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

Sols, biodéchets traités et boues — Dosage des dioxines, des furanes et des polychlorobiphényles de type dioxine par chromatographie en phase gazeuse avec détection sélective de masse (spectrométrie de masse à haute résolution ou SMHR, et spectrométrie de masse en tandem ou SM/SM)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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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 Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical and physical characterization.*

This third edition cancels and replaces the second edition (ISO 13914:2023), which has been technically revised.

The main changes are as follows:

— tandem mass spectrometry (MS/MS) has been added as an alternative gas chromatographic analysis to high resolution mass spectrometry (HRMS);

— validation results for MS/MS have 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 of which 17 have chlorine substitution in the 2,3,7,8-positions.

A group of chlorinated aromatic compounds similar to polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) is known as polychlorinated biphenyls (PCBs) which consists 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 mainly 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 uses in open and closed systems, e.g. as electrical insulators or dielectric fluids in capacitors and transformers, as specialized hydraulic fluids and 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, for example 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 (dl-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.

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

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 — Tests conducted according to this document shall be carried out by suitably trained staff.

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 gas chromatography/high resolution mass spectrometry (GC/HRMS). Detection by tandem mass spectrometry (MS/MS) can be used in an equivalent way.

The analytes that can be determined with the method specified in 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 [a]	TCB
Tetrachlorobiphenyl	TeCB
Pentachlorobiphenyl	PeCB
Hexachlorobiphenyl	HxCB
Heptachlorobiphenyl	HpCB
Decachlorobiphenyl [a]	DecaCB

[a] Groups containing no dl-PCB, given for informative purpose only.

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.

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

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 such as 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.

ISO 11465, *Sludge and solid environmental matrices — Determination of dry residue or water content and calculation of the dry matter fraction on a mass basis*

3 Terms and definitions

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

ISO and IEC maintain terminology 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 analyte analogue added to samples prior to extraction against which the concentrations of native analytes are calculated

[SOURCE: ISO 18073:2004, 3.1.5, modified — “2,3,7,8-PCDD/PCDF” and “PCDDs and PCDFs” were changed to “analytes”.]

3.2
recovery standard
$^{13}C_{12}$-labelled dl-polychlorinated biphenyl (dl-PCB) and polychlorinated dibenzo-p-dioxin/polychlorinated dibenzofuran (PCDD/PCDF), added before injection into the GC

[SOURCE: ISO 18073:2004, 3.1.12, modified — “2,3,7,8-chloro-substituted PCDD/PCDF” was changed to “dl-PCB and PCDD/PCDF”.]

4 Abbreviated terms

dl-PCB	dioxin-like polychlorinated biphenyls
MRM	multiple reaction monitoring
PCDD/PCDF or PCDD/F	Polychlorinated dibenzo-p-dioxins/dibenzofurans
I-TEQ	International toxic equivalent
WHO-TEF	Toxic equivalent factor proposed by WHO in 2005
WHO-TEQ	Toxic equivalent obtained by multiplying the mass determined with the corresponding WHO-TEF including PCDD, PCDF and dl-PCB

NOTE 1 The I-TEQ is 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.

NOTE 2 For detailed description of WHO-TEF, see Annex A.

For detailed description of WHO-TEQ, see Annex A. WHO-TEQPCB and WHO-TEQPCDD/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, at least 16 out of 17 labelled PCDD/PCDF and 12 labelled PCB internal standards are used. The extracts for the GC-MS measurements contain at least one recovery standard per group, i.e. at least one $^{13}C_{12}$-PCDD/PCDF and one $^{13}C_{12}$-PCB. The use of more recovery standards is recommendable. 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 GC/MS (HRMS or MS/MS) 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 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 can overload the separation method, disturb the quantification or otherwise severely impact the performance of the identification and quantification method and the separation of PCDD/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 GC/MS (HRMS or MS/MS).

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 such as 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

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

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

— calibration solutions PCDD/PCDF;

— calibration solutions PCB;

— $^{13}C_{12}$-spiking solution as recovery standard for PCDD/PCDF (remark: typical ^{13}C-congeners used as recovery standards are $^{13}C_{12}$-123789-HexaCDD, $^{13}C_{12}$-1234-TetraCDD, $^{13}C_{12}$-1234-TetraCDD or -DF);

— $^{13}C_{12}$-spiking solution as recovery standard for dl-PCB.

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

7 Apparatus

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 can 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.

7.2.4 Snyder column.

7.3 Soxhlet extractor

Alternatively, other automated Soxhlet-like devices can be used, if applicable. It is the responsibility of the user of this document to validate the application for these alternative devices.

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

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

7.3.3 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:

— 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.

7.4.3 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

Alternatively, other evaporation devices can be used, if applicable. It is the responsibility of the user of this document to validate the application for these alternative evaporation devices.

7.5.1 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.

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

7.5.3 Kuderna-Danish[1)] concentrator.

7.5.4 Sample vials, of the following types:

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

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

7.6 Other equipment

7.6.1 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.

7.6.2 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).

7.6.3 Mass spectrometer, 28 eV to 80 eV electron impact ionization, capable of repetitively selectively monitoring of twelve exact masses minimum during a period of approximately 1 s either at high mass resolution (≥10 000 at 10 % peak valley or equivalent) or 70 eV electron impact ionization following analysis utilizing a triple quad MS/MS system on specific multiple reaction monitoring (MRM) (see Table 5).

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

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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.

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 cool and dark where necessary in order to avoid alteration of sample constitution e.g. in combination with water content determination.

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 ISO 11465 or any equivalent method which is internationally standardized.

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 B or any suitable alternative procedures.

The determination of PCDD/PCDF and dl-PCB is based on quantification by the isotope-dilution technique using GC/MS (HRMS or MS/MS). $^{13}C_{12}$-labelled dl-PCB and 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 GC/MS analysis. However, due to possible differences in the binding and adsorption characteristics between the native analytes 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 B. Annex C gives examples of operation of GC/HRMS determination.

The main purpose of the clean-up procedure of the raw sample extract is the removal of sample matrix components, which can overload the separation method, disturb the quantification or otherwise severely impact the performance of the identification and quantification method and to separate dioxin-like PCB from PCDD/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.