CEN/TC BT/TF 151

Date: 2006-08

prEN xxxxx:2006

CEN/TC BT/TF 151

Secretariat:

Soils, sludges and treated biowaste — Determination of dioxines and furans and dioxin-like polychlorinated biphenyls by gaschromatography with high resolution mass spectrometra (GCC/HRMS)

Einführendes Element — Haupt-Element — Ergänzendes Element

Élément introductif — Élément central — Élément complémentaire

ICS:

Descriptors:

Document type: European Standard
Document subtype:
Document stage: Working Document
Document language: E

D:\AdLib eXpress\Special\StandardQuality\NoSecurity\Input\Dioxin_Furan_DL-PCB_Standard_ for _validation.doc STD Version 2.2

Contents

Page

Foreword

The European project HORIZONTAL is focused on the standardisation of analytical methods for the analyses of various inorganic and organic contaminants in soil, sludge and bio waste. During the preparation of the project, several desk studies have been started to elaborate the possibility of horizontal standardisation on specific subjects. One of these subjects was the horizontal standardisation of Polychlorinated Dibenzo-p-dioxins and Polychlorinated Dibenzofurans (PCDD/F) together with the dioxin-like Polychlorinated Biphenyls (DL-PCB).

This document has been developed in the framework of the project 'Horizontal-ORG' based on the results of a desk study. Numerous established test methods published in literature, available CEN and ISO standards and various guidelines from official bodies have been reviewed, compiled and described in this study. After discussion with the project partners and other interested parties the draft-standard has been developed as an modular horizontal method.

This standard is applicable for the determination of PCDD/F and dioxin-like PCBs in soil, sludge and treated biowaste. It may also be applicable for other sample categories of plant origin and mineral origin.

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 approx. 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 specialised hydraulic fluids, as a plasticizer in sealing material etc. World-wide more than one million tons of PCBs were produced.

PCDD/F as well as PCBs are emitted during thermal processes as e.g. waste incineration. In 1997 a group of experts of the World Health Organisation (WHO) fixed toxicity equivalent factors (TEFs) for PCDDs/PCDFs and 12 PCBs, known as dioxin-like PCBs (see Annex C). 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 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 Standard.

1 Scope

This European 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 soil, sludge and biowaste using liquid column chromatographic clean-up methods and GC/HRMS.

The limit of detection is depends on the kind of sample, the congener, the equipment used, the quality of chemicals used for extraction and clean-up. Under the quality requirements specified in this European standard, limits of detection better than 1 ng/kg should 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 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 standard to validate the application for these matrices.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

The following normative documents contain provisions which, through reference in this text, constitute provisions of this European standard.

ISO 14507 Soil quality - Guidance for sample pretreatment for the determination of organic contaminants in soil.

ISO/DIS 16720 Soil quality - Pretreatment of samples by freeze drying for subsequent analysis

EN 1948-2 Stationary source emissions - Determination of the mass concentration of PCDDs/PCDFs and dioxin-like PCBs - Part 2: Extraction and clean-up of PCDDs/PCDFs

EN 1948-3 Stationary source emissions - Determination of the mass concentration of PCDDs/PCDFs and dioxin-like PCBs - Part 3: Identification and quantification of PCDDs/PCDFs

US EPA Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

3 Definitions

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

3.1
analytical blank
a blank sample covering the complete analytical procedure including extraction, clean-up, identification and quantification including all the relevant reagents and materials

3.2
congener
any one of the 210 individual PCDD/Fs or any one of the 209 individual PCBs

3.3
dioxin-like PCBs
non- and mono-ortho PCB having a affinity to the Ah-receptor, showing similar toxical effects as the 2,3,7,8-substituted PCDDs/PCDFs

NOTE See [5]

3.4
internal standard
$^{13}C_{12}$-labelled PCDD/Fs and PCBs, added before extraction and used for calculation of results

3.5
keeper
high boiling point solvent added to the sampling standard solution

3.6
limit of detection (LOD)
The limit of detection is expressed as the mean sample blank value plus three times the standard deviation (3s) of the blank. (EUROCHEM Guide)

3.7
limit of quantification (LOQ)
The limit of quantification is expressed as the mean sample blank value plus, either, five, six or ten times the standard deviation of the blank. (EUROCHEM Guide)

3.8
marker PCBs
The six (or seven) PCBs 28, 52, 101, (118,) 138, 153, 180 depending on regulation

3.9
operational performance characteristics
measures which deal with the influence of the physical and chemical environment and maintenance problems, for example; mains voltage, temperature, supply of certain substances, set-up time, period of unattended operation (ISO 6879:1995)

3.10
pattern
defined as a chromatographic print of any series of PCDD, PCDF or PCB isomers

3.11
PCB isomers
PCBs with identical chemical composition but different structure

3.12
PCDD/PCDF isomers
PCDDs or PCDFs with identical chemical composition but different structure

3.13
profile
graphic representation of the analysed concentrations.

3.14
recovery standard
$^{13}C_{12}$-labelled PCDD/Fs and PCBs, added before injection into the GC and used for calculation of recovery rates of internal standards

3.15
spiking
addition of $^{13}C_{12}$-labelled PCDD/F or PCB standards

3.16
statistical performance characteristics
measures which quantify, for measured values, the possible deviations resulting from the random part of the measuring process; these are, for example, repeatability or instability (ISO 6879:1995)

4 Symbols and abbreviations

4.1 I-TEF

International toxic equivalent factor proposed by NATO-CCMS in 1988 (for detailed description, see Annex C).

4.2 I-TEQ

International toxic equivalent obtained by multiplying the mass determined with the corresponding I-TEF including PCDDs and PCDFs (for detailed description, see Annex C). Should only be used for comparison with older data

4.3 WHO-TEF

Toxic equivalent factor proposed by WHO in 1997 (for detailed description, see Annex C)

4.4 WHO-TEQ

Toxic equivalent obtained by multiplying the mass determined with the corresponding WHO-TEF including

PCDDs, PCDFs and PCBs (for detailed description, see Annex C). WHO-TEQ $_{PCB,}$ WHO-TEQ $_{PCDD/F,}$ should be used to distinguish different compound classes.

4.5 Substances and their abbreviations

Substances	Abbreviation
Polychlorinated Dibenzo-p-dioxins/Dibenzofurans	**PCDD/PCDF or PCDD/F**
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**

5 Principle

This European Standard is based on the use of the 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 soil, sediments, sludge, biowaste and waste. 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 isomers (position of chlorine substituents) whereas the mass spectrometric parameters enable the differentiation between congeners with different numbers of chlorine substituents and between dibenzo-p-dioxins, furans and PCB.

Soil, sludge or biowaste is sampled according to EN yyyy. $^{13}C_{12}$-labelled PCDD/F and PCB congeners are added 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 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 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/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/F and PCBs 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 alumina oxide, silica gel, Celite and others used for clean-up shall be of analytical grade quality or better and precleaned and activated if necessary.

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

6.2 Standards

^{13}C-spiking solution for PCDD/F (internal Standard)

^{13}C-spiking solution for PCB (internal Standard)

Calibration solutions PCDD/F

Calibration solutions PCB

Recovery standard PCDD/F

Recovery standard PCB

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

7 Apparatus and materials

7.1 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.

7.2 Equipment for sample preparation

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

7.2.2 Desiccator

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

7.3 Extraction apparatus

Soxhlet extractor consisting of:

a) Soxhlet, 50 mm internal diameter, 150 ml or 250 ml capacity with 500 ml round bottom flask;

b) thimble, 43 x 123 to fit Soxhlet;

c) hemispherical heating mantle, to fit 500 ml round-bottom flask.

7.4 Clean-up apparatus

7.4.1 Disposable pipettes, either disposable Pasteur pipettes, or disposable serological pipettes

7.4.2 Glass chromatographic columns of the following sizes:

a) 150 mm long x 8 mm internal diameter, with coarse-glass frit or glass-wool plug, 250 ml reservoir and glass or PTFE stopcock;

b) 200 mm long x 15 mm internal diameter, with coarse-glass frit or glass-wool plug, 250 ml reservoir and glass or PTFE stopcock;

c) 300 mm long x 25 mm internal diameter, with coarse-glass frit or glass-wool plug, 300 ml reservoir and glass or PTFE stopcock.

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

7.5 Concentration Apparatus

7.5.1 General

Solvent recovery apparatus is recommended for use in methods that require the use of any type of evaporative concentrators. Incorporation of this apparatus may be required by national legislation that governs air emissions of volatile organics. It is recommended to incorporate such type of reclamation system as a method to reduce solvent emissions to the atmosphere. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

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

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

b) 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);

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

7.5.3 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.

7.5.4 Kuderna-Danish concentrator consisting of a concentrator tube (10ml), an evaporation flask (500ml), snyder columns of appropriate size and a water bath capable of maintaining a temperature within ±2°C, installed in a fume hood

7.5.5 Sample vials, of the following types:

a) amber glass 2 ml to 5 ml with PTFE-lined screw-cap;

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

7.6 Other equipment

7.6.1 Gas chromatograph, equipped with a split-less or on-column or temperature programmed injection port for the use with capillary columns, and an oven temperature program which enables isothermal hold.

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

7.6.3 Mass spectrometer, 28 eV to 80 eV electron impact ionization, capable of repetitively selectively monitoring of 12 exact masses minimum at high resolution (> 6 000) during a period of approximately one second.

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

8 Sampling and sample pre-treatment

8.1 Sampling

Sampling should be carried out in accordance with EN yyyy. See Annex A for the specific information assuring the coherence and linkage between the different steps of measurement.

In addition to this European Standard the following requirements apply. Samples should be stored in suitable containers with an appropriate closure material such as 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 pre-treatment should take place within 3 days of sampling. Alternatively, samples may be frozen (–18 °C) directly after sampling and kept frozen before sample pre-treatment.

8.2 Sample pre-treatment

Drying and homogenization should be carried out according to EN wwww:

Store the ground material in a desiccator or a tightly closed glass container.

9 Extraction and Clean-up

9.1 General

In the European Standard 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 example procedures below and in Annex A or any suitable alternative procedures.

The determination of PCDDs/PCDFs 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 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 guaranteed. Therefore, in addition the applied methods have to be validated thoroughly. Examples of well-proven extraction and clean-up methods are given in Annex A.

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/F. 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 have to be tested thoroughly and have to pass a set of method validation requirements before they can be employed. In addition, the verification of the method performance for each single sample has to be part of the applied quality assurance protocol. This Standard describes a framework of method validation and quality control requirements which have to be fulfilled by any applied method.

9.2 Extraction

The sample amount used for extraction may 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 1 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.

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

Table 1 — ^{13}C labelled congeners included in the internal standard

^{13}C-spiking solution – Internal standard	
PCDD/F congeners	**PCB congeners**
2378-$^{13}C_{12}$-TCDD	$^{13}C_{12}$-PCB – 77
12378-$^{13}C_{12}$-PeCDD	$^{13}C_{12}$-PCB – 81
123478-$^{13}C_{12}$-HxCDD	$^{13}C_{12}$-PCB – 126
123678-$^{13}C_{12}$-HxCDD	$^{13}C_{12}$-PCB – 169
123789-$^{13}C_{12}$-HxCDD	
1234678-$^{13}C_{12}$-HpCDD	$^{13}C_{12}$-PCB – 105
$^{13}C_{12}$-OCDD	$^{13}C_{12}$-PCB – 114
	$^{13}C_{12}$-PCB – 118

2378-$^{13}C_{12}$-TCDF	$^{13}C_{12}$-PCB – 123
12378-$^{13}C_{12}$-PeCDF	$^{13}C_{12}$-PCB – 156
23478-$^{13}C_{12}$-PeCDF	$^{13}C_{12}$-PCB – 157
123478-$^{13}C_{12}$-HxCDF	$^{13}C_{12}$-PCB – 167
123678-$^{13}C_{12}$-HxCDF	$^{13}C_{12}$-PCB – 189
234678-$^{13}C_{12}$-HxCDF	
123789-$^{13}C_{12}$-HxCDF	
1234678-$^{13}C_{12}$-HpCDF	
1234789-$^{13}C_{12}$-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 have to concentrate PCDDs/PCDFs and dioxin-like PCBs in the extracts and to remove interfering matrix components present in the raw extract.

Proven clean-up procedures shall be used containing normally 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 A.

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 PCDDs/PCDFs 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.

9.3.3 Multilayer column

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

9.3.4 Sulfuric 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 has to be carried out very carefully to avoid losses of PCDDs/PCDFs and dioxin-like PCBs on the formed carboniferous surfaces.

9.3.5 Activated carbon column

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

9.3.6 Alumina column

By column liquid chromatography on alumina of different activity grade and acidity/basicity interfering compounds with small differences in polarity or structure compared to PCDDs/PCDFs and dioxin-like PCBs can be removed.

Additionally alumina columns can be used to separate PCDDs/PCDFs from dioxin-like PCBs.

9.3.7 Removal of sulfur

The removal of sulfur 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 b.pt. solvent.

Though PCDDs/PCDFs 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 have to 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

To avoid initial losses the column should be pre-wet with about 1ml of solvent. Boiling chips should be added and adjust the vertical position of the apparatus. 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 1ml 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. The recovery standards shall be added under following conditions:

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 Equipment

GC/MS analyses of PCDDs/PCDFs 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.

10.2 Gas chromatographic analysis

Gas chromatographic separation has to 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 items 10.4 and 10.5 are met.

For PCDD/F there is no capillary column available at present, which 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.

10.3 Mass spectrometric detection

A high resolution mass spectrometer at a resolution of 9 000 to 11 000 is used for the detection of PCDD/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. A mass resolution of 6 000 to 9 000 is possible if any interference is absent.

The mass spectrometer is used in the MID-Mode (multiple ion detection), the GC column is directly coupled to the man 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 2 and Table 3 have to be recorded in MID mode. For each PCDD/F or PCB congener of interest at least two ions of the molecular isotope cluster have to be recorded for both the native and the added $^{13}C_{12}$-labelled congeners.

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

The time slots for the MID windows have to 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 have to 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 2 — 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	303,9016	315,9419	319,8965	331,9368
	305,8987	317,9389	321,8937	333,9339
Penta-CDD/F	339,8598	351,9000	355,8547	367,8949
	341,8569	353,8970	357,8518	369,8919
Hexa-CDD/F	373,8208	385,8610	389,8157	401,8559

	375,8179	387,8580	391,8128	403,8529
Hepta-CDD/F	407,7818	419,8220	423,7767	435,8169
	409,7789	421,8190	425,7738	437,8140
Octa-CDD/F	441,7428	453,7830	457,7377	469,7779
	443,7399	455,7801	459,7348	471,7750

Table 3 — Masses for the dtection and quantification of PCB

Homologue groups	^{12}C	^{13}C
Trichloro-PCB	255,9613	**268,0016**
	257,9584	**269,9986**
Tetrachloro-PCB	289,9223	**301,9626**
	291,9194	**303,9597**
Pentachloro-PCB	325,8804	**337,9207**
	327,8775	**339,9177**
Hexachloro-PCB	359,8415	**371,8817**
	361,8385	**373,8788**
Heptachloro-PCB	393,8025	**405,8427**
	395,7995	**407,8398**
Octachloro-PCB	427,7635	**439,8038**
	429,7606	**441,8008**
Nonachloro-PCB	461,7245	**473,7648**
	463,7216	**475,7618**
Decachloro-PCB	497,6826	**509,7229**
	499,6797	**511,7199**

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 3).

Table 4 — 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-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 10 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/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 nature (polarity).

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.

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.

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 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

— 20 % to 150 % for the hepta- and octa-chlorinated 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 a 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 Quality assurance procedure

Analytical blank values of all congeners of interest shall be measured regularly. An analytical blank sample covers the complete analytical procedure including extraction, clean-up, identification and quantification including all the relevant reagents and materials

- Measurement of 1 blank sample per series (max. 20 samples in 1 series).
- Measurement of a blank sample if new or repaired equipment is used
- Measurement of a blank sample if new batches of solvents or adsorbents are used
- Measurement of a blank sample after the analysis of a sample with unusually high levels, exceeding average concentration levels by a factor 10.

A reference sample shall be analysed per series (max. 100 samples per series). A reference sample may be a certified material, but also a home made material from which the relevant concentrations are checked by other laboratories.

For the analysis of blank and reference samples the same quality requirements which are given in 10.4 and 10.5 are valid as for real samples.

10.7 Calibration of the HRGC/HRMS

10.7.1 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.

10.7.2 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 equation (1):

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

where:

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

$A_{i[^{12}C]}$ 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

10.7.3 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. The following Table 5 shows the relations between native congeners and $^{13}C_{12}$-labelled congeners.

Table 5 — 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
Tetrachlorohomologues	2,3,7,8	2,3,7,8	2,3,7,8	2,3,7,8
Pentachlorohomologues	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
Hexachlorohomologues	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
Heptachlorohomologues	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

10.8 Quantification of HRGC/HRMS results

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

The concentration of congener i in the sample is calculated using equation (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 (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 have to be repeated with smaller amounts of sample.

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

The recovery rates of the internal standards are quantified against the recovery standard using equation (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 $^{13}C_{12}$-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

10.8.3 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 equation (4):

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

where:

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

$\Sigma A_{i[^{12}C]}$ 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

10.8.4 Calculation of the toxic equivalent

The total TEQ concentration of PCDD/F is calculated using equation (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 C).

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

$$TEQ = \Sigma(c_{i}\left[^{12}C\right] \cdot TEF_{i} \quad (5)$$

where:

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

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

TEF_i is the toxic equivalency factor of congener I

10.8.5 Calculation of the limit of detection and the limit of quantification

10.8.5.1 Calculation of the limit of detection:

If no analytical blank can be detected the LOD 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 tree times the standard deviation of the analytical blank is defined as the LOD.

NOTE For PCDD/F usually no analytical blanks are detected if glassware and other laboratory equipment is 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 which detectable concentrations which leads to ubiquitous blank values.

10.8.5.2 Calculation of the limit of quantification:

If no analytical blank can be detected the LOQ is calculated by quantifying the virtual smallest possible peak as described in 10.8.5.1 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 ten times the standard deviation of the analytical blank value. The factor of five to ten depends on the accepted uncertainty of the results.

11 Test report

The test report shall contain the following information:

a) A reference to this European Standard including its date of publication;

b) Sampling report including precise identification of the sample;

c) Pre-treatment report;

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

e) A short description of the method used for extraction and sample clean-up;

f) The recoveries of the individual internal standards;

g) any deviation from this standard, and any facts which may have influenced the result. Where the test is not carried out in accordance with this standard, reference may only be made to EN xxxx in the report in case all deviations from the procedures prescribed in this standard are indicated in the report stating the reason for deviation.

Annex A
(informative)

Examples of operation of extraction and clean-up methods

A.1 Example A (as performed by Umweltbundesamt GmbH, Austria)

A.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 have to 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 have to be repeated with smaller amounts of sample.

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

A.1.1.1 Chemicals

— Acetone;

— Benzene;

— Celite 545 (e.g. Roth 0011.1);

— Dichloromethane;

— Ethanol absolut, analytical grade;

— Extraction thimbles, pure cellulose;

— Glass balls, 5 mm (e.g. LG 9.012.405);

— n-Hexane;

— Basic alumina;

— Silica gel 63-200 mesh, active;

— Sodium chloride, analytical grade;

— Sodium sulfate, analytical grade;

— Sodium hydroxide solution, 1 mol/l;

— Sulfuric acid, analytical grade, 95 % to -97 %;

— Seasand, analytical grade;

— Toluene.

A.1.2 Procedure

A.1.2.1 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-solution “sewage sludge” and 100 µl of ^{13}C-solution “WHO” (PCB). The compositions of these spiking solutions are listed in the following tables A.1 and A.2.

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

Table A.1 — Spiking solution "sewage sludge"

^{13}C- spiking solution „sewage sludge“	
	pg /100 µl
2378-$^{13}C_{12}$-TCDD	20
12378-$^{13}C_{12}$-PeCDD	40
123478-$^{13}C_{12}$-HxCDD	40
123678-$^{13}C_{12}$-HxCDD	140
123789-$^{13}C_{12}$-HxCDD	80
1234678-$^{13}C_{12}$-HpCDD	2500
$^{13}C_{12}$-OCDD	8500
2378-$^{13}C_{12}$-TCDF	60
12378-$^{13}C_{12}$-PeCDF	40
23478-$^{13}C_{12}$-PeCDF	40
123478-$^{13}C_{12}$-HxCDF	40
123678-$^{13}C_{12}$-HxCDF	40
234678-$^{13}C_{12}$-HxCDF	80
123789-$^{13}C_{12}$-HxCDF	20
1234678-$^{13}C_{12}$-HpCDF	500
1234789-$^{13}C_{12}$-HpCDF	40
$^{13}C_{12}$-OCDF	800

Table A.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	1000
$^{13}C_{12}$-PCB – 114	1000
$^{13}C_{12}$-PCB – 118	1000
$^{13}C_{12}$-PCB – 123	1000
$^{13}C_{12}$-PCB – 156	1000
$^{13}C_{12}$-PCB – 157	1000
$^{13}C_{12}$-PCB – 167	1000
$^{13}C_{12}$-PCB – 189	1000

A.1.2.2 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 x 130 mm for 150 ml adaptor and 33 mm x 205 mm for 250 ml adaptor).

The core will be set in a right dimensioned beaker.

The spiked and homogenised sample of clearing sludge or compost is filled into the core and some flask resisting particles will be flushed with a small amount of toluene and also put into the core. Flushing the flask will be repeated 3 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 be 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 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 (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.

A.1.2.3 Clean-up

A.1.2.3.1 Schematics of clean-up procedure

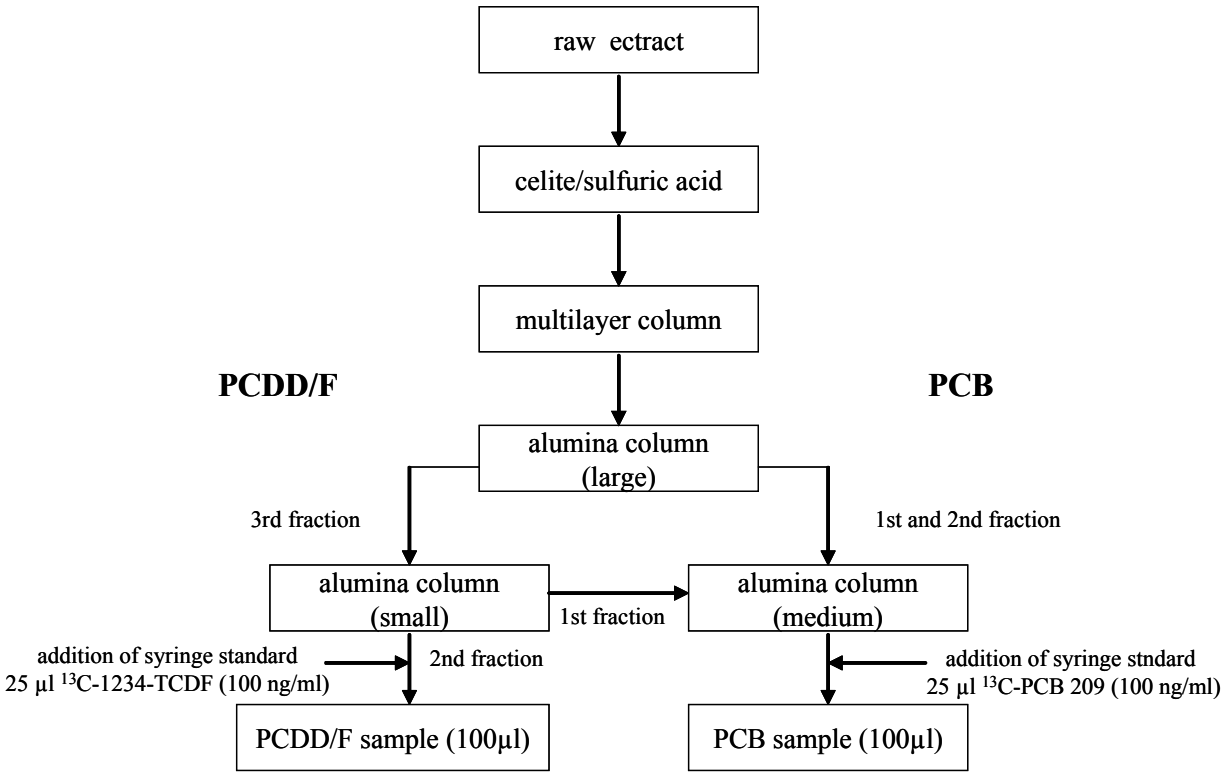

Figure A.1 — Schematic

A.1.2.3.2 Preparation of adsorbents

A.1.2.3.2.1 Celite/sulfuric acid

For preparing 200 g clean-up material weigh 100 g of celite and the same amount of sulphuric acid 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.

A.1.2.3.2.2 Silica gel/ sulfuric acid (44%)

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

A.1.2.3.2.3 Silica gel/ sodium hydroxide (33%)

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

A.1.2.3.3 Preparation of the clean-up columns

A.1.2.3.3.1 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,

— 30 g celite/sulfuric acid (1/1),

— 5 g silica gel.

The column will be conditioned with 70 ml of n-hexane/dichloromethane (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 and add this too.

Repeat the washing 3 times and elute the sample with 200 ml of n-hexane.

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

A.1.2.3.3.2 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,

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

— 2 g silica gel,

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

— 2 g silica gel,

— 10 g anhydrous sodium sulfate.

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

Repeat the washing for 3 times and elute the sample with 250 ml of n-hexane.

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

A.1.2.3.3.3 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,

— 20 g anhydrous sodium sulphate.

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

Repeat the washing 3 times.

Elute the sample with:

— 80 ml of benzene,

— 20 ml n-hexane/dichloromethane (98/2),

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

The first and second fraction contains 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.

Remark: alternatively elution with n-Heptan/Ethylacetate = 99/1 (first fraction) and n-Heptan/Ethylacetate = 9/1 (second fraction) should be possible as described by J.Höckel et al, Organohalogen Compounds 22, 433 (1996)

A.1.2.3.3.4 Small aluminium oxide column

The column consisting of glass (150 mm long x 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,

— 2 g anhydrous sodium sulphate.

The column will be conditioned with 40 ml of n-hexane. Add the sample (3rd fraction of A.1.2.3.3.3) to the column and after infiltration, rinse the flask, where the sample was kept with a small amount of n-hexane/dichloromethane (98/2) and add this too.

Repeat the washing 3 times.

Elute the sample with:

— 40 ml of n-hexane/dichloromethane (98/2),

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

The first fraction contains PCB and is combined with the first and second fraction of A.1.2.3.3.3. 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 40-50°C under vacuum down to app. 5ml

A.1.2.3.4 Midi aluminium oxide column

The column consisting of glass (200 mm long x 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,

— 4 g anhydrous sodium sulfate.

The column will be conditioned with 60 ml of n-hexane. Add the sample (combined PCB eluates from of A.1.2.3.3.4) to the column and after infiltration, rinse the flask, where the sample was kept with a small amount of n-hexane and add this too.

Repeat the washing 3 times.

Elute the sample with:

— 60 ml of n-hexane,

— 40 ml n-hexane/dichloromethane (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.

A.1.2.4 Preparation of sample solution for measurement

A.1.2.4.1 PCDD/F

The concentrated eluate from the clean-up procedure, see Figure A.1, is quantitatively transferred to a graduated conical vial (e.g. Suppelco Receiving Vessel Cat. No. 64723). 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 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 has to be stored in a refrigerator at approximately + 5 °C.

A.1.2.4.2 PCB

The concentrated eluate from the clean-up procedure, see Figure A.1, is quantitatively transferred to a graduated conical vial (e.g. Suppelco Receiving Vessel Cat. No. 64723). 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 has to be stored in a refrigerator at approximatela + 5 °C.

A.2 Example B: Approved clean-up methods

Table 3 includes a non comprehensive list of available international and national standard methods, which 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 A.3 — International and national standard methods containing approved clean-up methods

Method	Analyte	Matrix	Origin
EN 1948 – 2,3	PCDD/F	Emission	CEN
ISO/DIS 18073	PCDD/F	Water	INT
Guideline „Determination of Polychlorinated dioxins and urans in Soil" BUWAL, 2001	PCDD/F	Soil	CH
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
EPA Method 8290	PCDD/F	Soil, water, ash, waste, chemical product, distillation residue, fuels, sludge, biota	USA
EPA Method T0 9A	PCDD/F	Ambient Air	USA

Annex B
(informative)

Examples of operation of GC/HRMS determination

B.1 Example (Unweltbundesamt GmbH, Austria)

B.1.1 General

GC/MS analyses of PCDDs/PCDFs and dioxin-like PCBs is carried out on a high resolution GC/MS instrument equipped with a high resolution gas chromatograph, an autosampler, a cold injection system, a high resolution mass spectrometer and a data system for instrument control, data acquisition and processing.

B.1.2 Gas chromatographic analysis

Gas chromatographic separation has to be carried out in such a way, that sufficient separation of all PCDD/F 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/F congeners can be achieved. For dioxine-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.

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

Separation columns:

a) Total PCDDs/PCDFs and dioxin-like PCBs:

   DB-5[1)] fused silica capillary column, length 60 m x 0,25 mm internal diameter with a film thickness of 0,25 µm.

b) Isomer specific PCDD/PCDF analysis:

   DBDIOXIN [1)] fused silica capillary column, length 60 m x 0,25 mm inner diameter with a film thickness of 0,25 µm.

Oven temperature programmes:

---

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

PCDD/F:

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)
DBDIOXIN:	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)

B.1.3 Mass spectrometric detection

A high resolution mass spectrometer at a resolution of 9 000 to 11 000 is used for the detection of PCDD/F 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/F 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/F 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 have to be recorded in the different MID Windows.

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

— for the PCDD/F 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/F 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 will be 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 B.1 — MID-windows and masses for the detection and quantification of PCDD/F

MID-window	Dibenzofurans		Dibenzo-p-dioxins	
	^{12}C	^{13}C	^{12}C	^{13}C
MID-window 1 (Tetras)	303,9016 305,8987	315,9419 317,9389	319,8965 321,8937	331,9368 333,9339
MID-window 2 (Pentas)	339,8598 341,8569	351,9000 353,8970	355,8547 357,8518	367,8949 369,8919
MID-window 3 (Hexas)	373,8208 375,8179	385,8610 387,8580	389,8157 391,8128	401,8559 403,8529
MID-window 4 (Heptas)	407,7818 409,7789	419,8220 421,8190	423,7767 425,7738	435,8169 437,8140
MID-window 5 (Octas)	441,7428 443,7399	453,7830 455,7801	457,7377 459,7348	469,7779 471,7750

Table B.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	255,9613 257,9584	268,0016 269,9986
	Tetrachloro-PCB	289,9223 291,9194	301,9626 303,9597
MID-window 2	Tetrachloro-PCB	289,9223 291,9194	301,9626 303,9597
	Pentachloro-PCB	325,8804 327,8775	337,9207 339,9177
MID-window 3	Pentachloro-PCB	325,8804 327,8775	337,9207 339,9177
	Hexachloro-PCB	359,8415 361,8385	371,8817 373,8788
MID-window 4	Hexachloro-PCB	359,8415 361,8385	371,8817 373,8788
	Heptachloro-PCB	393,8025 395,7995	405,8427 407,8398
MID-window 5	Heptachloro-PCB	393,8025 395,7995	405,8427 407,8398
	Octachloro-PCB	427,7635 429,7606	439,8038 441,8008
MID-window 6	Octachloro-PCB	427,7635 429,7606	439,8038 441,8008
	Nonachloro-PCB	461,7245 463,7216	473,7648 475,7618
MID-window 7	Decachloro-PCB	497,6826 499,6797	509,7229 511,7199

Annex C
(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 strength.

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 normalisation 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 represent the total toxicity of the mixture.

For PCDD/Fs 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 PCBs only the WHO-TEF concept includes toxic equivalency factors. The TEF values for both schemes are given in Table C.1.

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

CONGENER	WHO-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	0,0001	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
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	0,0001	0,001
3,4,4',5-TCB (81)	0,0001	---
3,3',4,4'-TCB (77)	0,0001	---
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)	0,0001	---
2,3,4,4',5-PeCB (114)	0,0005	---
2,3',4,4',5-PeCB (118)	0,0001	---
2',3,4,4',5-PeCB (123)	0,0001	---
2,3,3',4,4',5-HxCB (156)	0,0005	---
2,3,3',4,4',5'-HxCB (157)	0,0005	---
2,3',4,4',5,5'-HxCB (167)	0,00001	---
2,3,3',4,4',5,5'-HpCB (189)	0,0001	---

Annex D
(informative)

Validation

D.1 General

The validation within the standardisation process has to be undertaken after finalizing of the R & D project HORIZONTAL consultation process.

The following validation data will be replaced after finalization of the R & D project HORIZONTAL validation process.

The validation data given in the following chapters are derived from validation work carried out within the accreditation process of the Umweltbundesamt GmbH, Austria, using the analytic method described in annexes A and B.

D.2 Example 1 (Umweltbundesamt GmbH)

D.2.1 Validation data for soil

For soil samples the validation data were calculated from manifold analyses of soil samples with different contamination levels. The results and statistical data are given for PCDD/F in Table D.1 and Table D.2 and for dioxin-like PCB in Table D.3 and Table D.4.

Table D.1 — Fourfould analysis of a relatively low level contaminated soil sample

Soil A	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Mean	Standard-deviation	Standard-deviation
	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	%
2378-TCDD	n.d.	n.d.	0,1	0,1	0,05	0,06	115
12378-PeCDD	n.d.	n.d.	0,1	0,1	0,05	0,06	115
123478-HxCDD	0,1	0,1	0,1	0,1	0,10	0,00	0
123678-HxCDD	0,2	0,2	0,4	0,3	0,28	0,10	34
123789-HxCDD	0,2	0,3	0,2	0,3	0,25	0,06	23
1234678-HpCDD	3,0	2,9	3,3	2,9	3,03	0,19	6
OCDD	13,9	13,4	12,3	12,0	12,90	0,90	7
total TCDD	0,5	0,8	1,2	0,9	0,85	0,29	34
total PeCDD	0,5	0,3	1,0	1,5	0,83	0,54	65
total HxCDD	1,1	2,1	3,1	3,0	2,33	0,93	40
total HpCDD	5,8	5,2	5,4	5,4	5,45	0,25	5
2378-TCDF	0,8	0,7	0,6	0,7	0,70	0,08	12
12378-PeCDF	0,3	0,2	0,2	0,2	0,23	0,05	22
23478-PeCDF	0,3	0,2	0,2	0,2	0,23	0,05	22
123478-HxCDF	0,8	0,6	0,3	0,4	0,53	0,22	42
123678-HxCDF	0,3	0,2	0,2	0,2	0,23	0,05	22
234678-HxCDF	0,2	0,2	0,2	0,2	0,20	0,00	0
123789-HxCDF	0,2	n.d.	n.d.	n.d.	0,05	0,10	200
1234678-HpCDF	1,7	1,5	1,7	1,7	1,65	0,10	6
1234789-HpCDF	0,3	0,2	0,2	0,2	0,23	0,05	22
OCDF	2,6	2,1	2,5	2,4	2,40	0,22	9
total TCDF	2,3	2,9	3,9	3,4	3,13	0,68	22
total PeCDF	1,6	1,1	2,8	2,6	2,03	0,81	40
total HxCDF	2,3	1,7	1,9	2,0	1,98	0,25	13
total HpCDF	3,0	2,5	2,9	3,2	2,90	0,29	10
Sum PCDD	**21,8**	**21,8**	**23,0**	**22,8**	**22,35**	**0,64**	**3**
Sum PCDF	**11,8**	**10,3**	**14,0**	**13,6**	**12,43**	**1,71**	**14**
Sum PCDD/PCDF	**33,6**	**32,1**	**37,0**	**36,4**	**34,78**	**2,32**	**7**
Sum 2378-Isomers	**24,9**	**22,8**	**22,6**	**22,0**	**23,08**	**1,26**	**5**
TEQ (I-TEF)	**0,5**	**0,4**	**0,5**	**0,5**	**0,50**	**0,06**	**13**

n.d. not detected

Table D.2 — Threefold analysis of a high level contaminated soil sample

Soil B	Experiment 1	Experiment 2	Experiment 3	Mean	Standard-deviation	Standard-deviation
	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	%
2378-TCDD	2,3	2,3	2,5	2,37	0,12	5
12378-PeCDD	7,0	7,6	5,9	6,83	0,86	13
123478-HxCDD	10,6	10,0	10,1	10,23	0,32	3
123678-HxCDD	22,0	21,0	17,9	20,30	2,14	11
123789-HxCDD	17,6	13,3	13,6	14,83	2,40	16
1234678-HpCDD	151,4	175,3	162,0	162,90	11,98	7
OCDD	257,2	312,9	282,1	284,07	27,90	10
total TCDD	127,2	140,6	119,6	129,13	10,63	8
total PeCDD	125,4	125,7	132,1	127,73	3,78	3
total HxCDD	206,6	213,3	200,0	206,63	6,65	3
total HpCDD	282,4	288,9	290,6	287,30	4,33	2
2378-TCDF	44,1	47,2	38,6	43,30	4,36	10
12378-PeCDF	66,0	72,2	61,4	66,53	5,42	8
23478-PeCDF	47,4	49,5	44,7	47,20	2,41	5
123478-HxCDF	137,9	137,7	136,1	137,23	0,99	1
123678-HxCDF	66,6	66,0	55,8	62,80	6,07	10
234678-HxCDF	58,1	56,3	52,3	55,57	2,97	5
123789-HxCDF	12,2	11,8	10,8	11,60	0,72	6
1234678-HpCDF	538,8	552,5	511,1	534,13	21,09	4
1234789-HpCDF	97,0	108,0	91,4	98,80	8,45	9
OCDF	727,1	705,1	785,2	739,13	41,38	6
total TCDF	270,0	312,1	352,6	311,57	41,30	13
total PeCDF	425,2	400,1	428,5	417,93	15,53	4
total HxCDF	650,4	729,0	646,9	675,43	46,42	7
total HpCDF	834,6	828,8	840,2	834,53	5,70	1
Sum PCDD	**998,8**	**1081,4**	**1024,4**	**1034,87**	**42,28**	**4**
Sum PCDF	**2907,3**	**2975,1**	**3053,4**	**2978,60**	**73,11**	**2**
Sum PCDD/PCDF	**3906,1**	**4056,5**	**4077,8**	**4013,47**	**93,59**	**2**
Sum 2378-Isomers	**2263,3**	**2348,7**	**2281,5**	**2297,83**	**44,98**	**2**
TEQ (I-TEF)	**78,6**	**80,2**	**73,1**	**77,28**	**3,70**	**5**

Table D.3 — Twofold analysis of a low level contaminated soil sample

Soil/Sediment		Experiment 1	Experiment 2	Mean	Standard-deviation	Standard-deviation
		ng/kg_{lyo}	ng/kg_{lyo}	ng/kg_{ly}	ng/kg_{lyo}	%
dioxin-like PCB (WHO 1998)						
non-ortho PCBs						
	#77	739,8	634,2	687,00	74,67	11
	#81	15,5	18,8	17,15	2,33	14
	#126	26,0	25,8	25,90	0,14	1
	#169	4,2	4,2	4,20	0,00	0
mono-ortho PCBs						
	# 105	1882,4	1722,1	1802,25	113,35	6
	# 114	69,7	68,2	68,95	1,06	2
	# 118	3872,9	3542,9	3707,90	233,35	6
	# 123	59,7	63,4	61,55	2,62	4
	# 156	1100,7	982,3	1041,50	83,72	8
	# 157	173,0	163,6	168,30	6,65	4
	# 167	484,8	466,7	475,75	12,80	3
	# 189	143,6	121,5	132,55	15,63	12
Σ TEQ		**4,0**	**3,8**	**3,92**	**0,10**	**3**

Table D.4 — Twofold analysis of a medium level soil sample

Soil/Sediment		Experiment 1	Experiment 2	Mean	Standard-deviation	Standard-deviation
		ng/kg_{lyo}	ng/kg_{lyo}	ng/kg_{lyo}	ng/kg_{lyo}	%
dioxin-like PCB (WHO 1998)						
non-ortho PCBs						
	#77	26620,6	28191,0	27405,80	1110,44	4
	#81	1031,7	940,5	986,10	64,49	7
	#126	97,0	95,0	96,00	1,41	1
	#169	1,2	4,0	2,60	1,98	76
mono-ortho PCBs						
	# 105	20374,6	17764,0	19069,30	1845,97	10
	# 114	876,5	766,0	821,25	78,14	10
	# 118	21163,6	19473,5	20318,55	1195,08	6
	# 123	664,3	602,5	633,40	43,70	7
	# 156	1000,4	972,5	986,45	19,73	2
	# 157	155,3	160,5	157,90	3,68	2
	# 167	390,1	402,5	396,30	8,77	2
	# 189	92,3	84,0	88,15	5,87	7
Σ TEQ		**17,7**	**17,2**	**17,46**	**0,37**	**2**

D.2.2 Validation data for sludge

The validation data for sludge are derived from a fourfold analyses of a sludge sample.

Table D.5 — Fourfold analysis of a sludge sample

Sludge A	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Mean	Standard-deviation	Standard-deviation
	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	ng/kg_{Lyo}	%
2378-TCDD	1,2	1,2	1,4	1,3	1,27	0,10	8
12378-PeCDD	2,3	2,4	2,3	2,6	2,39	0,12	5
123478-HxCDD	2,1	2,2	2,6	2,6	2,38	0,24	10
123678-HxCDD	14,8	14,6	12,8	14,4	14,15	0,92	6
123789-HxCDD	7,6	6,6	8,2	8,8	7,80	0,90	12
1234678-HpCDD	336,6	332,2	353,4	329,3	337,87	10,77	3
OCDD	3434,4	3050,7	2420,7	2261,7	2791,87	547,34	20
total TCDD	22,7	21,0	26,0	22,4	23,03	2,13	9
total PeCDD	39,3	38,2	37,3	36,0	37,71	1,40	4
total HxCDD	119,8	120,3	101,6	133,8	118,88	13,23	11
total HpCDD	608,5	719,9	644,0	591,0	640,85	57,10	9
2378-TCDF	6,1	6,4	5,9	6,1	6,13	0,21	3
12378-PeCDF	2,2	2,5	2,2	2,6	2,38	0,21	9
23478-PeCDF	3,9	4,9	4,3	4,9	4,52	0,48	11
123478-HxCDF	2,4	3,8	3,6	4,0	3,43	0,69	20
123678-HxCDF	3,6	3,5	4,3	3,9	3,82	0,39	10
234678-HxCDF	4,0	3,9	4,4	4,2	4,12	0,26	6
123789-HxCDF	0,3	0,6	0,4	0,3	0,39	0,13	34
1234678-HpCDF	46,5	44,6	44,8	47,5	45,86	1,39	3
1234789-HpCDF	3,9	4,7	3,6	4,2	4,07	0,48	12
OCDF	104,5	109,1	106,6	121,8	110,47	7,76	7
total TCDF	59,2	61,1	51,3	51,4	55,77	5,14	9
total PeCDF	54,0	57,2	55,1	50,8	54,26	2,63	5
total HxCDF	55,5	60,9	50,9	48,8	54,02	5,36	10
total HpCDF	100,9	102,0	91,1	84,4	94,58	8,36	9
Sum PCDD	**4224,7**	**3950,2**	**3229,6**	**3044,9**	**3612,34**	**564,95**	**16**
Sum PCDF	**374,1**	**390,2**	**354,9**	**357,3**	**369,09**	**16,44**	**4**
Sum PCDD/PCDF	**4598,7**	**4340,3**	**3584,5**	**3402,2**	**3981,43**	**578,20**	**15**
Sum 2378-Isomers	**3976,2**	**3593,9**	**2981,4**	**2820,0**	**3342,89**	**537,94**	**16**
TEQ (I-TEF)	**15,9**	**16,1**	**15,6**	**15,8**	**15,84**	**0,23**	**1**

D.2.3 Validation data for bio waste

In the following Table D.6 validation data from a threefold analysis of a bio-waste sample are shown.

Table D.6 — Threefold analysis of a bio waste sample

Bio waste B	Experiment 1	Experiment 2	Experiment 3	Mean	Standard-deviation	Standard-deviation
	ng/kg$_{Lyo}$	ng/kg$_{Lyo}$	ng/kg$_{Lyo}$	ng/kg$_{Lyo}$	ng/kg$_{Lyo}$	%
2378-TCDD	0,3	0,4	0,5	0,40	0,10	25
12378-PeCDD	1,1	1,1	1,0	1,07	0,06	5
123478-HxCDD	2,3	2,4	1,8	2,17	0,32	15
123678-HxCDD	10,3	10,6	9,1	10,00	0,79	8
123789-HxCDD	4,9	5,2	4,9	5,00	0,17	3
1234678-HpCDD	722,1	734,8	634,1	697,00	54,84	8
OCDD	3193,7	3721,7	3811,5	3575,63	333,80	9
total TCDD	9,5	6,4	8,6	8,17	1,59	20
total PeCDD	11,3	11,7	11,2	11,40	0,26	2
total HxCDD	81,4	84,1	82,7	82,73	1,35	2
total HpCDD	1089,8	1099,4	1039,7	1076,30	32,06	3
2378-TCDF	2,6	2,8	2,7	2,70	0,10	4
12378-PeCDF	1,0	1,1	1,0	1,03	0,06	6
23478-PeCDF	1,6	1,8	1,5	1,63	0,15	9
123478-HxCDF	3,2	1,0	1,3	1,83	1,19	65
123678-HxCDF	1,7	2,2	1,7	1,87	0,29	15
234678-HxCDF	1,8	1,7	1,7	1,73	0,06	3
123789-HxCDF	0,2	0,2	0,2	0,20	0,00	0
1234678-HpCDF	25,4	29,6	23,1	26,03	3,30	13
1234789-HpCDF	2,1	2,0	1,9	2,00	0,10	5
OCDF	49,3	55,7	50,2	51,73	3,46	7
total TCDF	23,3	25,2	21,6	23,37	1,80	8
total PeCDF	25,2	25,3	24,0	24,83	0,72	3
total HxCDF	28,3	31,2	24,5	28,00	3,36	12
total HpCDF	57,7	65,7	60,5	61,30	4,06	7
Sum PCDD	**4385,7**	**4923,3**	**4953,7**	**4754,23**	**319,52**	**7**
Sum PCDF	**183,8**	**203,1**	**180,8**	**189,23**	**12,10**	**6**
Sum PCDD/PCDF	**4569,5**	**5126,4**	**5134,5**	**4943,47**	**323,89**	**7**
Sum 2378-Isomers	**4023,6**	**4574,3**	**4548,2**	**4382,03**	**310,69**	**7**
TEQ (I-TEF)	**15,1**	**16,0**	**14,6**	**15,23**	**0,69**	**5**

D.3 International intercalibration studies

Since more than ten years international intercalibration studies are carried out for soil, sediment and sludge. Within these studies laboratories all around the world participate using their own methods. The results are comparable and the RSD is generally better than 20 %.

A summary of these international intercalibration studies was given by Mr. Bert van Bavel at the Dioxin conference in 2003, (see [8].

In 2005 the laboratory of the Umweltbundesamt GmbH, Austria, participated in this intercalibration study (see [9] using exactly the described method in this European standard. The evaluated results are given in the following Table D.7:

Table D.7 — Evaluation of the analytic results reported by the Umweltbundesamt GmbH, Austria (according to [9])

International Intercalibration Round 10				
	Sediment A	**Sediment B**	**Soil C**	**Sediment D**
TEQ PCDD/F + dioxin-like PCB	0,011 ng TEQ/kg	0,032 ng TEQ/kg	0,19 ng TEQ/kg	9,3 ng TEQ/kg
RSD	20 %	20 %	20 %	26 %
Z-Score Umweltbundesamt	- 1,8	- 0,63	- 0,53	- 1,05
No of laboratories	50	49	49	49

Bibliography

[1] BERNT JOHNKE, DORIS MENKE, JÜRGEN BÖSKE: WHO Revision of the Toxic Equivalency Factors for Dioxins and Furans and its Impact on the Emissions of Waste Incineration Plants in Germany. Newsletter WHO June 2003

[2] M. VAN DEN BERG, L. BIRNBAUM, ATC. Bosveld, B. Brunström, et al.: Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106, 775-92, 1998.

[3] Commission Directive 2002/70/EC of 26 July 2002 establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feeding stuffs (Text with EEA relevance)

[4] EN 1948-1,2,3: Stationary Source Emissions – Determination of the mass concentration of PCDDs/ PCDFs.
Part 1: Sampling,
Part 2: Extraction and clean up,
Part 3: Identification and quantification (May 1997)

[5] NATO/CCMS International Toxicity Equivalency Factor (I-TEF) Method of Risk Assessment for Complex Mixtures of Dioxin and Related Compounds. Report Number 176, August 1988, North Atlantic Treaty Organization, Committee on Challenges of Modern Society

[6] H. HAGENMAIER: Abschlußbericht zum Forschungs- und Untersuchungsvorhaben „Belastung der Umwelt mit Dioxinen“, Tübingen 1987

[7] K. BALLSCHMITER, R. BACHER, A. MENNEL: The Determination of Chlorinated Biphenyls, Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans by GC-MS; Journal of High Resolution Chromatography, Vol. 15, 260-270, 1992

[8] BERT VAN BAVEL, Ten Years of QA/QC: The international intercalibration study of PCDD/DF and WHO TEF assigned PCBs in incineration and soil/sediment/sludge samples, Organohalogen Compounds Vol 60, p.122-125 (2003).

[9] BERT VAN BAVEL, Final Report Tenth of the International Intercalibration Study, December 2005

[10] Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, U.S. Environmental Protection Agency, Office of Water Engineering and Analysis Division, Washington, October 1994

[11] J.Höckel, L.Düsterhöft, W.Körner and H.Hagenmaier, Modifiziertes Clean-up für PCDD/PCDF unter toxikologischen, ökologischen und ökonomischen Gesichtspunkten, Organohalogen Compounds 22, 433 (1996)