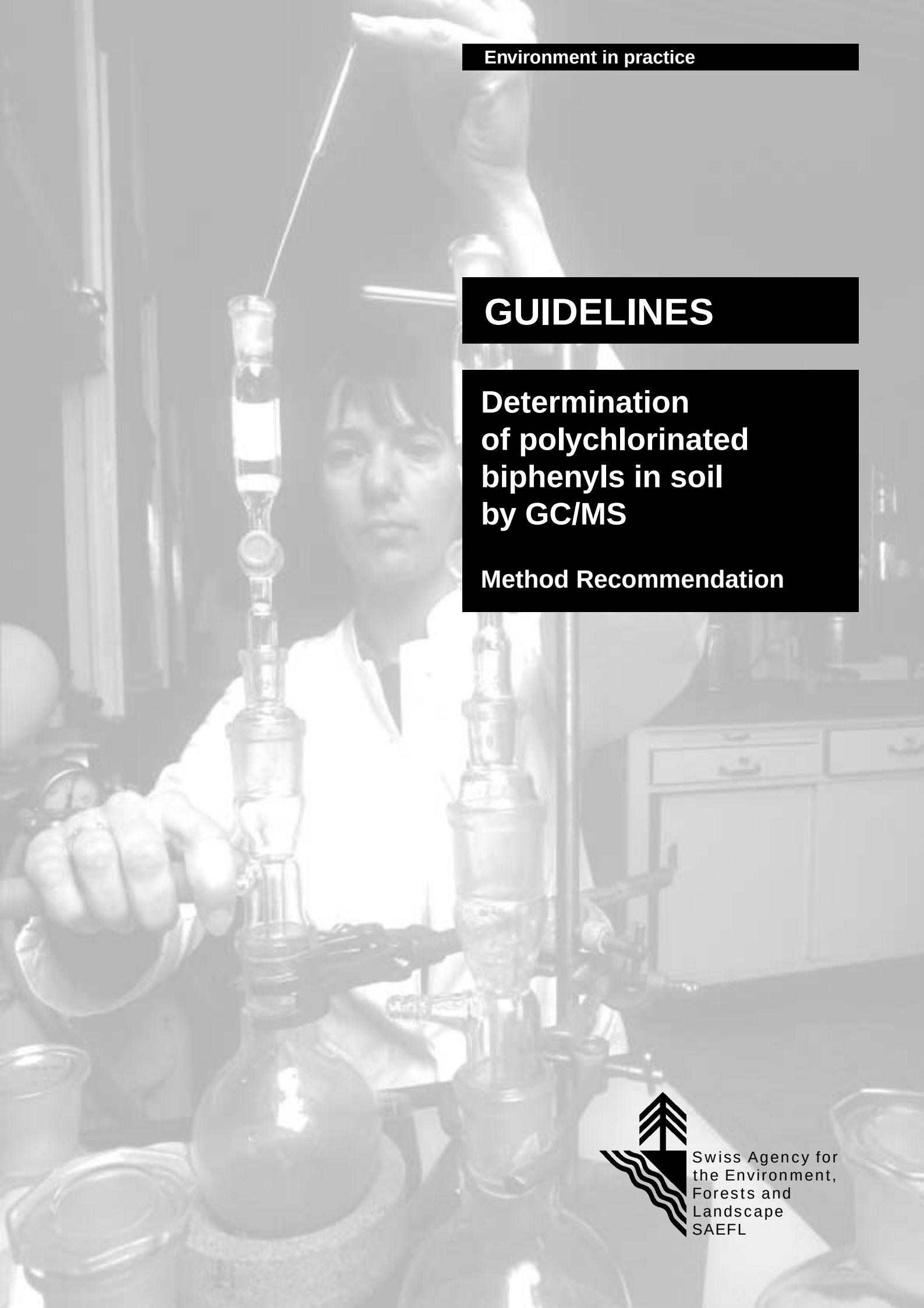

Environment in practice

GUIDELINES

Determination of polychlorinated biphenyls in soil by GC/MS

Method Recommendation

Swiss Agency for the Environment, Forests and Landscape SAEFL

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ABSTRACTS

A method is described for the determination of *polychlorinated biphenyls (PCB)* in soil, which 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 presented method also fulfils the requirements of the quality assurance norm *ISO/IEC 17'025*. It is based on soxhlet extraction of the dried soil samples followed by sample clean-up using column chromatography. The addition of internal standards prior to extraction (so-called extraction standards) allows the automatic correction of compound losses which are calculated for each sample by addition of a recovery standard to the sample extract before quantification. The separation of PCB is carried out by high resolution gas chromatography. Quantification is based on the internal standard method and low resolution mass spectrometry (MS). Detailed working procedures are given as well as information about quality control measures.

Es wird eine Methode zur Bestimmung von *polychlorierten Biphenylen (PCB)* in Böden beschrieben, welche das vom BUWAL veröffentlichte Qualitätssicherungskonzept für die Analytik organischer Schadstoffe im Boden erfüllt. Diese Methode erfüllt auch die Anforderungen der Qualitätssicherungsnorm *ISO/IEC 17'025*. Sie beruht auf Soxhletextraktion der getrockneten Bodenproben, gefolgt von einer Probenaufarbeitung mittels Säulenchromatografie. Der Zusatz von internen Standards vor der Probenextraktion (sogenannte Extraktionsstandards) erlaubt die automatische Korrektur von allfälligen Verlusten, die mit Hilfe der Zugabe von Wiederfindungsstandards zum Probenextrakt vor der Quantifizierung für jede einzelne Probe berechnet werden können. Die Trennung der PCB wird mit hochauflösender Gaschromatografie durchgeführt. Die Quantifizierung wird mit niedrigauflösender Massenspektrometrie (MS) in Bezug auf die internen Standards durchgeführt. Es werden sowohl detaillierte Arbeitsvorschriften als auch Informationen über Massnahmen der Qualitätskontrolle vermittelt.

Cette publication décrit une méthode de détection des *biphényles polychlorés (PCB)* dans le sol, qui remplit les exigences posées par le *système d'assurance de la qualité* élaboré par l'OFEFP pour l'analyse des polluants organiques du sol. Cette méthode répond également aux exigences de la norme d'assurance de la qualité *ISO/IEC 17'025*. Elle se base sur une extraction Soxhlet des échantillons de sols séchés, suivie d'une préparation des échantillons réalisée au moyen d'une chromatographie sur colonne. L'addition d'étalons internes (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 PCB est effectuée à l'aide d'une chromatographie en phase gazeuse à haute résolution. La quantification se fait par spectrométrie de masse à basse résolution, en se référant aux étalons internes. La publication présente une méthode de travail détaillée ainsi que des informations sur les mesures de contrôle de la qualité.

In questa pubblicazione viene descritto un metodo per la determinazione dei *bifenili policlorurati (PCB)* nel suolo, che soddisfa il *Concetto di "Quality Assurance"* elaborato dall'UFAFP per l'analisi di sostanze organiche inquinanti presenti nel suolo. Il metodo soddisfa pure le esigenze della norma di *"Quality Assurance" ISO/CEI 17'025*. Esso è basato sull'estrazione secondo Soxhlet di campioni di suolo essiccati, cui fa seguito una purificazione del campione mediante cromatografia su colonna. L'aggiunta di standard interni prima dell'estrazione del campione (i cosiddetti standard per l'estrazione) consente la correzione automatica di eventuali perdite, che possono essere calcolate per ogni singolo campione. La separazione dei PCB viene eseguita con la cromatografia in fase gassosa ad alta risoluzione. La quantificazione viene effettuata mediante spettrometria di massa a bassa risoluzione con riferimento agli standard interni. Vengono fornite sia prescrizioni dettagliate concernenti l'attività pratica in laboratorio sia informazioni sulle misure inerenti il controllo della qualità.

FOREWORD

Like the dioxins, furans and polycyclic aromatic hydrocarbons, polychlorinated biphenyls are organic pollutants that can contaminate the soil. The Swiss *Ordinance relating to impacts on the soil (OIS) of 1 July 1998 therefore* sets standards for these compounds in the form of trigger and clean-up values. The standards are also treated in the Stockholm Convention on persistent organic pollutants (POPs) signed in 2001 by 127 countries.

The analysis of PCB is methodically demanding. Therefore, such analyses are only carried out if specific indications about hazardous environmental impacts are to be assumed, e.g. contamination of soils of industrial sites. As for other groups of organic pollutants, it is important to have an analytical method available that can be reproduced at any time, and which also allows for comparable results.

After publishing the quality assurance concept, the guidelines for the determination of polychlorinated dioxins and furans as well as for PAH, Prof. M. Oehme 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.

Swiss Agency for the Environment,
Forests and Landscape

Georg Karlaganis
Head of the Department of Substances,
Soil and Biotechnology

ABBREVIATIONS AND DEFINITIONS

CEN	*Comité Européen de Normalisation* (European Committee for Standardisation 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 (Norm).
extraction standard	A compound that is added prior to sample extraction, allowing compensation for losses during extraction and clean-up.
GC-ECD	Gas chromatography-"electron capture detection".
GC-MS	Gas chromatography-mass spectrometry.
GPC	Gelpermeation chromatography.
HRGC	High resolution gas chromatography.
ISO	International Organization for Standardization.
isomers	Compounds with an identical carbon skeleton and the same number of substituents (e.g. chlorine) at different positions.
ISTD	Internal standard.
MS	Mass spectrometry.
OIS	Ordinance of 1 July 1998 relating to impacts on the soil.
PCB	Polychlorinated biphenyls.
ppb	*"parts-per-billion":* Concentration unit, corresponds to 1 nanogram (10^{-9} g) per g or 1 µg/kg.
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.
u	Abbreviation for atomic mass unit on the basis of the Carbon atom, ^{12}C = 12.00000 u.

1 Principle of Determination

11 Preliminary remarks

Polychlorinated biphenyls (PAH) are Soxhlet-extracted from the dried soil sample. ^{13}C-isotope-labelled PCB are added as internal standards prior to sample extraction. Any interfering sample matrix is removed by different column liquid chromatographic methods. After concentrating the sample extract and the addition of a recovery standard, all relevant PCB-compounds are separated by high resolution gas chromatography and quantified by low resolution mass spectrometry (MS) in the electron ionisation mode.

This method of measurement is suitable for all types of soil and levels of contamination. According to the OIS[1] the guide value for the sum of 7 PCB (cf. *Tab. 1)* is 0.1–0.2 mg/kg dry weight. It has to be quantified reliably with a sufficient limit of quantification. Depending on sample size, a limit of quantification in the order of 0.1–0.3 µg/kg (0.1–0.3 ppb) can be achieved for single congeners which is about 2 orders of magnitude below the guide value.

Specific manufacturers are only named if their products have unique properties necessary for successfully applying the best available techniques.

12 Safety information

PCB are very toxic carcinogenic substances. Therefore, the highest possible level of safety measures currently in force in Switzerland must be observed when handling PCB.

2 Equipment, chemicals and instruments

21 Glassware and equipment

211 Glassware

The following glassware made from high quality borosilicate glass is needed:

- *Round bottom flasks:* volumes 100, 250, 500 and 1'000 mL, joint size 24/29.
- *Pasteur pipettes:* length 150 mm and 250 mm.
- *Glass bottles with wide opening:* 500 and 1'000 mL (polypropylene screw cap GL 45) for sample storage.
- *Volumetric flasks with glass stopper:* 10 mL, quality A, precision ±0.025 mL at 20 °C.
- *Erlenmeyer flasks:* 250 mL with glass stopper.
- *Glass funnel:* 30, 150 mm diameter.
- *Chromatography column:* 200 mm long, 15 mm ID.
- *Chromatography column:* 600 mm long, 25 mm ID, with compression plunger (e.g. Omnifit).

[1] Ordinance of 1 July 1998 relating to impacts on the soil (OIS, SR 814.12).

- *Vibrator:* For dry-packing of chromatographic columns.
- *Petri dishes:* 150 mm diameter.

212 Equipment for Soxhlet extraction

- *Soxhlet extractor:* 200 mL volume, length 250 mm, joint size 34/35, fitting joint 24/29.
- *Soxhlet extractor:* 2'000 mL with sleeve lid and fitting joint 34/35.
- *Ball cooler:* length 330 mm, joint size 24/29.
- *Socket/cone adapter:* joint size 24/29 to 34/35.
- *Soxhlet thimbles:* cellulose, diameter 28 mm, length 80 mm (for pre-treatment, cf. *section 218).*
- *Polyurethane foam sheets:* size 35 mm, 210x210 mm - for lagging of Soxhlet extractor.

213 Sample vials

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

214 Other equipment for extract clean-up

- *Porcelain dish:* diameter 180 and 250 mm.
- *Pressure reduction valve:* metal-bellow sealed, pre-set to 3–5 bar.
- *Rotavapor:* with automatic pressure regulation.
- *Turbovap 500:* volume reduction apparatus and corresponding vials (Zymark).
- *Gelpermeation chromatographic system:* Chromatographic column 600 mm long, 25 mm ID, filled with 50 g Biobeads SX-3, 200–400 mesh, HPLC pump with flow rate 5 mL/min at a counter pressure of ca. 6 bar and injection port with 10 mL sample loop.
- *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.
- *Micro balance:* range 0–3'000 mg, precision ±1 µg.
- *Furnace:* 200–1'000 °C, precision ±10 °C.
- *Ultrasonic bath:* power 100 W.
- *Membrane vacuum pumps:* resistant to solvents, with Teflon membrane, 4 or 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.
- *Heating jackets:* for 500 mL and 1'000 mL round bottom flasks.
- *Porcelain mortar:* diameter 130 mm, pestle 145x38 mm.
- *Sieve:* made from stainless steel, mesh size 2 mm (according to DIN 4188).

215 Syringes for dilutions

- *With fixed needle and steel plunger:* 10, 25 and 1'000 µL.
- *Transfer pipette:* 1 mL, precision ±0.01 mL.
- *Glass syringe:* 5 mL, with scale, and Luer connector.
- *GC syringe:* 10 µL, with fixed needle and steel plunger, for autosampler.
- *Calibrated micro pipettes:* 10, 20, 50 and 100 µL, precision ±0.25–1 %.

216 Other equipment

- *Solvent resistant gloves*.
- *Disposable gloves made from polyethylene*.
- *Cork rings*.
- *Boiling stones:* pre-cleaned.
- *Joint clamps:* for joint size 14 and 29.

217 Cleaning of glassware

After each extract clean-up, all types of round bottom flasks, beakers, centrifuge tubes and chromatography columns are soaked for 24 h in a 2.5 % (v/v) solution of RBS 25 (cf. *section 222).* These items are then rinsed twice 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 350–450 °C. Pasteur pipettes are rinsed before use with the solvent that is to be used.

218 Cleaning of Soxhlet thimbles

Up to eight Soxhlet thimbles are extracted for 8 h with toluene in a 2'000 mL Soxhlet extractor. After drying in a vacuum exsiccator (80 kPa respectively 0.8 bar reduced pressure at 100 °C) they are wrapped in aluminium foil.

22 Chemicals, adsorbents and gases

221 Solvents

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

- Cyclohexane;
- n-Hexane;
- Dichloromethane;
- Diethylether;
- Ethylacetate;
- n-Nonane;
- *iso*-Octane.

222 Various chemicals, materials and preparatory steps

222.1 Basic materials

- *Cotton wool:* chemically clean (cleaning; cf. *section 222.2).*
- *Aluminium foil:* thickness 0.018 mm, size 450 mm.
- *Aluminium oxide:* basic, pH 10, activity grade I, 50–200 µm.
- *Gelpermation phase:* Biobeads S-X3, GPC-gel, 200–400 mesh Bio Rad.
- *Silica:* 0.063–0.20 mm (pre-treatment; cf. *sections 222.3).*
- *Silanised glass wool:* pre-treated with dimethyldichlorosilane.
- *Sodium sulphate:* p.a. (pre-treatment; cf. *section 222.4).*
- *RBS 25 Laboratory detergent:* Chemical Products, Brussels, Belgium.

222.2 Cleaning of cotton wool

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

222.3 Pre-treatment of silica

Activation is carried out for 8 h in an oven at 130° C by placing 100 g silica on a porcelain dish (diameter 180 mm). Then the silica is stored in a glass bottle with an air-tight Teflon-lined screw cap. The storage period is four weeks.

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 Gases and gas cleaning

223.1 Basic materials

Helium, 99.995 % (further cleaning, cf. *section 223.2).*	Nitrogen, 99.99 % (further cleaning, cf. *section 223.3).*
O_2/activated charcoal filter.	Molecular sieve filter.
Loose molecular sieve of 0.5–2.0 mm, if needed (further cleaning cf. *section 223.4).*	Activated charcoal, 1.5 mm particle size.
Empty metal cartridges, Whitey 304L-HDF4-50 and 340L-HDF4-75, or equivalent made from stainless steel.	

223.2 Additional cleaning of helium

Helium serving as 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.
- Two metal cartridges in series are mounted directly before each gas chromatograph. The first one is filled with molecular sieve (cf. *section 223.4)* and the second one with activated charcoal. Both cartridges are replaced only if irregularities or problems occur (e.g. comple-

te emptying of a pressure tank), or after three years at the latest. Pressure tanks must not be emptied below 1'500 kPa (15 bar). The oxygen-activated charcoal filter is then disposed of. The contents of the molecular sieve filter are replaced by regenerated material (cf. *section 223.4),* and the activated charcoal in the metal cartridges is replaced with new material.

223.3 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).

223.4 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 214).* 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

Whenever possible, calibration and reference compounds of certified quality and available as crystalline solids should be used. Their purity should be minimum 99 %. The compounds listed in *Table 1* are needed.

Table 1: List of applied PCB and internal standards.

Compounds	Abbreviation and numbering according to IUPAC
2,4,4'-Trichlorobiphenyl	PCB 28*
2,2',5,5'-Tetrachlorobiphenyl	PCB 52*
2,2',4,5,5'-Pentachlorobiphenyl	PCB 101
2,3',4,4',5-Pentachlorobiphenyl	PCB 118*
2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153*
2,2',3,4,4',5'-Hexachlorobiphenyl	PCB 138
2,2',3,4,4',5,5'-Heptachlorobiphenyl	PCB 180*
1,2,3,4-Tetrachloronaphthaline (recovery standard)	TCN

Annotation: * Also as ^{13}C-isotope labelled compounds required.

Certified reference compounds are available from e.g. *"Institute for Reference Materials and Measurements, Geel, Belgium, IRMM"* (earlier *Bureau of Reference, BCR*), from *"Dr. Ehrenstorfer"* (Augsburg, Germany) or from *"LGC-Promochem"* (Wesel, Germany).

All solid reference standards are stored at 4–6 °C in the dark. Due to their chemical stability, PCB have an unlimited storage period, if stored in that way. Before weighing all necessary reference standards have to be store at room temperature for at least four hours.

32 Preparation of the primary standard

The primary standard STD 1 contains the compounds listed in *Table 1* without internal and recovery standards. Some of the reference compounds are carcinogenic. Therefore, the weighing procedure has to be carried out with the greatest possible care. The instructions for use of the analytical balance have to be followed. In addition disposable gloves have to be worn and the area around the balance covered with paper or aluminium foil.

Before use, the weighing boats and the spatula have to be cleaned thoroughly with n-hexane in the ultrasonic bath. The primary standard including internal standards is prepared in a 10 mL measuring flask. The concentration of the single compounds listed in *Table 1* should be in the range of 100±20 ng/µL. This corresponds to a weight of 1'000±200 µg per compound. Depending on the applied mass spectrometer, the response factors of the PCB-congeners might vary requiring an adaptation of the concentrations. After weighing of each compound the spatula is rinsed with a pasteur pipette filled with n-hexane and dried with a paper tissue.

The content of the weighing boats is transferred with n-hexane to the10 mL volumetric flask which is nearly filled to the mark. Then the volumetric flask is placed into an ultrasonic bath until all is dissolved. After cooling to room temperature missing solvent is added. The volumetric flask is labelled and weighed. All details are noted in the reference solution catalogue.

33 Preparation of internal standard solutions

Before use, the weighing boats and the spatula must be cleaned thoroughly with n-hexane in the ultrasonic bath. The internal standard is set up in a 10 mL volumetric flask. The concentrations of the ^{13}C-isotope-labelled internal standards should be in the range of 100±20 ng/µL. This corresponds to a weight of 1'000±200 µg for each compound. After weighing of each compound the spatula is rinsed with a Pasteur pipette filled with n-hexane and dried with a paper tissue.

The content of the weighing boats is transferred with *iso*-octane to the 10 mL volumetric flask which is nearly filled to the mark. Then the volumetric flask is placed into an ultrasonic bath until all is dissolved. After cooling to room temperature missing solvent is compensated. The volumetric flask is labelled and weighed. All details are noted in the reference solution catalogue.

Before preparing a dilution 1:10 or 1:100, the volumetric flask is equilibrated at room temperature and its weight controlled. Loss of solvent is compensated to the final control weighing. 1 mL or 0.1 mL is transferred to a 10 mL a volumetric flask and diluted with *iso*-octane. The concentration is now 10±2 ng/µL or 1±0.2 ng/µL. The storage period is 24 months. 1 mL each is transferred to a 1.5 mL a sample vial with a capillary opening (CERTAN®) and used as working standards.

34 Preparation of recovery standard solution

1,2,3,4-Tetrachloronaphthalin is employed as recovery standard.

Before use, the weighing boats and the spatula have to be cleaned thoroughly with n-hexane in the ultrasonic bath. The recovery standard is prepared in a 10 mL volumetric flask. The concentrations of the recovery standard should be in the range of 100±20 ng/µL. This corresponds to a weight of 1'000±200 µg. After weighing of each compound the spatula is rinsed with a Pasteur pipette filled with n-hexane and dried with a paper tissue.

The content of the weighing boats is transferred with *isio*-octane to the 10 mL volumetric flask which is nearly filled to the mark. Then the volumetric flask is placed into an ultrasonic bath until all is dissolved. After cooling to room temperature missing solvent is compensated. The volumetric flask is labelled and weighed. All details including the mass loss are noted in the reference solution catalogue.

Before preparing a dilution 1:10 or 1:100, the volumetric flask is equilibrated at room temperature and its weight controlled. Loss of solvent is compensated to the final control weighing. 1 mL or 0.1 mL is transferred to a 10 mL a volumetric flask and diluted with *iso*-octane. The concentration is now 10±2 ng/µL or 1±0.2 ng/µL. 1 mL each is transferred to a 1.5 mL a sample vial with a capillary opening (CERTAN®) and used as working standards.

35 Preparation of the quantification standard solutions

The volumetric flask with the primary standard Std 1 is equilibrated at room temperature and its weight controlled. Then it is ultrasonicated for 5 min and ensured that everything is dissolved. Otherwise, the procedure has to be repeated. Loss of solvent is compensated to the final control weighing.

0.5 mL of the primary standard Std 1 is transferred to a 10 mL a volumetric flask and diluted with n-hexane (dilution 1:20). The concentration of single compounds is 5±1 ng/µL. The volumetric flask is marked with Std 2 and stored at –20 °C. The storage period is 12 months. All details including the mass loss are noted in the reference solution catalogue.

Five quantification standard solutions are prepared for a multipoint calibration in the concentration range 5–500 pg/µL, e.g. 10, 20, 50, 200 and 500 pg/µL. The concentration of the internal standards and the recovery standard should be around 100 pg/µL (cf. *section 34* and *35).* This corresponds to an addition of 0.1 mL, 0.4 mL or 1 mL of the standard solution Std 2 for a volumetric flask of 10 mL and concentrations of 50, 200 and 500 pg/µL. For the remaining quantification standard solution, the standard solution Std 2 has to be diluted once more by a factor of 10 (Std 3). 1 mL are added of the dilutions 1:100 of the internal and recovery standards with a concentration of 1 ng/mL. 1 mL each of the calibration solutions is transferred to a 1.5 mL a sample vial with a capillary opening (CERTAN®) and used as working standards.

A single-point calibration is permissible, if the linearity of the mass spectrometer fulfils the requirements of *section* 4 of the guidelines "*Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil*".

36 Storage of standard solutions

All solid reference standards are stored at 4–6 °C in the dark. They have a unlimited storage period. All primary, internal and recovery standard solutions are stored at –20 °C. They have a storage period of 24 months. All other standards are kept at 4–6 °C. Their storage period is limited to maximum 12 months. The weight of working standards stored in sample vials with a capillary opening (CERTAN®) has not necessarily to be checked (loss within six months due to evaporation <1 mg). They can be used for maximum 6 months.

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).* Bottles already in use are cleaned according to *section 217.* New bottles are cleaned by heating at 350–450 °C.

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

10–25 g of the ≤2 mm fraction are weighed into a pre-cleaned Soxhlet thimble (28x80 mm; cf. *section 218),* and 10–50 µL of the internal standard solution is added in the centre part of the sample *(*cf. *section 34* and *45).* A small amount of cleaned cotton wool is placed on top of the thimble. The sample is extracted for 24 h with 300 mL of n-hexane 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. Furthermore, elemental sulphur is efficiently removed by gelpermeation chromatography (GPC). If this clean-up step is omitted, eventually one of the methods for sulphur removal should be used as proposed in the guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil* (SAEFL; cf. *section 73).* Since the method described here was validated with the GPC step, it must be revalidated if another technique is used.

45 Quantity of internal standards added to the sample

The quantity of internal standards 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 internal standards should correspond to about the average concentration to be expected of single PCB.

Guide values

- ***sample not contaminated:*** Amount of soil 40–50 g (possibly two Soxhlet extractions); added amount of internal standard 20 µL of the dilution 1:10 with 10 ng/µL (ca. 200 ng per PCB); added amount of recovery standard 20 µL of the dilution 1:10 with 10 ng/µL (ca. 200 ng);
- ***sample contaminated:*** Amount of soil 5–10 g (eventually a larger amount but only clean-up of an aliquot of extract; added amount of internal standard 20 µL of the dilution 1:10 with 10 ng/µL (ca. 200 ng per PCB); added amount of recovery standard 20 µL of the dilution 1:10 with 10 ng/µL (ca. 200 ng).

5 Extract clean-up

51 Preliminary remarks

Soil may contain matrix components interfering the detection of PCB by GC-ECD or GC-MS. Their main fraction can be removed by gelpermeation chromatography (GPC). This method removes disturbing components with molecular weights differing from that of PCB. Examples are elemental sulphur and humic compounds.

52 Gelpermeation chromatography

521 Packing of GPC column

50 g Bio Beads S-X3 (cf. *section 222)* are weighed into an erlenmeyer flask, the adsorbent is completely covered with cyclohexane/ethylacetate 1+1 and soaked for 24 h. This suspension is filled into a column of 600 mm length and 25 mm ID equipped with the lower compression plunger. After settlement of the adsorbent, the excess of solvent is drained and the procedure repeated until the column is completely filled. Then the upper compression plunger is mounted and the packing compressed until no void volume is any longer visible between piston and adsorbent. The column should never run dry! It is conditioned by pumping cyclohexane/ethylacetate 1+1 through the column at a flow rate of ca. 2 mL/min for 2 h. Afterwards, usually the packing material can be compressed further with the plunger.

522 Calibration of GPC column

Each newly packed column has to be tested concerning separation properties and sample capacity as well as recovery of PCB. For this purpose 500 mg of a sample very rich in matrix such as fish oil (e.g. cod liver oil) are dissolved in 5 mL of cyclohexane/ethylacetate 1+1. The complete sample is then injected with a 5 mL glass syringe equipped with a Luer-connector. It is eluted with cyclohexane/ethylacetate 1+1 at a flow of 5 mL/min. 20 fractions of 10 mL each (ca. 2 min for each fraction) are collected in weighed test tubes. All fractions are evaporated at 50 °C and weighed. 90 % of the fat should be eluted after 20 min. Otherwise, the separation of the column is too poor, and it has to be repacked.

The recovery of PCB is controlled with a total amount of ca. 10–50 ng of each PCB-congener (corresponds to ca. 1–5 ng/g for 10 g of soil) and a similar quantity of internal standards. Four fractions of 5 min are collected, the first one after 20 min. Each fraction is quantified after addition of the recovery standard (10–50 ng). The sum of the total recoveries in all fractions should be >90 %. Depending on the distribution of the PCB-congeners in the single fractions, the time window of the final PCB fraction is selected and confirmed by a repeated recovery test.

523 Clean-up of sample extract by GPC

The raw extract is concentrated at the Turbovap to ca. 1 mL, and cyclohexane/ethylacetate 1+1 added to a volume of 5 mL. Then it is cleaned with the parameters determined by the calibration described before.

53 Further clean-up with silicagel

531 Preliminary remarks

After pre-cleaning by GPC, the sample extract is further cleaned with silicagel. For samples containing little matrix and sulphur, this step can be carried out directly after extraction without GPC.

The cyclohexane/ethylacetate extract is transferred to a round bottom flask. Then 50 µL of n-nonane are added as keeper, and the extract concentrated to ca. 0.5–1 mL with the Turbovap. It should not smell any longer of ethylacetate. Otherwise, cyclohexane has to be added and the procedure repeated.

532 Clean-up of sample extracts

The bottom of a glass column of 200 mm length and 15 mm ID is covered with a bit of cotton wool. Then it is filled to about 50 % with n-hexane. and 4 g of silicagel (cf. *section 222.3).* the packing material is compressed with a vibrator, and 1 g of sodium sulphate is added. The the column is washed with 30 mL of 10 % diethylether in n-hexane. The column shall never run dry! The sample extract is then transferred to the column. The sample flask is rinsed with 2–3 mL of 10 % diethylether in n-hexane; this volume is brought to the column as well.

Then the column is eluted with 30 mL of 10 % diethylether in n-hexane. This fraction is collected in a Turbovap glass, and 20 µL of n-nonane is added as keeper. The sample is

concentrated to 0.5 mL with a Turbovap system and transferred to a sample vial. The Turbovap glass is rinsed three times with a volume of 0.15 mL of n-hexane which is transferred to the sample vial as well. The volume may be reduced further to the desired final volume (ca. 200 µL) by a gentle flow of N_2. The surface of the solvent should not be disturbed by the gas flow. 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 or syringe before quantification. If necessary, the sample volume is reduced to about 200 µL with a gentle flow of nitrogen. Then, the sample aliquots as defined in *section 624* may be injected.

62 Gas chromatographic separation

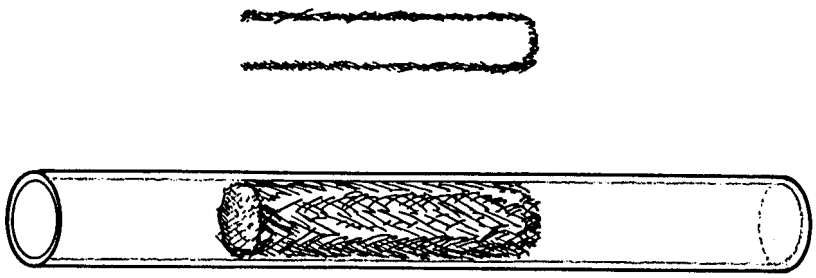
621 Preliminary remarks

The separation of PCB 28 from 31 as well as PCB 138 from 163 is particularly critical. The latter is only possible on special stationary phases (cf. comments and literature in the guidelines *"Quality Assurance Concept, Analysis of PAH, PCB and Dioxins in Soil"*).

622 Instrumentation

- Gas chromatograph.
- Injector for splitless injection, alternatively with auto injector. Volume of glass insert minimum 1 mL. 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. 1).*

Figure 1: Positioning and structure of glass wool to be used in the injector insert in connection with an auto injector.



623 Injection syringes

For manual injections, 5 or 10 µL syringes with fixed needle and metal plunger are used. The same holds true for the auto injector.

624 Separation capillary

The following separation capillary is used:

- *5-%-Phenyl-95-%-methylpolysiloxane:* For example DB5, Ultra 2, CP-Sil 8, RTx5 or equivalent, immobilised, film thickness 0.1 µm.
- *14-%-Cyanopropylphenyl-86-%-methylpolysiloxane:* For example DB1701, HP-1701, CP-Sil 19, RTx1701 or equivalent, immobilised, film thickness 0.1 µm.
- *90-%-Biscyanopropyl-10-%-phenylcyanopropylpolysiloxane:* For example RTx2330, SP-2330, CP-Sil 84 or equivalent, immobilised, film thickness 0.1 µm.
- *Capillary dimensions:* Polyimide coated quartz capillary, length 25 m or 30 m, 0.20 mm or 0.25 mm internal diameter.

625 Injection and separation conditions

- *Carrier gas:* He, flow velocity 35–45 cm/s.
- *Gas flow at split outlet:* 50±10 mL He/min.
- *Septum purge:* 0.8–1.0 mL He/min.
- *Injector temperature:* 280 °C.
- *GC/MS-Interface temperature:* 260 °C.

Injection conditions: Splitless injection (auto injector or hot needle injection) of 1–3 µL sample, 2 min waiting period before opening of the split valve.

Temperature programme: Only informative, has to be adapted depending on capillary dimension and phase. 60 °C, 2 min waiting period, 60 °C to 150–190 °C with 30 °C/min, 150–190°C with 3 °C/min to 230–280 °C, isothermal (6.5–15 min).

Manual hot needle injection: Even PCB of very low volatility are transferred nearly quantitatively to the capillary (>90 %) with this technique:

- Pull up the sample into the syringe until the needle is empty (air volume of 2–3 mm visible).
- Penetrate the septum with the empty needle and wait 5–10 s (needle is heated up).
- Injection of sample, the overpressure due to evaporating solvent ejects the sample out of the needle as fine aerosol droplets.

Alternatively, an on-column injection can be used into a deactivated, but non-coated pre-column *("retention gap")* of about 2 m length and 0.32–0.53 mm ID.

The correct range of retention times for each group of ions (cf. *section 632)* has to be checked with a true (spiked) sample or a reference standard. 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 of ion groups correspondingly to the time difference for PCB 118.

63 Mass spectrometric quantification

631 Instrumentation

A mass spectrometer suitable for electron ionisation (EI) is required. The following typical detection limits have to be attained when applying the detection conditions described in *section 632* (signal-to-noise ratio 3:1 for the GC-signal in the corresponding mass chromatogram): About 10–20 pg when injecting 1 µL of the sample extracts. This corresponds to a total amount of 2–4 ng per sample at a sample volume of e.g. 200 µL.

632 Optimisation and detection conditions

- 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 219.0, 264.0 and 414.0. The signal width at half height is adjusted to 0.55±0.03 u and the mass scale calibrated to an accuracy of ±0.05 u.

  Electron energy: 70 eV (EI), Ion source temperature 200 °C.

- Detection of the $M^{+\cdot}$- and $[M+2]^{+\cdot}$- or $[M+2]^{+\cdot}$- and $[M+4]^{+\cdot}$-ion. The mass spectrometer is operated in the *selected ion monitoring (SIM)* mode. Dwell time 50 ms/ion or at least 10–12 measuring points per signal – in total ≤11 ions per group

The SIM programme listed in *Table 2* is used for quantification. The exact mass resulting in the best signal-to-noise ratio, has to be determined with an dynamic mass calibration (signal-to-noise ratios at masses of –0.2 to +0.2 u of the nominal mass).

Table 2: Masses of ions used for quantification of PCB by GC/MS. The ion with the highest mass is used for quantification. Depending on the stationary phase of the separation capillary, it might be necessary to adapt the groups.

Group no.	PCB no.	$M^{+\cdot}$	$[M+2]^{+\cdot}$
1	PCB 28, PCB 31	255,9	257,9
	1,2,3,4-TCN	263,9	265,9
	^{13}C-PCB 28	267,9	269,9
2	PCB 52	289,9	291,9
	^{13}C-PCB 52	301,9	303,9
	1,2,3,4-TCN	263,9	265,9
3	PCB 101, PCB 118	325,8*)	327,8*)
	^{13}C-PCB 118	337,9*)	339,9*)
4	PCB 138, PCB 153	359,8*)	361,8*)
	^{13}C-PCB 153	371,8*)	373,8*)
5	PCB 180	393,8*)	395,8*)
	^{13}C-PCB 180	405,7*)	407,7*)

*) $[M+2]^{+\cdot}$- respectively $[M+4]^{+\cdot}$-ion

64 Quantification procedure

After completion of the GC/MS analysis, all integrated signal areas and mass chromatograms are printed out for each PCB. The quality of the analysis is then evaluated according to the following points (cf. *section 7*, quality assurance):

- Are the mass chromatograms without disturbances or interferences? Are PCB missing which should be part of the sample, or are extra signals present not belonging to a characteristic pattern?
- Are the retention times of the PCB correct compared to those of the calibration standard (cf. *section 76)* ?
- Is the gas chromatographic separation sufficient (see SAEFL guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil)?*
- Is the signal-to-noise ratio sufficient for a quantitative determination?
- Do the abundance ratios of the mass fragmentograms agree within ±15 % with those of the corresponding isotope ratios determined in calibration solutions?

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 is used.

All calculations are carried out according to the following principle:

- The response factors rf_i of all single PCB-congeners i are calculated relative to the corresponding internal standard (ISTD). The integrated single signal areas and concentrations of the quantification standard are used as follows:

$$rf_i = \frac{\text{Conc. } PCB_i \times \text{Area ISTD}}{\text{Conc. ISTD} \times \text{Area } PCB_i}$$

rf_i:	Response factor relative to PCB_i and the internal standard ISTD
Conc.:	Concentration in quantification standard

- The total amount of PCB-congener i in the sample is calculated. For this purpose, the integrated areas of the single PCB_i and the internal standard ISTD are used as well as the total amount of ISTD added to the sample:

$$M_i = \frac{\text{Amount ISTD} \times \text{Area } PCB_i \times rf_i}{\text{Area ISTD}}$$

M_i:	Total amount of PCB i in the sample
Amount ISTD:	Total amount ISTD added to the sample

- The mass concentration is calculated as the ratio between total amount M_i and sample amount.
- The calculation of the recovery rate R_i of the ISTD (added before extraction) in % is carried out relative to the recovery standard (Rec.STD). The latter is added to the sample prior to quantification:

$$rf_w = \frac{\text{Conc. ISTD} \times \text{Area Rec.STD}}{\text{Conc. Rec.STD} \times \text{Area ISTD}}$$

rf_w: Response factor of ISTD relative to recovery standard

$$R(\%)_i = \frac{\text{Amount Rec.STD} \times \text{Area ISTD} \times rf_w \times 100}{\text{Added tot.amount ISTD} \times \text{Area Rec.STD}}$$

$R(\%)_i$:	Recovery in % of the added ISTD
Amount Rec.STD:	Total amount of recovery standards added to the sample
Added tot amount ISTD:	Total amount of ISTD added to the sample

7 Quality assurance

71 Checking standard solutions for quantification

Before they are used, all freshly prepared primary and calibration standards have to be compared with the preceding standard generation. Deviations that are within the repeatability of the quantification method (±10 %) are acceptable. At least once a year, primary standards have to be compared with a certified reference standard (e.g. NIST SRM 1492 "Chlorinated pesticides in hexane" or BCR CRM 365 "Polychlorinated biphenyls in iso-octane") or the reference standard of a well-reputed intercalibration. They are handled in the same way as reference standards and stored at –20 °C.

Both certified crystalline reference compounds are available with a purity of >99.5 % as well as certified reference standard solutions with an accuracy of ca. ±5 %.

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

72 Frequency of injection of quantification standard

The quantification standard (assuming a sufficient linearity) or the calibration series has to be injected before each sample series and at least after each tenth sample. For sample series with less than ten samples, the quantification standard has to be re-injected after the last sample.

73 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*[2]. Blanks of all PCB to be quantified shall be checked for the complete analysis method (extraction, clean-up, separation, quantification), in the following cases:

- When analysing samples with concentration differences <20 after a series of no more than 10 samples using the same clean-up system.
- When changing to another sample matrix and expecting at least ten times lower concentration levels.
- After a complete cleaning/maintenance of the separation system.
- After the analysis of a sample with unusually high concentrations exceeding average concentration levels of previously analysed samples by a factor of >100.
- The blank of the clean-up system used has always to be controlled for very important samples with completely unknown concentration level.

The results of a blank may only be accepted if all criteria given here are met:

- The blank of all PCB to be quantified shall either correspond to the detection limit at a signal-to-noise ratio of 3:1, or the values found shall be lower by at least a factor of 10 than the lowest measured concentration.
- The recovery rate of the internal standards shall be within 70 and 110 %.

74 Analysis of control samples

The analysis and quantification procedure for PAH is based on the application of internal standards employed as extraction and recovery standards. This technique has the advantage that a complete quality control is available for each sample on the basis of calculated recovery rates of the added internal standards. In addition, the quality assurance described here requires a rather frequent control of blanks (after about every 10^{th} sample). The analysis method for a blank is identical with that of a real sample. Only the sample matrix is missing.

The reproducibility of the quantification is controlled regularly by analysing control standards. They are injected after each 20^{th} sample or else after the last one of a series. For each PCB the results are registered in a control diagram.

Due to the quality control measures mentioned before, only a limited additional control is necessary of the performance of the analysis method. Four times annually, a certified reference sample is analysed such as BCR 481 PCB in industry soil, BCR-536 PCB in fresh water habour sediment (both are reference material from the *Institute for Reference Materials and Measurements, IRMM*, Geel, Belgium), RT 910 PCB in soil, RT 912 organics in contaminated soil or SRM 1939 PCB in river sediment (the last three and more available from LGC-Promochem, Wesel, Germany). The difference between analysis result and certified values shall not exceed ±10 %.

PCB intercalibrations are rather expensive and labour intensive. Therefore, only a very limited number of such exercises are organised for different sample matrices. Nevertheless, the aim should be to annually participate at an PCB intercalibration.

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

75 Archiving of quality assurance information

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

76 Acceptance of results

Details are given in the guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil*. Particularly, the following requirements are valid:

- The retention time of a PAH must be within a retention time window of ±3 s with reference to the quantification standard.
- The signal area ratio between the two ions measured for each PCB must be within ±20 % of the value determined for the quantification standard.
- The signal-to-noise ratio must be minimum 3:1 for detection, and 10:1 for quantification.
- The recovery rate of the added internal standards has to be within 50 to 110 % with reference to the recovery standard.

8 Accuracy and reproducibility of the method

- The accuracy of the concentration of the available reference standards is ±5 %.
- The standard deviation of at least five analyses in parallel of a homogeneous sample is within ±10 %. Deviations of ±20 % are acceptable for samples that are difficult to homogenise.
- The analysis of long-term series of control samples indicates a reproducibility of ±10–25 %.

9 Literature

Section 74 of the guidelines *Quality Assurance Concept, Analysis of PAH, PCB and dioxins in soil* contains detailed literature to the methods applied in this method recommendation. In addition, it gives information about alternative techniques and critical points which might cause problems.