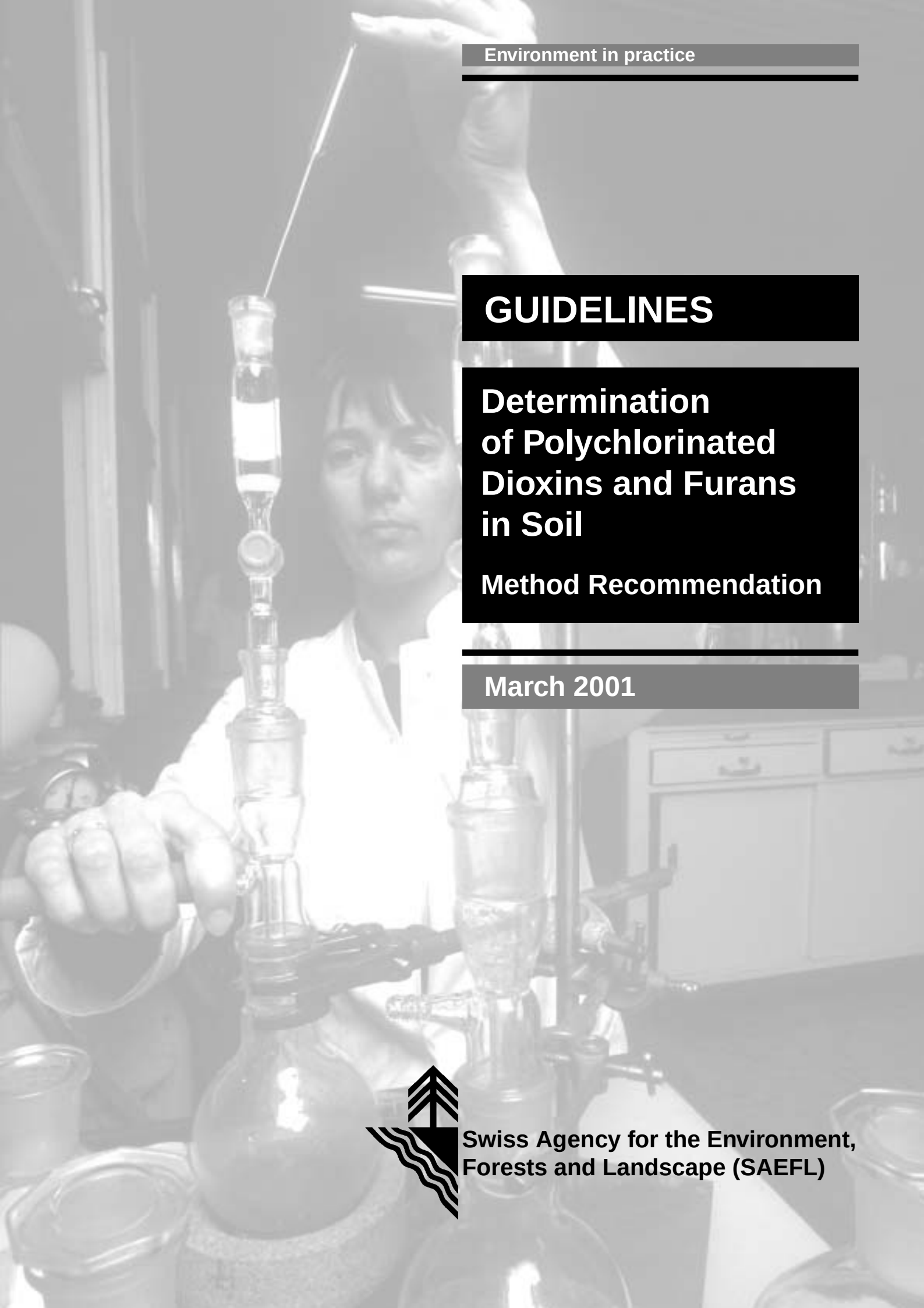

Environment in practice

GUIDELINES

Determination of Polychlorinated Dioxins and Furans in Soil

Method Recommendation

March 2001

Swiss Agency for the Environment,
Forests and Landscape (SAEFL)

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ABSTRACTS

Es wird eine Methode zum Nachweis von polychlorierten Dibenzo-*p*-dioxinen und Dibenzofuranen in Böden beschrieben, welche das vom BUWAL ausgearbeitete *"Qualitätssicherungskonzept"* für die Analytik organischer Schadstoffe im Boden erfüllt. Diese Methode hat in verschiedenen Ringversuche die Vergleichbarkeit mit anderen Techniken bewiesen und erfüllt die Anforderungen der Qualitätssicherungsnorm EN 45'000. Sie beruht auf Soxhletextraktion der getrockneten Bodenproben - gefolgt von einer Probenaufarbeitung mit einem Mehrsäulensystem, welches eine Aktivkohlesäule enthält. Der Zusatz von ^{13}C-isotopenmarkierten 2,3,7,8-chlorsubstituierten Standards vor der Probenextraktion (sogenannte Extraktionsstandards) erlaubt die automatische Korrektur von allfälligen Verlusten, die mit Hilfe der Zugabe von Wiederfindungsstandards vor der Quantifizierung für jede einzelne Probe berechnet werden können. Die Trennung der PCDD/PCDF-Kongenere wird mit hochauflösender Gaschromatographie durchgeführt. Die isotopenmarkierten Standards erlauben die Quantifizierung durch die Isotopenverdünnungstechnik mittels niedrig- oder hochauflösender Massenspektrometrie. Es werden sowohl detaillierte Arbeitsvorschriften, als auch Informationen über Qualitätskontrollmassnahmen vermittelt.

Cette publication décrit une méthode de détection des polychlorodibenzo-*p*-dioxines et des polychlorodibenzofuranes dans le sol. La méthode remplit les conditions posées par le *"système d'assurance de la qualité"* élaboré par l'OFEFP pour l'analyse des polluants organiques du sol. Il a été démontré lors de divers tests interlaboratoires qu'elle était comparable à d'autres techniques ; en outre, elle remplit les exigences de la norme d'assurance de la qualité EN 45'000. Elle se base sur une extraction Soxhlet des échantillons de sol séchés, suivie d'une préparation des échantillons réalisée au moyen d'un système à plusieurs colonnes incluant une colonne de charbon actif. L'addition d'étalons de 2,3,7,8-tétrachlorodioxine ou de 2,3,7,8-tétrachlorofurane marqués au moyen d'isotopes ^{13}C (appelés étalons d'extraction) avant l'extraction de l'échantillon permet de corriger automatiquement les éventuelles pertes, qui peuvent être calculées pour chaque échantillon grâce à l'adjonction d'étalons de récupération avant la quantification. La séparation des congénères PCDD/PCDF est effectuée à l'aide d'une chromatographie en phase gazeuse à haute résolution. Les étalons marqués au moyen d'isotopes permettent de procéder à la quantification grâce à la spectrométrie de masse par dilution isotopique. On recourt à des techniques à basse résolution et à haute résolution. La publication présente des procédures de travail détaillées ainsi que des informations sur les mesures de contrôle de la qualité.

Nella presente pubblicazione viene descritto un metodo per la determinazione delle dibenzo-*p*-diossine e dei dibenzofurani policlorurati presenti nel suolo, il quale soddisfa il concetto di *"Quality Assurance"* elaborato dall'UFAFP per l'analisi degli inquinanti organici nel suolo. Il metodo ha dimostrato la sua comparabilità con altre tecniche in diverse intercalibrazioni e adempie le esigenze della norma EN 45'000 sulla Quality Assurance. Esso si basa sull'estrazione mediante Soxhlet di campioni di suolo seccati, seguita da una preparazione dei campioni mediante un sistema a più colonne che comprende una colonna di carbone attivo. L'aggiunta, prima dell'estrazione, di composti clorurati nelle posizioni 2,3,7,8 e marcati con l'isotopo ^{13}C, quali standard di controllo (i cosiddetti standard per l'estrazione), consente la correzione automatica di eventuali perdite, le quali possono essere calcolate per ogni singolo campione mediante l'aggiunta di uno standard per il calcolo del tasso di ricupero. La separazione dei congeneri PCDD/PCDF viene effettuata mediante la cromatografia in fase gassosa ad alta risoluzione. Usando la spettrometria di massa, il metodo della diluizione isotopica consente la determinazione quantitativa degli standard marcati con isotopi. Vengono impiegate sia le tecniche a bassa risoluzione che quelle ad alta risoluzione. Sono pure forniti i procedimenti analitici dettagliati nonché le informazioni relative alle misure per il controllo della qualità.

A method is described for the determination of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in soil. This method fulfils the criteria of the "quality assurance concept for the analysis of organic pollutants in soil" published by the Swiss Agency for the Environment, Forests and Landscape. The method presented has proved to be comparable with other methods when different comparisons are made, and also fulfils the requirements of the EN 45'000 quality assurance norm. It is based on Soxhlet extraction of dried soil samples, followed by sample clean-up with a multiple column system including an activated charcoal column. The addition of ^{13}C-isotope-labelled 2,3,7,8-chlorine substituted reference compounds prior to extraction (so-called extraction standards) allows the automatic correction of compound losses, which are calculated for each sample by the addition of a recovery standard before quantification. The separation of PCDD/PCDF congeners is carried out by high resolution gas chromatography. The isotope-labelled standards enable quantification to be done by the isotope dilution method using low or high resolution mass spectrometry. Detailed working procedures are given, as well as information about quality control measures.

FOREWORD

Polychlorinated dibenzo-*p*-dioxins (dioxins) and dibenzofurans (furans) are substances of high environmental toxicity. Whereever they are found, be it in soils or in food, these substances are a cause for deep concern, and such events frequently give rise to political motions and reactions on the part of the press.

As a consequence, the federal *Ordinance relating to impacts on the soil (OIS) of 1 July 1998* sets standards for dioxins and furans by means of guide values, trigger values and clean-up values.

However, the chemical analysis of these substances is very expensive and technically demanding. Therefore, such analyses are only carried out if specific indications about hazardous environmental impacts are to be assumed.

For these reasons, it is importance to have an analytical method available that can be reproduced at any time, and which also allows for comparable results.

After publishing guidelines for a *Quality assurance concept*, Prof. M. Oehme of the *Institute of Organic Analytical Chemistry* of the University of Basel has produced a reference method corresponding to the current state of the art.

In making this paper available to those interested, we hope to contribute once again to reliability and accuracy of the results of chemical analyses. This also fulfils our obligations in relation to the aforementioned ordinance.

I wish to express my gratitude to all those who have contributed to the publication and success of this document.

Georg Karlaganis

Head of the Department for Substances,
Soil, and Biotechnology

ABBREVIATIONS AND DEFINITIONS

CEN	*Comité Européen de Normalisation* (European Committee for Standardization) in Brussels
Congeners	Compounds with an identical carbon skeleton but a different number of substituents (e.g. chlorine)
DIN	Deutsches Institut für Normung (German Standards Institute)
EI	Electron Ionisation
EN	European Standard
Extraction standard	A compound that is added prior to sample extraction, allowing compensation for losses during extraction and clean-up
HpCDD	Heptachlorodibenzo-*p*-dioxin
HpCDF	Heptachlordibenzofuran
HPLC	High-performance liquid chromatography
HRGC	High-resolution gas chromatography
HRMS	High-resolution mass spectrometry
HxCDD	Hexachlorodibenzo-*p*-dioxin
HxCDF	Hexachlorodibenzofuran
ISO	International Organization for Standardization
Isomers	Compounds with an identical carbon skeleton and the same number of substituents (e.g. chlorine) at different positions
I-TE	Toxicity equivalent factor according to the international model relative to 2,3,7,8-TCDD
I-TEQ	Toxicity equivalent according to the international model relative to 2,3,7,8-TCDD
"Keeper"	High boiling-point solvent, to be added to the sample extract to prevent evaporation losses
LRMS	Low resolution mass spectrometry
MS	Mass spectrometry
NICI	Negative Ion Chemical Ionisation
OCDD	Octachlorodibenzo-*p*-dioxin
OCDF	Octachlorodibenzofuran
OIS	Ordinance relating to impacts on the soil, of 1 July 1998
PCDD/PCDF	Polychlorinated dibenzo-*p*-dioxins and dibenzofurans
PeCDD	Pentachlorodibenzo-*p*-dioxin
PeCDF	Pentachlorodibenzofuran
PFK	Perfluorokerosene
ppt	*"parts per trillion"*, concentration unit, corresponds to 1 picogram (10^{-12} g) per g
PFTBA	Perfluorotributylamine
Recovery standard	A compound that is added prior to sample quantification, allowing losses in extraction standards and clean-up standards to be calculated
SIM	Selected ion monitoring
TCDD	Tetrachlorodibenzo-*p*-dioxin
TCDF	Tetrachlorodibenzofuran

1 Principle of determination

11 Preliminary remarks

Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) are Soxhlet-extracted from the dried soil sample. ^{13}C-isotope-labelled compounds of all 2,3,7,8-chlorine substituted congeners are added prior to sample extraction. Any interfering sample matrix is removed by multiple column chromatography. The sample clean-up procedure described here is a method generally suited to all kinds of sample matrices including heavily contaminated soils. In principle, soil with little organic content does not require clean-up column 1. However, the method has been validated with the complete clean-up procedure. After concentrating the sample extract to 10 µl and the addition of a recovery standard, all relevant PCDD/PCDF congeners are separated by high resolution gas chromatography and quantified by high or low resolution mass spectrometry.

This method of measurement is suitable for all types of soil and levels of contamination. Depending on sample size and the congener to be quantified, a limit of detection of 0.1-1 pg/g (0.1-1 ppt) can be achieved.

Specific manufacturers are only named if their products have unique properties necessary for the method to be applied successfully.

12 Safety information

2,3,7,8-chlorine substituted PCDD/PCDF compounds are amongst the most toxic substances known. Therefore, the highest possible level of safety measures currently in force in Switzerland must be observed when handling PCDD/PCDF compounds.

2 Equipment, chemicals and instruments

21 Glassware and equipment

211 Glassware

The following glassware made from high quality borosilicate glass ("Pyrex") is needed:

- *Round bottom flasks:* volumes 100, 500 and 2,000 mL, joint size 24/29.
- *Round bottom flasks:* 100 mL with a 5 mL centrifuge tube fused to the bottom, joint size 24/29.
- *Pasteur pipettes:* lengths 150 mm and 250 mm.
- *Glass bottles:* different volumes (polypropylene screw cap GL 45) for sample storage.
- *Centrifuge tubes:* conical, volume 10 mL, with scale.
- *Glass beakers:* 50 and 100 mL.
- *Volumetric flasks with glass stopper:* 1, 2, 5 and 10 mL, quality A, precision ±0.04 mL (20 °C).
- *Erlenmeyer flasks:* 250 mL with glass stopper.

- *Exsiccator:* 300 mm diameter, cover with sleeve (without grease) – without drying agent.
- *Measuring cylinders:* 100 and 1,000 mL, quality A, precision ±0.75 mL or ±5.0 mL (20 °C), respectively.
- *Watch glasses:* 60 mm diameter.

212 Equipment for Soxhlet extraction

- *Soxhlet apparatus:* volumes 200 and 500 mL, length 250 mm, joint size 60/48 and 34/35.
- *Soxhlet apparatus:* 2,000 mL with sleeve lid and fitting joint 34/35.
- *Ball cooler:* length 250 mm, joint size 34/35.
- *Soxhlet thimbles:* cellulose, diameter 28 mm, length 80 mm (for pre-treatment, cf. *section 218*).
- *Socket/cone adapters:* joint size 34/35 to 60/48 and 34/35 to 50/42.
- *Polyurethane foam sheets:* size 35 mm, 210x210 mm – for lagging of Soxhlet extractor.

213 Chromatography equipment

- *Chromatography column 1:* internal diameter (i.d.) 45 mm, length 1,000 mm, with ball joint KS 13/2 at outlet, glass plate made from glass at the column bottom (cf. *fig. 1*).
- *Chromatography column 2:* i.d. 21 mm, outer diameter (o.d.) 30 mm, length 250 mm, with smoothed, flame-polished ends for fitting the end pieces. These consist of Teflon stoppers with a steel capillary pressed into a drilled hole of exact size (o.d. 2 mm, i.d. 1 mm; cf. *fig. 2*).
- *Chromatography column 3:* 9.2 mm i.d., 13 mm o.d., length 80 mm, with smoothed, flame-polished ends. The fitting pieces are similar to those for column 2.
- *Chromatography column 4:* i.d. 40 mm, length 150 mm, sometimes with separate glass tap and joint connection.
- *Chromatography column 5:* i.d. 15 mm, length 150 mm, sometimes with separate glass tap and joint connection.
- *Connection unit for columns 1-3:* with Teflon tubes (i.d. 0.8 mm) and Hamilton valves type 86781 and 86779 (cf. *section 514*).

214 Sample vials

- *Sample vial:* 1.5 mL with 100 µL insert and septum cap, sample vial 1.5 mL with screw cap (Teflon sealed).
- *Certan® vial:* Promochem GmbH, 1.5 mL with capillary insert and screw cap with Teflon-lined seal.
- *Sample vial:* 8 mL with screw cap (Teflon sealed).

215 Other equipment for extract clean-up

- *Suction filter:* diameter 100 mm, porcelain, with Erlenmeyer flask.
- *Porcelain dish:* diameter 180 and 250 mm.
- *Pressure reduction valve:* BS300, metal-bellow sealed.
- *Rotavapor:* with automatic pressure regulation.

Figure 1: Assembly of chromatography column 1

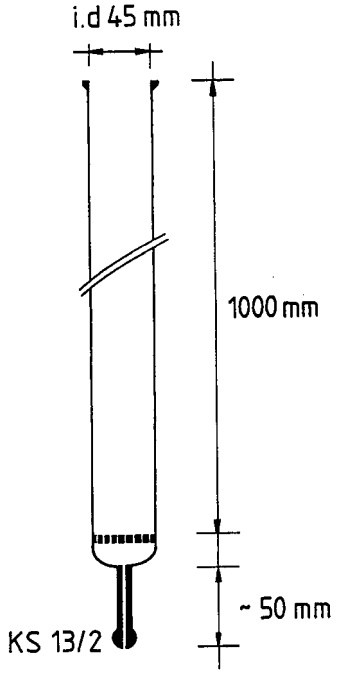
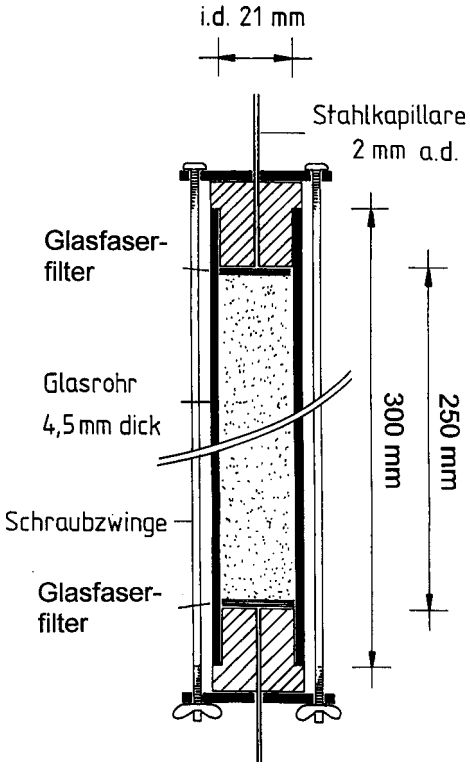


Figure 2: Assembly of chromatography column 2



(***Translation of diagram wordings:*** Glasfaserfilter = glass fibre filter, Glasrohr ... dick = glass tube ... wall thickness, Schraubenzwinge = clamp, Stahlkapillare = steel capillary).

- *Turbovap 500:* volume reduction apparatus (Zymark).
- *Oven:* temperature range 50-300 °C, precision ±3 °C.
- *Tube furnace:* temperature range 50-1,100 °C, precision ±5 °C.
- *Analytical balance:* range 0-160 g, precision ±0.001 g.
- *Balance:* 0-1,200 g, precision ±0.1 g.
- *Millipore MilliQ:* water cleaning system.
- *Furnace:* 200-1,000 °C, precision ±10 °C.
- *Ultrasonic bath:* power 100 W.
- *Centrifuge:* capacity 8x10 mL tubes, minimum 6,000 r.p.m.
- *Ultraturrax:* homogenisation apparatus.
- *Membrane vacuum pumps:* resistant to solvents, with Teflon membrane, 4-8 m^3/h, final vacuum 8 kPa (80 mbar) for 8 m^3/h, 1.5 kPa (15 mbar) for 4 m^3/h.
- *Porcelain mortar:* diameter 130 mm, pestle 145x38 mm.
- *Sieve:* made from stainless steel, mesh size 2 mm (according to DIN 4188).
- *Solvent resistant gloves*.

216 Syringes for dilutions

- *With fixed needle and steel plunger:* 25, 50, 100, 250 and 500 µL.
- *With replaceable needle and Teflon plunger:* 1,000 and 2,500 µL.
- *Calibrated micro pipettes:* 10, 20, 50 and 100 µL, precision ±0.25-1 %.

217 Cleaning of glassware

After each extract clean-up, all types of round bottom flasks, beakers, centrifuge tubes and chromatography columns are soaked for 16 h in a 2.5 % (v/v) solution of RBS 25 (cf. *section 222*); .These items are then rinsed ten times with warm tap water and twice with de-ionised water from a Millipore MilliQ-system. To remove any remaining traces of organic material the air-dried glassware is then heated for 6 h in a furnace at 480 °C. Pasteur pipettes are rinsed before use with the solvent that is to be used.

218 Cleaning of Soxhlet thimbles

Two to eight Soxhlet thimbles are extracted for 8 h with toluene in a 2,000 mL Soxhlet extractor. After drying in a fume hood they are wrapped in aluminium foil.

22 Chemicals, adsorbents and gases

221 Solvents

Acetone	Acetonitrile	Benzene
Cyclohexane	Diethylether	Hexane
Methanol	Methylene chloride	Toluene
Petroleum ether, 40-60 °C		Tetradecane, p.a.

All solvents are used without further cleaning and are, if not specified otherwise, of pesticide grade.

222 Various chemicals, materials and preparatory steps

222.1 Basic materials

- *Cotton wool:* chemically clean (cleaning; cf.*section 222.2*).
- *Glass fibre filter:* type AE, diameter 142 mm, Gelman no. 64878 (cleaning; cf.*section 222.3*).
- *Aluminium foil:* thickness 0.018 mm, size 450 mm.
- *Potassium hydroxide:* p.a.
- *Mercury:* purity 99.99 %.
- *Silanised glass wool:* pre-treated with dimethyldichlorosilane.
- *Sodium sulphate:* P.a. (pre-treatment; cf. *section 222.4*).
- *Sulphuric acid:* 96 %, p.a.
- *RBS 25 Laboratory detergent:* Chemical Products, Brussels, Belgium.
- *Azobenzene:* Tokyo Kasei Kogyo Ltd., Japan, no. A565.
- *Methylazobenzene:* Tokyo Kasei Kogyo Ltd., Japan, no. P142.

222.2 Cleaning of cotton wool

50 g cotton wool (chemically pure) are first Soxhlet-extracted for 8 h with 600 mL methylene chloride and dried in an exsiccator at room temperature under vacuum. This procedure is repeated using 600 mL hexane.

222.3 Cleaning of glass fibre filters

3x10 filters are heated in a furnace for 8 h at 450 °C, and then wrapped in aluminium foil.

222.4 Pre-treatment of sodium sulphate

Two batches of about 1,000 g of sodium sulphate are dehydrated for 8 h at 600 °C, each in a porcelain dish of 180 mm diameter, and stored in the original bottle. The maximum storage period is three months.

223 Adsorbents

223.1 Basic materials

- *Activated charcoal:* Anderson Development Company, AX21.
- *Aluminium oxide:* basic, pH 10, activity I, 50-200 μm, ICN Eschwege, no. 02072.
- *Silica:* 0.063-0.20 mm (pre-treatment; cf. *sections 223.2, 223.3 and 223.4*).

223.2 Pre-treatment of silica

Activation is carried out for 8 h in an oven at 130° C by placing 400 g silica on a porcelain dish (diameter 150 mm). Then the silica is stored in a glass bottle with an air-tight Teflon-lined screw cap. The storage period is four weeks.

Some batches of silica may show increased PCDD/PCDF blanks. In such cases, the silica has to be pre-cleaned with methylene chloride by Soxhlet extraction.

223.3 Preparation of potassium hydroxide coated silica

140 g of potassium hydroxide are dissolved in 600 mL of methanol in a 2 L round bottom flask, and 250 g of untreated silica are added. The mixture is homogenised for 90 min at 55 °C by rotating it with a rotary evaporator. The mixture is then poured into a chromatography column no. 4 with a glass frit at the bottom. The drained adsorbent is then washed with 500 mL methanol and 500 mL methylene chloride. The adsorbent is dried overnight in a fume cupboard, transferred to a porcelain dish, and activated in a furnace for 8 h by increasing the temperature gradually from 50 to 130 °C. After holding the temperature at 130 °C overnight, the adsorbent is then stored in a glass bottle with a Teflon-sealed screw cap. The storage period is four weeks.

223.4 Preparation of sulphuric acid coated silica

Pre-treated activated silica is mixed with sulphuric acid at a weight ratio of 3+2 by adding first 60 g silica to a 250 mL glass bottle and then 40 g of sulphuric acid. The bottle is shaken for about 30 min to obtain a homogenous mixture. If the preparation is kept in a glass stoppered Erlenmeyer flask the storage period is three weeks.

223.5 Aluminium oxide

Aluminium oxide is used without any special treatment. The activity grade is checked monthly (cf. *section 223.6*). The maximum storage period is one year.

223.6 Checking the activity grade of adsorbents

To ensure that the freshly activated adsorbents have the correct level of activity,the following test is carried out:

A glass column (i.d. 15 mm, length 150 mm) with a cotton wool plug at the bottom is dry-packed with an adsorbent layer of 10 cm and covered with 3 mm of sodium sulphate. 10 mL of petroleum ether/benzene 4+1 containing 4 mg of azobenzene (AB) and 4 mg of methylazobenzene (MAB) are placed on top of the column (5-6 portions) and eluted with 20 mL of the same solvent. The level of activity is correct (cf. *fig. 3*) if the following elution distance is observed from the top of the adsorbent layer to the midline of the coloured band:

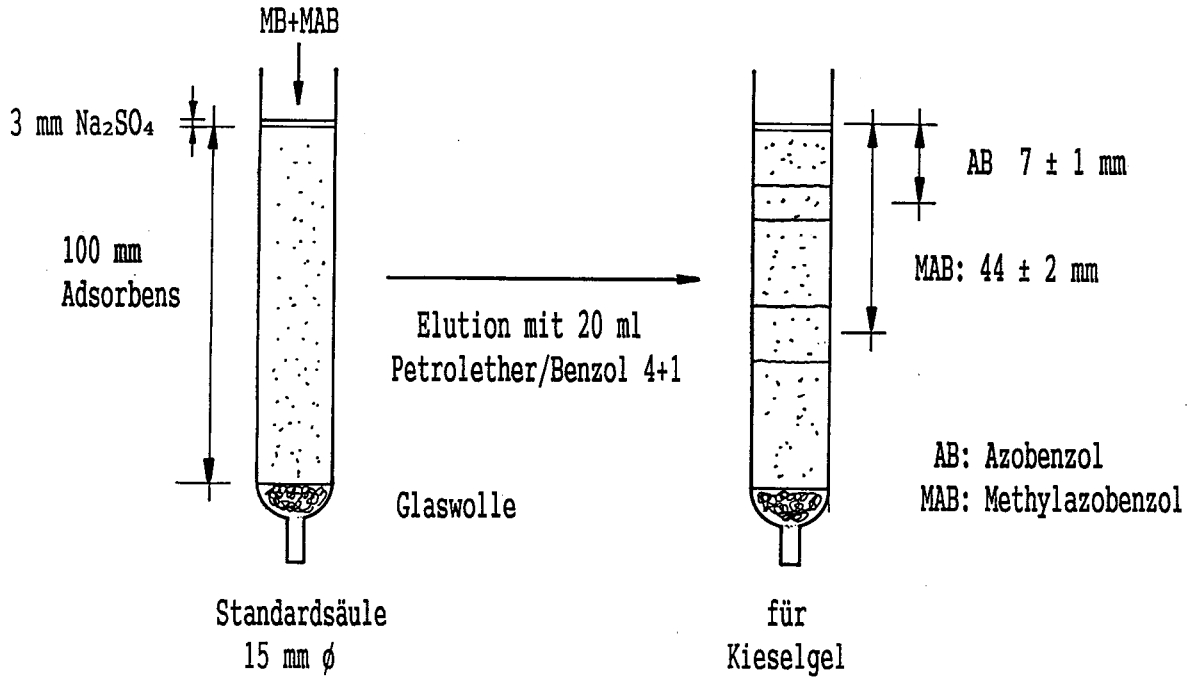
- *Aluminium oxide:* AB, 6±1 mm; MAB, 29±1.5 mm.
- *Silica:* AB, 7±1 mm; MAB, 44±2 mm.

224 Gases and gas cleaning

224.1 Basic materials

Helium, 99.995 % (further cleaning, cf. *section 224.2*)	Nitrogen, 99.99 % (further cleaning, cf.*section 224.3*)
Methane, grade 3.5, 99.95 %	O_2/activated charcoal filter
Molecular sieve filter	Loose molecular sieve of 0.5-2.0 mm, if needed (further cleaning cf. *section 224.4*)
Activated charcoal, 1.5 mm particle size	Empty metal cartridges, Whitey 304L-HDF4-50 and 340L-HDF4-75, or equivalent made from stainless steel

Figure 3: Checking the activity grade of adsorbents



(***Translation of diagram wordings:*** Adsorbens = adsorbent, Standartsäule = reference column, Glaswolle = glass wool, Elution mit ... = elution with ..., Benzol = benzene, für Kieselgel = for silicagel, Azobenzol = azobenzene, Methylazobenzol = methylazobenzene).

224.2 Additional cleaning of helium

Helium serving as the carrier gas for gas chromatography is cleaned as follows:

- A filter filled with molecular sieve and an oxygen-activated charcoal filter are mounted in series just after the pressure reduction valve. These two units are replaced after using up two 50 L pressure tanks, or else once per year. Pressure tanks must not be emptied below 1,500 kPa (15 bar).
- Two metal cartridges in series are mounted directly before each gas chromatograph. The first one is filled with molecular sieve (cf.*section 224.4*), and the second one with activated charcoal. Both cartridges are replaced only if irregularities or problems occur (e.g. complete emptying of a pressure tank), or after three years at the latest. The oxygen-activated charcoal filter is then disposed of. The contents of the molecular sieve filter are replaced by regenerated material (cf.*section 224.4*), and the activated charcoal in the metal cartridges is replaced with new material.

224.3 Additional cleaning of nitrogen

Nitrogen is used for reducing solvent volume, or as a pressure source. It is additionally cleaned with a metal cartridge filled (flow direction) in the first half with molecular sieve and then topped up with activated charcoal. The contents of the cartridge are replaced when the 50 L pressure tank is changed. The tank must never be emptied below 1,500 kPa (15 bar).

224.4 Regeneration of molecular sieve

The molecular sieve to be regenerated is filled into a metal cartridge and activated for 3 h in a tube furnace at 300 °C (cf. *section 215*). During this operation, the cartridge is flushed with a flow of 20 mL/min of purified nitrogen. After having been cooled under a flow of nitrogen, the cartridge may be used directly, or else its content may be transferred to another cartridge (a leak check is necessary).

3 Reference solutions for quantification

31 Reference standards

The following reference standards are used (all from Cambridge Isotope Laboratories CIL, Woburn, MA 0181, USA).

311 Non-isotope labelled 2,3,7,8-chlorine substituted PCDF congeners

CIL no. EF-909B-5, solvent n-nonane, volume 1.2 mL, concentration 5±0.5 µg/mL of the following congeners (purity >99 %):

2,3,7,8-tetrachlorodibenzofuran	1,2,3,7,8,9-hexachlorodibenzofuran
1,2,3,7,8-pentachlorodibenzofuran	2,3,4,6,7,8-hexachlorodibenzofuran
2,3,4,7,8-pentachlorodibenzofuran	1,2,3,4,6,7,8-heptachlorodibenzofuran
1,2,3,4,7,8-hexachlorodibenzofuran	1,2,3,4,7,8,9-heptachlorodibenzofuran
1,2,3,6,7,8-hexachlorodibenzofuran	

Non-isotope labelled *octachlorodibenzofuran (OCDF):* CIL no. EF-982C, crystalline (purity >99.9 %).

312 Non-isotope labelled 2,3,7,8-chlorine substituted PCDD congeners

CIL no. ED-906B-5, solvent n-nonane, volume 1.2 mL concentration 5±0.5 µg/mL of the following congeners (purity >99 %):

2,3,7,8-tetrachlorodibenzo-*p*-dioxin	1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin
1,2,3,7,8-pentachlorodibenzo-*p*-dioxin	1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin
1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin	1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin

Non-isotope labelled *octachlorodibenzo-p-dioxin (OCDD):* CIL no. ED-982-L, crystalline (purity >99 %).

313 Preparation of non-isotope labelled OCDD/OCDF reference standard

10 mL of a solution with OCDD and OCDF, each at a concentration of 25±0.1 µg/mL in toluene is prepared in a 10 mL volumetric flask. OCDD and OCDF are of low solubility, so the solution has to be treated in an ultrasonic bath until all visible crystals have dispersed. The standard is weighed and stored in the deep freezer at -20 °C.

314 $^{13}C_{12}$-2,3,7,8-chlorine substituted PCDD/PCDF congeners

Each congener is delivered in a sealed ampoule at a concentration of 50±5 µg/mL (OCDD, 10 µg/mL), dissolved in n-nonane (^{13}C-enriched to 99 %, solvent volume 1.2 mL):

Catalogue number	Congener
CIL ED-900	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
CIL ED-912	1,2,3,4-tetrachlorodibenzo-*p*-dioxin
CIL ED-955	1,2,3,7,8-pentachlorodibenzo-*p*-dioxin
CIL ED-966	1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin
CIL ED-972	1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin
CIL ED-981	octachlorodibenzo-*p*-dioxin (only 10 µg/mL)
CIL EF-904	2,3,7,8-tetrachlorodibenzofuran
CIL EF-952	1,2,3,7,8-pentachlorodibenzofuran
CIL EF-958	2,3,4,7,8-pentachlorodibenzofuran
CIL EF-963	1,2,3,4,7,8-hexachlorodibenzofuran
CIL EF-974	1,2,3,4,6,7,8-heptachlorodibenzofuran
CIL EF-983	1,2,3,4,6,7,8,9-octachlorodibenzofuran

32 Preparation of primary standards

321 Preliminary remarks

The unopened ampoules of reference standards are stored at 4-6 °C. The contents of the opened ampoules are transferred to 1.5 mL Certan® vials, which are stored at -20 °C. All reference vials are weighed at room temperature before and after each sample withdrawal, and the weight and date are entered into the reference standard book. In view of the chemical stability of the PCDD/PCDFs, the reference standards may be stored unlimitedly, if evaporation of the solvent, contamination, and exposure to light are prevented.

Prior to dilution, all necessary reference standards are kept at room temperature for at least 2 h. They are then put in an ultra sonic bath for 5 min to dissolve any possible micro-crystalline precipitates (30 min for OCDD/OCDF solutions).

The weight of all standards must then be checked (analytical balance; cf. *section 215*). For deviations of more than 2-3 mg, the weight is adjusted with solvent, and re-checked. Aliquots are removed using clean, calibrated syringes, which are kept only for the preparation of standards. The syringes are weighed when empty and after filling, and the volume is

calculated as a check. The reference standards are weighed immediately after sample withdrawal, and the weight is entered in the reference standard book.

322 $^{13}C_{12}$-isotope labelled primary standards

322.1 Primary standard 13C1G

The following volumes of the reference standards listed below (cf. *section 31*) are transferred to a 5 mL volumetric flask:

Congener	**Concentration of the commercially available reference standards** [ng/µL]	**Volume taken** [µL]	**Concentration in the primary standard 13C1G** [ng/µL]
$^{13}C_{12}$-2,3,7,8-TCDF	50	50	0.50
$^{13}C_{12}$-2,3,4,7,8-PeCDF	50	50	0.50
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	50	100	1.00
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	50	150	1.50
$^{13}C_{12}$-2,3,7,8-TCDD	50	50	0.50
$^{13}C_{12}$-1,2,3,7,8-PeCDD	50	100	1.00
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	50	120	1.20
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	50	180	1.80
$^{13}C_{12}$-OCDD	10	1,500[1)]	3.00

1) More than one of the commercially available ampoules of 1.2 mL volume

The volumetric flask is filled with n-nonane to the marked level. This primary standard named 13C1G serves to prepare internal standards 13C1I and 13C2I.

322.2 Primary standard 13C2G

A primary standard is made, which serves to prepare the recovery standards. The following volumes are transferred to a 1 mL volumetric flask:

Congener	**Concentration of the reference standards** [ng/µL]	**Volume taken** [µL]	**Concentration in the primary standard 13C2G** [ng/µL]
$^{13}C_{12}$-1,2,3,4-TCDD	50	100	5.0
$^{13}C_{12}$-1,2,3,7,8-PeCDF	50	100	5.0

The volumetric flask is filled with n-nonane to the marked level. Its content is then transferred to a 1.5 mL Certan® vial.

33 Preparation of quantification standards

331 Quantification standard 13C12C1Q

The following volumes of the reference and primary standards listed below are transferred to a 1.5 mL sample vial with a capillary opening using clean, calibrated syringes that are kept for this purpose only:

Standard	**Volume taken** [µL]	**Concentration in the quantification standard 13C121Q** [pg/µL]
ED-906-B5 ^{12}C	45	150
EF-909-B5 ^{12}C	45	150
OCDD/OCDF ^{12}C (cf. *section 313*)	42	700
Standard 13C1G	300	100-600
Standard 13C2G	45	150

With a syringe 1,023 µL of n-nonane are added, so that the total volume determined gravimetrically will be 1,500±10 µL. The standard is named 13C12C1Q.

332 Quantification standard 13C12C2Q

100 µL of quantification standard 13C12C1Q are transferred by means of a calibrated syringe to a sample vial with a capillary opening, and 900 µL n-nonane are added. The weight is checked and the standard named 13C12C2Q (concentration range 15-70 pg/µL).

333 Quantification standard 13C12C3Q

100 µL of quantification standard 13C12C2Q are transferred by means of a calibrated syringe to a 5 mL volumetric flask, which is then filled with n-nonane to the marked level. The weight is checked and the standard named 13C12C3Q (concentration range 3-14 pg/µL).

34 Dilution of internal standards

341 Internal standard 13C1I

1 mL of primary standard 13C1G are transferred to a 10 mL volumetric flask, which is then filled with n-nonane to the marked level. The weight is checked and the standard named 13C1I (concentration range 50-300 pg/µL).

342 Internal standard 13C2I

2 mL of internal standard 13C1I are transferred to a 10 mL volumetric flask, which is then filled with n-nonane to the marked level. The weight is checked and the standard named 13C2I (concentration range 10-60 pg/µL).

35 Dilution of recovery standards 13C1W

100 µL of primary standard 13C2G are transferred by means of a calibrated syringe to a 1 mL volumetric flask, which is then filled with n-nonane to the marked level. From this flask once again 100 µL are taken and transferred to another 1 mL volumetric flask. Again this is then filled with n-nonane to the marked level. Finally, this standard is stored in a 1.5 mL Certan® vial and named 13C1W (concentration 50 pg/µL).

36 Quality assurance

All reference standards are stored at -20 °C. They have an unlimited storage period, if evaporation of the solvent and contamination are prevented. All primary standards are stored at 4-6 °C. Their storage period is limited to two years. The weight of primary standards stored in sample vials with a capillary opening does not necessarily have be checked (loss within six months due to evaporation <1 mg).

Before use, all freshly prepared basic standards must be compared with both the preceding standard generations. Deviations that are within the precision of the quantification method (±15 %) are acceptable. At least once a year, the primary standards have to be checked against a certified reference standard, or against the reference standard of an official intercalibration. These standards are treated like the reference standards, and stored at -20 °C.

4 Sample preparation

41 Preliminary remarks

Soil samples should not contain biological material (roots, residue from grass). The samples are stored in wide opening bottles of 0.5-1 L (cf. *section 211*). New bottles are cleaned by heating at 350-450 °C; bottles already in use are cleaned according to *section 217*.

42 Drying and sieve fractionation of samples

All samples are dried on Petri dishes in an oven at 40 °C until a constant weight is attained (after 24-72 h). The water content is calculated. Lumpy samples are crushed in a porcelain mortar with a pestle. Then, all sample are sieved to a particle size of 2 mm.

43 Sample extraction

5-50 g of the ≤2 mm fraction are weighed into a pre-cleaned Soxhlet thimble (28x80 mm; cf. *section 218*), and the ^{13}C-labelled standard is added in the centre part of the sample (cf. *section 45*). A small amount of cleaned cotton wool is placed on top of the thimble. The sample is extracted for 24 h with toluene in a 200 mL Soxhlet extractor (≥6 cycles per hour). The extractor is lagged with a sheet of polyurethane foam.

44 Removal of elemental sulphur and of sulphur compounds

Soil often contains only small amounts of sulphur, and this does not interfere with the mass spectrometric detection. The following procedure is only needed if interference is observed. The method proposed is very efficient. However, the use of mercury should be avoided whenever possible, and alternative techniques should be favoured as proposed in the guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil* (SAEFL; cf. *section 73* of the guidelines). The method described here was validated with mercury. It must be revalidated if another technique is used.

The sample extract is concentrated to about 15 mL in a round bottom flask on the rotary evaporator (water temperature 37 °C, pressure 10 kPa, i.e. 100 mbar), and transferred to a 50 mL beaker. As much metallic mercury is added as to cover about 10 % of the bottom of the flask. A watch glass is placed on the beaker, and about 20 min treatment in an ultrasonic bath are given. If the metallic surface is no longer visible when agitating the sample, the procedure has to be repeated with some more mercury.

The final extract including mercury and precipitate are transferred to a centrifuge tube and centrifuged for 15 min at 2,000 r.p.m.. The toluene phase is transferred to a turbovap unit. The precipitate is washed with a few mL of toluene and, after renewed centrifugation, the toluene phase is added to the extract. Three droplets of tetradecane are added. Then, the sample is concentrated using the turbovap and then with nitrogen until it no longer smells of toluene. Finally, the residue is dissolved in 20 mL of methylene chloride/cyclohexane 1+1. The sample is now ready for further clean-up.

45 Quantity of ^{13}C internal standard added to the sample

The quantity of ^{13}C-isotope-labelled 2,3,7,8-chlorine substituted congeners added to the sample material is dependent on both the amount of sample and the concentration to be expected. The following quantities are guide values and should be adapted if the need arises. In general, the amount of ^{13}C-isotope-labelled 2,3,7,8-chlorine substituted congeners should correspond to about the concentrations to be expected of ^{12}C-PCDD/PCDF. At low concentrations (<5 pg/g), the quantity added may be up to one order of magnitude higher.

Guide values	***Not contaminated:*** Amount of soil 40-50 g (possibly two Soxhlet extractions); added amount of internal standard 13C2I 50 µL (500-3,000 pg per PCDD/PCDF); added amount of recovery standard 13C1W 10-20 µL (500-1,000 pg).
	Contaminated: Amount of soil 5-10 g; added amount of internal standard 13C1I 20 µL (1,000-6,000 pg per PCDD/PCDF); added amount of recovery standard 13C1W 20 µL (1,000 pg).

5 Extract clean-up

51 Extract clean-up with 3-column system

511 Packing of column 1 (3-column system)

Chromatography column 1 (cf. *section 213*) is fixed vertically in a support. A glass fibre filter (diameter 48 mm, punched out from a filter with a diameter of 142 mm) is placed on the perforated glass plate using a rod with a Teflon plate mounted at the end (40 mm diameter). Then, the column is filled with the following adsorbents (order from bottom to top):

- 2 cm sodium sulphate (cf. *section 222.4*).
- 30 g silica (cf. *section 223.2*).
- 30 g potassium hydroxide coated silica (cf. *section 223.3*).
- 4 cm sodium sulphate (cf. *section 222.4*).

512 Packing of column 2 (3-column system)

Several layers of Teflon tape are wrapped around one of the fitting pieces of chromatography column 2 (cf. *section 213*). Glass fibre filters are placed on the fitting piece, which is carefully pressed into the column. It is first filled with a 12.5 cm thick layer of potassium hydroxide coated silica (cf. *section 223.3*). The remaining volume of the column is filled up with silica (cf. *section 223.2*) to 1 cm from the top. Both the layers are compressed with a vibrator. Then, the second fitting piece (Teflon tape wrapped) and 4 glass fibre filters are pressed into the column. Both links are safely secured to the support. The column is mounted in such a manner that the potassium hydroxide coated layer comes first, seen in the direction of flow.

513 Packing of column 3 (3-column system)

200 mg of activated charcoal AX21 (cf. *section 223)* are dried overnight in an oven at 105 °C. They are then transferred to a 100 mL beaker and suspended in 20 mL of methylene chloride. The smallest particles floating on the surface are decanted into a 50 mL beaker until 50 mg (dry weight) have been collected. Using a pair of scissors, 600 mg of a glass fibre filter (cf. *section 222*) are cut into small pieces, and homogenised using an Ultraturrax for 30 s in a 100 mL beaker with 60 mL of methylene chloride.

50 mg of activated charcoal are added, and the mixture is blended manually. A fitting piece wrapped with Teflon tape is placed into the end of chromatography column 3. A water jet pump with collecting bottle for the solvent is fitted to the capillary. 15-20 glass fibre filters are placed at the bottom of the column and the activated charcoal/glass fibre mixture is poured into the column. The remaining void volume is filled with about 20 glass fibre filters. Then, the inlet plug is mounted. After removing the water jet pump, both links are safely secured to the support.

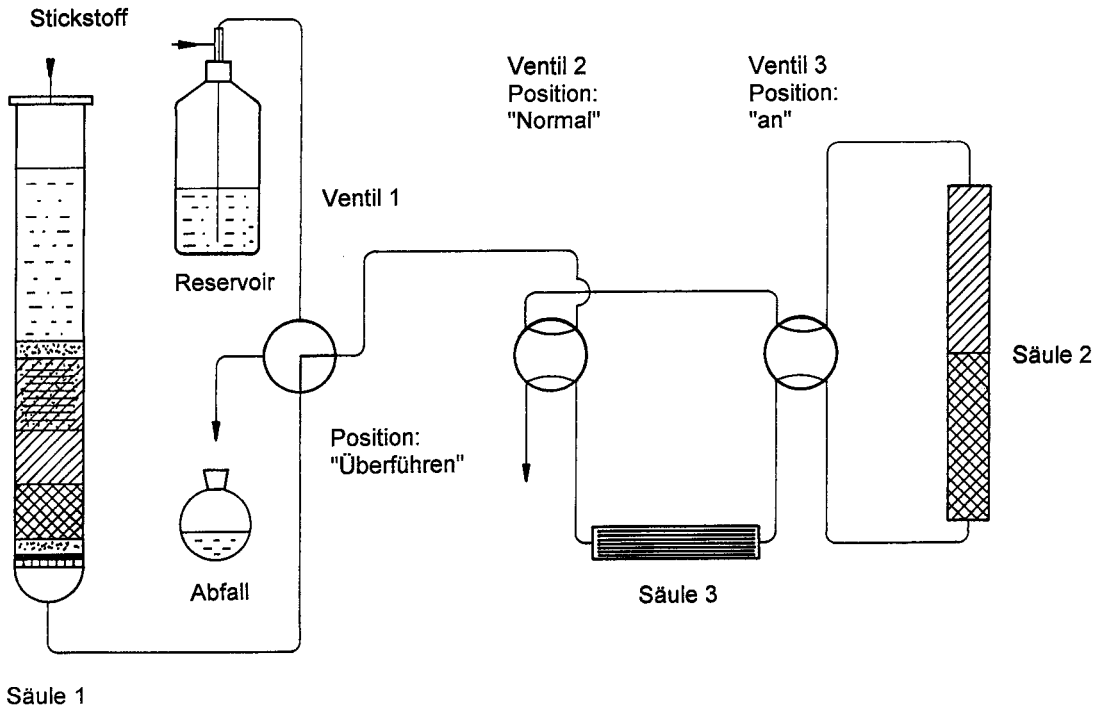
Next, the column is flushed with 200 mL of methylene chloride followed by 100 mL of toluene. The same set-up is used as shown in *figure 4*; but without columns 1 and 2.

514 Assembly and cleaning of the 3-column system

The three columns are linked together as shown in *figure 4*. Columns 2 and 3 are pre-cleaned as follows (cf. *figs 5, 6 and 7*):

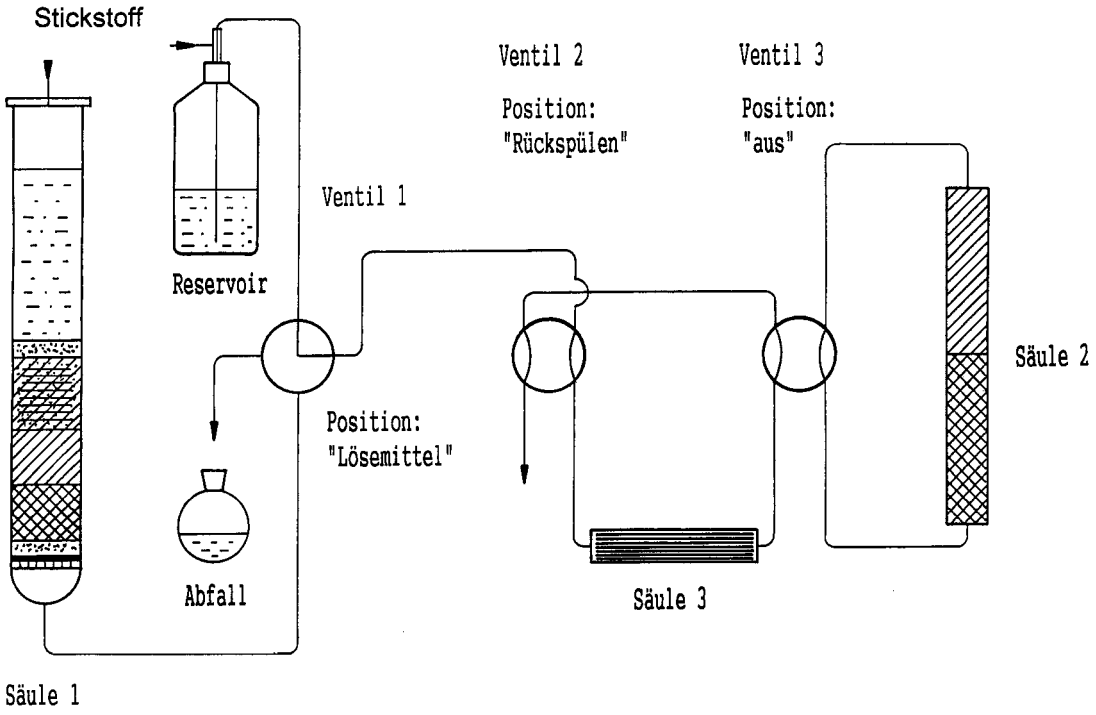
- **Cleaning step 1:** The solvent reservoir is filled with toluene and valve 1 is set to position "solvent", valve 2 to "reverse" and valve 3 to "off" (cf. *fig. 7*). The reservoir is pressurised with N_2 (maximum 50 kPa, i.e. 0.5 bar). Column 3 is flushed with 100 mL methanol and then with 100 mL toluene.
- **Cleaning step 2a:** The solvent reservoir is filled with cyclohexane/methylene chloride 1+1 (solvent A). The valves 1, 2 and 3 are set in such a manner that the reservoir is linked with column 3 in reverse direction (valve 2, "reverse") and the latter again with column 2 (valve 3, "on"; cf. *fig. 8*). The columns are flushed with 100 mL of solvent A.
- **Cleaning step 2b:** Valve 2 is set to position "normal", so that the solvent moves in a normal flow direction through column 2 and then through column 3. Once again the columns are flushed with 100 mL solvent. The system is now ready for use.

Figure 4: Assembly of the 3-column system



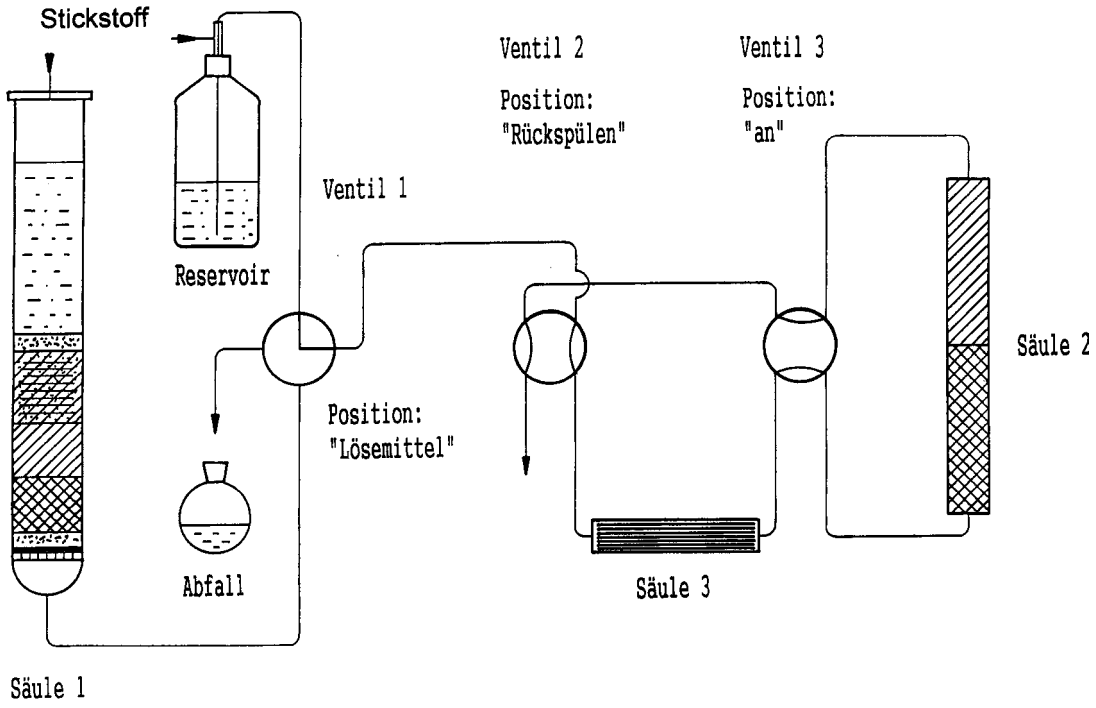
(***Translation of diagram wordings:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Position "Überführen" = position "transfer", Ventil 2 - Position "Normal" = valve 2 - position "normal", Ventil 3 - Position "an" = valve 3 - position "on")

Figure 5: Cleaning step 1 for column 3



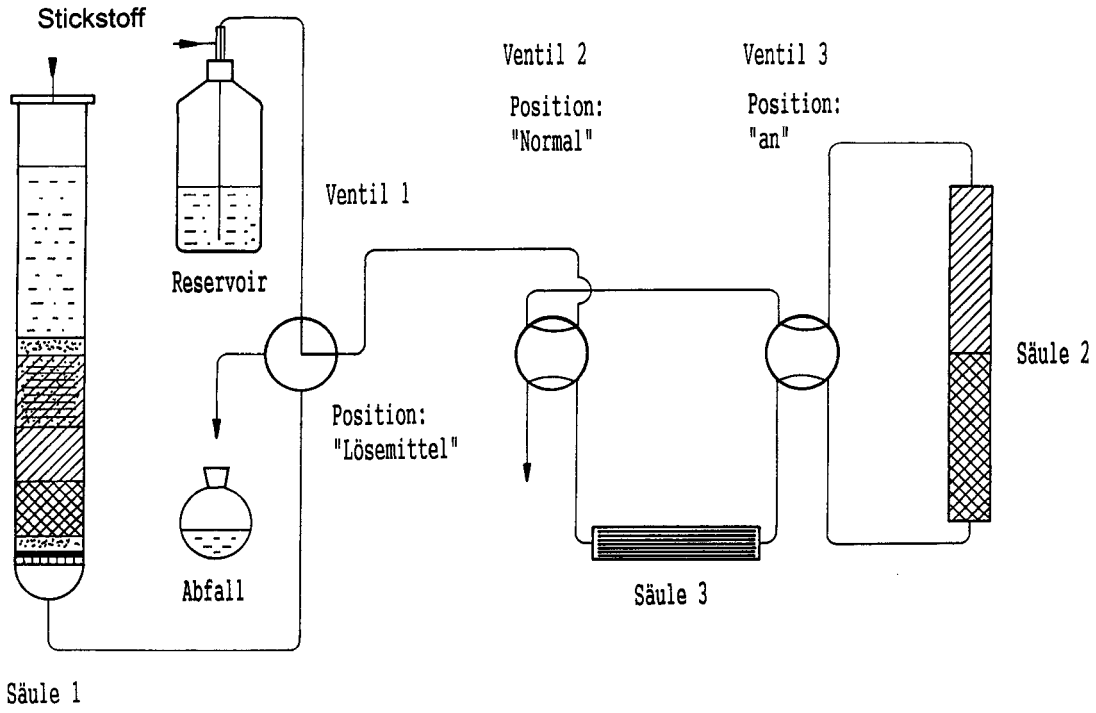
(***Translation of diagram:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Position "Lösemittel" = position "solvent", Ventil 2 - Position "Rückspülen" = valve 2 - position "back flush", Ventil 3 - Position "aus" = valve 3 - position "off").

Figure 6: Cleaning step 2a for columns 2 and 3



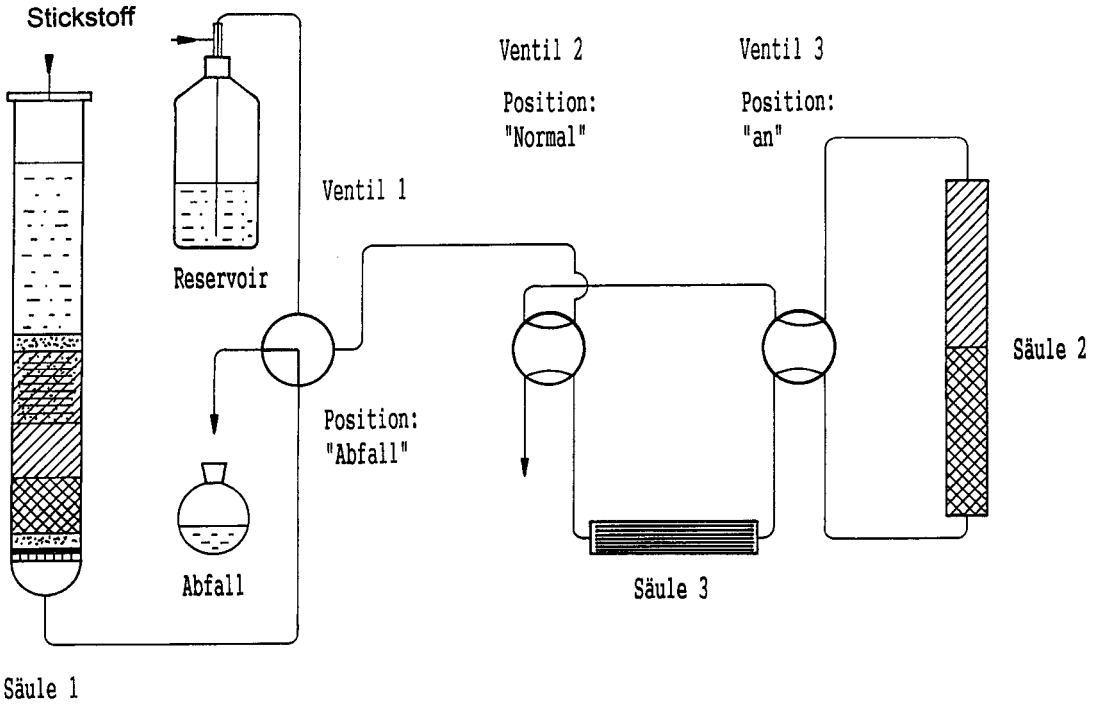
(***Translation of diagram:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Position "Lösemittel" = position "solvent", Ventil 2 - Position " Rückspülen" = valve 2 - position "back flush", Ventil 3 - Position "an" = valve 3 - position "on").

Figure 7: Cleaning step 2b for columns 2 and 3



(***Translation of diagram wordings:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Position "Lösemittel" = position "solvent", Ventil 2 - Position "Normal" = valve 2 - position "normal", Ventil 3 - Position "an" = valve 3 - position "on").

Figure 8: De-aeration of column 1



(***Translation of diagram wordings:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Position "Abfall" = position "waste", Ventil 2 - Position "Normal" = valve 2 - position "normal", Ventil 3 - Position "an" = valve 3 - position "on").

515 Extract clean-up

The sample extract is transferred to the sodium sulphate layer of column 1 just before clean-up (Pasteur pipette), and the sample vial is rinsed with 2-3 mL of solvent A (methylene chloride/cyclohexane 1+1).

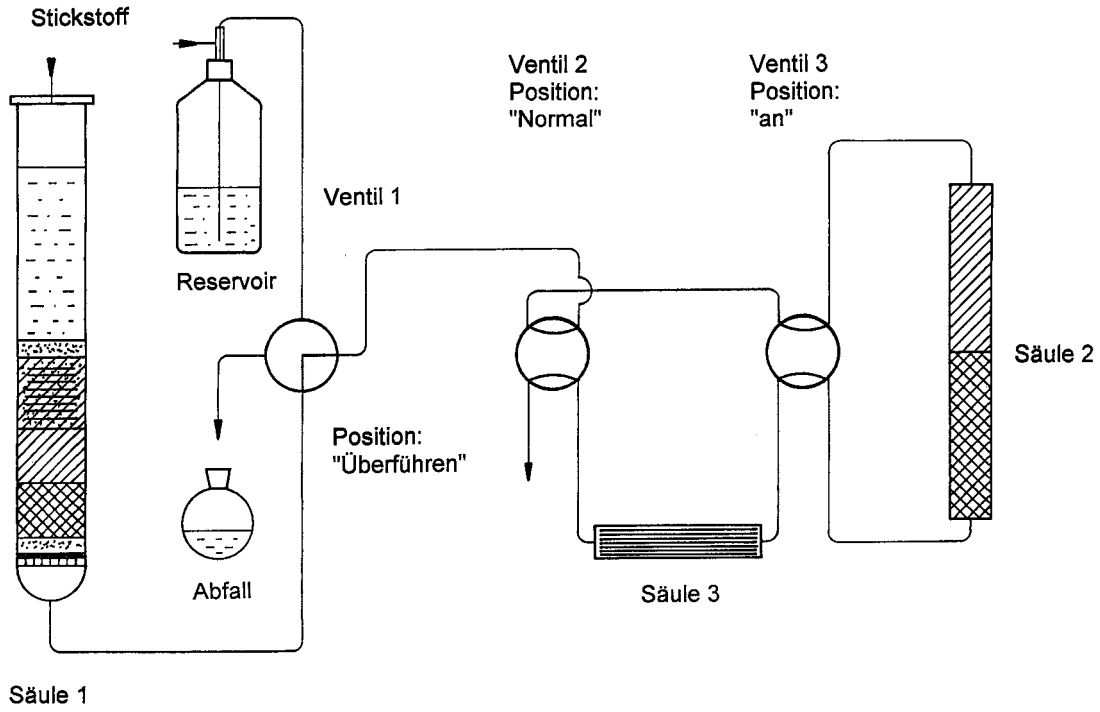
Column 1 containing the sample extract is linked to valve 1 using the ball joint. Valve 1 is set to the position "waste" and valve 2 to "normal" (cf. *fig. 10*). 650 mL of solvent A (methylene chloride/cyclohexane 1+1) are filled into the upper part of column 1.

When the solvent has passed the ball joint, valve 1 is set to position "transfer". The connector is fixed to column 1 with a clamp, and the column pressurised with N_2 (maximum 50 kPa, i.e. 0.5 bar; wear a mask!) to obtain a flow velocity of about 5 mL/min. As shown in *figure 9* the sample is transferred from column 2 to column 3.

The pressure is released at the very moment when the last of the solvent has passed the ball joint (takes about 2-3 h). Then, valve 1 is switched to position "waste" and the connector removed from column 1.

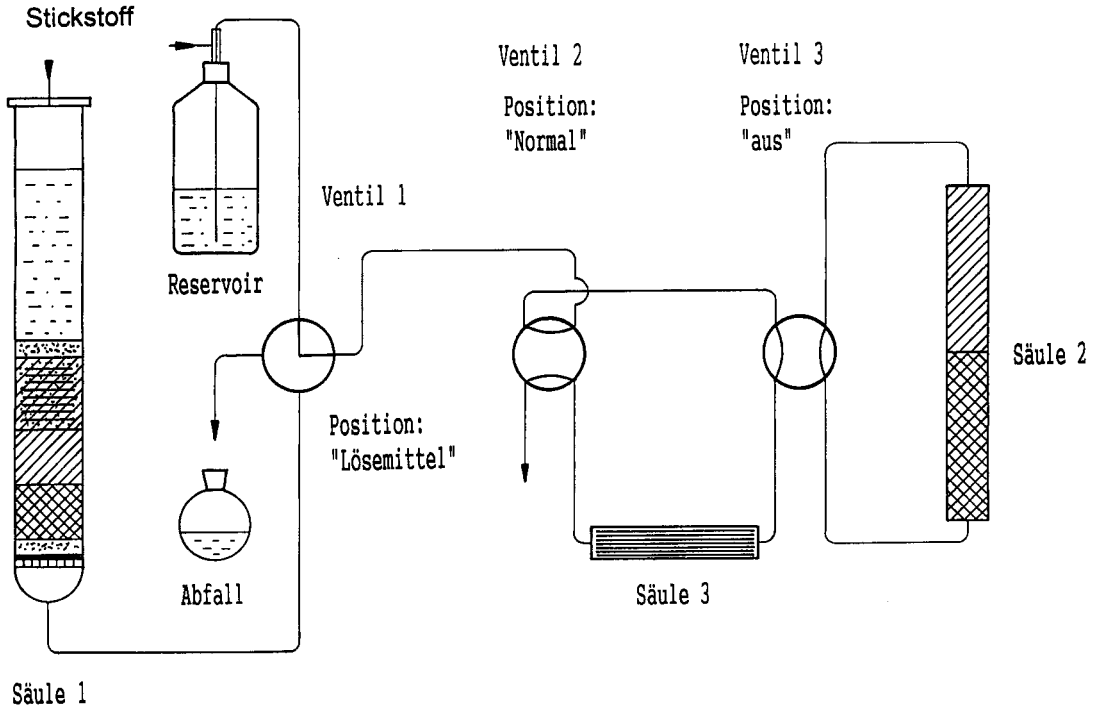
The solvent reservoir is then filled with solvent A (cyclohexane/methylene chloride 1+1) and the nitrogen tube is fitted. Valve 3 is switched to "off" and valve 1 to "solvent". The system is pressurised (maximum 70 kPa, i.e. 0.7 bar), and column 3 is eluted with 75 mL of solvent A (cf. *fig. 10*).

Figure 9: Transfer of sample from column 1 via column 2 to column 3



(***Translation of diagram wordings:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Position "Überführen" = position "transfer", Ventil 2 - Position "Normal" = valve 2 - position "normal", Ventil 3 - Position "an" = valve 3 - position "on").

Figure 10: Elution of column 3 in normal flow direction with solvents A and B



(***Translation of diagram wordings:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Ventil 2 - Position "Normal" = valve 2 - position "normal", Ventil 3 - Position "aus" = valve 3 - position "off").

The pressure is then released and the solvent reservoir filled with solvent B is connected (methylene chloride/methanol/benzene 75+20+5). After renewed application of pressure, column 3 is rinsed with 50 mL of solvent B.

After releasing the pressure, the solvent reservoir containing toluene is opened. Valve 2 is set to "reverse" and valve 1 to "waste". The system then is pressurised (maximum 30 kPa, i.e. 0.3 bar). When toluene begins to drip from valve 1 it is switched to the "solvent" position. A toluene fraction of 40 mL containing the PCDD/PCDF is collected in a 100 mL round bottom flask with attached centrifuge tube (cf. *fig. 11*).

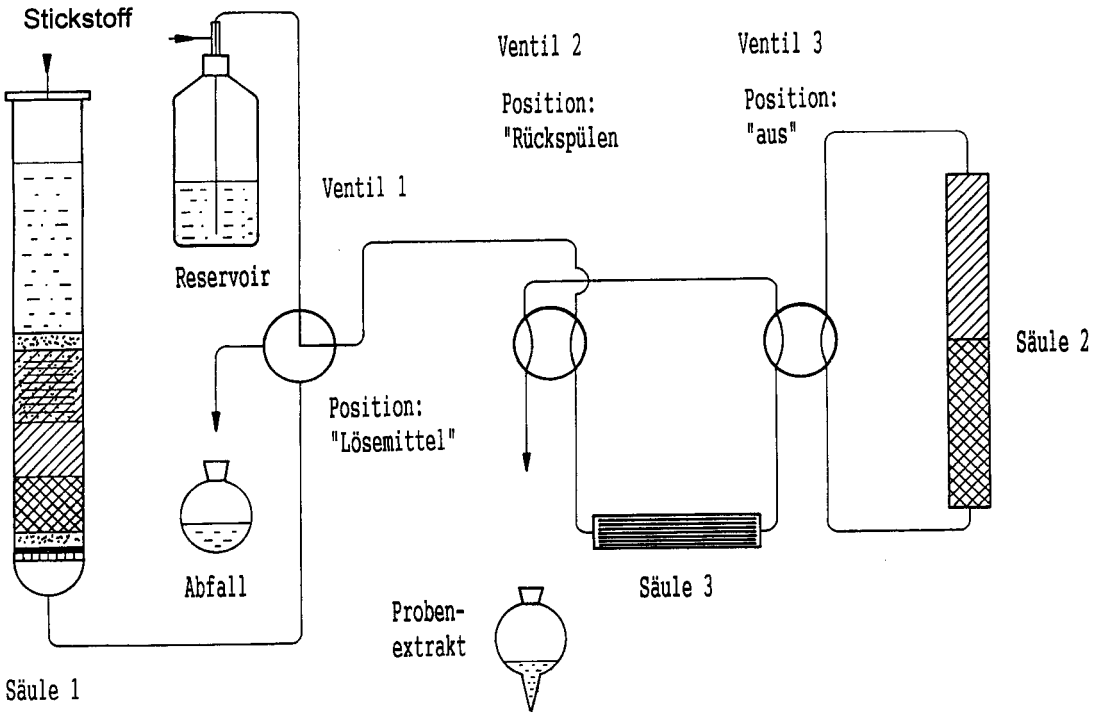
Then, the toluene fraction is reduced to a volume of 0.5 mL using the Turbovap. Three droplets of tetradecane are added and the extract is evaporated to dryness with nitrogen. The extract should no longer smell of toluene. 1 mL of hexane is added. The sample is now ready for the next clean-up step (2-column system).

516 Additional cleaning of charcoal column 3 and the valve unit

If blanks of the clean-up are nominally too high (cf. *section 75*) an additional cleaning of the charcoal column as well as of the connecting system (tubes/valves) is carried out as follows:

- Chromatography column 1 without adsorbents is filled with 500 mL methylene chloride. The Teflon tube normally linked with column 2 is fitted with the tube between column 2 and 3 (charcoal column). The system is rinsed with about 200 mL solvent, and valve 2 is set to the “normal” position. The remaining 300 mL are used for rinsing. Valve 2 is set to the “reverse” position. This procedure is repeated with 500 mL methanol and with 500 mL toluene.

Figure 11: Elution of column 3 using a reverse flow of toluene



(***Translation of diagram wordings:*** Stickstoff = nitrogen, Säule = column, Abfall = waste, Ventil 2 - Position "Rückspülen" = valve 2 - position "back flush", Ventil 3 - Position "aus" = valve 3 - position "off", Probenextrakt = sample extract).

52 Extract clean-up with 2-column system

521 Packing of Pasteur pipettes

A small cotton wool plug (cf. *section 222.2*) is placed at the bottom of each of two Pasteur pipettes. One pipette is filled with a 4 cm layer of aluminium oxide on top of the cotton wool plug (cf. *section 223.5*) and the other one with a 4 cm layer of sulphuric acid coated silica (cf. *section 223.4*). Both columns are mounted in a support and rinsed with 10 mL of hexane. To save time, the pipettes can be pressurised with a rubber balloon.

522 Fractionation of sample extracts

The outlet of the pipette filled with silica is placed over the aluminium oxide layer of the other one. A 50 mL beaker is placed under the combined columns. The sample extract dissolved in 1 mL of hexane is transferred to the silica with a Pasteur pipette so that the extract is adsorbed in the uppermost layer.

The pipette combination is eluted first with 5 mL of hexane using the first 2 mL for an additional rinse of the round bottom flask with the centrifuge tube. The solvent is transferred with the same pipette used for the sample extract. The silica column is removed after running dry.

The pipette with aluminium oxide is eluted first with 5 mL of hexane/methylene chloride 98+2. The flow rate is increased by pressurising it with a pipette balloon. The beaker is removed and a round bottom flask with centrifuge tube is placed under the outlet. The PCDD/PCDF-fraction is eluted with 10 mL of methylene chloride/hexane 50+50.

The volume of the sample extract is reduced to 0.5 mL using the Turbovap, and the walls of the flask are rinsed with a few droplets of hexane. Thee extract is then transferred to a 1.5 mL sample vial and the volume is reduced to 50 µL with a gentle flow of nitrogen.

The sample is then transferred with a long Pasteur pipette to a 1.5 mL vial with an insert of 100 µL volume (cf. *section 214*). The walls of the previous vial are rinsed with a few droplets of hexane that are added as well using the same pipette. The volume may be reduced by slow evaporation at room temperature (screw cap loose on the vial) or by a gentle flow of N_2. The required volume of recovery standard is added (cf. *section 45*) and the volume, if necessary, reduced once more (normally to about 10 µL). The sample is now ready for quantification.

523 Clean-up with 5 g sulphuric acid coated silica

523.1 Preliminary remarks

Alternatively, a column filled with 5 g sulphuric acid coated silica is used for samples with a high content of organic material. A small plug of cotton wool (cf. *section 222.2*) is placed on the bottom of chromatography column 5. The column is filled to about two thirds, with hexane, and the sulphuric acid coated silica (5 g in total) is added in small portions. Then, the layer is compacted by tapping the glass wall (wet-packing procedure). The column is then washed with 50 mL hexane. It must not run dry.

A second column is packed in the same way with aluminium oxide.

523.2 Sample fractionation

The large silica column is mounted on a support so that its outlet is above the surface of the second one filled with aluminium oxide. A 100 mL beaker is placed under the combined columns. The sample extract dissolved in 1 mL of hexane is transferred to the silica surface with a long Pasteur pipette so that it is adsorbed just on the column surface.

The pipette combination is eluted first with 25 mL hexane, of which the first 2 mL are used for rinsing the sample vial. They are transferred with the same pipette as used for the sample extract. Once the column has run dry, it is removed.

The pipette containing aluminium oxide is eluted first with 25 mL hexane/methylene chloride 98+2. The flow rate is increased by pressurising it with a pipette balloon. The beaker is removed and a round bottom flask with centrifuge tube fused to the bottom is placed under the outlet. The PCDD/PCDF fraction is eluted with 50 mL methylene chloride/hexane 50+50.

The volume of the sample extract is reduced to 0.5 mL using the Turbovap, and the walls of the flask are rinsed with a few droplets of hexane. The extract is then transferred to a 1.5 mL sample vial and the volume reduced to 50 µL with a gentle flow of nitrogen.

The sample is then transferred with a long Pasteur pipette to a 1.5 mL vial with an insert of 100 µL volume (cf. *section 214*). The walls of the previous vial are rinsed with a few droplets of hexane that are also added using the same pipette. The volume may be reduced by slow evaporation at room temperature (screw cap loose on the vial) or by a gentle flow of nitrogen. The required volume of recovery standard is added (cf. *section 45*) and the volume, if necessary, reduced once more (normally to about 10 µL). The sample is now ready for quantification.

6 Quantitative Analysis

61 Preliminary remarks

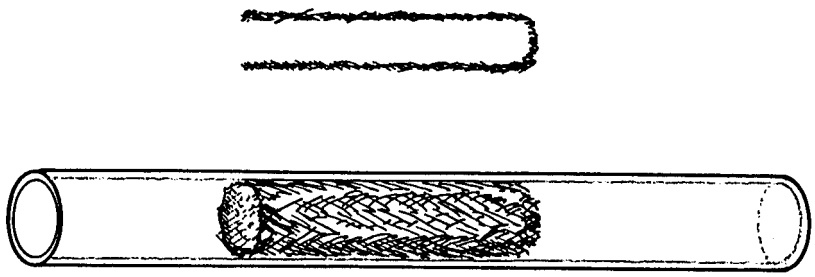
The amount of recovery standard given in *section 45* has to be added to the sample using a disposable pipette before quantification. If necessary, the sample volume is reduced to about 10-50 µL with a gentle flow of nitrogen. Then, the sample aliquots as defined in *section 624* may be injected.

62 Gas chromatographic separation

621 Instrumentation

- Hewlett-Packard 5980 (I or II) or another equivalent gas chromatograph.
- Hewlett-Packard 7673A or B auto injector or another equivalent product.
- Injector for splitless injections with glass insert, i.d. 3.8 mm (Hewlett-Packard no. 19251-60340), or another equivalent product. If the auto injector is used, the glass insert has to be filled with silanised glass wool. The glass wool is moulded into a small Soxhlet thimble (cf. *fig. 12*).

Figure 12: Positioning and shape of the glass wool plug used for the auto injector



622 Injection syringes

For manual injections, 5 µL syringes with fixed needle and metal plunger are used. The same holds true for the auto injector.

623 Separation capillary

The following separation capillary is used:

- *Polycyanopropyl polymethylsiloxane:* 90+10 (e.g. SP2330, SP2331, RTx2330), immobilised, film thickness 0.1 µm.
- *Capillary dimensions:* Polyimide coated quartz capillary, length 30 or 60 m, internal diameter 0.25 mm.

624 Injection and separation conditions

- *Carrier gas:* He, 80 kPa (0.8 bar) at 30 m (guide length).
- *Gas flow at split outlet:* 50±10 mL He/min.
- *Septum purge:* 0.8-1.0 mL He/min.
- *Injector temperature*: 250 °C.
- *GC/MS-Interface temperature:* 260-280 °C.

- **Injection conditions:** Splitless injection (auto injector or hot needle injection) of 2-4 µL sample (maximum 4 µL hexane or 2 µL toluene), 2 min waiting period before opening the split valve.
- **Temperature programme:** 100 °C, 2 min waiting period, 100-180 °C with 20 °C/min, 180-260 °C with 3 °C/min, 260 °C isothermal (5-10 min).

The correct range of retention times for each group of ions (cf. *section 62*) has to be checked with a true sample or a reference standard containing the first and last eluting isomers listed in *table 1*.

Table 1: Retention times of the first and last eluting compounds of each isomer group

Isomer group	Compound elutes	
	first	Last
TCDD	1,3,6,8-	1,2,8,9-
PeCDD	1,2,4,7,9-	1,2,3,8,9-
HxCDD	1,2,4,6,7,9-	1,2,3,4,6,7-
HpCDD	1,2,3,4,6,7,9-	1,2,3,4,6,7,8-
TCDF	1,3,6,8-	1,2,8,9-
PeCDF	1,3,4,6,8-	1,2,3,8,9-
HxCDF	1,2,3,4,6,8-	2,3,4,6,7,8-
HpCDF	1,2,3,4,6,7,8-	1,2,3,4,7,8,9-

A precise check of all retention time ranges is only necessary when using a new capillary the first time, or after significant changes in the retention behaviour (>30 s). Normally, it is only necessary to correct the retention time ranges correspondingly to the time difference for 2,3,7,8-TCDF.

63 Mass spectrometric quantification

631 Low resolution mass spectrometry (LRMS)

631.1 Instrumentation

- Mass spectrometer with electron ionisation (EI).
- Mass spectrometer that can be operated in both the EI and the chemical ionisation modes with negative ion detection (NICI). The conditions given below are valid for a Hewlett-Packard 5989 instrument and have to be adjusted for other mass spectrometers.

LRMS is combined with both EI and NICI. EI-LRMS is used for quantification of concentrations >2 pg/µL. The range of application of NICI-LRMS is between >0.1 pg/µL and about 250 pg/µL. However, this technique is not suitable for the determination of 2,3,7,8-TCDD (very poor sensitivity), and that has to be quantified separately by EI-LRMS. The following typical detection limits (signal-to-noise ratio 3:1) expressed on the basis of total sample amount have to be attained when applying the detection conditions described in *section 631.2*:

- **EI-LRMS**: About 4 pg when injecting 60 % of the sample extract, otherwise 15 pg if injecting 20 % of the sample extract.
- **NICI-LRMS**: About 2.5 pg if injecting 10 % of the sample extract.

631.2 Optimisation and detection conditions

- The position of the outlet of the separation capillary in the ion source has a considerable influence on the sensitivity achieved. The length measured from the connection nut depends on the type of instrument, and has to be respected precisely or optimised.
- Manual optimisation of the ion yield of the ion source and the transmission of the mass filter (quadrupole) with perfluorotributylamine (PFTBA) applying the fragment masses m/z 312, 414 and 464. The signal width at half height is adjusted to 0.55±0.03 amu and the mass scale calibrated to an accuracy of ±0.05 amu.

  Electron energy: 70 eV (EI) or about 100-120 eV (NICI), CH_4 as reagent gas for NICI, ion source pressure 60 Pa (0.6 mbar), ion source temperature 200 °C.
- Detection of the $M^{+\cdot}$- and $(M+2)^{+\cdot}$-ions for each ^{12}C-PCDF/PCDD isomer group as well as each added ^{13}C-labelled isomer (internal standard and recovery standard), dwell time 50 ms/ion (octachloro congeners, 100 ms/ion) or at least 10-12 measuring points per signal - in total ≤11 ions per group. The mass spectrometer is operated in the selected ion monitoring (SIM) mode.

The SIM programmes listed in *table 2* are used for quantification. The masses listed in *tables 2* and *3* are the molecular or fragment ions of the isomer groups given in *table 4*.

Table 2: Ion groups used for quantification with EI-LRMS

Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
m/z	m/z	m/z	m/z	m/z	m/z
303.9	303.9	337.9	337.9	371.8	441.7
305.9	305.9	339.9	339.9	373.8	443.7
317.9	337.9	351.9	351.9	387.8	453.7
319.9	339.9	353.9	355.9	389.8	455.7
321.9	351.9	355.9	371.8	405.8	457.7
333.9	353.9	371.8	373.8	407.8	459.7
351.9	355.9	373.8	385.8	419.8	469.7
337.9	367.9	385.8	387.8	421.8	471.7
339.9	371.8	387.8	389.8	423.8	
353.9	373.8	389.8	401.8	435.8	
355.9					

Table 3: Ion groups used for quantification with NICI-LRMS

Group 1 m/z	Group 2 m/z	Group 3 m/z	Group 4 m/z	Group 5 m/z	Group 6 m/z
303.9	303.9	337.9	337.9	371.8	422.8
305.9	305.9	339.9	339.9	373.8	424.8
317.9	337.9	351.9	351.9	386.8	434.7
333.9	339.9	353.9	355.9	387.8	441.7
337.9	351.9	355.9	371.8	388.8	443.7
339.9	353.9	371.8	373.8	389.8	
351.9	355.9	373.8	385.8	400.8	
353.9	367.9	385.8	387.8	405.8	
355.9	371.8	387.8	389.8	407.8	
367.9	373.8	389.8	401.8	419.8	

632 High resolution mass spectrometry (HRMS)

632.1 Instrumentation

A high resolution mass spectrometer has to be selected that can be operated routinely at a resolution of 10,000 (cf. *section 632.2*). The following details are valid for a Micromass Autospec I System. They have to be partially adapted for other mass spectrometers.

High resolution mass spectrometry (HRMS) is suitable for the determination of concentrations from about 50 fg/µL to about 5 ng/µL (injection volume 1 µL). At higher concentrations the sample has to be diluted. The detection conditions given in *section 632.2* allow the following typical detection limit to be attained for the whole sample extract (signal-to-noise ratio 3:1, tetrachloro to hexachloro isomers):

- **EI-HRMS**: About 500 fg if injecting 10 % of the sample extract.

632.2 Optimisation and detection conditions

- The position of the outlet of the separation capillary in the ion source has a considerable influence on the sensitivity achieved. The length measured from the connection nut depends on the type of instrument, and has to be respected precisely or optimised.
- Manual optimisation of the ion yield, the transmission of the mass filter (magnet and electrostatic sector) with perfluoro kerosene (PFK, boiling point 70-210 °C) at a temperature of the separation capillary of 240 °C using the fragment m/z 330.9792 in the TUNE mode. The resolution is adjusted to R = 10,000. It is defined as m/Δm = 10,000 at 5 % valley:
  - *Electron energy:* About 19-30 eV, depending on the age of the filament;
  - *Ion source temperature:* 240 °C.
- Calibration of the mass scale with 5 PFK fragments using the programme LOCATION DATA. The signal abundance on the oscilloscope is reduced to about 50-100 mV for m/z 330.9792. The optimisation of the ion source and the resolution are checked and if necessary adjusted for each of the PFK fragments listed in *table 5*.

Table 4: Molecular or fragment mass selected for the different isomer groups

Isomer group	^{12}C		^{13}C	
	$M^{+\cdot}/M^-$	$(M+2)^{+\cdot}/^-$	$M^{+\cdot}/M^-$	$(M+2)^{+\cdot}/^-$
TCDF	303.9	305.9	315.9	317.9
PeCDF	337.9	339.9	349.9	351.9
HxCDF	371.8	373.8	383.8	385.8
HpCDF	405.8	407.8	417.8	419.8
TCDD	319.9	321.9	331.9	333.9
PeCDD	353.9	355.9	365.9	367.9
HxCDD	387.8	389.8	399.8	401.8
HpCDD (EI)	421.8	423.8	433.8	435.8
	$(M+2)^{+\cdot}/^-$	$(M+4)^{+\cdot}/^-$	$(M+2)^{+\cdot}/^-$	$(M+4)^{+\cdot}/^-$
OCDF	441.7	443.7	453.7	455.7
OCDD(EI)	457.7	459.7	469.7	471.7
	$(M-Cl)^-$	$(M+2-Cl)^-$		$(M+2-Cl)^-$
HpCDD (NICI)	386.8	388.8		400.8
	$(M+2-Cl)^-$	$(M+4-Cl)^-$		$(M+4-Cl)^-$
OCDD (NICI)	422.8	424.8		434.8

Table 5: PFK fragments used for calibration of the mass scale applied for each congener group

Isomer group	m/z				
TCDF/TCDD	292.9824	304.9824	318.9792	330.9792	342.9792
PeCDF/PeCDD	330.9792	342.9742	354.9792	366.9792	380.9761
HxCDF/HxCDD	366.9792	380.9761	392.9761	404.9761	416.9761
HpCDF/HpCDD	404.9761	416.9761	430.9729	442.9729	454.9729
OCDF/OCDD	430.9729	442.9729	454.9729	466.9729	480.9697

- Detection of the $M^{+\cdot}$- and $(M+2)^{+\cdot}$ or $(M+2)^{+\cdot}$- and $(M+4)^{+\cdot}$-ions for each ^{12}C-PCDF/PCDD isomer group as well as each added ^{13}C-labelled isomer (internal standard and recovery standard). Dwell time per ion: 50 ms for ^{12}C-isomers, 20 ms for ^{13}C-isomers and 10 ms for PFK fragments used for mass scale control. The mass spectrometer is operated in the SIM mode. The total cycle time per ion group including PFK control ions has to be within 400-600 ms, which corresponds to a total number of about 10 ions.

The mass of the ion to be measured within one group is selected by varying the acceleration voltage. At the same time the magnetic field is fixed to one of the PFK control masses. Larger deviations from the maximum possible acceleration voltage of 8,000 V result in poorer detection limits. The difference between the PFK control mass and that of the ion to be determined is usually within 50-80 amu.

The retention times of isomer groups with different degrees of chlorination overlap strongly when using polycyanopropyl phases (cf. *section 623*), so that the sample has to be analysed with two different SIM programmes. These are listed in *tables 6* and *7*.

Table 6: Ion groups used for quantification with HRMS on a polar GC stationary phase - injection 1. The most abundant ion of each isomer group is used for quantification.

Group no.	**Isomer group**	**Non-labelled PCDD/PCDF**		**$^{13}C_{12}$-PCDD/PCDF**	
1	TCDF	303.9016	305.8987	315.9419	317.9389
	TCDD	319.8965	321.8936	331.9368	333.9339
	PFK	316.9824			
2	HxCDF	373.8207	375.8178	383.8642	385.8610
	HxCDD	389.8160	391.8127	401.8559	403.8530
	PFK	380.9728			
3	OCDF	441.7428	443.7398		
	OCDD	457.7377	459.7348	469.7780	471.7750
	PFK	454.9728			

Table 7: Ion groups used for quantification with HRMS on a polar GC stationary phase - injection 2

Group no.	**Isomer group**	**Non-labelled PCDD/PCDF**		**$^{13}C_{12}$-PCDD/PCDF**	
1	PeCDF	339.8597	341.8568	351.9000	353.8970
	PeCDD	353.8576	355.8546	365.8978	367.8949
	PFK	366.9792			
2	HpCDF	407.7818	409.7788	417.8253	419.8220
	HpCDD	423.7767	425.7737	435.8169	437.8140
	PFK	430.9728			
3	OCDF	441.7428	443.7398		
	OCDD	457.7377	459.7348	469.7780	471.7750
	PFK	454.9728			

64 Quantification procedure

After completion of the GC/MS analysis, all integrated signal areas and mass fragmentograms are printed out for each congener group. The quality of the analysis is then evaluated according to the following points:

- Are the mass chromatograms without disturbances or interferences - are isomers missing, or are extra signals present not belonging to a characteristic pattern?

- Are the retention times of the 2,3,7,8-chlorine substituted congeners correct compared to those of the ^{13}C-labelled congeners?
- Is the gas chromatographic separation sufficient?
- Are abundance differences between single GC signals in the $M^{+\cdot}$-, $(M+2)^{+\cdot}$- or $(M+4)^{+\cdot}$-mass fragmentograms similar, or do changed ratios indicate a wrong chlorine isotope ratio?
- Is the signal-to-noise ratio sufficient for a quantitative determination?

In addition, all requirements listed in the SAEFL guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil* have to be fulfilled The calculation of the sample concentrations is only carried out if all the points mentioned above have been checked and found to be correct. A spread-sheet programme is used for this purpose, based on a commercial programme, or the quantification programme supplied by the instrument manufacturer. All calculations are carried out according to the following principle:

- The response factors f_i of all single ^{12}C-2,3,7,8-chlorine substituted congeners are calculated relative to the ^{13}C-labelled congeners. The integrated single signal areas and concentrations of the quantification standard are used as follows:

$$f_i = \frac{\text{Conc.}^{12}\text{C}-\text{isomer} \times \text{area}^{13}\text{C}-\text{isomer}}{\text{Conc.}^{13}\text{C}-\text{isomer} \times \text{area}^{12}\text{C}-\text{isomer}}$$

f_i: Response factor relative to ^{13}C-isomer i
Conc.: Concentration in quantification standard

- The total amount of ^{12}C-2,3,7,8-chlorine substituted isomers in the sample is calculated. For this purpose, the integrated areas of the single ^{12}C- and ^{13}C-congeners are used as well as the total amount of the corresponding ^{13}C-2,3,7,8-chlorine substituted congener added to the sample:

$$M_i = \frac{\text{Amount}^{13}C_i \times \text{area}^{12}C_i \times f_i}{\text{Area}^{13}C_i}$$

M_i: Total amount of ^{12}C-isomer i in the sample
Amount $^{13}C_i$: Total amount added to the sample

- The mass concentration is calculated as the ratio between total amount M_i and sample amount.

- The sum of all homologues with the same number of chlorine atoms (e.g. ΣTCDD, ΣPeCDF) is obtained by adding all the areas of the single signals. This assumes that the response factor of all isomers corresponds to that of the 2,3,7,8-chlorine substituted isomer(s). For congener groups containing several ^{13}C-2,3,7,8-chlorine substituted congeners, the average of their response factors is employed. An exception is the group ΣHpCDF. Here, only the response factor of 1,2,3,4,6,7,8-HpCDF is used (about 80 % of ΣHpCDF), since that of 1,2,3,4,7,8,9-HpCDF is about 50 % lower - otherwise the Σ HpCDF values would become unrealistically high.

- The calculation of the recovery rate R_i (in %) of the ^{13}C-labelled 2,3,7,8-chlorine substituted congener i (added before extract clean-up as an internal standard) is carried out relative to the recovery standard (Rec.STD). The latter is added to the sample prior to quantification:

$$f_{rc} = \frac{\text{Conc.}^{13}C_i \times \text{area Rec.STD}}{\text{Conc. Rec.STD} \times \text{area } ^{13}C\text{ –isomer}}$$

f_{rc}: Response factor of ^{13}C-isomer i relative to recovery standard Rec.STD
Conc.: Concentration in internal standard

$$R(\%)_i = \frac{\text{Amount Rec.STD} \times \text{Area } ^{13}C_i \times f_{rc} \times 100}{\text{Added tot. amount} ^{13}C_i \times \text{Area Rec.STD}}$$

$R(\%)_i$: Recovery in % of the added ^{13}C-isomer i
Amount Rec.STD: Total amount of recovery standard added to the sample
Added tot. amount $^{13}C_i$: Total amount of the ^{13}C-isomer i added to the sample

The calculation programme of the high resolution MS analysis also automatically checks whether retention time, isotope ratio and signal-to-noise ratio are within the range required by the quality control.

The concentrations of the 2,3,7,8-chlorine substituted congeners are converted into 2,3,7,8-tetrachlorodioxin equivalents (I-TEQ). 2,3,7,8-Tetrachloro dibenzo-*p*-dioxin is the most toxic congener of all 210 PCDD/PCDF. The concentrations of all other, less toxic 2,3,7,8-chlorine substituted congeners are weighted according to the toxicity differences. Factors of different toxicity models can be used. All weighted concentrations are then summed to give the I-TEQ concentration. *Table 8* shows the toxicity equivalent factors of the international model.

Table 8: Toxicity equivalent factors (I-TE) of the international model for calculating 2,3,7,8 I-TEQ concentrations

Congener	Toxicity equivalent factors (I-TE)
2,3,7,8-TCDD	1
1,2,3,7,8-PeCDD	0.5
2,3,7,8,X,X-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.001
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
2,3,7,8,X,X-HxCDF	0.1
2,3,7,8,X,X,X-HpCDF	0.01
OCDF	0.001

7 Quality assurance

71 Checking standard solutions for quantification

All reference standards are stored at -20 °C and have an unlimited storage period. All primary standards are stored at 4-6 °C. Their storage period is limited to a maximum of two years. Primary standards in sample vials with capillary opening have evaporation losses of <1 mg within six months. Primary standards in normal sample vials can be used for a maximum of two months.

Before they are used, all freshly prepared primary and calibration standards have to be compared with the two preceding standard generations. Deviations that are within the repeatability of the quantification method (±15 %) are acceptable. At least once a year, primary standards have to be compared with a certified reference standard or the reference standard of a well-reputed intercalibration. At present, only reference standard solutions are available with an indicated accuracy of ±10 % that are prepared by companies with known high quality standards. International certification authorities can still only offer a limited number of certified 2,3,7,8-chlorine substituted congeners.

Therefore, concentration differences of up to 10 % between laboratories are to be considered as normal and acceptable.

72 Adsorption test gas chromatography

The quantification standard automatically performs an adsorption test at each injection for the whole chromatography system and the GC/MS interface system. OCDF is very sensitive to adsorption effects in the injector as well as on the separation capillary and the GC/MS interface. The gas chromatography system is running in a satisfactory manner if the signal intensity of the quantifying ion of OCDF is at least 40 % that of OCDD.

73 Test of mass spectrometry detection limit

731 Low resolution mass spectrometry (LRMS)

- **Electron ionisation (EI):** The detection limit is checked daily before quantification. The injection of 2 µL of a standard containing 15 pg/µL 2,3,7,8-TCDD (e.g. 13C12C2Q; cf. *section 332*) has to result in a signal-to-noise ratio of >10:1 (noise smoothing over three measuring points is allowed), if the separation capillary and the corresponding temperature programme are used as given in *section 623*.

- **Negative ion chemical ionisation (NICI):** The detection limit is checked daily before quantification. The injection of 2 µL of a standard containing 2 pg/µL 2,3,7,8-TCDF has to result in a signal-to-noise ratio of >15:1 (noise smoothing over three measuring points is allowed), if the separation capillary and the corresponding temperature programme are used as given in *section 623*.

732 High resolution mass spectrometry (HRMS)

- **Electron ionisation:** The mass spectrometric resolution R is checked daily before starting quantification. It should be R=10,000±1,000, and is determined with mass m/z 330.9792 at 5 % valley.

The detection limits are also checked daily before quantification. The injection of 1 µL of a standard containing 3 pg/µL 2,3,7,8-TCDD (e.g. 13C12C3Q; cf. *section 333*) has to result in a signal-to-noise ratio of >100:1 (without noise smoothing), if the separation capillary and the corresponding temperature programme are used as given in *section 623*.

74 Frequency of injection of quantification standards

The quantification standard has to be injected before each sample series and after each fifth sample. For sample series with less than five samples, the standard has to be re-injected after the last sample.

75 Blanks for extraction and extract clean-up

Details are given in the SAEFL guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil*[1].

76 Archiving of quality assurance information

Details are given in the guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil*.

77 Acceptance of results

Details are given in the guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil*.

8 Accuracy and reproducibility of the method

- The accuracy of the available reference standards is ±10 %.
- The standard deviation of at least five analyses in parallel of a homogeneous sample is within ±15 %. Deviations of ±25 % are possible for samples that are difficult to homogenise.
- In addition, the complete method was validated in an intercalibration *(Hagenmaier and Lindig 1993)*. The deviation from the average of the 22 participating laboratories was typically 10-20 %.

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[1] SAEFL, 27 pages, Berne, January 2000 - in German, French, Italian, and English.

- The analysis of long-term series of control samples indicates a reproducibility of ±15-25 %.

The bibliography should be consulted concerning interferences due to other polychlorinated aromatic compounds (e.g. *Bacher and Ballschmiter 1992* or *Oehme 1998)*.

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9 Literature

The literature listed below contains further information about the methods employed in the present guidelines. It also includes material about alternative techniques and critical points that might cause problems:

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