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Soil, treated biowaste and sludge — Determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high resolution mass selective detection (HR GC-MS)

Sols, biodéchets traités et boues — Dosage des dioxines, des furanes et des polychlorobiphényles de type dioxine par chromatographie en phase gazeuse avec spectrométrie de masse à haute résolution (HR CG-SM)

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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 of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by CEN/TC 444, *Environmental characterization of solid matrices* (as EN 16190:2018) and drafted in accordance with its editorial rules. It was assigned to Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical and physical characterization* and adopted under the "fast-track procedure".

This second edition cancels and replaces the first edition (ISO 13914:2013), which has been technically revised.

The main changes are as follows:

— technical content of EN 16190:2018 has been adopted;

— comprehensive validation results for soil, treated biowaste and sludge have been included;

— calculation of toxicity factors on the basis of interlaboratory data has been added.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 PCDD and 135 PCDF.

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.

PCDD and PCDF 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. PCDD/PCDF 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.

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

PCDD/PCDF as well as PCB are emitted during thermal processes as e.g. waste incineration. In 1997 a group of experts of the World Health Organization (WHO) fixed toxicity equivalent factors (TEF) for PCDD and twelve PCB, known as dioxin-like PCB (see Annex A). These twelve dioxin-like PCB consist of four non-ortho PCB and eight mono-ortho PCB (no or only one chlorine atoms in 2-, 2'-, 6- and 6'-position), having a planar or mostly planar structure. Dioxin-like PCB 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 document.

This document is applicable for several types of matrices and validated for municipal sludge (see also Annex A) for the results of the validation).

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

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

Soil, treated biowaste and sludge — Determination of dioxins and furans and dioxin-like polychlorinated biphenyls by gas chromatography with high resolution mass selective detection (HR GC-MS)

1 Scope

This document 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 document 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 document, limits of detection better than 1 ng/kg (expressed as dry matter) can be achieved.

This method is “performance based”. The method can be modified 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 document 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 are referred to in the text in such a way that some or all of their content constitutes requirements 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.

EN 15934, *Sludge, treated biowaste, soil and waste — Calculation of dry matter fraction after determination of dry residue or water content*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
internal standard
$^{13}C_{12}$-labelled 2,3,7,8-PCDD/PCDF analogue added to samples prior to extraction against which the concentrations of native PCDD and PCDF are calculated

[SOURCE: ISO 18073:2004, 3.1.5]

3.2
recovery standard
$^{13}C_{12}$-labelled 2,3,7,8-chloro-substituted PCDD/PCDF, added before injection into the GC

[SOURCE: ISO 18073:2004, 3.1.12]

4 Abbreviated terms

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

5 Principle

This document 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/PCDF 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 PCB.

$^{13}C_{12}$-labelled PCDD/PCDF and PCB congeners are added to the sample prior to extraction and HRGC/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 HRGC/HRMS analysis. For the determination of these substances it is necessary to separate PCB from PCDD/PCDF and vice versa.

The main purpose of the clean-up procedure of the raw sample extract is the removal of sample matrix components, which may overload the separation method, disturb the quantification or otherwise severely impact the performance of the identification and quantification method and the separation of PCDD/PCDF 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/PCDF and PCB is based on quantification by the isotope-dilution technique using HRGC/HRMS.

6 Reagents

6.1 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 B for a specific list of solvents and chemicals.

6.2 Standards

— ^{13}C-spiking solution for PCDD/PCDF (internal standard);

— ^{13}C-spiking solution for PCB (internal standard);

— calibration solutions PCDD/PCDF;

— calibration solutions PCB;

— recovery standard PCDD/PCDF;

— recovery standard PCB.

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

7 Apparatus and materials

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.

7.1 Equipment for sample preparation

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

7.1.2 Desiccator.

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

7.2 Soxhlet extractor

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

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

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

7.3 Clean-up apparatus

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

7.3.2 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.

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

7.4 Concentration apparatus

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

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

— 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.

7.4.2 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 or of another suitable inert gas, installed in a fume hood.

7.4.3 Kuderna-Danish[1] **concentrator.**

7.4.4 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.

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

7.5 Other equipment

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

7.5.2 GC column for PCDD/PCDF 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).

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

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

8 Sample storage and sample pretreatment

8.1 Sample storage

Samples should be stored in suitable containers with an appropriate closure material such as polytetrafluoroethylene (PTFE). Samples to be frozen may be stored in aluminium containers pre-cleaned by heating to 450 °C for minimum 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 three days of sampling. If not achievable, samples may be frozen (–18 °C) directly after sampling and kept frozen before sample pretreatment.

8.2 Sample pretreatment

Drying and homogenization should be carried out according to EN 16179, if not otherwise specified. Store the ground material in a desiccator or a tightly closed glass container.

Determination of water content shall be carried out according to EN 15934.

9 Extraction and clean-up

9.1 General

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

The determination of PCDD/PCDF is based on quantification by the isotope-dilution technique using HRGC/HRMS. $^{13}C_{12}$-labelled 2,3,7,8-chlorine substituted PCDD/PCDF 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 HRGC/HRMS analysis. However, due to possible differences in the binding and adsorption characteristics between the native PCDD/PCDF 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 may 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/PCDF. Furthermore, an enrichment of the analytes in the final sample extract is achieved. Extraction procedures are normally 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 (see Annex D). In addition, the verification of the method performance for each single sample shall be part of the applied quality assurance protocol.

9.2 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 onto the sample before extraction.

The extraction procedure is carried out using Soxhlet extraction with toluene. Duration of extraction should be adjusted according to 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 labelled congeners included in the internal standard

^{13}C-spiking solution – Internal standard	
PCDD/PCDF 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	

9.3 Clean-up

9.3.1 General

Clean-up methods shall prepare the sample extract in an appropriate manner for the subsequent quantitative determination. Clean-up procedures shall concentrate PCDD/PCDF and dioxin-like PCB in the extracts and to 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 D.

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

9.3.2 Gel permeation chromatography

The interesting molecular weight range for PCDD/PCDF and dioxin-like PCB 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 sulphur.

9.3.3 Multilayer column

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

9.3.4 Sulphuric acid treatment

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

9.3.5 Activated carbon column

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

9.3.6 Aluminium oxide column

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

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

9.3.7 Removal of sulphur

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

9.4 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 high boiling solvent.

Though PCDD/PCDF 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 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 around 200 µl.

d) Kuderna Danish[2)]:

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

9.5 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.

10 HRGC/HRMS analysis

10.1 General

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

10.2 Gas chromatographic analysis

Gas chromatographic separation shall be carried out in such a way that sufficient separation of all PCDD/PCDF and dioxin-like PCB congeners is achieved and the quality criteria specified in 10.4 and 10.5 are met.

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

For PCDD/PCDF 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 twelve dioxin-like PCB congeners from all other non-dioxin-like PCB congeners.

10.3 Mass spectrometric detection

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

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

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

Table 4 *(continued)*

Homologue groups	12_C	13_C
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

10.4 Minimum requirements for identification of PCDF/PCDD and PCB

10.4.1 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

10.4.2 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.

10.4.3 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 ten times the signal width at half height. Peak-to-peak values are taken.

10.5 Minimum requirements for quantification of PCDF/PCDD and PCB

10.5.1 For PCDD/PCDF 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 nature (polarity), or by using a GC-QQQ apparatus.

Single column data may 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, or using a GC-QQQ apparatus.

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 twelve 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.

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

10.5.3 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.

10.5.4 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 hexachlorinated congeners;

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

If the above ranges are exceeded for one or more congeners, then the ranges given below are acceptable for congeners with recoveries not within those 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 hexachlorinated congeners;

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

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

10.5.6 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.

10.5.7 An analytical blank shall be analysed as defined in 10.6. 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.

10.6 Calibration of the HRGC/HRMS system

10.6.1 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.

10.6.2 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):

$$rrf_i = \frac{A_{i[^{12}C]}}{A_{i[^{13}C]}} \times \frac{c_{i[^{13}C]}}{c_{i[^{12}C]}} \quad (1)$$

where

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

$A_{i[12C]}$ is the area of native congener i;

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

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

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

10.6.3 Calibration for sum of homologue groups

The calibration of the mass spectrometer is done in the same way and with the same calibration solutions than for single congeners. The relative response factors for each homologue group is calculated by 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

10.7 Quantification of HRGC/HRMS results

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

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

$$c_{i[^{12}C]} = \frac{A_{i[^{12}C]}}{A_{i[^{13}C]}} \times \frac{c_{i[^{13}C]}}{rrf_i} \quad (2)$$

where

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

$A_{i[12C]}$ is the area of native congener i;

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

$c_{i[12C]}$ is the concentration of native congener i in the sample, on the basis of the dry matter;

$c_{i[13C]}$ 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 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 (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.

10.7.2 Quantification of recovery rates of ^{13}C-labelled standards

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

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

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 $^{13}C_{12}$-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.

10.7.3 Quantification of 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):

$$C_{h[12C]} = \frac{\Sigma A_{i[12C]}}{A_{i[13C]}} \times \frac{c_{i[13C]}}{rrf_i} \quad (4)$$

where

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

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

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

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

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

10.7.4 Calculation of the toxic equivalent

The total TEQ concentration of PCDD/PCDF is calculated using Formula (5) by the addition of the concentrations of the 17 individual 2,3,7,8-chlorine substituted PCDD/PCDF 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 twelve individual coplanar and monoortho PCB congeners multiplied by the appropriate TEF (see Annex D).

$$TEQ = \sum (c_{i\left[^{12}C\right]} \times TEF_i) \quad (5)$$

where

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

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

TEF_i is the toxic equivalency factor of congener i.

10.7.5 Calculation of the limit of detection and the limit of quantification

10.7.5.1 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 10.4.3). Otherwise the mean analytical blank value adding three times the standard deviation of the analytical blank is defined as the X_{LD}.

NOTE For PCDD/PCDF 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 but also the indoor air may be contaminated with detectable concentrations which leads to ubiquitous blank values.

10.7.5.2 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 10.4.3 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.

11 Expression of results

Report mass concentration, in nanograms per kilogram (ng/kg) on the basis of the dry matter, of individual compounds, with two significant figures shall be provided.

EXAMPLES	OCDD	11 ng/kg DM
	1,2,3,6,7,8-HxCDF	1,2 ng/kg DM
	DL PCB 81	0,14 ng/kg DM

This applies also for the calculation of sum of homologue groups (10.7.3), and for limits of detection (10.7.5.1) and of quantification (10.7.5.2).

TEF are calculated and expressed as given in 10.7.4.

12 Precision

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

13 Test report

The test report shall contain the following information:

a) a reference to this document, i.e. ISO 13914:yyyy;

b) complete identification of the sample;

c) 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/PCDF and PCB congeners (see Clause 11);

f) the recoveries of the individual internal standards;

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

Annex A
(informative)

Toxic equivalency factor (TEF)

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/PCDF congeners have many of the same biological effects but with different strength.

In the environment PCDD/PCDF practically never appear as single compounds but always as a complex mixture associated with other structurally related ("dioxin-like") compounds such as PCB.

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/PCDF 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 represent the total toxicity of the mixture.

For PCDD/PCDF currently two different TEF-concepts are 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 PCB 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/PCDF congeners and dioxin-like PCB congeners according I-TEF and WHO-TEF concepts

CONGENER	WHO 2005 TEF WHO_{Humans}	I-TEF 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 3	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

Table A.1 *(continued)*

CONGENER	WHO 2005 TEF WHO_{Humans}	I-TEF NATO-CCMS
1,2,3,7,8,9-HxCDF	0,1	0,1
2,3,4,6,7,8-HxCDF	0,1	0,1
1,2,3,4,6,7,8-HpCDF	0,01	0,01
1,2,3,4,7,8,9-HpCDF	0,01	0,01
OCDF	0,000 3	0,001
3,4,4',5-TCB (81)	0,000 3	--
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)	0,03	--
2,3,3',4,4'-PeCB (105)	0,000 03	--
2,3,4,4',5-PeCB (114)	0,000 03	--
2,3',4,4',5-PeCB (118)	0,000 03	--
2',3,4,4',5-PeCB (123)	0,000 03	--
2,3,3',4,4',5-HxCB (156)	0,000 03	--
2,3,3',4,4',5'-HxCB (157)	0,000 03	--
2,3',4,4',5,5'-HxCB (167)	0,000 03	--
2,3,3',4,4',5,5'-HpCB (189)	0,000 03	--

Annex B
(informative)

Examples of extraction and clean-up methods

B.1 Example A

B.1.1 General

This method is applicable for the determination of PCDD/PCDF 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 detection limit and upper end of 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/PCDF or PCB solely. In this case clean-up steps can be reduced accordingly.

B.1.2 Chemicals

B.1.2.1 Propanone (acetone), C_3H_6O.

B.1.2.2 Benzene, C_6H_6.

B.1.2.3 Celite™[3] 545.

B.1.2.4 Dichloromethane, CH_2Cl_2.

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

B.1.2.6 Extraction thimbles, pure cellulose.

B.1.2.7 Glass balls, 5 mm.

B.1.2.8 n-Hexane, C_6H_{14}.

B.1.2.9 Basic aluminium oxide, Al_2O_3.

B.1.2.10 Silica gel 63, mesh to 200 mesh, active.

B.1.2.11 Sodium chloride, NaCl, analytical grade.

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

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

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

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

B.1.2.15 Sea sand, analytical grade.

B.1.2.16 Toluene, $C_6H_5CH_3$.

B.1.3 Procedure

B.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 is spiked with 100 µl of ^{13}C-solution "sewage sludge" and 100 µl of ^{13}C-solution "WHO" (PCB). The compositions of these spiking solutions are listed in the following Tables B.1 and B.2.

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

Table B.1 — Spiking solution "sewage sludge"

^{13}C-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 B.2 — Spiking solution "WHO"

^{13}C-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

Table B.2 *(continued)*

^{13}C-spiking solution "WHO"	pg/100 µl
$^{13}C_{12}$-PCB – 157	1 000
$^{13}C_{12}$-PCB – 167	1 000
$^{13}C_{12}$-PCB – 189	1 000

B.1.3.2 Extraction

Depending on 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 is set in a right dimensioned beaker.

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

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

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

Now the adaptor is filled twice with toluene 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.

B.1.3.3 Clean-up

B.1.3.3.1 Schematics of clean-up procedure

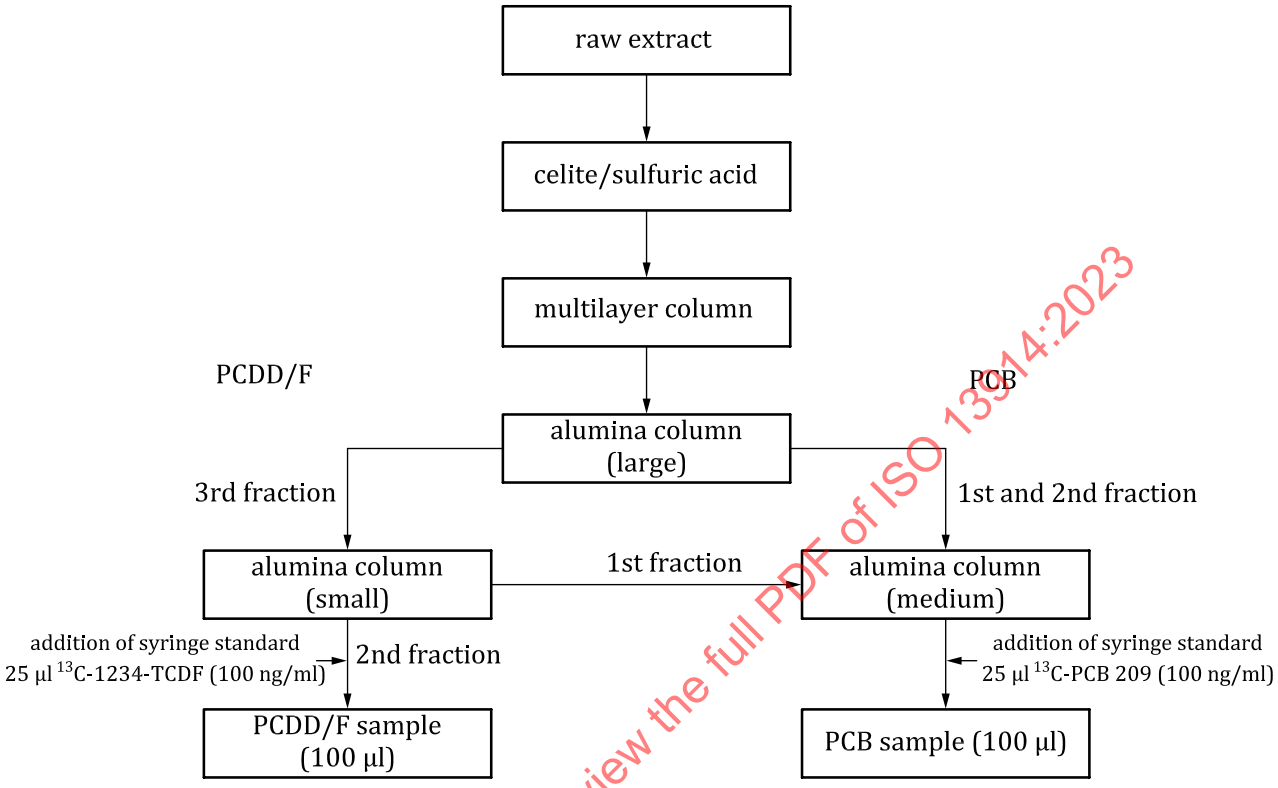

Figure B.1 — Schematic clean-up procedure

B.1.3.3.2 Preparation of adsorbents

B.1.3.3.3 Celite™/sulphuric acid

For preparing 200 g clean-up material weigh 100 g of Celite™ (B.1.2.3) and the same amount of sulphuric acid (B.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 closed position.

B.1.3.3.4 Silica gel/sulphuric acid (44 %)

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

B.1.3.3.5 Silica gel/sodium hydroxide (33 %)

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

B.1.3.3.6 Preparation of the clean-up columns

B.1.3.3.7 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 (B.1.2.10);

— 30 g Celite™ (B.1.2.3)/sulfuric acid (B.1.2.14) (1/1);

— 5 g silica gel (B.1.2.10).

The column is conditioned with 70 ml of n-hexane (B.1.2.8)/dichloromethane (B.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 (B.1.2.8) and add this too.

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

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

B.1.3.3.8 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 (B.1.2.10);

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

— 2 g silica gel (B.1.2.10);

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

— 2 g silica gel (B.1.2.10);

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

The column is conditioned with 150 ml of n-hexane (B.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 (B.1.2.8) and add this too.

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

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

B.1.3.3.9 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 (B.1.2.9);

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

The column is conditioned with 150 ml of n-hexane (B.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 (B.1.2.2) and add this too.

Repeat the washing three times.

Elute the sample with:

— 80 ml of benzene (B.1.2.2);

— 20 ml n-hexane (B.1.2.8)/dichloromethane (B.1.2.4) (98/2);

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

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

B.1.3.3.10 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 (B.1.2.9);

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

The column is conditioned with 40 ml of n-hexane (B.1.2.8). Add the sample (third fraction of B.1.3.3.3) to the column and after infiltration, rinse the flask, where the sample was kept with a small amount of n-hexane (B.1.2.8)/dichloromethane (B.1.2.4) (98/2) and add this too.

Repeat the washing three times.

Elute the sample with:

— 40 ml of n-hexane (B.1.2.8)/dichloromethane (B.1.2.4) (98/2);

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

The first fraction contains PCB and is combined with the first and second fraction of B.1.3.3.3 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/PCDF and is concentrated on a rotating evaporator at 40 °C to 50 °C under vacuum down to approximately 5 ml.

B.1.3.3.11 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 (B.1.2.9);

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

The column is conditioned with 60 ml of n-hexane (B.1.2.8). Add the sample (combined PCB eluates from of B.1.3.3.4) to the column and after infiltration, rinse the flask, where the sample was kept with a small amount of n-hexane (B.1.2.8) and add this too.

Repeat the washing three times.

Elute the sample with:

— 60 ml of *n*-hexane (B.1.2.8);

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

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

B.1.3.4 Preparation of sample solution for measurement

B.1.3.4.1 PCDD/PCDF

The concentrated eluate from the clean-up procedure, see Figure B.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 auto sampler vial with conical 100 µl insert and seal it with a PTFE lined crimp cap. The vial should be labelled with sample number and 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 approximately +5 °C.

B.1.3.4.2 PCB

The concentrated eluate from the clean-up procedure, see Figure B.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 conical 100 µl insert and seal it with a PTFE lined crimp cap. The vial should be labelled with sample number and 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 approximately +5 °C.

B.2 Example B: Approved clean-up methods

Table B.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 may chose an appropriate combination of these clean-up steps according to the nature of the sample matrix and the available equipment.

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

Method	Analyte	Matrix	Origin
EN 1948-2 EN 1948-3	PCDD/PCDF	Emission	CEN
ISO 18073	PCDD/PCDF	Water	ISO
Guideline "Determination of Polychlorinated dioxins and furans in Soil" BUWAL, 2001	PCDD/PCDF	Soil	Switzerland
JIS K 0311	PCDD/PCDF coplanar PCB	Emission	Japan
EPS 1/RM/19	PCDD/PCDF	Paper industry products	Canada
EPA Method 1668	Coplanar PCB	Soil, water, sludge, sediment, biota and other samples	USA
EPA Method 1613	PCDD/PCDF	Soil, water, ash, waste, chemical products, food, feeds, biota and other matrices	USA
EPA Method 8280	PCDD/PCDF	Soil, water, ash, waste, chemical product, distillation residue, fuels, sludge	USA
EPA Method 8290	PCDD/PCDF	Soil, water, ash, waste, chemical product, distillation residue, fuels, sludge, biota	USA
EPA Method T0 9A	PCDD/PCDF	Ambient Air	USA

Annex C
(informative)

Examples of operation of GC/HRMS determination — Example

C.1 General

GC-MS analyses of PCDD/PCDF and dioxin-like PCB are carried out on a high resolution GC-MS instrument equipped with a high resolution gas chromatograph, an auto sampler, a cold injection system, a high resolution mass spectrometer and a data system for instrument control, data acquisition and processing.

C.2 Gas chromatographic analysis

Gas chromatographic separation shall be carried out in such a way that sufficient separation of all PCDD/PCDF and dioxin-like PCB congeners is achieved.

The following conditions can be used as a starting point for optimizing a method. With the given specifications the complete separation of all 2,3,7,8-substituted PCDD/PCDF congeners can be achieved. For dioxin-like PCB the sufficient separation of all congeners of interest except for PCB-123 can be achieved. Different columns and parameters can be used if all quality requirements are fulfilled.

a) Injector temperature:

Split/splitless:	270 °C to 320 °C
Cold Injection System:	40 °C, Injection
	2 °C/s to 60 °C
	60 °C, 90 s, solvent vent
	12 °C/s to 320 °C
	320 °C, 10 min

b) Separation columns:

1) Total PCDD/PCDF and dioxin-like PCB:

DB-5[4] fused silica capillary column, length 60 m × 0,25 mm internal diameter with a film thickness of 0,25 µm.

2) Isomer specific PCDD/PCDF analysis:

DB-Dioxin[4] fused silica capillary column, length 60 m × 0,25 mm inner diameter with a film thickness of 0,25 µm.

c) Oven temperature programmes:

4) Fused silica capillary columns DB-5 and DB-Dioxin are trade names of products supplied by Agilent J&W, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named. Equivalent products may be used if they can be shown to lead to the same results.

PCDD/PCDF:

DB5:	60 °C, 5 min
	20 °C/min to 200 °C
	1 °C/min to 220 °C
	220 °C, 16 min
	3 °C/min to 320 °C
	320 °C, 3 to 10 min (depending on matrix load)
DB-Dioxin:	60 °C, 5 min
	20 °C/min to 220 °C
	220 °C, 40 min
	5 °C/min to 270 °C
	270 °C, 57 min

Dioxin-like PCB:

DB5:	60 °C, 5 min
	20 °C/min to 190 °C
	1 °C/min to 220 °C
	220 °C, 16 min
	3 °C/min to 300 °C
	300 °C, 3 to 10 min (depending on matrix load)
Carrier gas:	Helium, 1,7 ml/min, „constant flow“
MS-Interface temperature:	270 °C (DBDIOXIN), 320 °C (DB-5)

C.3 Mass spectrometric detection

A high resolution mass spectrometer at a resolution of 9 000 to 11 000 is used for the detection of PCDD/PCDF and dioxin-like PCB.

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 is adjusted to 250 °C. To achieve appropriate sensitivity the mass spectrometer is adjusted to a sensitivity better than 200 fg for 2,3,7,8-TCDD.

For each PCDD/PCDF or PCB congener of interest two ions of the molecular isotope cluster are recorded for both the native and the added $^{13}C_{12}$-labelled congeners. In addition an appropriate lock and a calibration mass are detected for the quality control of the mass calibration during the analysis of a sample or a standard. For PCDD/PCDF and PCB measurement therefore 10 masses are detected repetitively with a cycle time of 0,5 s.

For identification and quantification the masses given in Table B.1 and Table B.2 shall be recorded in the different MID Windows (Table C.1 and C.2).

The time slots for the MID windows are defined as follows:

— for the PCDD/PCDF analysis on a DB-DIOXIN column by measuring a calibration standard and setting the MID windows in a way that all congeners of interest elute within the related MID-window;

— for the PCDD/PCDF analysis on a DB-5 column by measuring a standard mixture containing the first and last eluting isomers of each isomer group. The MID windows are set in a way that all isomers of a homologue group are detected;

— for the PCB analysis on a DB-5MS column by measuring a calibration standard and setting the MID windows in a way that all congeners of interest elute within the related MID-window.

Table C.1 — MID-windows and masses for the detection and quantification of PCDD/PCDF

MID-window	Dibenzofurans		Dibenzo-p-dioxins	
	^{12}C	^{13}C	^{12}C	^{13}C
MID-window 1 (Tetras)	303,901 6 305,898 7	315,941 9 317,938 9	319,896 5 321,893 7	331,936 8 333,933 9
MID-window 2 (Pentas)	339,859 8 341,856 9	351,900 0 353,897 0	355,854 7 357,851 8	367,894 9 369,891 9
MID-window 3 (Hexas)	373,820 8 375,817 9	385,861 0 387,858 0	389,815 7 391,812 8	401,855 9 403,852 9
MID-window 4 (Heptas)	407,781 8 409,778 9	419,822 0 421,819 0	423,776 7 425,773 8	435,816 9 437,814 0
MID-window 5 (Octas)	441,742 8 443,739 9	453,783 0 455,780 1	457,737 7 459,734 8	469,777 9 471,775 0

Table C.2 — MID-windows and masses for the detection and quantification of PCB

MID-Window	Homologue groups	^{12}C	^{13}C
MID-window 1	Trichloro-PCB	255,961 3 257,958 4	268,001 6 269,998 6
	Tetrachloro-PCB	289,922 3 291,919 4	301,962 6 303,959 7
MID-window 2	Tetrachloro-PCB	289,922 3 291,919 4	301,962 6 303,959 7
	Pentachloro-PCB	325,880 4 327,877 5	337,920 7 339,917 7
MID-window 3	Pentachloro-PCB	325,880 4 327,877 5	337,920 7 339,917 7
	Hexachloro-PCB	359,841 5 361,838 5	371,881 7 373,878 8
MID-window 4	Hexachloro-PCB	359,841 5 361,838 5	371,881 7 373,878 8
	Heptachloro-PCB	393,802 5 395,799 5	405,842 7 407,839 8
MID-window 5	Heptachloro-PCB	393,802 5 395,799 5	405,842 7 407,839 8
	Octachloro-PCB	427,763 5 429,760 6	439,803 8 441,800 8

Table C.2 *(continued)*

MID-Window	Homologue groups	^{12}C	^{13}C
MID-window 6	Octachloro-PCB	427,763 5 429,760 6	439,803 8 441,800 8
	Nonachloro-PCB	461,724 5 463,721 6	473,764 8 475,761 8
MID-window 7	Decachloro-PCB	497,682 6 499,679 7	509,722 9 511,719 9

Annex D
(informative)

Repeatability and reproducibility data

D.1 Materials used in the interlaboratory comparison study

The interlaboratory comparison for the determination of dioxins, furans and dioxin-like polychlorinated biphenyls by gas chromatography with high resolution mass spectrometry (HRGC-MS) in sludge, treated biowaste and soil was carried out by 16 laboratories on three materials listed in Table B.1 Mixing of different materials was partly applied to obtain the desired concentration levels.

Table D.1 provides a list of the materials chosen for testing and the selected components.

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

Sample	Grain size	Material tested
Sludge	< 0,5 mm	Mix of municipal waste water treatment plant sludges from Berlin, Germany
Compost	< 0,25 mm	Compost from the vicinity of Berlin, Germany
Soil	< 2,0 mm	Mix of soil from Brandenburg, Germany and PAH-free German reference soil, spiked.

D.2 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The mean values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table D.2).

Table D.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 (HRGC-MS) in sludge, treated biowaste and soil

Matrix	l	n	n_o	$\bar{\bar{x}}$ ng/kg DM	s_R ng/kg DM	CV_R %	s_r ng/kg DM	CV_r %	BD
DL PCB 77									
Sludge	10	30	1	225 453,433	29 045,000	12,88	14 646,380	6,50	0
Compost	10	30	0	66,293	21,194	31,97	6,412	9,67	1

l is the number of laboratories after outlier rejection
n is the number of analytical results after outlier rejection
n_o is the number of outliers (laboratories)
$\bar{\bar{x}}$ is the total mean of analytical results (without outliers)
s_R is the reproducibility standard deviation
s_r is the repeatability standard deviation
CV_R is the coefficient of variation of reproducibility
CV_r is the coefficient of variation of repeatability
BD is the number of laboratories providing measurements below detection limit