INTERNATIONAL STANDARD

ISO 23646

First edition
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Soil quality — Determination of organochlorine pesticides by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)

Qualité du sol — Détermination des pesticides organochlorés par chromatographie en phase gazeuse avec détection sélective de masse (CG-SM) et chromatographie en phase gazeuse avec détection par capture d'électrons (GC-ECD)

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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on the 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 the following URL: 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 first edition cancels and replaces ISO 10382:2002, which has been technically revised.

The main changes are as follows:

— polychlorinated biphenyls have been deleted from the Scope;

— modern extraction techniques and commonly used methods with optimized extraction time, proven clean-up methods and state of the art quantification methods 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.

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Introduction

Organochlorine pesticides (OCPs) are organic synthetic substances which are globally used. The vast majority of OCPs have been released directly in the environment as agricultural insecticides but they have been also used as by-products for different applications, e.g. as a wood preserver. OCPs are persistent, bioaccumulating and prone to long-range atmospheric transport and deposition. They are ubiquitous in the environment (water, soil, sediment and waste) and their presence is regularly monitored and controlled.

This document describes the determination of OCPs in soil and sediments. At present, determination of OCPs is carried out in these matrices in most of the routine laboratories following the preceding steps for sampling, pretreatment, extraction and clean-up by measurement of a specific OCP by means of gas chromatography in combination with mass spectrometric detection (GC-MS) or gas chromatography with electron capture detector (GC-ECD). GC-MS/MS is also applicable (see Annex C for an example of GC-MS/MS measurement conditions for OCPs). The described analytical steps are also applicable for the determination of polychlorinated biphenyls (PCBs). However, for the determination of PCBs, a specific European Standard, EN 17322, is available. Both standards are very similar; differences exist especially in a broader variety of clean-up steps for PCBs.

Considering the different matrices and possible interfering compounds, this document does not contain one single possible way of working. Several choices are possible, in particular relating to clean-up. Detection with both mass spectrometry and electron capture is possible. Three different extraction procedures and four clean-up procedures are described. The use of internal and injection standards is described in order to have an internal check on the choice of the extraction and clean-up procedure.

This document is applicable and validated for several types of matrices as indicated in Table 1 (see also Annex A for the results of the validation).

Table 1 — Matrices for which this document is applicable and validated

Matrix	Materials used for validation
Soil	Sandy soil, contaminated with OCPs Soil from the vicinity of Berlin
Humic rich soil	Humic rich soil Mix of soil from the vicinity of Berlin, Germany and PCB-free German reference soil
Sediment	Validation results from ISO 10382 (WC 102 and WC 106)

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Soil quality — Determination of organochlorine pesticides by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD)

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document are carried out by suitably trained staff.

1 Scope

This document specifies a method for quantitative determination of organochlorine pesticides (OCPs) and semi-volatile chlorobenzenes in soil and sediment, using GC-MS and GC-ECD (see Table 2).

Table 2 — Target analytes of this document

Target analyte	CAS-RN	Formula
Aldrin	309-00-2	$C_{12}H_8Cl_6$
Dieldrin	60-57-1	$C_{12}H_8Cl_6O$
Endrin	72-20-8	$C_{12}H_8Cl_6O$
Isodrin	465-73-6	$C_{12}H_8Cl_6$
Telodrin	297-78-9	$C_9H_4Cl_8O$
Heptachlor	76-44-8	$C_{10}H5Cl_7$
Heptachloro epoxide (exo-, cis-isomer)	1024-57-3	$C_{10}H_5Cl_7O$
Heptachloro epoxide (endo-, trans-isomer)	28044-83-9	$C_{10}H_5Cl_7O$
α-Endosulfan	959-98-8	$C_9H_6Cl_6O_3S$
β-Endosulfan	33213-65-9	$C_9H_6Cl_6O_3S$
Endosulfan sulfate	1031-07-8	$C_9H_6Cl_6O_3S$
p,p′-DDE (1,1-bis-(4-chlorophenyl)-2,2-dichloroethen)	72-55-9	$C_{14}H_8Cl_4$
o,p′-DDD (1-(2-Chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethan)	53-19-0	$C_{14}H_{10}Cl_4$
o,p′-DDT (1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethan)	789-02-6	$C_{14}H_9Cl_4$
p,p′-DDD (1,1-Dichloro-2,2-bis(4-chlorophenyl)ethan)	72-54-8	$C_{14}H_{10}Cl_4$
o,p′-DDE (2-(2-Chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethen)	3424-82-6	$C_{14}H_8Cl_4$
p,p′-DDT (1,1,1-Trichlor-2,2-bis-(4-chlorophenyl)ethan)	50-29-3	$C_{14}H_9Cl_4$
Methoxychlor	72-43-5	$C_{16}H_{15}Cl_3O_2$
HCB Hexachlorobenzene	118-74-1	C_6Cl_6
α-HCH (α-Hexachlorocyclohexane)	319-84-6	$C_6H_6Cl_6$
β-HCH (β-Hexachlorocyclohexane)	319-85-7	$C_6H_6Cl_6$
γ-HCH (γ-Hexachlorocyclohexane)	58-89-9	$C_6H_6Cl_6$
δ-HCH (δ-Hexachlorocyclohexane)	319-86-8	$C_6H_6Cl_6$
Hexachloro-1,3-butadiene	87-68-3	C_4Cl_6
α-Chlordane	5103-71-9	$C_{10}H_6Cl_8$
γ-Chlordane	5103-74-2	$C_{10}H_6Cl_8$
1,2,4-Trichlorobenzene	120-82-1	$C_6H_3Cl_3$
1,2,3-Trichlorobenzene	87-61-6	$C_6H_3Cl_3$
1,3,5-Trichlorobenzene	108-70-3	$C_6H_3Cl_3$

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Table 2 *(continued)*

Target analyte	CAS-RN	Formula
1,2,3,4-Tetrachlorobenzene	634-66-2	$C_6H_2Cl_4$
1,2,3,5-Tetrachlorobenzene	634-90-2	$C_6H_2Cl_4$
1,2,4,5-Tetrachlorobenzene	95-94-3	$C_6H_2Cl_4$
Pentachlorobenzene	608-93-5	C_6HCl_5

The limit of detection and the limit of application depends on the determinants, the sample intake, the equipment used, the quality of chemicals used for the extraction of the sample and the clean-up of the extract.

Under the conditions specified in this document, lower limits of application from 1 µg/kg (expressed as dry matter) for soils to 10 µg/kg (expressed as dry matter) for sediments can be achieved. The necessity to achieve these lower limits of application depends on the analyses order and the current limit values.

Soils and sediments can differ in properties as well as in the expected contamination levels of OCPs and the presence of interfering substances. These differences make it impossible to describe one general procedure. Based on the properties of the samples, this document contains decision tables regarding drying-, extraction- and clean-up procedures. This method is performance based. The method can be modified if all performance criteria given in this method are met.

The method can be applied to the analysis of other chlorinated compounds not specified in the scope in cases where suitability has been proven by proper in-house validation experiments.

NOTE The validation data are shown in Annex A. This document is validated only for α-HCH, β-HCH, γ-HCH, δ-HCH, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD, o,p′-DDT and p,p′-DDT. For sediments, data are displayed measured using an ECD detection. The comparability of ECD and MS data in terms of the approach of this document was demonstrated on additional matrices.

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 5667-15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods — Part 1: Linear calibration function*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

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

ISO 18512, *Soil quality — Guidance on long and short term storage of soil samples*

ISO 22892, *Soil quality — Guidelines for the identification of target compounds by gas chromatography and mass spectrometry*

EN 16179, *Sludge, treated biowaste and soil — Guidance for sample pretreatment*

3 Terms and definitions

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

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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
calibration standard
solution of organochlorine pesticides (OCPs) prepared from a secondary standard and/or stock solutions of native OCPs and used to calibrate the response of the instrument with respect to analyte concentration

3.2
internal standard
labelled organochlorine pesticide (OCP) or other OCP that is unlikely to be present in the sample, added to the sample prior to extraction and used for quantification of OCP content

3.3
extraction standard
chemical substance which is only used for checking the extraction efficiency and not used for quantification purposes

3.4
injection standard
labelled organochlorine pesticide (OCP) or other OCP that is unlikely to be present in the sample, added to the extract before injection into the gas chromatograph, and used to monitor variability of instrument response and the recovery of the *internal standards* (3.2)

3.5
performance standard
one calibration solution used for the determination of *performance criteria* (3.6), which contains the same amount of internal, extraction and *injection standards* (3.4) used in the samples

3.6
performance criterion
value for the recovery of standards which describes the capacity of the analytical method or parts of the analytical method

4 Principle

Due to the multi-matrix character of this document, different procedures for different steps (modules) are allowed. Which modules should be used depends on the sample. A recommendation is given in this document. Performance criteria are described and it is the responsibility of the laboratories applying this document to show that these criteria are met. Use of spiking standards (internal standards) allows an overall check on the efficiency of a specific combination of modules for a specific sample. But it does not necessarily give the information regarding the extensive extraction efficiency of the native OCPs bonded to the matrix.

After pre-treatment, the test sample is extracted with a suitable solvent or solvent-mixture.

The extract is concentrated by evaporation. If necessary, interfering compounds are removed by a clean-up method suitable for the specific matrix, before this concentration step.

The extract is analysed by gas chromatography. The compounds are separated using a capillary column with a stationary phase of low polarity. Detection takes place with mass spectrometry (MS) or with an electron capture detector (ECD). GC-MS/MS is also applicable if the described performance criteria (see 10.7.5) and performance characteristics (see Clause 11) are met.

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OCPs are identified and quantified by comparison of relative retention times and relative peak heights (or peak areas) with respect to internal standards added. The efficiency of the procedure depends on the composition of the matrix that is investigated.

5 Interferences

5.1 Interference with sampling and extraction

Use sampling containers of materials (preferably of steel, aluminium or glass) that do not affect the sample during the contact time. Avoid plastics and organic materials during sampling, sample storage or extraction. Keep the samples away from direct sunlight and prolonged exposure to light.

During storage of the samples, losses of OCPs can occur due to adsorption on the walls of the containers. The extent of the losses depends on the storage time.

5.2 Interference with GC

Substances that coelute with the target OCPs can interfere the determination. These interferences can lead to incompletely resolved signals and can, depending on their magnitude, affect accuracy and precision of the analytical results. Peak overlap does not allow an interpretation of the result. Asymmetric peaks and peaks being broader than the corresponding peaks of the reference substance suggest interferences.

Depending on the utilized stationary phase, some isomers (e.g. 1,2,4,5- and 1,2,3,5-Tetrachlorobenzene) can coelute or be not fully separated. In this case, a positive result should be reported as the sum of both isomers or a different stationary phase should be applied to ensure a separation, which allows to give results for both single isomers.

6 Safety remarks

Some OCPs are toxic and shall be handled with extreme care. Avoid contact with solid materials, solvent extracts and solutions of standard OCPs. It is strongly advised that standard solutions are prepared centrally in suitably equipped laboratories or are purchased from suppliers specialized in their preparation.

Solvent solutions and samples containing OCPs shall be disposed of in a manner approved for disposal of toxic wastes.

For the handling of hexane, precautions shall be taken because of its neurotoxic properties.

Precautions shall be taken with respect to all hazards associated with this method.

7 Reagents

7.1 General

All reagents shall be of recognized analytical grade. The purity of the reagents used shall be checked by running a blank determination as described in 10.1. The blank shall be less than 50 % of the lowest reporting limit.

7.2 Reagents for extraction

7.2.1 Acetone (2-propanone), $(CH_3)_2CO$.

7.2.2 n-heptane, C_7H_{16}.

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7.2.3 Petroleum ether, boiling range 40 °C to 60 °C.

7.2.4 Hexane-like solvents, boiling range between 30 °C and 89 °C.

7.2.5 Anhydrous sodium sulfate, Na_2SO_4. The anhydrous sodium sulfate shall be kept carefully sealed.

7.2.6 Distilled water or water of equivalent quality, H_2O.

7.2.7 Sodium chloride, NaCl, anhydrous.

7.2.8 Keeper substance. Non-polar organic solvent with high boiling point, i.e. octane, nonane.

7.3 Reagents for clean-up

7.3.1 Clean-up A using aluminium oxide

7.3.1.1 Aluminium oxide, Al_2O_3.

Basic or neutral, specific surface 200 m^2/g, activity Super I in accordance with Reference [6].

7.3.1.2 Deactivated aluminium oxide.

Deactivated with approximately 10 % water.

Add approximately 10 g of water (7.2.6) to 90 g of aluminium oxide (7.3.1.1). Shake until all lumps have disappeared. Allow the aluminium oxide to condition before use for about 16 h, sealed from the air. Use it for a maximum of two weeks.

NOTE The activity depends on the water content. It can be necessary to adjust the water content.

7.3.2 Clean-up B using silica gel 60 for column chromatography

7.3.2.1 Silica gel 60, particle size 63 µm to 200 µm.

7.3.2.2 Silica gel 60, water content: mass fraction $w(H_2O)$ = 10 %.

Silica gel 60 (7.3.2.1), heated for at least 3 h at 450 °C, cooled down and stored in a desiccator containing magnesium perchlorate or a suitable drying agent. Before use, heat at least for 5 h at 130 °C in a drying oven. Then allow cooling in a desiccator and add 10 % water (mass fraction) in a flask. Shake for 5 min intensively by hand until all lumps have disappeared and then for 2 h in a shaking device. Store the deactivated silica gel in the absence of air. Use it for a maximum of two weeks.

7.3.3 Clean-up C using gel permeation chromatography (GPC)

7.3.3.1 Bio-Beads®[1] S-X3.

7.3.3.2 Ethyl acetate, $C_4H_8O_2$.

1) Bio-Beads® 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. Equivalent products can be used if they can be shown to lead to the same results.

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7.3.3.3 Cyclohexane, C_6H_{12}.

Preparation of GPC, for example:

— put 50 g Bio-Beads®[1)] S-X3 (7.3.3.1) into a 500 ml Erlenmeyer flask and add 300 ml elution mixture made up of cyclohexane (7.3.3.3) and ethyl acetate (7.3.3.2) 1:1 (volume) in order to allow the beads to swell;

— after swirling for a short time until no lumps are left, maintain the flask closed for 24 h;

— drain the slurry into the chromatography tube for GPC;

— after approximately three days, push in the plungers of the column so that a filling level of approximately 35 cm is obtained;

— to further compress the gel, pump approximately 2 l of elution mixture through the column at a flow rate of 5 ml min^{-1} and push in the plungers to obtain a filling level of approximately 33 cm.

7.3.4 Clean-up D using Florisil®[2)]

7.3.4.1 Florisil®[2)], baked for 2 h at 600 °C. Particle size 150 µm to 750 µm.

7.3.4.2 Iso-octane, C_8H_{18}.

7.3.4.3 Toluene, C_7H_8.

7.3.4.4 Iso-octane/Toluene 95/5 (volume fraction).

7.3.4.5 Diethylether, $C_4H_{10}O$.

7.4 Reagents for gas chromatographic analysis

Operating gases for gas chromatography ECD or MS, of high purity and in accordance with the manufacturer's specifications.

7.5 Standards

7.5.1 General

Solvents for preparing standard solutions shall be free of OCPs. Hexane, cyclo-hexane, iso-hexane or other hexane-like solvents can be used. Verify the stability of the standards regularly.

7.5.2 Calibration standards

The calibration standards should contain the target compounds selected from Table 2.

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2) Florisil® is a trade name for a prepared diatomaceous substance, mainly consisting of anhydrous magnesium silicate. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products can be used if they can be shown to lead to the same results.

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7.5.3 Internal, extraction and injection standards

7.5.3.1 General

For internal, extraction and injection standards, choose substances whose physical and chemical properties (e.g. extraction behaviour, retention time) are similar to those of the compounds to be analysed.

The number of internal and extraction standards depends on the calibration strategy of the analyses. Three different possibilities can occur (see Annex B):

a) OCP analysis without any information about the sample:

   1) The internal standard is added to the sample prior to extraction and is used for quantification.

   2) At least three OCPs, covering the chromatogram, shall be used as internal standard.

   3) If required, an injection standard is added to the extract prior to injection.

b) High expected OCP concentrations:

   1) When highly contaminated samples are analysed, an aliquot of the extract is often used for further clean-up. This makes the costs of analyses caused by use of labelled standards very high. In these cases, add the extraction and internal standards in two steps:

      i) Step 1: An extraction standard is added to the sample prior to extraction. The extraction efficiency is checked by comparison with the performance standard.

      ii) Step 2: After extraction, add the internal standards to an aliquot of the extract. These internal standards are used for quantification.

   2) If required, an injection standard is added to the measuring solution prior to injection.

c) Proof of absence:

   1) An extraction standard is added to the sample prior to extraction. The extraction efficiency is checked by comparison with the performance standard.

   2) If required, an injection standard is added to the extract prior to injection.

   3) At least one OCP with a comparable sensitivity to the extraction procedure shall be used as extraction standard.

The substances to be considered as internal, extraction and injection standards are listed below.

For MS-detection, labelled OCPs are advised.

Other OCPs or similar substances, e.g. PCBs not present in the sample or $^{13}C_{12}$-labelled OCPs not used as internal standard, can be used as an injection standard.

7.5.3.2 Labelled analytes

From most of the target analytes listed in Table 2, ^{13}C-labelled standards are commercially available. Also, some deuterated standards are applicable.

α-Endosulfan-d4	(CAS-RN-203645-57-2)
β-Endosulfan-d4	(CAS-RN-203716-99-8)
p,p′-DDE-d4 or -d8	(CAS-RN-93952-19-3)

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o,p′-DDT-d4 or -d8	(CAS-RN-221899-88-3)
p,p′-DDD-d4 or -d8	(CAS-RN-93952-20-6)
o,p′-DDE-d8	(CAS-RN-1402834-57-4)
p,p′-DDT-d4 or -d8	(CAS-RN-93952-18-2)
Methoxychlor-d6	(CAS-RN-106031-79-2)
α-HCH-d6	(CAS-RN-86194-41-4)
γ-HCH-d6	(CAS-RN-60556-82-3)
1,2,4-Trichlorobenzene-d3	(CAS-RN-2199-72-6)
1,2,3-Trichlorobenzene-d3	(CAS-RN-3907-98-3)
1,3,5-Trichlorobenzene-d3	(CAS-RN-1198-60-3)
1,2,3,4-Tetrachlorobenzene-d2	(CAS-RN-2199-73-7)
1,2,3,5-Tetrachlorobenzene-d2	(CAS-RN-2199-74-8)
1,2,4,5-Tetrachlorobenzene-d2	(CAS-RN-1198-57-8)
PCB28-d4	
PCB52-d3	
PCB101-d3	
Phenanthrene-d10	(CAS-RN-1517-22-2)

7.5.3.3 Non-labelled analytes

PCB29	2,4,5-trichlorobiphenyl	(CAS-RN 15862-07-4)
PCB30	2,4,6-trichlorobiphenyl	(CAS-RN 35693-92-6)
PCB143	2,2′,3,4,5,6′-hexachlorobiphenyl	(CAS-RN 68194-15-0)
PCB155	2,2′,4,4′,6,6′-hexachlorobiphenyl	(CAS-RN 33979-03-2)
PCB198	2,2′3,3′,4,5,5′,6,-octachlorobiphenyl	(CAS-RN 68194-17-2)
PCB207	2,2′,3,3′,4,4′,5,6,6′-nonachlorobiphenyl	(CAS-RN 52663-79-3)
PCB209	2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl	(CAS-RN 2051-24-3)

7.5.3.4 Analytes for resolution check

If a resolution check of the GC-column is necessary, the following PCBs are recommended.

PCB28	2,4,4′-trichlorobiphenyl	(CAS-RN 7012-37-5)
PCB31	2,4′,5-trichlorobiphenyl	(CAS-RN 16606-02-3)

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7.5.3.5 Analytes for liner check

Some OCPs (dieldrin, endrin, p,p′-DDT, o,p′-DDT, p,p′-DDD, Methoxychlor) tend to degrade or be adsorbed in the GC liner. To monitor the liner condition, a regular check is highly recommended. Therefore, inject a standard solution containing the following OCPs:

p,p′-DDT (CAS-RN 50-29-3)

Endrin (CAS-RN 72-20-8)

This test is recommended to be performed to assess GC column performance and injection port inertness. During the test, the degradation of DDT to DDE and DDD should not exceed 20 %. Endrin degrades to form endrin aldehyde and endrin ketone. The degradation should also in this case not exceed 20 %. The degradation is calculated using Formula (1).

$$\%\ \text{degradation} = \frac{\text{sum of peak area of degradation products}}{\text{sum of peak area of substance and degradation products}} \times 100 \quad (1)$$

Alternatively, $^{13}C_{12}$-labelled OCPs should be used as internal or injection standards.

7.6 Preparation of standard solutions

7.6.1 Preparation of calibration standard solutions of OCPs

Prepare individual concentrated primary standard solutions of about 0,4 mg/ml in n-heptane (7.2.2) by weighing approximately 10 mg of each of the calibration standards (see 7.5.2) to the nearest 0,1 mg and dissolving them in 25 ml of n-heptane.

Combine small quantities (2 ml to 10 ml) of these individual primary standard solutions into a mixed standard solution of OCP.

WARNING — Because of the dangerous nature of the substances to be used, commercially available (preferably certified) standard solutions or mixed standard solutions should be used. Avoid skin contact.

The working standard solutions shall be in the same solvent as the extract.

The primary and diluted standard solutions have to be stored in a dark place at (5 ± 3) °C. The solutions are stable for at least one year, provided that evaporation of solvent is negligible.

Components present in mixed standard solutions shall be separated by the gas chromatographic columns and conditions used.

7.6.2 Preparation of internal standard solution

Prepare a concentrated primary internal standard solution, containing at least three different components (see 7.5.3.2 or 7.5.3.3) of about 0,4 mg/ml in n-heptane (7.2.2) by weighing approximately 10 mg of each of the chosen internal standards to the nearest 0,1 mg and dissolving them in 25 ml of n-heptane. Prepare from this a secondary internal standard solution with such a concentration that the added amount gives a peak with measurable peak area or peak height in the chromatogram (at least 10 times the detection limit).

If the two-step procedure for GC-MS is used, make two different internal standard solutions, one containing the non-labelled compounds. At least two unlabelled congeners shall be used in the first internal standard solution and at least three labelled congeners in the second solution.

Internal standard solutions have to be stored at (5 ± 3) °C.

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7.6.3 Preparation of injection standard solution

Prepare a concentrated primary injection standard solution, containing at least two different components (see 7.5.3.2 or 7.5.3.3) of about 0,4 mg/ml in an appropriate solvent by weighing approximately 10 mg of each of the chosen injection standards to the nearest 0,1 mg and dissolving them in 25 ml. Prepare from this a secondary injection standard solution with such a concentration that the added amount gives a peak with measurable peak area or peak height in the chromatogram (at least 10 times the detection limit).

Injection standard solutions have to be stored at (5 ± 3) °C.

7.6.4 Preparation of solution for liner checking

For liner checking, prepare a concentrated solution containing p,p′-DDT and endrin (see 7.5.3.5), of about 1 mg/ml in an appropriate solvent by weighing approximately 10 mg of each to the nearest 0,1 mg and dissolving them in 10 ml.

The solution for liner checking has to be stored at (5 ± 3) °C.

8 Apparatus

8.1 Extraction and clean-up procedure

Use customary laboratory glassware.

All glassware to be used shall be thoroughly cleaned, preferably in a dishwasher using a customary cleaning procedure, followed by rinsing with acetone and a subsequent rinsing with a hexane-like solvent.

8.1.1 Sample bottles, made of glass, stainless steel or aluminium, with glass stopper or screw top and polytetrafluoroethylene (PTFE) seal of appropriate volume.

8.1.2 Shaking device, with horizontal movement (200 strokes to 300 strokes per min).

8.1.3 Water bath, adjustable up to 100 °C.

8.1.4 Separating funnels of appropriate volume.

8.1.5 Conical flasks of appropriate volume.

8.1.6 Soxhlet extraction apparatus, consisting of round bottom flask, e.g. 100 ml, Soxhlet extractors and Soxhlet thimbles, e.g. 27 mm × 100 mm, vertical condensers, e.g. 300 mm, heating device.

8.1.7 Pressurized liquid extraction apparatus, including extraction cells and vials.

8.1.8 Ultrasonic bath, with adjustable temperature unit.

8.1.9 Concentrator, Kuderna Danish type.

Other evaporators, e.g. a rotary evaporator, can be used if found to be equally suitable.

8.1.10 Boiling chips, glass or porcelain beads.

8.1.11 Quartz wool or silanized glass wool.

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WARNING — Working with quartz wool imposes a risk to health through the release of fine quartz particles. Inhalation of these particles should be prevented by using a fume cupboard and wearing a dust mask.

8.1.12 Calibrated test tubes, with a nominal capacity of 10 ml to 15 ml and ground glass stopper.

8.1.13 Chromatography tubes. Chromatography column of glass, 5 mm to 10 mm inside diameter, length, e.g. 600 mm.

8.2 Gas chromatograph

8.2.1 General

Equipped with a capillary column, mass spectrometric detector (MS) or electron capture detector (ECD) based on ^{63}Ni.

NOTE Working with an encapsulated radioactive source as present in an ECD requires a licence according to national regulations.

8.2.2 Capillary columns

Comprising a non-polar stationary phase, e.g. 5 % phenyl-methyl silicone, coated onto a fused silica capillary column or an equivalent chemically bonded phase column. In general, column length should be 25 m to 60 m. Internal diameter 0,18 mm to 0,32 mm and film thickness 0,1 µm to 0,5 µm.

If necessary, check the resolution with PCB28 and PCB31. A column is suitable if the chromatographic peaks of PCB28 and PCB31 resolves sufficiently (resolution at least 0,8).

9 Sample storage and pre-treatment

9.1 Sample storage

The samples shall be analysed as soon as possible after sampling.

Wet sediment or suspended matter samples shall be stored in accordance with ISO 5667-15.

Dried samples can be stored at room temperature in a dark place for up to one month. Soil samples shall be stored in accordance with ISO 18512.

9.2 Sample pretreatment

Pretreat samples in accordance with ISO 14507 and/or EN 16179, if not otherwise specified, to obtain a test sample.

NOTE EN 16179 includes sampling, particle size reduction and sample pretreatment. ISO 14507 contains only sample pretreatment.

Pre-treatment is necessary to reduce the moisture content to enable extraction of the OCPs and to increase the homogeneity.

Depending on the nature of the sample material and the extraction solvent to be used, a drying step can be needed. If it is necessary, air-dry the complete sample, dry it in a ventilated drying oven at 40 °C or in a freeze dryer. The drying time depends on the technique chosen and the nature of the sample.

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Complete drying of the sample is essential if Soxhlet is used for extraction. Complete drying is also recommended if it is required that the sample is stored for a long period. The drying time depends on the technique chosen and the nature of the sample.

NOTE Complete drying can cause losses of volatile trichlorobenzenes.

10 Procedure

10.1 Blank test

Perform a blank test with an OCP-free soil following the applied procedure (selected extraction and clean-up procedure) using the same amount of reagents that are used for the pre-treatment, extraction, clean-up and analysis of a sample. Analyse the blank immediately prior to analysis of the samples to demonstrate sufficient freedom from contamination. The blank shall be less than 50 % of the lowest reporting limit.

10.2 Extraction

10.2.1 General

Depending on the sample (matrix and moisture content), choose a suitable extraction procedure (see Table 3).

Extraction procedures 1 (see 10.2.2) and 2 (see 10.2.3) are recommended if it is important to break up aggregates in the sample to access the OCPs. With wet samples, these procedures shall be applied in order to eliminate the presence of water.

Extraction procedure 3 (see 10.2.4) is recommended if the sample is dry and dissolving the OCPs is the most important step (organic rich materials).

Other extraction procedures, e.g. microwave extraction, can be used provided the laboratory can show that the extraction efficiency is equivalent to one of the extraction procedures 1, 2 or 3 as described in this document.

The extraction procedures described in this document are suitable to extract 2 g to 20 g of dry sample. If the sample has a low density (i.e. humic rich soil) or the sample is homogeneous, depending on the expected OCP content, less sample can be used.

The amount of sample shall be weighed with an accuracy of at least 1 %.

Table 3 — Extraction procedures to be used for different matrices

Moisture status of the test sample	Matrix	Extraction solvent	Extraction technique	Extraction procedure
Dry	Soil, sediment	Acetone/hexane-like solvent	Agitation, sonication	Procedure 1 (see 10.2.2)
			PLE	Procedure 2 (see 10.2.3)
		Hexane-like solvent	Soxhlet	Procedure 3 (see 10.2.4)
			PLE	Procedure 2 (see 10.2.3)

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Table 3 *(continued)*

Moisture status of the test sample	Matrix	Extraction solvent	Extraction technique	Extraction procedure
Wet	Soil, sediment	Acetone/hexane-like solvent	Agitation, sonication	Procedure 1 (see 10.2.2)
			PLE	Procedure 2 (see 10.2.3)

10.2.2 Extraction procedure 1 — Agitation or sonication

Place the sample in a bottle (8.1.1). Add a definite volume of the internal standard solution (see 7.6.2). Add 50 ml of acetone (7.2.1) to the test sample and extract by shaking or sonication thoroughly to break up aggregates for 30 min.

Then add 50 ml of petroleum ether (7.2.3) or hexane-like solvent (7.2.4) and shake again thoroughly or use sonication during at least 1 h. Use a horizontal shaking device (8.1.2) and have the solvent movement in the sample bottle as long as possible (horizontal position).

After the solids have been settled, decant the supernatant. Wash the solid phase with 50 ml petroleum ether (7.2.3) or hexane-like solvent (7.2.4) and decant again.

Collect the extracts in a separating funnel (8.1.4) and remove the acetone by shaking twice with 400 ml of water (7.2.6).

Dry the extract over anhydrous sodium sulfate (7.2.5). Rinse the sodium sulfate with petroleum ether (7.2.3) or hexane-like solvent (7.2.4) and add the rinsing to the extract.

NOTE 1 Tap water has shown to be applicable for removal of the acetone, because target compounds are not present.

If the sample contains water up to 25 %, the same procedure can be used. If the water content of the sample is greater than 25 %, this procedure is less effective and the amount of acetone shall be increased. The ratio acetone:water should be at least 9:1. The ratio acetone:petroleum ether or hexane-like solvent should be kept constant to 1:2.

The definite amount of the internal standard added in all extraction procedures shall have such a quantity that their concentrations in the final extract are within the working range of the measurement method. Typically, the concentration of the individual internal standards in the final extract is 0,1 µg/ml. In order to wet the complete sample, a minimum amount of 100 µl of internal standard is recommended.

NOTE 2 In matrices with a high organic matter content, longer extraction procedures can be necessary.

10.2.3 Extraction procedure 2 — Pressurized liquid extraction (PLE)

The apparatus (8.1.7) is prepared according to the instructions of the manufacturer. The volume of the PLE-cell chosen is dependent upon the level of contamination and the volume of the sample to be analysed. Place a cellulose filter at the bottom of the cell and add the sample or a mixture of the sample with diatomaceous earth or sea sand in the extraction cell. Add the definite amount of the secondary internal standard solution (see 7.6.2). If necessary, add further diatomaceous earth or sea sand so that the cell is completely filled. Close the cell tightly and follow the manufacturer's instructions. Depending on the moisture content, choose hexane-like solvent (7.2.4) or a mixture of hexane-like solvent (7.2.4) and acetone (7.2.1).

Example for setting the PLE equipment:

— temperature: 100 °C;

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— static phase: 10 min;

— number of cycles: 2;

— flush: 100 %.

When the extraction is finished, the volume of the extract is measured or filled up to a defined volume.

10.2.4 Extraction procedure 3 — Soxhlet

Place the dried sample in the extraction thimble (8.1.6). Add the definite amount of the secondary internal standard solution (see 7.6.2) and approximately 70 ml of the hexane-like solvent (7.2.4) to the extraction vessel. Extract the sample with the Soxhlet extraction apparatus (8.1.6). The duration of the extraction should be calculated with a minimum of 100 extraction cycles.

If the sample is hygroscopic, add sodium sulfate to the sample to get a free-flowing material.

10.3 Concentration

Add a boiling chip (8.1.10) to the extract and concentrate the extract to approximately 10 ml by evaporation using a concentrator (8.1.9). Transfer the concentrated extract to a calibrated test tube (8.1.12) and concentrate to 1 ml using a gentle stream of nitrogen or another inert gas at room temperature. Record the final volume of the extract.

Concentration is not always required if compounds of interest are present in high concentrations or if large volume injection is used.

In heavily contaminated samples, an aliquot is used for further clean-up. Establish the fraction *f* of the extract used for further clean-up. If non-labelled substances have been used as internal standard added to the sample, add a definite amount of the secondary internal standard solution (see 7.6.2).

To prevent losses of the most volatile OCPs, it is not allowed to evaporate till complete dryness. It is advisable to add a small amount (one drop) of keeper substance (7.2.8).

10.4 Clean-up of the extract

10.4.1 General

Clean-up shall be used if compounds are present that can interfere with the OCPs of interest in the gas chromatogram, or if those compounds can influence the GC-procedure (i.e. contamination of the chromatographic system). If no or negligible interfering substances are present, no clean-up is necessary. Depending on the target substances and the substances to be removed, Table 4 shall be used. If polar compounds shall be removed, take special care on the recoveries of polar OCPs.

Table 4 — Clean-up methods

Method	Clean-up	For removal of	Remarks
Clean-up A	Aluminium oxide	Polar compounds	Take care to adjust water content and keep it constant
Clean-up B	Silica gel	Polar compounds	
Clean-up C	Gel permeation	High molecular compounds, lipids	
Clean-up D	Florisil®[2)]	Polar compounds	

Other clean-up procedures can also be used, provided they remove the interfering peaks in the chromatogram and recoveries after use of the clean-up are at least 80 % for all relevant compounds (including internal standards).

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Transfer the extract obtained in 10.3 quantitatively to the clean-up system. Alternatively, an aliquot can be used.

10.4.2 Clean-up A — Aluminium oxide

Prepare an adsorption column by placing a small plug of quartz wool (8.1.11) in the chromatography tube (8.1.13) and packing it dry with 2,0 g ± 0,1 g of aluminium oxide (7.3.1.1).

Apply the extract to the dry packed adsorption column. Rinse the test tube twice with 1 ml of petroleum ether (7.2.3) or hexane-like solvent (7.2.4) and quantitatively transfer the rinsings to the column with the same pipette as soon as the liquid level reaches the upper side of the column packing. Elute with approximately 20 ml of petroleum ether or hexane-like solvent. Collect the entire eluate.

Keeper substance (7.2.8) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

If a new batch of aluminium oxide is used, the solvent volume to eluate the OCPs completely from the column shall be determined using a proper OCP standard solution.

NOTE Commercially available disposable aluminium oxide cartridges can be used as an alternative if found suitable. A column is suitable if the performance of the method is in agreement with 10.7.5 and 10.8.6.

10.4.3 Clean-up B — Silica gel

Put glass wool (8.1.11) and 10 g silica gel (7.3.2.2) into the chromatographic tube (8.1.13). A layer of sodium sulfate (7.2.5) is added if necessary. Condition with 20 ml petroleum ether (7.2.3) or hexane-like solvent (7.2.4). Apply the extract to the column when the level of the solvent mixture is drained to approximately 0,5 cm above the column packing.

Elution is performed using a total of 10 ml petroleum ether (7.2.3) or hexane-like solvent (7.2.4). Keeper substance (7.2.8) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

10.4.4 Clean-up C — Gel permeation chromatography

The extract is carefully reduced under a gentle nitrogen flow. The residue is immediately dissolved in 5 ml solvent mixture [(ethyl acetate (7.3.3.2) and cyclohexane (7.3.3.3) (1:1)]. The dissolved residue is put into the GPC column filled with Bio-Beads®[1)] (7.3.3.1).

The solvent mixture for GPC is used for elution.

The GPC system-settings should be:

— flow rate: 5 ml/min;

— volume of the sample loop: 5 ml;

— first fraction: 120 ml (24 min);

— OCP elution: 155 ml (31 min);

— last fraction: 20 ml (4 min).

The elution volumes of the first fraction, eluate and last fraction shall be considered recommended values and shall be regularly verified by means of the multi-component OCP standard solution.

Keeper substance (7.2.8) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

NOTE During use of the gel permeation column, a small shift in volume to be collected can occur. This is visible in a decrease of recoveries of the internal standards. If this occurs, readjustment of the sampled volume can be necessary.

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10.4.5 Clean-up D — Florisil®[2)]

Put glass wool (8.1.11) into a chromatographic tube (8.1.13) and add 5 mm sodium sulfate (7.2.5), 1,5 g Florisil®[2)] (7.3.4.1), and again 5 mm sodium sulfate. To fix the mixture, place glass wool (8.1.11) on the top. Rinse the column with approximately 50 ml iso-octane (7.3.4.2). Apply the extract to the column. Rinse the extraction tube/vessel twice with 1 ml iso-octane/toluene (95/5 volume fraction) (7.3.4.4) and quantitatively transfer it onto the column. Afterwards elute with 7 ml iso-octane/toluene and then with 10 ml hexane like solvent (7.2.4)/diethylether (7.3.4.5) (90/10 volume fraction). One drop of keeper substance (7.2.8) is added to the eluate, and then the eluate is reduced to the desired volume (see 10.3).

Commercially available Florisil®[2)] solid phase extraction (SPE) cartridges can also be used. In all cases, the suitability for the clean-up procedure shall be evaluated by checking the method performance.

10.5 Addition of the injection standard

If addition of an injection standard is necessary, add an appropriate amount of injection standard (see 7.6.3) to the extract obtained after clean-up (this amount shall be in line with the concentration of the calibration standard). Record the final volume, *V*.

10.6 Gas chromatographic analysis (GC)

10.6.1 General

Both MS and ECD detectors are allowed. In general, MS is recommended. For both detection techniques, the internal standard method is used for quantification.

Some OCPs are thermolabile and tend to adsorb on the surface of the injection liner. Programmed temperature vaporizing (PTV) injection is recommended. A regular check of the injection system is necessary (control chart).

10.6.2 Setting the gas chromatograph

Set the gas chromatograph (8.2) in such a way that sufficient separation of the OCPs is achieved (see 5.2). Optimize the gas chromatograph starting, for example, from the following conditions.

Separation column: Capillary column (see 8.2.2).

Oven temperature programme: 60 °C, 2 min; 30 °C/min to 120 °C; 5 °C/min to 300 °C; 300 °C, 15 min.

Injector temperature: 260 °C or PTV: 40 °C, 0,1 min; 300 °C min-1 to 250 °C, 5 min at 250 °C.

Splitless injection: 1 µl, keep the split 1,8 min closed.

Carrier gas: Helium 0,8 ml/min to 1 ml/min.

10.7 Mass spectrometry (MS)

10.7.1 Mass spectrometric conditions

Tune the mass spectrometer in accordance with the manufacturer's instructions. Chromatograms are recorded in full scan or selected ion monitoring/recording mode (SIM/SIR). The ions to be selected are given in Table 5. For each target compound, two ions of the chlorine isotope cluster (of the molecular ion) and one specific fragment ion are chosen.

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Table 5 — Diagnostic ions for OCPs to be used with MS detection

Compound	Diagnostic ion 1 *m/z*	Diagnostic ion 2 *m/z*	Diagnostic ion 3 *m/z*
Aldrin	263	265	261
Dieldrin	263	345	381
Endrin	263	261	265
Isodrin	263	227	193
Telodrin	311	206	240
Heptachlor	100	65	272
Heptachloroepoxide (exo-, cis-isomer)	253	183	289
Heptachloroepoxide (endo-, trans-isomer)	253	81	263
α-Endosulfan	195	159	265
β-Endosulfan	195	241	159
Endosulfan sulfate	387	389	253
p,p′-DDE	246	318	176
o,p′-DDD	235	165	199
o,p′-DDT	235	165	199
p,p′-DDD	235	165	199
o,p′-DDE	246	318	176
p,p′-DDT	235	165	199
Methoxychlor	227	228	274
HCB	284	286	282
α-HCH	181	219	109
β-HCH	181	219	109
γ-HCH	181	219	109
δ-HCH	109	219	183
Hexachloro-1,3-butadiene	225	260	190
α-Chlordane	373	375	377
γ-Chlordane	373	375	377
1,2,4-Trichlorobenzene	180	182	145
1,2,3-Trichlorobenzene	180	182	145
1,3,5-Trichlorobenzene	180	182	145
1,2,3,4-Tetrachlorobenzene	216	214	108
1,2,3,5-Tetrachlorobenzene	216	214	108
1,2,4,5-Tetrachlorobenzene	216	214	108
Pentachlorobenzene	250	252	215

Key

m = mass of ion

z = charge number of ion

NOTE The mentioned ions can differ depending on the MS system used and its conditions.

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10.7.2 Calibration of the method using an internal standard

10.7.2.1 General

This is an independent method for the determination of the mass concentrations and is not influenced by injection errors, the volume of water present in the sample or matrix effects in the sample, provided that recovery of the compounds to be analysed is about equal to that of the internal standard.

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Add a specific mass of the internal standard (see 7.6.2) and injection standard (see 7.6.3) to dilutions of the mixed calibration solution (see 7.6.1). The mass concentration of both standards shall be the same for all calibration solutions and comparable with the concentration of both standards in the final extract. Run the GC-MS analysis with the calibration solutions. Calculate the relative response ratio for the target OCPs and the internal standards after obtaining a calibration curve by plotting the ratio of the mass concentrations against the ratio of the peak areas (or peak heights) using Formula (2):

$$\frac{A_{\mathrm{n}}}{A_{\mathrm{IS}}} = s \times \frac{\rho_{\mathrm{n}}}{\rho_{\mathrm{IS}}} + b \tag{2}$$

where

A_{n} is the measured response of the target OCPs, e.g. peak area;

A_{IS} is the measured response of the internal standard, e.g. peak area;

s is the slope of the calibration function;

ρ_{n} is the mass concentration of the target OCPs in the calibration solution, expressed in micrograms per litre (µg/l);

ρ_{IS} is the mass concentration of the internal standard in the calibration solution, expressed in micrograms per litre (µg/l);

b is the intercept of the calibration curve with the ordinate.

Two types of calibration are distinguished: the initial calibration (see 10.7.2.2) and the daily calibration (validity check of the initial calibration), which is known as the "calibration verification" (see 10.7.2.3).

Nonlinear calibration methods can be applied.

10.7.2.2 Initial calibration

The initial calibration serves to establish the linear working range of the GC-MS. This calibration is performed when the method is used for the first time and after maintenance and/or repair of the equipment.

Take a gas chromatogram of a series of at least five standard solutions with equidistant concentrations, including the solvent blank. Identify the peaks, using MS or the gas chromatograms of the individual compounds. Prepare a calibration graph for each compound.

Check for linearity in accordance with ISO 8466-1.

Nonlinear calibration using all five standards is allowed. In that case, the same five standards shall be used for re-calibration.

10.7.2.3 Calibration verification

The calibration verification checks the validity of the linear working range of the initial calibration curve and shall be performed before each series of samples.

For every series of samples, inject at least two calibration standards with concentrations of (20 ± 10) % and (80 ± 10) % of the established linear range and calculate the straight line from these measurements. If the straight line falls within ±10 % of the reference values of the initial calibration line, the initial calibration line is assumed to be valid. If not, a new calibration line shall be established according to 10.7.2.2.

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10.7.3 Measurement

Analyse the extracts according to 10.6. With the aid of the absolute retention times, identify the peaks to be used to calculate the relative retention times. Use the internal standard or injection standard as close as possible to the target peak to be quantified. For the other relevant peaks in the gas chromatograms, determine the relative retention times.

If the concentration is above the level for proper identification or quantification, a diluted extract shall be injected for proper identification or quantification of the relevant target OCPs or re-extract the sample using a lower amount of sample.

If as a result of dilution, the internal standard is outside the linear range, Formula (4) does not give the proper quantification and the deviation from linearity shall be taken into account.

10.7.4 Identification

Apply ISO 22892 for the identification of the OCPs. In ISO 22892, the chromatographic criteria and MS-criteria are described, which are necessary for proper identification. Use the diagnostic ions as given in Table 5.

10.7.5 Check on method performance

Because this document allows using different modules, it is essential to ensure quality criteria specified in performance characteristics (see Clause 11). For that, a blank material (e.g. see sand) is spiked with the performance standard solution. The performance standard can be one of the calibration standards, provided that the ratio of the volumes (internal-, extraction- and/or injection standard) used is the same. The performance standard sample runs through the total analytical procedure like the matrix samples. The recovery of the internal standard is calculated using Formula (3).

Use for this analysis:

— the same final volume;

— the same definite volume of internal standard;

— the same definite volume of injection standard.

Calculate for each internal standard the ratio between sample and performance standard solution using the closest injection standard with Formula (3):

$$U = \frac{A_1(S)}{A_2(S)} \times \frac{A_2(p_s)}{A_1(p_s)} \times 100 \quad (3)$$

where

U is the recovery rate, expressed in per cent (%);

A_1 is the measured response of the internal standard, e.g. peak area;

A_2 is the measured response of the injection standard, e.g. peak area;

p_s is the performance standard;

S is the sample.

The recovery of the internal standard shall be at least 50 %. If the recovery of the internal standard is lower than 50 %, this standard is not applicable.

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If the two-step procedure for addition of the internal standard has been used, calculate the extraction ratio between the non-labelled extraction standard added to the sample and the internal standard added to the extract (quantification standard) using Formula (4):

$$E = \frac{A_{1,\text{mean}}(S) \times f}{A_3(S)} \times \frac{A_3(p_s)}{A_1(p_s)} \times 100 \tag{4}$$

where

E is the extraction recovery rate in per cent (%);

$A_{1,\text{mean}}$ is the average measured response of the labelled OCP internal standard, e.g. peak area;

A_3 is the measured response of the non-labelled OCP extraction standard, e.g. peak area;

f is the fraction of the original extract used for clean-up;

p_s is the performance standard;

S is the sample.

The values calculated for the concentrations of target OCPs in the sample are only considered to be acceptable if the recoveries of the internal standards fulfil the requirement described above. In other cases, the values should be reported as indicative.

10.7.6 Calculation

Calculate the mass content of each target compound from the multipoint calibration of the total method by using Formula (5).

$$w_\text{n} = \frac{(A_\text{n} / A_\text{IS}) - b}{s \cdot m \cdot d_\text{s}} \times \rho_\text{IS} \times f_\text{e} \times f_\text{t} \times V \times 100 \tag{5}$$

where

w_n is the content of the target compound found in the sample, expressed in milligrams per kilogram (mg/kg) on the basis of the dry matter;

A_IS is the measured response of the labelled OCP internal standard in the sample extract;

A_n is the measured response of the target compound in the sample extract;

ρ_IS is the mass of the labelled OCP internal standard added to the sample, expressed in micrograms per millilitre (µg/ml);

m is the mass of the test sample used for extraction, expressed in grams (g);

d_s is the dry matter fraction in the field moist sample, determined in accordance with ISO 11465, expressed in per cent (%);

f_e is the ratio of the total organic solvent volume used for extraction to that of the aliquot used for the analysis; $f = 1$ if the whole extract is used;

f_t is the addition factor;

V is the volume of the final solution, expressed in millilitres (ml);

s is the slope of the recalibration function;

b is the intercept of the recalibration curve with the ordinate.

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The result shall be expressed in milligrams per kilogram (mg/kg) dry matter and rounded to two significant figures.

10.8 Electron capture detection (ECD)

10.8.1 General

Using ECD, the same procedure as for MS can be followed, except the points described below for all specific steps in the measurement. For ECD, the same order in paragraphs has been used as in 10.7 (MS-detection). Only the differences are described.

The analyst has to pay attention to the presence of sulfur in the extract, and use the suitable clean-up before injection.

10.8.2 ECD conditions

The ECD shall be operated at temperatures of 300 °C to 350 °C. Use the manufacturer's recommended settings to give the best conditions for linearity of the detector response.

The make-up gas flow rate shall be selected to give the best sensitivity.

10.8.3 Calibration of the method using an internal standard

Using an ECD, internal and injection standards are not labelled OCP but standards described in 7.5.3.3. Calibration is carried out in accordance to 10.7.2.

10.8.4 Measurement

Refer to 10.7.3.

10.8.5 Identification

Three identification points shall be obtained in accordance with ISO 22892. Check the presence of any assigned compound by repeating the gas chromatographic analysis using GC-MS (see above) or using a column with a moderate polar phase in combination with ECD. The results using the second column should be within 10 %. If both are correct, the three identification points (see ISO 22892) for identification are obtained. If one is missing, only indication can be reported.

An individual compound is assumed to be present if the retention time of the substance in the chromatogram of the sample agrees with the retention time in the chromatogram obtained from a reference substance in a reference solution, measured under the same conditions (tolerance ±1 %, maximum 10 s).

If there is no peak at the characteristic retention time, and the chromatogram is normal in all other aspects, assume that the compound is not present.

10.8.6 Check on ECD method performance

Mistakes are probable when a peak of an interfering compound appears at the same position in the chromatogram as that of the internal standard. Therefore, the following procedure is used to check if interfering compounds are present.

The presence or absence of interfering compounds is determined from the measured responses of the injection standards. When no interfering compounds are present in the extract, the ratio between the responses of the injection standards in the extracts is equal to that ratio in the standard solutions. The quotient of these ratios is the relative response ratio, R_{relr}. When no interfering compounds are present in the extract, the value of R_{relr} is in principle 1,00. In this document, it is assumed that no interfering compounds are present in the extract when R_{relr} = 1,00 ± 0,05.

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When the value of R_{relr} deviates from 1,00 ± 0,05, it is assumed that the response of one of the injection standards is influenced by an interfering compound present in the extract. In this case, the performance of the method is calculated using the undisturbed injection standard.

Verify the correctness of the response of the injection standards as follows.

Calculate the relative response ratio, R_{relr}, for the PCB injection standards by using Formula (6):

$$R_{relr} = \frac{R_{e,198}}{R_{e,2}} \times \frac{R_{s,2}}{R_{s,209}} \tag{6}$$

where

$R_{e,198}$ is the response of PCB198 in the extract;

$R_{e,2}$ is the response of the selected second internal standard in the extract;

$R_{s,209}$ is the response of PCB209 in the working standard solution;

$R_{s,2}$ is the response of the selected second injection standard in the working standard solution.

NOTE PCB198 or PCB209 are recommended as injection standards for ECD detection because of fewer interferences. Other internal standards can be used.

The theoretical value of R_{relr} is 1,00. If R_{relr} = 1,00 ± 0,05, regard the injection standards as correctly quantified and enter the value 1,00 for R_{relr} in Formula (6). If R_{relr} < 0,95 or R_{relr} > 1,05, the gas chromatogram shall be checked for correct quantification of both injection standards. Take particular note of the peak shapes and peak widths. If the quantification has been correctly carried out, use both standards if R_{relr} = 1,00 ± 0,05. Use only the injection standard PCB198 if R_{relr} < 1,05 and use only PCB209 if R_{relr} > 1,05.

Calculate the ratio between sample and performance standard solution for each internal standard using the closest injection standard according to Formula (3).

If multiple clean-up is necessary, lower ratios can be found, because with each clean-up step losses are accepted by this standard. Lower ratios are acceptable if this can be explained by the accepted losses in each clean-up step. The minimum recovery of internal standards shall be 50 %. If the recovery of the internal standard is lower than 50 %, this standard is not applicable.

10.8.7 Calculation

Refer to 10.7.6.

11 Performance characteristics

The method is performance based. It is permitted to modify the method to overcome interferences not specified in this document, provided that the performance criteria are met. Internal standards shall be used to check the pre-treatment, extraction and clean-up procedures. Recoveries of these standards shall be 50 % to 110 %. If the recovery is lower or outside the limits (i.e. 50 % to 110 %), the method shall be modified using other modules described in this document.

Some samples can require multiple clean-up in case of lower recoveries. In this case it is essential to choose a higher number of internal standards or use labelled standards.

12 Precision

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

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13 Test reports

The test report shall contain at least the following information:

a) a reference to this document, i.e. ISO 23646, and to the used detector, ECD or MS;

b) complete identification of the sample;

c) the results of the determination according to 10.7 (GC-MS) and 10.8 (GC-ECD);

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

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Annex A
(informative)

Repeatability and reproducibility data

A.1 Interlaboratory comparison for soil

For the determination of repeatability and reproducibility, data for the determination of OCPs in soil results from two different interlaboratory comparisons were used. One intercomparison was part of the interlaboratory comparison programme carried by the Federal Institute for Materials Research and Testing, Germany (Bundesanstalt für Materialforschung und -prüfung (BAM)). Three sandy soils with different contamination levels were analysed. The second intercomparison was also organized by BAM. In the framework of the project "Ruggedness test study to investigate optimum extraction conditions for the determination of organic substances in soil", one compost sample was analysed. In both intercomparisons, the variety of the extraction and clean-up procedures were applied, and the samples were quantitated by gas chromatography with mass selective detection (GC-MS) and gas chromatography with electron-capture detection (GC-ECD).

Organization and implementation were carried out in accordance with the guidelines given in ISO 13528 and ISO/IEC 17043.

Table A.1 lists the types of materials tested.

Table A.1 — Materials tested in the interlaboratory comparison for the determination of OCPs in soil

Sample	Grain size	Material tested
Soil 1	< 0,125 mm	Sandy soil from an industrial contaminated site, Berlin, Germany
Soil 2	< 0,25 mm	Sandy soil from an industrial contaminated site, Berlin, Germany
Soil 3	< 0,25 mm	Sandy soil from an industrial contaminated site, Berlin, Germany
Compost	< 2 mm	Mixture of compost from green waste, spiked compost and silty sand

The statistical evaluation was conducted in accordance with ISO 5725-2. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (see Table A.2).

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