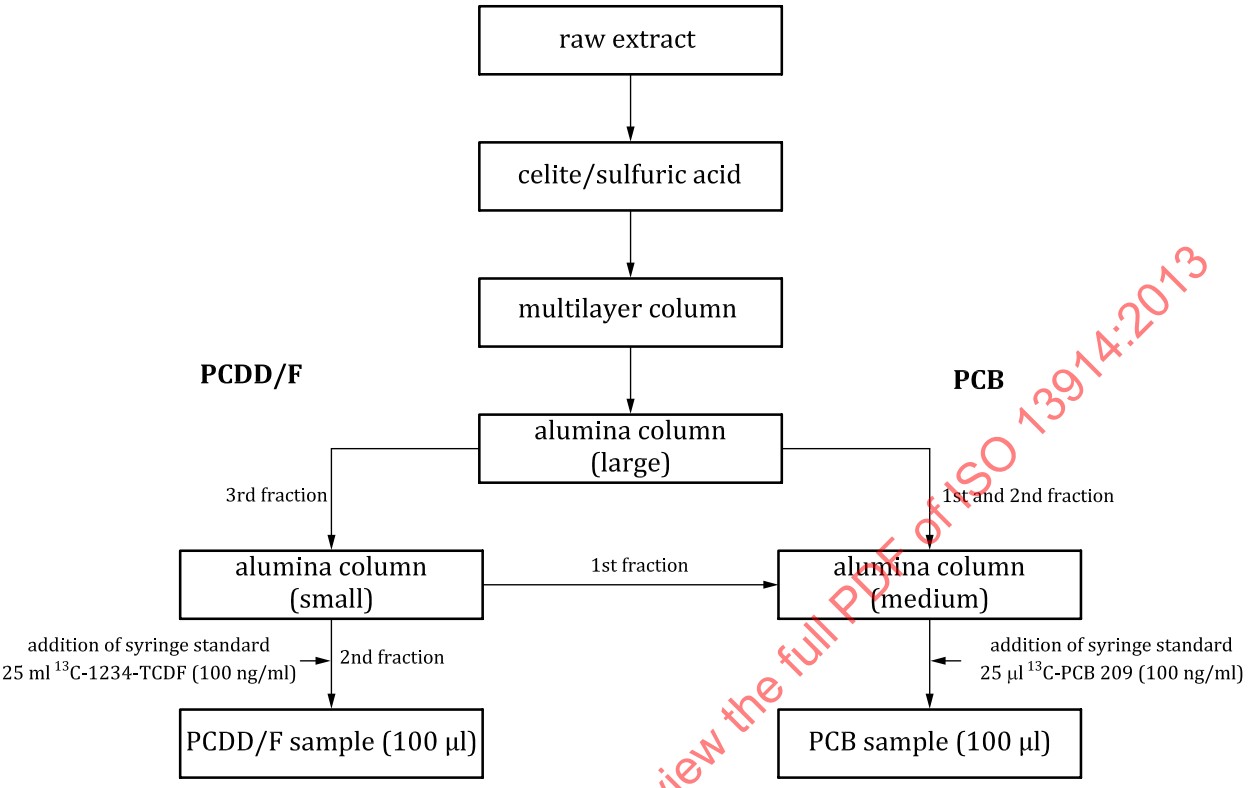

Figure C.1 — Schematic of clean-up procedure

C.1.3.3.2 Preparation of adsorbents

C.1.3.3.2.1 Celite/sulfuric acid

For preparing 200 g clean-up material, weigh 100 g of celite (C.1.2.3) and the same amount of sulfuric acid (C.1.2.14) into a conical flask of 1 000 ml volume. Close the flask and shake briefly by hand until everything is mixed up steadily.

Agitate it for 1 h using mechanical shaking.

Leave it in a closed position.

C.1.3.3.2.2 Silica gel/sulfuric acid (44 %)

For preparing 100 g clean-up material, weigh 56 g silica gel (C.1.2.10) and 46 g sulfuric acid (C.1.2.14) (95 % to 97 %) into a conical flask, close the flask, and extract it for 1 h using mechanical shaking.

C.1.3.3.2.3 Silica gel/sodium hydroxide (33 %)

For preparing 100 g clean-up material, weigh 67 g silica gel (C.1.2.10) and 33 g 1 mol/l sodium hydroxide (C.1.2.13) into a conical flask, close the flask, and extract it for 1 h using mechanical shaking.

C.1.3.3.3 Preparation of the clean-up columns

C.1.3.3.3.1 Celite column

The column consisting of glass (25 mm diameter, 300 mm length, coarse-glass frit, 300 ml reservoir, PTFE stopcock) is filled with (top-down)

— 5 g silica gel (C.1.2.10),

— 30 g celite (C.1.2.3)/sulfuric acid (C.1.2.14) (1/1), and

— 5 g silica gel (C.1.2.10).

The column will be conditioned with 70 ml of n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (80/20). Add the sample to the column and after infiltration, rinse the flask where the sample was kept with a small amount of n-hexane (C.1.2.8) and add this too.

Repeat the washing three times and elute the sample with 200 ml of n-hexane (C.1.2.8).

The eluate will be concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum down to approximately 5 ml.

C.1.3.3.3.2 Multilayer column

The column consisting of glass (25 mm diameter, 300 mm length, coarse-glass frit, 300 ml reservoir, PTFE stopcock) is filled with (top-down)

— 2 g silica gel (C.1.2.10),

— 5 g silica gel (C.1.2.10)/sodium hydroxide (C.1.2.13) (33 % 1 mol/l),

— 2 g silica gel (C.1.2.10),

— 10 g silica gel (C.1.2.10)/sulfuric acid (C.1.2.14) (44 % conc.),

— 2 g silica gel (C.1.2.10), and

— 10 g anhydrous sodium sulfate (C.1.2.12).

The column will be conditioned with 150 ml of n-hexane (C.1.2.8). Add the sample to the column and after infiltration, rinse the flask where the sample was kept with a small amount of n-hexane (C.1.2.8) and add this too.

Repeat the washing three times and elute the sample with 250 ml of n-hexane (C.1.2.8).

The eluate will be concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum down to approximately 5 ml.

C.1.3.3.3.3 Large aluminium oxide column

The column consisting of glass (25 mm diameter, 300 mm length, coarse-glass frit, 300 ml reservoir, PTFE stopcock) is filled with (top-down)

— 25 g basic aluminium oxide (C.1.2.9) and

— 20 g anhydrous sodium sulfate (C.1.2.12).

The column will be conditioned with 150 ml of n-hexane (C.1.2.8). Add the sample to the column and after infiltration, rinse the flask where the sample was kept with a small amount of benzene (C.1.2.2) and add this too.

Repeat the washing three times.

Elute the sample with

— 80 ml benzene (C.1.2.2),

— 20 ml n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (98/2), and

— 150 ml n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (1/1).

The first and second fractions contain the PCBs, whereas the third fraction contains the PCDD/F. The eluates will be concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum to approximately 5 ml.

C.1.3.3.3.4 Small aluminium oxide column

The column consisting of glass (150 mm long × 8 mm internal diameter, with coarse-glass frit or glass-wool plug, 250 ml reservoir, and glass or PTFE stopcock) is filled with (top-down)

— 2,5 g basic aluminium oxide (C.1.2.9) and

— 2 g anhydrous sodium sulfate (C.1.2.12).

The column will be conditioned with 40 ml of n-hexane (C.1.2.8). Add the sample (third fraction of C.1.3.3.3.3) to the column and after infiltration, rinse the flask where the sample was kept with a small amount of n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (98/2) and add this too.

Repeat the washing three times.

Elute the sample with

— 40 ml n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (98/2) and

— 25 ml n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (1/1).

The first fraction contains PCB and is combined with the first and second fractions of C.1.3.3.3.3. The combined eluates are concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum to approximately 5 ml.

The second fraction contains PCDD/F and will be concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum down to approximately 5 ml.

C.1.3.3.4 Midi aluminium oxide column

The column consisting of glass (200 mm long × 15 mm internal diameter, with coarse-glass frit or glass-wool plug, 250 ml reservoir, and glass or PTFE stopcock) is filled with (top-down)

— 6 g basic aluminium oxide (C.1.2.9) and

— 4 g anhydrous sodium sulfate (C.1.2.12).

The column will be conditioned with 60 ml of n-hexane (C.1.2.8). Add the sample (combined PCB eluates from C.1.3.3.3.4) to the column and after infiltration, rinse the flask where the sample was kept with a small amount of n-hexane (C.1.2.8) and add this too.

Repeat the washing three times.

Elute the sample with

— 60 ml n-hexane (C.1.2.8) and

— 40 ml n-hexane (C.1.2.8)/dichloromethane (C.1.2.4) (7/3).

The second fraction contains PCB and will be concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum to approximately 5 ml.

C.1.3.4 Preparation of sample solution for measurement

C.1.3.4.1 PCDD/F

The concentrated eluate from the clean-up procedure (see Figure C.1) is quantitatively transferred to a graduated conical vial. Rinse the larger vial with toluene and add the rinse to the conical vial. Concentrate the sample by applying a gentle N_2-stream down to 100 µl and add 25 µl 1,2,3,4-$^{13}C_{12}$-TCDF (concentration = 100 ng/ml). Adjust the final volume to 100 µl. Transfer the sample to an autosampler vial with a conical 100 µl insert and seal it with a PTFE-lined crimp cap. The vial should be labelled with the sample number and the type of analyte. The sample can be stored in the dark at room temperature until measurement. For longer storage, the sample shall be stored in a refrigerator at (4 ± 3) °C.

C.1.3.4.2 PCB

The concentrated eluate from the clean-up procedure (see Figure C.1) is quantitatively transferred to a graduated conical vial. Rinse the larger vial with toluene and add the rinse to the conical vial. Concentrate the sample by applying a gentle N_2-stream down to 100 µl and add 25 µl $^{13}C_{12}$-PCB-209 (concentration = 100 ng/ml). Adjust the final volume to 100 µl. Transfer the sample to an autosampler vial with a conical 100 µl insert and seal it with a PTFE-lined crimp cap. The vial should be labelled with the sample number and the type of analyte. The sample can be stored in the dark at room temperature until measurement. For longer storage, the sample shall be stored in a refrigerator at (4 ± 3) °C.

C.2 Example B: Approved clean-up methods

Table C.3 includes a non-comprehensive list of available international and national standard methods that contain descriptions of approved clean-up methods. Due to the modular design of the described methods, laboratories can chose an appropriate combination of these clean-up steps according to the nature of the sample matrix and the available equipment.

Table C.3 — International and national standard methods containing approved clean-up methods

Method	Analyte	Matrix	Origin
EN 1948–2 EN 1948–3	PCDD/F	Emission	CEN
ISO 18073	PCDD/F	Water	ISO
Guideline “Determination of Polychlorinated dioxins and furans in Soil” BUWAL, 2001	PCDD/F	Soil	Switzerland
JIS K 0311	PCDD/F, coplanar PCBs	Emission	Japan
EPS 1/RM/19	PCDD/F	Paper industry products	Canada
EPA Method 1668	Coplanar PCBs	Soil, water, sludge, sediment, biota, and other samples	USA
EPA Method 1613	PCDD/F	Soil, water, ash, waste, chemical products, food, feeds, biota, and other matrices	USA
EPA Method 8280	PCDD/F	Soil, water, ash, waste, chemical product, distillation residue, fuels, sludge	USA