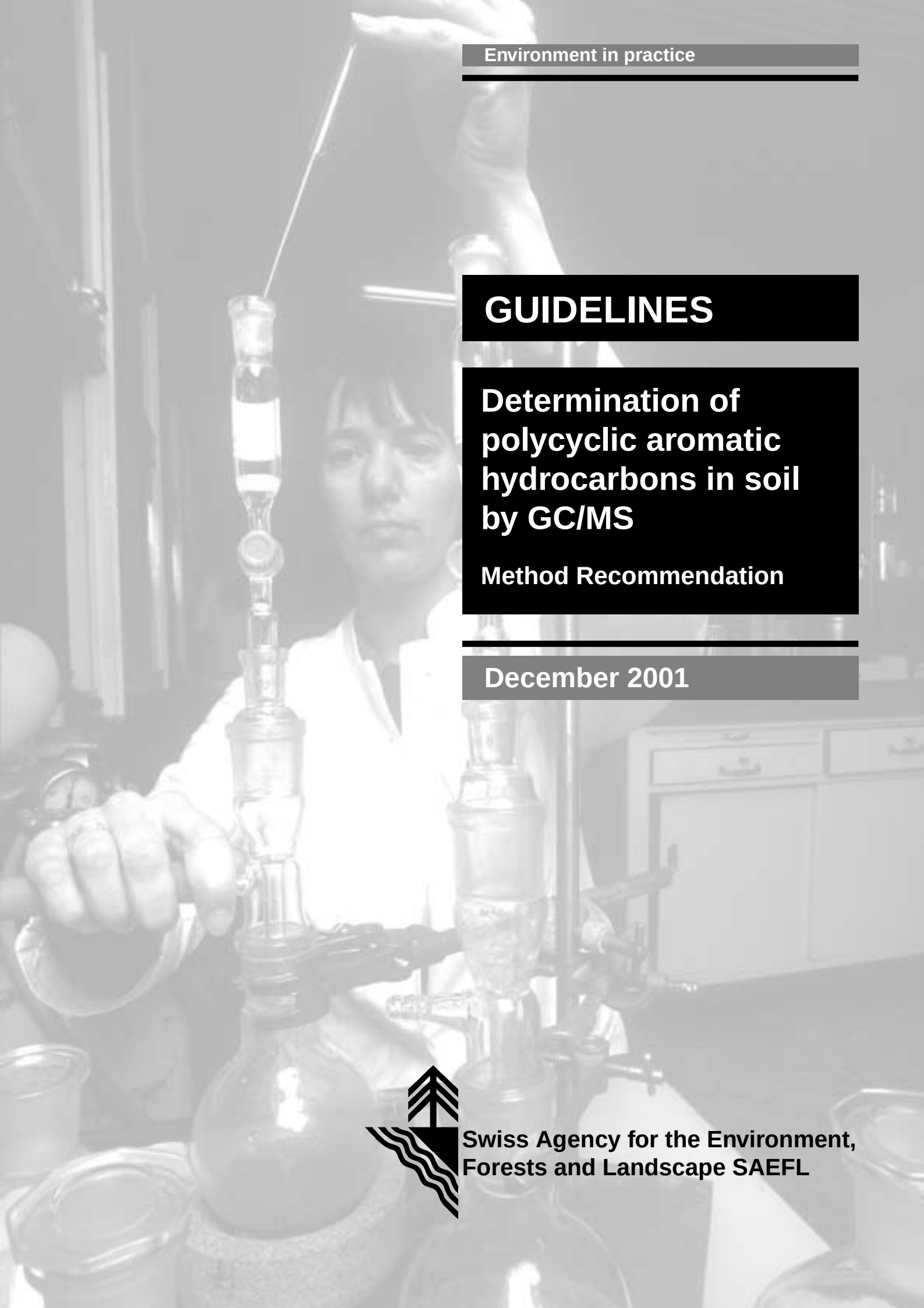

Environment in practice

GUIDELINES

Determination of polycyclic aromatic hydrocarbons in soil by GC/MS

Method Recommendation

December 2001

Swiss Agency for the Environment,
Forests and Landscape SAEFL

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ABSTRACTS

A method is described for the determination of *polycyclic aromatic hydrocarbons (PAH)* in soil, which fulfils the criteria of the *quality assurance concept* for the analysis of organic pollutants in soil published by Swiss Agency for the Environment, Forests and Landscape. The presented method has proven its comparability with other methods at intercomparisons and also fulfils the requirements of the quality assurance norm *EN 45'000*. It is based on soxhlet extraction of the dried soil samples followed by sample clean-up using liquid/liquid extraction and 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 PAH is carried out by high resolution gas chromatography. Quantification is based on the internal standard method and low resolution mass spectrometry. Detailed working procedures are given as well as information about quality control measures.

Es wird eine Methode zur Bestimmung von *polyzyklischen aromatischen Kohlenwasserstoffen (PAK)* in Böden beschrieben, welche das vom BUWAL veröffentlichte *Qualitätssicherungskonzept für die Analytik organischer Schadstoffe im Boden* erfüllt. Diese Methode hat in Ringversuchen 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 mittels Flüssig-/Flüssigextraktion und Säulenchromatographie. 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 PAK wird mit hochauflösender Gaschromatographie durchgeführt. Die Quantifizierung wird mit niedrigauflösender Massenspektrometrie 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 *hydrocarbures aromatiques polycycliques (HAP)* 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'une extraction de la phase liquide/liquide et 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 HAP 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 des méthodes de travail détaillées ainsi que des informations sur les mesures de contrôle de la qualité.

Viene descritto un metodo per la determinazione degli *Idrocarburi policiclici aromatici (PAH)* nel suolo, che rispetta il Concetto di *Quality Assurance* per l'analisi di sostanze organiche presenti nel suolo, pubblicato dall'UFAFP. Il metodo ha rivelato la comparabilità con altre tecniche nelle intercalibrazioni e soddisfa le esigenze della norma di Quality Assurance *EN 45'000*. Esso è basato sull'estrazione mediante Soxhlet dei campioni di suolo essiccati - cui fa seguito una purificazione del campione mediante estrazione in controcorrente e cromatografia su colonna. L'aggiunta di standard interni prima dell'estrazione del campione (i cosiddetti standard di estrazione) consente la correzione automatica di eventuali perdite che possono essere calcolate per ogni singolo campione mediante l'aggiunta di tassi di recupero al campione estratto prima della quantificazione. La separazione dei PAH viene eseguita mediante cromatografia in fase gassosa ad alta risoluzione. La quantificazione viene effettuata mediante spettrometria di massa a bassa risoluzione in relazione agli standard interni. Vengono fornite sia prescrizioni dettagliate relative al lavoro che informazioni sulle misure inerenti alla Quality Assurance.

FOREWORD

Polycyclic aromatic hydrocarbons (PAH) are substances capable of contaminating the soil. Accordingly, the Swiss *Ordinance relating to impacts on the soil (OIS) of 1 July 1998* sets guide, trigger and clean-up values for these pollutants.

The chemical analysis of these substances is technically very demanding. In practice, therefore, PAH analyses tend to be carried out only if there is specific evidence of problematic environmental impacts, along transport routes for example. As with other groups of organic pollutants, it is important to have an available method that provides reproducible and comparable results.

Having published guidelines for a quality assurance concept and for the determination of dioxins and furans, Prof. M. Oehme of the Institute of Organic Analytical Chemistry at the University of Basel has now produced a reference method for PAH that reflects the current state of the art.

In making this paper available to interested parties, we hope to make a further contribution to the accuracy and comparability of the available data and we are fulfilling an obligation under the *OIS* ordinance.

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

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)
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
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 relating to impacts on the soil, of 1 July 1998
PAH	Polycyclic aromatic hydrocarbons
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

Polycyclic aromatic hydrocarbons (PAH) are Soxhlet-extracted from the dried soil sample. Internal standards are added prior to sample extraction. Any interfering sample matrix is removed by liquid/liquid extraction combined with multiple column liquid chromatography. After concentrating the sample extract to 200-300 µL and the addition of a recovery standard, all relevant PAH-compounds are separated by high resolution gas chromatography and quantified by low resolution mass spectrometry in the election 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 16 PAH (cf. *Table 1*) is 1 mg/kg dry weight and for benzo(a)pyrene 0.2 mg/kg. Both have to be quantified reliably with a sufficient limit of quantification. Depending on sample size, a limit of quantification in the order of 0.3-3 µg/kg (0.3-3 ppb) can be achieved for single compound which is 2-3 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

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

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 and 500 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:* length 150 mm and 250 mm.
- *Glass bottles with wide opening:* different volumes, e.g. 0.5-1 L (polypropylene screw cap GL 45) for sample storage.
- *Centrifuge tubes:* conical, volume 15-20 mL, with scale and glass stopper.
- *Glass beakers:* 50 mL.
- *Volumetric flasks with glass stopper:* 10 and 25 mL (amber glass), quality A, precision ±0.025 mL and ± 0.04 mL, respectively at 20 °C.

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

- *Erlenmeyer flasks:* 250 mL with glass stopper.
- *Exsiccator:* 300 mm diameter, cover with sleeve (without grease) – without drying agent.
- *Glass funnel:* 100 mm diameter.
- *Chromatography column:* 250 mm long, 12.5 mm ID.
- *Weighing boats.*
- *Watch glasses:* 60 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).
- *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).
- *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.
- *Millipore MilliQ:* water cleaning system.
- *Furnace:* 200-1'030 °C, precision ±10 °C.
- *Ultrasonic bath:* power 100 W.
- *Centrifuge:* capacity 8x10 mL tubes, minimum 6'000 r.p.m.
- *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 round bottom flasks.
- *Porcelain mortar:* diameter 130 mm, pestle 145x38 mm.
- *Porcelain dishes:* diameter 180 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.
- *Pipette:* 5 and 10 mL, with scale, precision ±0.03 mL.
- *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;
- Methanol;
- Toluene;
- Dimethylformamide *(exception:* analytical grade);
- Water, distilled in glass and further cleaned with a Millipore MilliQ water cleaning system.

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.
- *Silica:* 0.063-0.20 mm (pre-treatment; cf. *sections 222.3*).
- *Mercury:* purity 99.99 %.

- *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. complete 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 PAH and internal standards.

Compounds	
naphthaline	benzo(a)anthracene
acenaphthylene	chrysene
acenaphthene	benzo(b)fluoranthene
fluorene	benzo(k)fluoranthene
phenanthrene	benzo(a)pyrene
anthracene	indeno(1,2,3-cd)pyrene
fluoranthene	dibenzo(a,h)anthracene
pyrene	benzo(ghi)perylene
3,6-dimethylphenanthrene	2,2’-binaphthyl
(internal standard I)	(internal standard II)
	indeno(1,2,3-cd)fluoranthene (recovery standard)

Annotation: 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 *"Promochem"* (Wesel, Germany). 3,6-Dimethylphenanthrene can be bought from *"Tokyo Kasei Kogyo Ltd."* (Japan).

All solid reference standards are stored at 4-6 °C in the dark. Due to their chemical stability, PAH 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 contains the compounds listed in *Table 2* according to their elution order on the applied gas chromatographic separation column (cf. *section 623*).

Table 2: PAH-compounds contained in the primary standard.

No.	Compounds	*Remarks*
1 2 3 4 5	naphthaline acenaphthylene acenaphthene fluorene phenanthrene	
6 7 8 9 10	anthracene 3,6-dimethylphenanthrene fluoranthene pyrene benzo(a)anthracene	 *Internal standard I*
11 12 13 14 15	chrysene 2,2'-binaphthyl benzo(b)fluoranthene benzo(k)fluoranthene benzo(a)pyrene	*Co-elution with triphenylene* *Internal standard II* *Co-elution with benzo(j)fluoranthene*
16 17 18 19	indeno(1,2,3-cd)fluoranthene indeno(1,2,3-cd)pyrene dibenzo(a,h)anthracene benzo(ghi)perylene	*Recovery standard* *Co-elution with dibenzo(a,c)anthracene*

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 cyclo-hexane in the ultrasonic bath. The primary standard including internal standards is prepared in a 10 mL measuring flask (amber glass, or protected from light). The concentration of the single compounds listed in *Table 2* should be in the range of 50±20 ng/µL. This corresponds to a weight of 500±200 µg per compound. After weighing of each compound the spatula is rinsed with a pasteur pipette filled with cyclohexane and dried with a paper tissue.

The content of the weighing boats is transferred with cylcohexane 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, weighed and stored at -20 °C in the freezer. The storage period is 24 months. All details are noted in the reference solution catalogue.

33 Preparation of calibration standards

The volumetric flask with the primary standard is removed from the freezer and the frozen cyclohexane thawed. Then, it is placed in the ultrasonic bath for 5 min and controlled that everything is dissolved. Otherwise, the ultrasonic treatment has to be repeated. Then, the volumetric flask is equilibrated at room temperature and its weight controlled. Loss of solvent is compensated until the final control weighing.

Variable volumes of the primary standard are transferred to a 10 mL volumetric flask which is filled with cyclohexane to the marked level. Five calibration standards should cover a concentration range of about 0.1-20 ng/µL for each compound. Eventually, repeated dilutions are necessary. The volumetric flasks are labelled, weighed and stored in the freezer at -20 °C. Their storage period is 12 months. All details are noted in the reference solution catalogue including the loss of weight due to volume removal.

The volumetric flasks are equilibrated at room temperature and the weight is controlled. Loss of solvent is compensated to the final control weighing. 1 mL each is transferred to a 1.5 mL a sample vial with a capillary opening (CERTAN®) and used as working standards. They are stored in the refrigerator at 4-6 °C. These working standards are replaced after 6 months.

Quantification by a single point calibration can only be carried out, if the linearity of the mass spectrometer fulfils the requirements given in *section* 4 of the guidelines *"Quality assurance concept - Analysis of PAH, PCB and dioxins in soil"*.

34 Preparation of internal standard solution

Before use, the weighing boats and the spatula must be cleaned thoroughly with cyclohexane in the ultrasonic bath. The internal standard is set up in a 25 mL volumetric flask. The concentrations of both internal standards 3,6-dimethylphenanthrene and 2,2'-binaphthyl should be in the range of 320±30 ng/µL. This corresponds to a weight of 8±0.8 mg for each compound if a 25 mL volumetric flask is employed. After weighing of each compound the spatula is rinsed with a Pasteur pipette filled with cyclohexane and dried with a paper tissue.

The content of the weighing boats is transferred with cylcohexane to the 25 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, weighed and stored at -20 °C in the freezer. The storage period is 24 months. All details are noted in the reference solution catalogue.

The volumetric flask is equilibrated at room temperature and its weight controlled. Loss of solvent is compensated to the final control weighing. 1 mL is transferred to a 1.5 mL a sample vial with a capillary opening (CERTAN®) and used as working standard. It is stored in the refrigerator at 4-6 °C. This working standard is replaced after 6 months.

35 Preparation of recovery standard solution

Before use, the weighing boats and the spatula have to be cleaned thoroughly with cyclohexane in the ultrasonic bath. The recovery standard is prepared in a 25 mL volumetric flask.

The concentrations of the recovery standard indeno(1,2,3-cd)fluoranthene should be in the range of 320±30 ng/µL. This corresponds to a weight of 8±0.8 mg for each compound if a 25 mL volumetric flask is used. After weighing of each compound the spatula is rinsed with a Pasteur pipette filled with cyclohexane and dried with a paper tissue.

The content of the weighing boats is transferred with cyclohexane to the 25 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, weighed and stored at -20 °C in the freezer. The storage period is 24 months. All details are noted in the reference solution catalogue.

The volumetric flask is equilibrated at room temperature and its weight controlled. Loss of solvent is compensated to the final control weighing. 1 mL is transferred to a 1.5 mL a sample vial with a capillary opening (CERTAN®) and used as working standard. It is stored in the refrigerator at 4-6 °C. This working standard is replaced after 6 months.

36 Preparation of the control standard solution

This control standard is used to control the measuring uncertainty and repeatability of the quantification (cf. *section 74*). It is quantified regularly against the calibration curve as an unknown solution and contains the following compounds:

2,2'-binaphthyl (ISTD I)	3,6-dimethylphenanthrene (ISTD II)
benzo(a)anthracene	benzo(a)pyrene
phenanthrene	fluoranthene

A solution of the control standard with a concentration of 5±2 ng/µL is prepared and stored in the same way as described in *section 33* for calibration standards.

37 Quality assurance

All primary, internal and recovery standards are stored at 4-6 °C. They have a storage period of 24 months, if evaporation of the solvent and contamination are prevented. The weight of working standards stored in sample vials with a capillary opening does not necessarily have to be checked (loss within six months due to evaporation <1 mg). They can be used for a maximum of six months.

Before use, all freshly prepared basic standards must be compared with the preceding standard generation. Deviations that are within the precision of the quantification method (±10 %) 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*). 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 cyclohexane 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 of the GC separation 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 those 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 and the rinse solution (3x3 mL cyclohexane) are transferred to centrifuge tubes and centrifuged for 15 min at 2'000 r.p.m. The cyclohexane phase is transferred to a Turbovap unit and then concentrated to about 1 mL with a Turbovap 500 unit. The sample is now ready for extract clean-up.

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 PAH.

Guide values

- ***sample not contaminated:*** Amount of soil 40-50 g (possibly two Soxhlet extractions); added amount of internal standard 30 µL (ca. 10'000 ng per PAH); added amount of recovery standard 20 µL (ca. 6'400 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 30 µL (ca. 10'000 ng per PAH); added amount of recovery standard 20 µL (ca. 6'400 ng).

5 Extract clean-up

51 Preliminary remarks

Dependent on the matrix content of the sample, not all steps of extract clean-up described below have to carried out. However, the liquid/liquid extraction procedure has to be performed in any case.

52 Liquid/liquid extraction

The first step of extract clean-up is a liquid/liquid extraction to remove aliphatic hydrocarbons and other non-polar compounds. These substances cannot undergo weak charge transfer complexes with dimethylformamide such as PAH due to their delocalised π-electron systems.

A mixture of dimethylformamide (DMF)/water 9+1 (e.g. 180 mL DMF and 20 mL MilliQ water) is prepared and the following procedure applied:

- The sample extract of about 4 mL is transferred to a 15 mL centrifuge tube. 3.2±0.1 mL DMF/H_2O 9+1 are added with a scaled pipette of 5 mL. After plugging with a glass stopper, the mixture is shaken vigorously (30 s).
- The centrifuge tube is centrifuged at 2'500 rpm for 5 min. The cyclohexane phase is sucked off with a pipette and transferred to a new centrifuge tube. 1.2±0.1 mL DMF/H_2O 9+1 are added with a scaled pipette. Shaking and centrifuging are repeated. Then, the DMF/H_2O-phase is transferred to the first centrifuge tube.
- 5.2±0.2 mL of water (10 mL scaled pipette) and 3.2±0.1 mL of cyclohexane are added to the centrifuge tube with the DMF/H_2O-phase (total volume about 13 mL). After plugging with a glass stopper the mixture is vigorously shaken (30 s). The cyclohexane phase is removed with a Pasteur pipette and transferred to a centrifuge tube.
- 1.0±0.1 mL of cyclohexane are added to the remaining DMF/H_2O-phase which is extracted once more as described above (cf. pre-previous paragraph).

- The cyclohexane extracts are washed with 2 mL of water, transferred to a new centrifuge tube and dried with 1 g of Na_2SO_4.
- Then, the sample extract is transferred to a Turbovap vial and the Na_2SO_4 washed with 1 mL of cyclohexane.
- The sample extract is adjusted to the desired sample volume (guide volume 1 mL) by the Turbovap and eventually cleaned further with a silica column.

53 Further clean-up with silicagel

531 Packing of chromatographic column

The chromatographic column described in *section 211* is dry-packed from bottom to top as follows and compacted with a vibrator:

- A little bit of pre-cleaned cotton wool (cf. *section. 222.2*);
- 2 g Na_2SO_4 (cf. *section. 222.4*);
- 5 g silica (cf. *section 222.3*);
- 2 g Na_2SO_4 (cf. *section 222.4*).

Finally the column is filled twice with n-hexane and rinsed.

532 Fractionation of sample extracts

The whole sample extract, or at more contaminated soils an aliquot corresponding to ca. 2 g of soil are placed on top of the column with a Pasteur pipette. In the latter case, the extract volume has to be determined precisely to correct recovery rates correspondingly. After transfer of the whole extract, the sample vial is rinsed twice with 1 mL of n-hexane.

Finally, the PAH-fraction is eluted with 40 mL of n-hexane/toluene 7+3 and transferred to a Turbovap container and concentrated to about 1 mL with a Turbovap 500 system. The volume may be reduced further to minimum 200-300 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.

If signal disturbances are observed in the gas chromatogram due to a too high residue of toluene (shoulders, split signal etc.), a solvent change to cyclohexane should be carried out. 5-10 mL are added in 2-3 portions; in between the volume is reduced again to 1 mL.

6 Quantitative analysis

61 Preliminary remarks

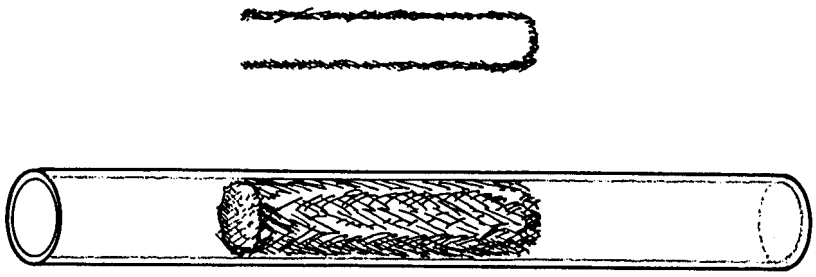
The amount of recovery standard given in *section 45* has to be added to the sample using a disposable pipette before quantification. If necessary, the sample volume is reduced to about 200-300 µL with a gentle flow of nitrogen. Then, the sample aliquots as defined in *section 624* may be injected.

62 Gas chromatographic separation

621 Instrumentation

- 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.



622 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.

623 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.
- *Capillary dimensions:* Polyimide coated quartz capillary, length 25 m, 0.25 mm internal diameter.

624 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: 40 °C, 2 min waiting period, 40-100 °C with 30 °C/min, 100-300 °C with 10 °C/min, 300 °C isothermal (5-10 min).

Manual hot needle injection: Even PAH 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 injector 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 correspondingly to the time difference for fluoranthene.

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 10-100 pg when injecting 1 µL of the sample extracts. This corresponds to a total amount of 0.5-5 ng per sample at a sample volume of e.g. 50 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 119.0, 219.0, 264.0 or 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 $M^{+\cdot}$-, $[M-2H]^{+\cdot}$- or $[M-26]^{+}$-fragments for non-substituted PAH, or $M^{+\cdot}$- and [M-15]-ion for methyl substituted PAH. 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 3* 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). The total dwell time per ion

group should be selected so that each gas chromatographic signal is defined with at least 10-12 measuring points.

Table 3: Rounded masses of ions used for quantification of PAH by GC/MS. The exact masses are maximally 0.1 u higher. The ion with the highest mass is used for quantification.

Group 1 m/z	**Group 2** m/z	**Group 3** m/z
128/126 resp. 102	178/176 resp. 152	254/252 resp. 226
142/141 resp. 127	202/200 resp. 176	276/274 resp. 250
152/150 resp. 126	216/201	278/276 resp. 252
154/152 resp. 128	228/226 resp. 202	
166/164 resp. 140		

64 Quantification procedure

After completion of the GC/MS analysis, all integrated signal areas and mass chromatograms are printed out for each PAH. The quality of the analysis is then evaluated according to the following points (cf. also *section 7* of the SAEFL guidelines *Quality assurance concept - Analysis of PAH, PCB and dioxins in soil*):

- Are the mass chromatograms without disturbances or interferences? Are PAH 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 PAH 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?

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 PAH 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:

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

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

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

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

M_i: Total amount of PAH_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

All reference standards and primary standards are stored at 4-6 °C in the dark, and have an unlimited storage period. Working standards in sample vials with capillary opening stored at 4-6 °C show evaporation losses within six months of <1 mg, they can be stored for six months. Working 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 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 or the reference standard of a well-reputed intercalibration.

At present, only reference standard solutions are available that are prepared by companies with known high quality standards. They report an accuracy of ±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 PAH 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 PAH compounds 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 PAH the results are registered in a control diagram.

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

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 SRM 1941 PAH in sediments (NIST), CRM 524 PAH in contaminated soil or CRM 535 PAH in contaminated freshwater sediments (the latter are reference material from the *Institute for Reference Materials and Measurements, IRMM,* Geel, Belgium). The difference between analysis result and certified values shall not exceed ±10 %.

PAH 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 PAH intercalibration.

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 PAH 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.
- In addition, the complete method was validated in intercalibrations, last time in 1995 by participation at the intercalibration of *"International Atomic Energy Agency"*[3], however, organised for the more complex matrix “sediments”. The deviation from the average of the 22 participating laboratories was typically 15 %.
- The analysis of long-term series of control samples indicates a reproducibility of ±10-25 %.

[3] International Atomic Energy Agency, Marine Environment Laboratory, MC 98012 Monaco, Report of December 11, 1995.

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.