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BSI Standards Publication

# Animal feeding stuffs — Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

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The UK participation in its preparation was entrusted to Technical Committee AW/10, Animal feeding stuffs.

A list of organizations represented on this committee can be obtained on request to its secretary.

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# Animal feeding stuffs - Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS

Aliments des animaux - Dosage des dioxines, des PCB de type dioxine et des PCB indicateurs par GC/HRMS

Futtermittel - Bestimmung von Dioxinen und dioxinähnlichen PCBs mittels GC/HRMS und von Indikator-PCBs mittels GC/HRMS

This European Standard was approved by CEN on 9 March 2012.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

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# Foreword

This document (EN 16215:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2012, and conflicting national standards shall be withdrawn at the latest by October 2012.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

# 1 Scope

This European Standard is applicable to the determination of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), (together termed 'dioxins' (PCDD/Fs)) and dioxin-like PCBs and non dioxin-like PCBs (dl-PCBs and ndl-PCBs) in animal feeding stuffs. Collaborative studies have been carried out. The method is suitable for the determination of dioxins, dl-PCBs and ndl-PCBs at the appropriate MRL in compound feed and ingredients e.g. oil, mineral clay. The method is applicable to samples containing residues of one or more of the following dioxins, dioxin-like PCBs and indicator PCBs. The limit of quantification (LOQ) for the relevant individual congeners of dioxins/furans is 0,05 pg/g (OCDD/F = 0,1 pg/g), of non-ortho PCBs 0,05 pg/g, of mono-ortho PCBs 10 pg/g and of indicator PCBs 100 pg/g.

For determination of dioxins and dioxin-like PCBs, the procedure can be used as confirmatory method as defined by Commission Regulation (EC) No 152/2009 for dioxins and dl-PCB in feed [6]. Confirmatory methods are high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) methods. If only the analysis of indicator PCBs is required, a GC-LRMS method can be used (e.g. EN 15741 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/MS [1] and EN 15742 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/ECD [2]) provided that appropriate analytical performance criteria are met in the relevant range for the matrix of interest.

This European Standard is split into four modules each describing a part of the whole procedure (see Figure 1 and Figure 2) to be followed:

a) Module A: Description of standards which might be used;

b) Module B: Description of extraction procedures;

c) Module C: Description of clean up procedures;

d) Module D: GC/HRMS determination.

Each module describes a part of the whole method as well as, when applicable, alternatives which should be equivalent. Each module has to be regarded as an example. Combining modules and/or alternatives gives a highly flexible procedure which is "performance based". It is permitted to modify the method if all performance criteria laid down in Commission Regulation (EC) No 152/2009 [6] are met.

Any deviation of the described method, combination of modules needs to be recorded as part of the QA/QC procedures of accredited laboratories and should be available on request.

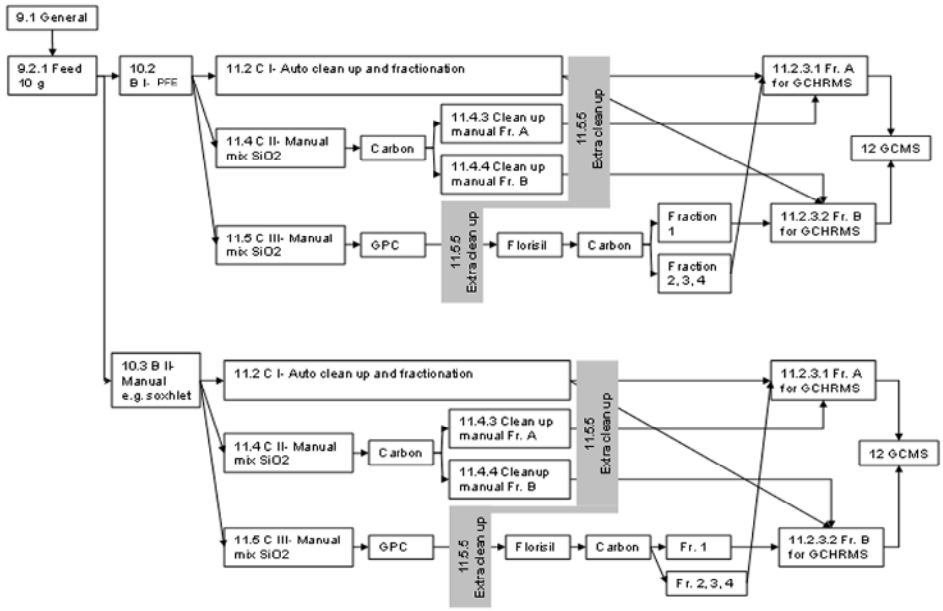


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**Figure 1 — Flow scheme for the determination of Dioxins, dl-PCBs and Indicator PCBs in feed**

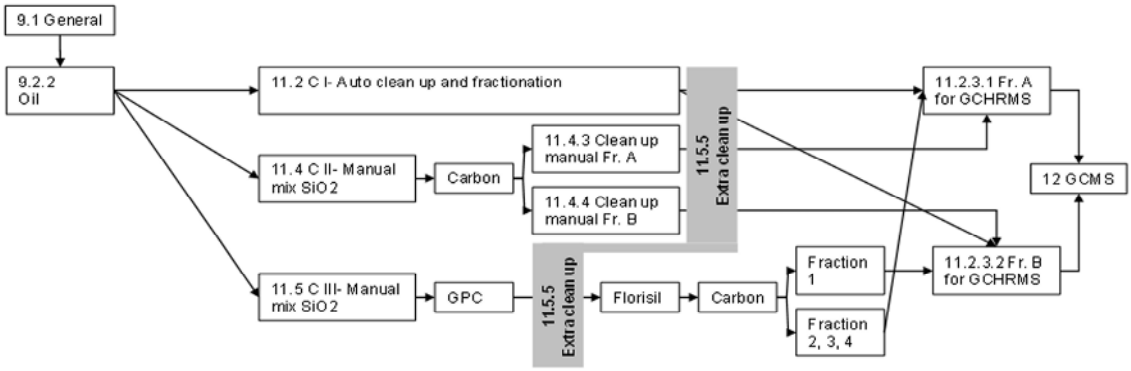


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**Figure 2 — Flow scheme for the determination of Dioxins, dl-PCBs and Indicator PCBs in oil / fat**

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

prEN ISO 6498, *Animal feeding stuffs — Guidelines for sample preparation (ISO/DIS 6498)*

## 3 Terms and definitions

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

**3.1**
**limit of detection**
smallest measured content, from which it is possible to deduce the presence of the analyte with reasonable statistical certainty

Note 1 to entry: The limit of detection is numerically equal to three times the standard deviation of the mean of blank determinations (n > 10).

**3.2**
**limit of quantification**
lowest content of the analyte that can be measured with reasonable statistical certainty

Note 1 to entry: If both accuracy and precision are constant over a concentration range around the limit of detection, then the limit of quantification is numerically equal to six times the standard deviation of the mean of blank determinations (n > 10).

Note 2 to entry: For dl-PCBs and PCDD/F: Use correct definition for limit of quantification for each congener as in Commission Regulation (EC) No 152/2009 [6].

Note 3 to entry: Limit of quantification should be in the range of about one fifth of the level of interest.

**3.3**
**feed additives**
substances that comply with the definition of feed additives given in the Commission Regulation (EC) No 1831/2003 [7]

**3.4**
**upper, middle and lower bound**
upper, middle and lower bound results for WHO-PCDD/F-TEQ and WHO-PCB-TEQ are defined as follows: upper bound concentrations are calculated assuming that all values of the different congeners less than the limit of quantification are equal to the limit of quantification; for lower bound: LOQ = 0 and middle bound: ½ LOQ is used

## 4 Principle

A test portion of animal feeding stuff or ingredient is fortified with $^{13}C$ labelled internal standards (dioxins, furans, dioxin-like PCBs and indicator PCBs) and extracted using a manual or an automated method.

After automated or manual clean up an aliquot of the extract is concentrated and injected into a GC-HRMS using a split less injector (an alternative here is PTV injection (Programmed Temperature Vaporizer injection) see NOTE.

Quantification is based on isotope dilution. If only indicator PCBs are required, they can be determined with GC-LRMS (e.g. according to EN 15741 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/MS [1] and EN 15742 Animal feeding stuffs - Determination of OC-pesticides and PCBs by GC/ECD [2])

Preconditions of combining modules for extraction and clean-up are:

a) for each extraction module an equal sample intake of 10 g for feed or feed ingredients with a fat content ≤ 25 % or 2,5 g fat or oil is required;

b) in order to achieve the required LOQ for dioxins a final volume of 10 µl in combination with an injection volume of 2 µl is required. If a different injection volume is applied, the final volume has to be adjusted directly proportional.

NOTE In case more sensitivity is necessary or less volume reduction is wanted, injection of a larger volume by PTV (an example is described in Annex A) or higher sample intake is possible (see also 9.2.2 NOTE).

## 5 Reagents

Use only reagents of recognized analytical grade and with purity suitable for dioxin and PCB residue analysis. Check the purity of the reagents and reference materials (e.g. standard solutions) by performing a blank test under the same conditions as used in the method. The chromatogram should not show any interfering impurity at the retention time of compounds of interest.

**WARNING — The use of this European Standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European Standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.**

### 5.1 Dioxins, furans, non-ortho PCBs, mono-ortho PCBs and indicator PCBs and their labelled analogues:

— $^{13}$C-spiking solution for PCDD/F (internal standard);

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

— calibration solutions PCDD/F;

— calibration solutions PCB;

— recovery standard PCDD/F;

— recovery standard PCB.

See Annex B: Description of standards and concentration of the standard solutions.

# 6 Apparatus

All technical descriptions are examples of possible system setups and parameters and have to be scaled or adopted to the user's equipment.

**6.1 Evaporator,** suitable for volumes up to 200 ml and inlet for nitrogen gas.

**6.2 Evaporator tubes,** endpoint about 0,5 ml.

**6.3 Homogeniser**

**6.4 Pasteur pipette,** borosilicate glass, 150 mm.

**6.5 Vortexmixer**

**6.6 Measuring cylinder,** borosilicate glass, 100 ml, 2 ml graduations with a precision of ± 0,5 ml.

**6.7 Measuring cylinder,** borosilicate glass, glass-stoppered, 25 ml, 1 ml graduation with a precision of ± 0,5 ml graduation and 50 ml, 2 ml graduation with a precision of ± 0,5 ml graduation.

# 7 Sampling

The sample should be truly representative and not damaged or changed during transport or storage. Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497 [3].

# 8 Preparation of test sample

Prepare the test sample in accordance with prEN ISO 6498.

Dry or low moisture products such as cereals and cereal products, mixed feeds, and hay should be ground carefully so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

High moisture products such as grasses and silages and liquid feed should be (freeze-)dried and after that ground carefully so that it passes completely through a sieve with 1 mm apertures. Mix thoroughly.

Oil / fat are directly dissolved in n-hexane.

# 9 Procedure

## 9.1 General

Analyse the following samples in each series:

— procedure blank (n = 1);

— (certified) reference material at appropriate level or a home made reference sample;

— all samples (maximum 20).

The procedure blank should be free of contaminants at or above the limits of quantification.

### 9.2 Animal feed stuff sample and oil/fat sample

#### 9.2.1 10 g animal feed stuff sample (12 % moisture content)

Weigh an appropriate amount, e.g. 10,0 g (± 0,10 g) of the prepared test sample into a 100 ml glass vial. Sample amount is based on 12 % moisture content. If extracting by Pressurized Fluid Extraction (PFE), add 3 g diatomaceous earth and mix thoroughly. Fortify the sample with 500 µl $^{13}$C-DIOXNOP-2 (Annex B, B.2.31) and 500 µl $^{13}$C-MOPIP-2 (Annex B, B.2.33) and incubate until solvent has been evaporated and continue at 10.2 (module BI) or 10.3 (module BII). For samples with more than 25 % fat, the sample intake has to be reduced proportionally.

#### 9.2.2 2,5 g oil/fat sample

Weigh an appropriate amount, e.g. 2,5 g (± 0,10 g) of the oil/fat sample into graduated cylinder of 25 ml (6.7). Fortify the sample with 500 µl $^{13}$C-DIOXNOP-2 (Annex B, B.2.31) and 500 µl $^{13}$C-MOPIP-2 (Annex B, B.2.33). Fill the graduated cylinder to 25 ml with n-hexane. Close the graduated cylinder with a glass stopper and mix thoroughly. Continue sample clean up procedure at paragraph 11.2 (module CI) or 11.3 (module CII) or 11.5 (module CIII).

NOTE Calculation in 13.4 is based on sample intake of 10 g for feed with fat content of ≤ 25 % and 2,5 g for fat and oil. Deviations of sample intake should be taken into account in the formulas in 13.4 (M = sample intake in gram).

## 10 Extraction

### 10.1 General

The sample amount used for extraction may vary from 5 g to 50 g depending on the expected level of contamination. However calculation in 13.4 is based on sample intake of 10 g for feed with fat content of ≤ 25 % and 2,5 g for fat and oil. Deviations of sample intake should be taken into account in the formula's in 13.4 (M = sample intake in gram).

The internal standard consisting of $^{13}$C-labelled congeners listed in Annex B, Table B.1 shall be added directly onto the sample before extraction, or onto the oil sample before clean up.

The extraction procedure is carried out using Pressurized Fluid Extraction (PFE) with consecutively toluene and a mixture of toluene/ethanol (module BI) or Soxhlet extraction (module BII). Duration of extraction should be adjusted according to kind and amount of sample used. The minimum requirement for Soxhlet extraction is 50 extraction cycles.

Other extraction techniques like microwave assisted extraction can also be used but shall be of proven equal performance.

### 10.2 Module BI: Extraction using automated Pressurized Fluid Extraction (PFE) system

#### 10.2.1 Reagents and materials

**10.2.1.1 Diatomaceous earth**

**10.2.1.2 n-Hexane,** for dioxin and PCB analysis.

**10.2.1.3 Toluene,** for dioxin and PCB analysis.

**10.2.1.4 Ethanol,** for dioxin and PCB analysis.

**10.2.1.5 Toluene/ethanol,** in volume portions of 9/1.

Mix 900 ml toluene (10.2.1.3) with 100 ml ethanol (10.2.1.4) thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 1 year.

**10.2.1.6 Anhydrous sodium sulphate,** heated at 160 °C for at least 24 h.

**10.2.1.7 Nitrogen**

**10.2.1.8 Silanised glass wool**

**10.2.2 Apparatus**

**10.2.2.1 Pressurized Fluid Extraction apparatus**

The apparatus shall be able to extract the samples at 100 °C and 10 MPa.

**10.2.2.2 Pressurized Fluid Extraction cell,** e.g. 30-40 ml.

**10.2.2.3 Measuring cylinder,** borosilicate glass, 25 ml, 1 ml graduation with a precision of ± 0,5 ml.

**10.2.2.4 Funnel**

**10.2.2.5 Evaporator,** suitable for volumes up to 200 ml and inlet for nitrogen gas.

**10.2.2.6 Evaporator tubes,** 0,5 ml endpoint.

**10.2.3 Procedure**

Put the sample (9.2.1) in a Pressurized Fluid Extraction cell (10.2.2.2) and fill with diatomaceous earth (10.2.1.1) and place the extraction cell into the Pressurized Fluid Extraction apparatus (10.2.2.1). For the extraction the following parameters might be used:

— temperature 100 °C;

— pressure 10 MPa;

— preheat 0 min;

— heat 5 min;

— static 15 min;

— flush 40 vol. % of extraction cell, e.g. for a 33 ml extraction cell = 13,2 ml;

— purge 300 s;

— cycles 3;

— solvent cycle 1 toluene (10.2.1.3);

— solvent cycle 2 and 3 toluene/ethanol in volume portions of 9/1 (10.2.1.5).

Combine solvent obtained with each cycle and filter over a funnel (10.2.2.4) equipped with a glass wool plug (10.2.1.8) and 5 g pre-dried sodium sulphate (10.2.1.6). Evaporate the filtrate using an evaporator (10.2.2.5)

until an end volume of 0,5 ml. Take the sample extract from the evaporator tube and place in a glass-stoppered graduated 25 ml cylinder (6.7) and wash the evaporator tube 5 times with 4 ml n-hexane each time (i.e. 20 ml total) (10.2.1.2). The n-hexane is added to graduated cylinder containing the sample extract. Bring the volume to 25 ml with n-hexane (10.2.1.2), close the graduated cylinder with the glass stopper and mix thoroughly. Continue sample clean up procedure using the automated procedure (module CI, 11.2) or at paragraph using the manual method (module CII, 11.3 or module CIII, 11.5).

NOTE Comparable techniques in combination with appropriate parameters can be used provided that Commision Regulation (EC) No 152/2009 [6] is obeyed.

## 10.3 Module BII: Manual extraction procedure

### 10.3.1 Reagents and materials.

**10.3.1.1 DCM,** dichloromethane, for dioxin and PCB analysis.

**10.3.1.2 Toluene,** for dioxin and PCB analysis.

**10.3.1.3 Ethanol,** for dioxin and PCB analysis.

**10.3.1.4 n-Hexane,** for dioxin and PCB analysis.

**10.3.1.5 Toluene/ethanol,** in volume portions of 9/1.

Mix 900 ml toluene (10.2.1.3) with 100 ml ethanol (10.2.1.4) thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 1 year.

**10.3.1.6 Anhydrous sodium sulphate,** heated at 160 °C for at least 24 h.

**10.3.1.7 Measuring cylinder,** borosilicate glass, 25 ml, 1 ml graduation with a precision of ± 0,5 ml.

**10.3.1.8 Silanised glass wool**

### 10.3.2 Apparatus

**10.3.2.1 Extraction thimbles,** cleaned by extracting in soxhlet extractor for 2 h with DCM (10.3.1.1).

**10.3.2.2 Recirculating cooler**

**10.3.2.3 Soxhlet extractor**

**10.3.2.4 Heating apparatus,** e.g. mantle.

**10.3.2.5 Anti-bumping granules**

**10.3.2.6 Round bottom flask (RBF),** borosilicate glass, 500 ml.

**10.3.2.7 Funnel,** standard, 150 mm, 80 mm diameter, borosilicate glass.

**10.3.2.8 Evaporator,** suitable for volumes up to 200 ml and inlet for nitrogen gas.

**10.3.2.9 Evaporator tubes,** 0,5 ml endpoint.

#### 10.3.3 Procedure

Wash the soxhlet extractor (10.3.2.3) including round bottom flasks (RBF) (10.3.2.6) subsequently with toluene (10.3.1.2) and dichloromethane (DCM) (10.3.1.1). Switch on the refrigerated recirculator (10.3.2.2) and leave to cool. Put sample (9.2.1) in the prepared extraction thimble (10.3.2.1) and place thimbles (10.3.2.1) plugged with silanised glass wool (10.3.1.8) into soxhlet extractor (10.3.2.3). Add 200 ml toluene (10.3.1.2) and 3 - 6 anti-bumping granules (10.3.2.5) to the RBF (10.3.2.6) and place into heating mantle (10.3.2.4). Connect all the soxhlet extractor together including the condenser and check all seals are tight. Set the soxhlets running at a rate of 5 cycles per hour to 7 cycles per hour. Leave the soxhlets extracting for 4 h.

Switch off heating mantles and allow apparatus to cool and remove the RBF containing toluene from the soxhlet apparatus. Add 200 ml toluene/ethanol (10.3.1.5) and 3 anti-bumping granules to 6 anti-bumping granules (10.3.2.5) to a new RBF (10.3.2.6) and place into heating mantle (10.3.2.4). Connect all the soxhlet equipment together including the condenser and check all seals are tight. Set the soxhlets running at a rate of 5 cycles per hour to 7 cycles per hour. Leave the soxhlets extracting for 16 h until 20 h or overnight.

Switch off heating mantles and allow apparatus to cool and combine the toluene extract and the toluene/ethanol extract. Filter the soxhlet-extract through a funnel equipped with a silanised glass wool plug (10.3.1.8) and 5 g -anhydrous sodium sulphate (10.3.1.6). Evaporate the filtrate using an evaporator (6.1) until the solvent has evaporated (approximately 0,5 ml). Take the sample extract from the evaporator tube and place in a glass-stoppered graduated 25 ml cylinder (6.7) and wash the evaporator tube 5 times with 4 ml n-hexane each time (i.e. 20 ml total) (10.3.1.4). The n-hexane is added to graduated cylinder containing the sample extract. Bring the volume to 25 ml with n-hexane (10.3.1.4), close the graduated cylinder with the glass stopper and mix thoroughly. Continue sample clean up procedure using the automated procedure (module CI, 11.2) or at paragraph using the manual method (module CII, 11.3 or module CIII, 11.5).

NOTE Comparable techniques, e.g. twisselmann (hot extraction) in combination with appropriate parameters can be used provided that Commision Regulation (EC) No 152/2009 [6] is obeyed. In addition, the azeotropic mixture of toluene/ethanol in volume portions of 3/7 can be used.

## 11 Clean up

### 11.1 General

Clean up methods shall prepare the sample extract in an appropriate manner for the subsequent quantitative determination. Clean up procedures have to concentrate PCDDs/PCDFs and dioxin-like PCBs in the extracts and to remove interfering matrix components present in the raw extract.

Below principles of frequently used clean up techniques are briefly described.

— Gel permeation chromatography

  The interesting molecular weight range for PCDDs/PCDFs and dioxin-like PCBs of 200 g/mol to 500 g/mol can be isolated from larger molecules such as fat / oil and polymers which might overload other clean-up methods.

— Multi-layer column

  Multi-layer column liquid chromatography using silica with different activity grades and surface modifications. Compounds with different chemical properties than PCDDs/PCDFs and dioxin-like PCBs can be removed.

— Sulphuric acid treatment

  A direct treatment of the sample extract for removal of oxidizable coextractives with sulphuric acid is possible but is not recommended due to safety reasons. Furthermore, this has to be carried out very carefully to avoid losses of PCDDs/PCDFs and dioxin-like PCBs on the formed carboniferous surfaces.

This procedure is not described within this standard, but might be used for initial treatment of very dirty oil or fat samples e.g. frying oil.

— Activated carbon column

Column adsorption chromatography using activated carbon may be used to separate planar PCDD/PCDF and coplanar PCB molecules from mono-ortho PCB and other interfering non-planar molecules. Additionally activated carbon can also be used to clean the PCDD/PCDDF fraction or separate non-ortho PCBs, mono-ortho PCBs and indicator PCBs.

— Alumina column

Column fluid chromatography on alumina of different activity grade and acidity/basicity. Interfering compounds with small differences in polarity or structure compared to PCDDs/PCDFs and dioxin-like PCBs can be removed.

Additionally, alumina columns can be used to separate PCDDs/PCDFs from dioxin-like PCBs.

— Florisil column

Column fluid chromatography on Florisil of different activity grade can be used to separate PCDDs/PCDFs from PCBs, also dioxin-like PCBs and other interfering compounds with small differences in polarity or structure compared to PCDDs/PCDFs.

— Procedure sample clean up

Proven clean up procedures shall be used containing normally two or more of the above discribed techniques which can be combined in different orders. A detailed description of the automated clean up procedure is given in Module CI (automated method). In module CII and module CIII manual methods are described, where module CII describes the clean up over a mixed silica column followed by a activated naoh carbon column where non-ortho PCB, PCDDs/PCDFs are separated from mono-ortho PCBs and indicator PCBs. Module CIII describes the clean up procedure using mixed silica followed by GPC or Florisil. Other methods can also be used but shall be of proven equal performance as the methods described in modules C.

## 11.2 Module CI: Automated clean up

### 11.2.1 General

The purification method consists of a comprehensive automated system. Extracts are transferred by a pump to the system and purified consecutively on an acid silica column, a neutral silica column, a basic alumina column and an activated carbon/Celite column. For the elution of the columns, custom made solvents and mixtures are used: n-hexane, n-hexane/dichloromethane in volume portions of 1/1, ethylacetate/toluene in volume portions of 1/1 and toluene. The program can be downloaded from:
http://www.rikilt.wur.nl/UK/services/Analyses/Dioxine+analysis/ [11].

After the automated procedure two fractions are obtained: mono-ortho substituted PCBs and indicator PCBs (Fraction A) and planar PCDD/Fs and non-ortho substitute PCBs (Fraction B). For additional clean up especially in case of dirty complex samples (oxidized fat/oil) a small multi layer silica column might be used.

### 11.2.2 Reagents and materials

**11.2.2.1 Toluene,** for dioxin and PCB analysis.

**11.2.2.2 n-Hexane,** for dioxin and PCB analysis.

**11.2.2.3 Dichloromethane,** for dioxin and PCB analysis.

**11.2.2.4 Ethylacetate,** for dioxin and PCB analysis.

**11.2.2.5 Ethylacetate/toluene,** in volume portions of 1/1.

Mix 1 l ethylacetate (11.2.2.4) with 1 l toluene (11.2.2.1) thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 1 year.

**11.2.2.6 Dichloromethane/n-hexane,** in volume portions of 1/1.

Mix 1 l dichloromethane (11.2.2.3) with 1 l n-hexane (11.2.2.2) thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**11.2.2.7 Iso-octane,** HPLC-grade.

**11.2.2.8 Sulphuric acid $H_2SO_4$,** 96 %, 1,84 specific gravity.

**11.2.2.9 63-200 silica** mesh size 63 µm to 200 µm.

**11.2.2.10 NaOH pellets**

**11.2.2.11 NaOH,** 1 mol/l.

Dissolve 40,0 g NaOH pellets (11.2.2.10) in 1 l water.

**11.2.2.12 High capacity disposable acidic silica columns** (packed with 45 g of acidic silica).

High capacity disposable acidic silica is prepared by mixing concentrated $H_2SO_4$ (11.2.2.8) with 63-200 silica (11.2.2.9) in volume/mass portions of 11/25 and shaking until free flowing and free of clumps. Before acidifying silica is baked at 500 °C for 5 h.

**11.2.2.13 Neutral silica**

Neutral silica is prepared by mixing DI water with 63-200 silica (11.2.2.9) in volume/mass portions of 1/19 ratio and shaking until free flowing and free of clumps.

**11.2.2.14 Basic silica**

Base modified silica is prepared by mixing 1 mol/l NaOH (11.2.2.11) with 63-200 silica (11.2.2.9) in volume/mass portions of 3/10 and shaking until free flowing and free of clumps.

**11.2.2.15 Carbon AX-21**

**11.2.2.16 Celite**

**11.2.2.17 Methanol,** glass distilled.

### 11.2.3 Apparatus

#### 11.2.3.1 Automated sample clean up system

The system shall be able to clean samples automatically on respectively a silica, alumina and carbon column using different solvents.

**11.2.3.2 High capacity disposable acidic silica column** Fluid Management System, Watertown, USA e.g. (see 11.2.4.3 NOTE 1).

**11.2.3.3 Mixed bed silica column.**

**11.2.3.4 Alumina column,** 11 g of basic alumina.

Alumina is activated at 1 000 °C for 12 h.

**11.2.3.5 Carbon column,** 0,34 g of carbon AX-21/Celite mixture.

Carbon AX-21 (11.2.2.15) is washed with methanol (11.2.2.17) and let dry and then baked at 130 °C for 72 h. Mixture is prepared in mass portions of 2/23, carbon/Celite.

**11.2.3.6 Connection fitting for high capacity disposable acidic silica column**

**11.2.3.7 Connection fitting for mixed bed silica, alumina and carbon columns**

**11.2.3.8 GC-autosampler vial,** approximately 2 ml.

**11.2.3.9 Flat bottom micro inserts,** approximately 0,2 ml.

**11.2.3.10 Evaporator,** suitable for volumes up to 200 ml and inlet for nitrogen gas.

**11.2.3.11 Evaporator tubes,** 0,5 ml endpoint.

### 11.2.4 Procedure

**11.2.4.1** Use an automated clean up system which is designed to extract and clean up of toxic compounds such as Dioxins, Furans, dioxin-like PCBs, and indicator PCBs from environmental, food or feed samples.

To the 25 ml sample extracts (prepared according to paragraph (9.2.2) or (10.2) or (10.3)) 50 µl clean up standard $^{37}Cl_4$ – 2,3,7,8 TCDD (Annex B, B.2.3) is added and homogenized (6.3). The graduated cylinder with the n-hexane diluted sample extract is placed in the automated sample clean up system (11.2.3.1). In 24 automated steps the sample is cleaned and the mono-ortho PCBs and indicator PCBs are collected in fraction ‘A’ and in fraction ‘B’ the dioxins/furans and non-ortho PCBs. In Table C.1 the 24 automated sample clean up steps are described, see Annex C.

In the first 13 steps the system (columns and tubing) is washed with solvent. Step 1 to step 4 uses n-hexane (11.2.2.2) for washing and step 5 for conditioning the combination of the high capacity disposable acidic silica column (11.2.3.2) and the mixed bed silica column (11.2.3.3). In step 6 + step 7 toluene (11.2.2.1) used for cleaning the tubing and the carbon column. Furthermore, in step 8 + step 9 and step 10 + step 11 tubing and carbon column are flushed with mixtures of solvents with decreasing polarity to finish with n-hexane (11.2.2.2) in step 12 + step 13. Step 14 includes the transfer of sample extract trough the combined silica columns to the alumina column (11.2.3.4). Step 15 elutes the combined silica and alumina columns with n-hexane (11.2.2.2). The contaminants are trapped in the alumina column. In step 16 tubing is flushed with dichloromethane/n-hexane (11.2.2.6). Then (step 17) the alumina column is eluted with dichloromethane/n-hexane (11.2.2.6). The eluting compounds are directed to the carbon column (11.2.3.5). Mono-ortho PCBs and indicator PCBs go through the carbon column and are collected in fraction ’A’. Step 18 and step 19 are for

flushing the tubing and the carbon column with ethylacetate/toluene (11.2.2.5). Then (step 20 + step 21) the same tubing and column is flushed with n-hexane (11.2.2.2). Step 22 involves washing the tubing with toluene (11.2.2.1). In step 23 the carbon column is eluted in the reverse direction using toluene (11.2.2.1) and fraction 'B' is collected. At the end of the procedure (step 24), the tubing is washed with n-hexane (11.2.2.2).

**11.2.4.2** Fraction 'A' is collected in 200 ml graduated glass evaporator tube. Place the evaporator tube (6.2) containing the extract into the evaporator (6.1) unit with the bath temperature at 40 ºC ± 2 ºC, and set the nitrogen pressure to ~0,7 bar. Select sensor end-point detection and begin concentration. Be aware that the displayed pressure may fall below 0,7 bar during use. When the volume has reduced by about one quarter, one half and three quarters, wash the evaporator tube (6.2) interior walls each time with iso-octane (11.2.2.7, 5 ml to 10 ml) from a wash bottle and re-concentrate. When the endpoint is reached, wash the tube walls again with isooctane (11.2.2.7, 5 ml to 10 ml), swirl and concentrate. Repeat twice more to remove. Finally, when the endpoint is again reached, remove the tube from the evaporator. To fraction 'A' 100 µl TCDD-RS-VI (Annex B, B.2.20) is added and the evaporator tube is washed with 2 ml iso-octane (11.2.2.7) and mixed on a vortexmixer (6.5). Then fraction 'A' is concentrated to an appropriate volume, e.g. 25 µl to 50 µl and transferred to a GC auto sampler-vial (11.2.3.8) with a flat bottom micro insert (11.2.3.9). Continue at module D: paragraph 12, GC-HRMS.

For additional clean up especially in case of dirty complex samples (oxidized fat/oil) a small multi-layer silica column might be used as described in 11.5.6 or in Annex E provided that Commission Regulation (EC) No 152/2009 [6] is obeyed.

**11.2.4.3** Fraction 'B' is collected in 200 ml graduated glass evaporator tube. Place the evaporator tube (6.2) containing the extract into the evaporator (6.1) unit with the bath temperature at 40 ºC ± 2 ºC, and set the compressed air pressure to ~0,7 bar. Select sensor end-point detection and begin concentration. Be aware that the displayed pressure may fall below 0,7 bar during use. When the volume has reduced by about one quarter, one half and three quarters, wash the evaporator tube (6.2) interior walls each time with toluene (11.2.2.1, 5 ml to 10 ml) and re-concentrate. When the endpoint is reached, wash the tube walls again with toluene (11.2.2.1, 5 ml to 10 ml), swirl and concentrate. Repeat twice more. Finally, when the endpoint is again reached, remove the tube from the evaporator. To fraction 'B' 100 µl DIOX-RS-1 (Annex B, B.2.18) is added and washed with 2 ml toluene (11.2.2.1) and mixed on a vortexmixer (6.5). Fraction 'B' is concentrated to an appropriate volume, e.g. 10 µl to 25 µl and is also transferred to a GC autosampler-vial (11.2.3.8) with a flat bottom micro insert (11.2.3.9). Continue at module D: paragraph 12, GC-HRMS.

For additional clean up especially in case of dirty complex samples (oxidized fat/oil) a small multi layer silica column might be used as described in 11.5.6 or in Annex E provided that Commission Regulation (EC) No 152/2009 [6] is obeyed.

NOTE 1 Fluid Management System, Watertown, USA e.g. is a company producing hardware for and disposable columns for automated clean-up. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product.

NOTE 2 Comparable techniques in combination with appropriate parameters can be used provided that Commison Regulation (EC) No 152/2009 [6] is obeyed. The program given in Annex D has to be regarded as an example, different programs and solvents can be used, provided that the procedure (method + program) is validated.

## 11.3 Module CII: Manual sample clean up, removal of fat and group separation

### 11.3.1 General

This module details a manual procedure for the isolation, fractionation and purification of PCDD/F and PCB analytes prior to concentration and analysis by GC-HRMS. The analytes are isolated from the matrix using cold solvent extraction simultaneously with acid and base hydrolysis on multi-layer, mixed silica columns. The procedure allows for in-tandem fractionation of planar PCDD/Fs and non-ortho substitute PCBs from ortho substituted PCBs, by connecting the outflow of the multi-layer column directly to an activated carbon column, alternatively, the concentrated outflow of the multi-layer column may be fractionated separately by a carbon column method. The fractions – mono-ortho substituted PCBs and indicator PCBs (Fraction A) and planar PCDD/Fs and non-ortho substitute PCBs (Fraction B) – are purified on activated basic alumina.

### 11.3.2 Reagents and materials

**11.3.2.1 Toluene,** for dioxin and PCB analysis.

**11.3.2.2 n-Hexane,** for dioxin and PCB analysis.

**11.3.2.3 Dichloromethane,** for dioxin and PCB analysis.

**11.3.2.4 Silica,** mesh size 63 µm to 200 µm.

**11.3.2.5 KOH pellets**

**11.3.2.6 Methanolic KOH,** 5 mol/l (see 11.3.2.5 and 11.3.2.15).

Dissolve 36 g KOH pellets (11.3.2.5) in 1 l water.

**11.3.2.7 Sulphuric acid,** $H_2SO_4$, 96 %, 1,84 specific gravity.

**11.3.2.8 Basic silica,** prepared by mixing 5 mol/l methanolic KOH (11.3.2.6) and silica (11.3.2.4) in volume/mass portions of 3/1 (ml/g), allowing evaporation of the methanol and allowing stabilisation for 24 h.

**11.3.2.9 Sulphuric acidic silica,** prepared by roller-mixing sulphuric acid (11.3.2.7) and silica (11.3.2.4) in volume/mass portions of 1/1,5 (ml/g) for min. 6 h.

**11.3.2.10 Dichloromethane/n-hexane,** in volume portions of 4/6.

Mix 400 ml dichloromethane (11.3.2.3) with 600 ml n-hexane (11.3.2.2) thoroughly. Store at room temperature in a tightly closed bottle.

**11.3.2.11 Methanol,** glass distilled.

**11.3.2.12 Nitrogen**

**11.3.2.13 Toluene/dichloromethane,** in volume portions of 3/7.

Mix 600 ml toluene (11.3.2.1) with 1 400 ml dichloromethane (11.3.2.3) thoroughly.

Store at room temperature in a tightly closed bottle.

**11.3.2.14 Anhydrous sodium sulphate,** heated at 160 °C for at least 24 h.

**11.3.2.15 Methanol,** for dioxin and PCB analysis.

### 11.3.3 Apparatus

All technical descriptions are examples of possible system setups and parameters and have to be scaled or adopted to the user's equipment.

**11.3.3.1 Large glass column,** 650 mm x 55 mm ID

**11.3.3.2 Medium glass column,** 400 mm x 25 mm ID

**11.3.3.3 Silanised glass wool**

**11.3.3.4 Pressure regulator,** 0 bar to 1 bar.

**11.3.3.5 Frit,** glass fibre disc.

**11.3.3.6 Funnel,** standard, 150 mm, 80 mm diameter, borosilicate glass.

**11.3.3.7 #11 plug,** PTFE, with female e.g. Omnifit 1/8" fitting.

**11.3.3.8 #50 plug,** PTFE, with e.g. Omnifit 1/8" connection to nitrogen supply.

**11.3.3.9 Tubing,** PTFE, 1/8", with fittings, Omnifit.

**11.3.3.10 Connector,** male, threaded, 1/8", Omnifit.

**11.3.3.11 Measuring cylinder,** borosilicate glass, 250 ml, 2 ml graduations with a precision of ± 0,5 ml.

**11.3.3.12 Wash bottle,** PTFE, 500 ml.

**11.3.3.13 Measuring cylinder,** borosilicate glass, 500 ml, 5 ml graduations with a precision of ± 0,5 ml.

**11.3.3.14 Measuring cylinder,** borosilicate glass, 100 ml, 2 ml graduations with a precision of ± 0,5 ml.

**11.3.3.15 Pasteur pipette,** borosilicate glass, 150 mm.

**11.3.3.16 47 mm GF/D, Glass fibre Filter Discs.**

**11.3.3.17 Vial, tapered, 1,1 ml.**

**11.3.3.18 1 l glass bottle Duran /Amber.**

### 11.3.4 Procedure

#### 11.3.4.1 Preparation of mixed silica column

Take a large glass column (11.3.3.1) and wash with dichloromethane (11.3.2.3). Plug the column at the lower end (#11 fitting) with a #11 plug (11.3.3.7). Add two glass fibre discs (11.3.3.5) and a plug of silanised glass wool (11.3.3.3). Place on to this, in ascending order, anhydrous sodium sulphate (11.3.2.14, 10 g ± 1 g), basic silica (11.3.2.8, 50 g ± 5 g), sulphuric acidic silica (11.3.2.9, 10 g ± 2,5 g), 47 mm glass fibre disc (11.3.3.16) and anhydrous sodium sulphate (11.3.2.14, 30 g ± 3 g). Tap the column firmly to arrange the layers of powdered reagents evenly. Place a 1 l glass bottle (11.3.3.18) at the outfall of each silica column.

NOTE If the carbon column is to be used in tandem, it can be directly coupled at this stage to the bottom of the mixed silica column followed by the use of the procedure described below (11.3.4.2) to give Fraction A and the procedure described in (11.3.4.5) to give Fraction B. Condition the carbon columns using the solvents in reverse direction as described in (11.3.4.3). and ensure that the carbon column is marked indicating flow direction prior to use. Connect the top of the carbon column directly to the outflow of the mixed silica column and the bottom to a 1 l reservoir using 1/8 connectors and tubing.

#### 11.3.4.2 Sample clean up using mixed silica column

Transfer the sample (9.2.2) or extract (10.2) or extract (10.3) into a 1 l bottle (11.3.3.18). Add 200 ml ± 10 ml n-hexane (11.3.2.2) using a measuring cylinder (11.3.3.11) and mix. Add sulphuric acidic silica (11.3.2.9) 75 g to the bottle using small funnel and swirling during addition. Quantitatively transfer the slurry to the large

glass column (11.3.3.1) washing the bottle 3 times with 10 ml to 30 ml n-hexane (11.3.2.2) dispensed from a wash bottle (11.3.3.12). Check and twist the lower #11 plug to release any air lock and prevent channelling and re-tighten once the solvent front has reached the plug. Raise the large glass column in the frame and allow the sample to drain through into the 1 l bottle (11.3.3.18) at the outfall of each apparatus. Using a 500 ml measuring cylinder (11.3.3.13), add dichloromethane/hexane (11.3.2.10, 400 ml ± 40 ml), to each large glass extraction column once the initial 200 ml has passed the reagent bed level. Lower the column from initial draining to do this.

Carefully fit a #50 plug (11.3.3.8) to each column top and apply nitrogen (10.2.1.7) head pressure (approximately 0,1 bar to 0,2 bar). Check for solvent leaks at the base of the column, and carefully tighten any fitting which seems loose. Adjust the regulator of the nitrogen supply (0,3 bar to 0,5 bar). Leave to allow the solvent to pass through into the 1 l bottle (11.3.3.18) (typically overnight). Ensure solvent pressure does not allow any one sample to drip through faster than giving individual droplets. It is recommended to check at least 30 min after setting, for changes in pressure. Once all the extraction solvent has eluted through the column and the tubing into the receiving bottle starts to splutter, switch off the nitrogen pressure supply. Remove the #50 plug (11.3.3.8) and using a 100 ml measuring cylinder (11.3.3.14) wash the inner wall of the column with n-hexane (11.3.2.2, 100 ml ± 10 ml). Re-cap and pressurise the columns (up to 0,7 bar), collecting the additional n-hexane into the same bottle, and when the tubing into the receiving bottle starts to splutter, switch off the nitrogen pressure supply.

If the carbon column has been directly coupled, then this extract represents the mono-ortho substituted PCB and indicator PCB fraction (Fraction A). Proceed according to (11.3.4.3). Disconnect the mixed silica column and proceed to (11.3.4.5) to obtain the PCDD/Fs and non-ortho substituted PCB fraction. (Fraction B)

NOTE 1 Comparable carbon adsorbents might also be used in combination with appropriate parameters and elution solvents can be used provided that Commission Regulation (CE) No 152/2009 [6] is obeyed.

NOTE 2 Instead of the above described mixed silica column GPC can be used see (11.5) module CIII.

#### 11.3.4.3 Preparation and use of carbon column for separation of mono-ortho PCBs and indicator PCBs (fraction A) from PCDD/Fs and non-ortho PCBs (fraction B)

Condition the carbon columns using the following solvents in reverse direction:

a) toluene 60 ml ± 10 ml (11.3.2.1);

b) methanol 60 ml ± 10 ml (11.3.2.15);

c) toluene 60 ml ± 10 ml (11.3.2.1);

d) dichloromethane/n-hexane 60 ml ± 10 ml (11.3.2.10).

#### 11.3.4.4 Collection of non planar fraction (combined indicator PCBs and mono-ortho PCBs fraction)

Transfer the extract obtained with 11.3.4.2 on to the carbon column and elute with 400 ml dichloromethane/n-hexane (11.3.2.10). The fraction which passes straight through and collected in the 1 l bottle contains mono-ortho PCBs and indicator PCBs. Transfer the extract into evaporator tubes. Place the evaporator tube into the evaporator (6.1) unit with the bath temperature at 40 °C ± 2 ºC, and set the compressed air pressure to ~0,7 bar. Select sensor end-point detection and begin concentration. When the volume has reduced by about one quarter, one half and three quarters, wash the evaporator tube (6.2) interior walls each time with toluene/dichloromethane (11.3.2.13, 5 ml to 30 ml) and re-concentrate. When the endpoint is reached (≤ 0,5 ml approximately), wash the tube walls again with n-hexane (11.3.2.2, 15 ml to 20 ml), swirl and concentrate. Repeat twice more to remove any trace of DCM to leave a final volume of ~0,5 ml in n-hexane. This fraction is labelled fraction A and continue at (11.4).

#### 11.3.4.5 Collection of planar fraction (combined non-ortho PCB and PCDD/F fraction)

After elution of fraction A invert the carbon column so that the flow will now be in the reversed direction, and connect it to a medium glass column (11.3.3.2) to be used as a solvent reservoir. The other end of column is connected via a 1/8" tubing to a clean evaporator tube. Using a 250 ml measuring cylinder (11.3.3.11), elute the combined non-ortho PCBs and PCDD/Fs in the reverse direction with toluene (11.3.2.1, 170 ml ± 10 ml into the clean evaporator tube (6.2).

Place the evaporator tube into the evaporator (6.1) unit with the bath temperature at 40 °C ± 2 °C, and set the compressed air pressure to ~0,7 bar. Select sensor end-point detection and begin concentration. When the volume has reduced by about one quarter, one half and three quarters, wash the tube (6.2) interior walls each time with toluene/dichloromethane (11.3.2.13, 5 ml to 30 ml) and re-concentrate. When the endpoint is reached (≤ 0,5 ml approximately), wash the tube walls again with n-hexane (11.3.2.2, 15 ml to 20 ml), swirl and concentrate. Repeat twice more to remove any trace of toluene, to leave a final volume of ~0,5 ml in n-hexane.

Using a clean pasteur pipette (11.3.3.15), quantitatively transfer the extract from the evaporator tube (6.2) to a 1,1 ml vial. This fraction is labelled fraction B. For clean up continue at 11.4.

## 11.4 Additional clean up-steps for of fraction A and fraction B of module CII

### 11.4.1 Reagents and materials

**11.4.1.1 n-Hexane,** for dioxin and PCB analysis.

**11.4.1.2 Dichloromethane,** for dioxin and PCB analysis.

**11.4.1.3 63-200 silica,** mesh size 63 µm to 200 µm.

**11.4.1.4 KOH pellets**

**11.4.1.5 Methanol,** for dioxin analysis.

**11.4.1.6 Methanolic KOH,** 5 mol/l (see 11.4.1.4 and 11.4.1.5).

Dissolve 36 g KOH pellets (11.4.1.4) in 1 l water.

**11.4.1.7 Basic silica,** prepared by mixing 5 mol/l methanolic KOH (11.4.1.6) and silica (11.4.1.3), in volume/mass portions of 3/1 (ml/g), allowing evaporation of the methanol and allowing stabilisation for 24 h.

**11.4.1.8 Sulphuric acidic silica,** prepared by roller-mixing sulphuric/silica in volume/mass portions of 1/1,5 (ml/g) for min. 6 h.

**11.4.1.9 Sulphuric acid, $H_2SO_4$,** 96 %, 1,84 specific gravity.

**11.4.1.10 Dichlormethane/n-hexane,** in volume portions of 3/7.

Mix 300 ml dichloromethane with 700 ml n-hexane thoroughly. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 1 year.

**11.4.1.11 Anhydrous sodium sulphate,** heated at 160 °C for at least 24 h.

### 11.4.2 Apparatus

All technical descriptions are examples of possible system setups and parameters and have to be scaled or adopted to the user's equipment.

**11.4.2.1 Silanised glass wool**

**11.4.2.2 Pasteur pipette,** borosilicate glass, 150 mm.

**11.4.2.3 Alumina – basic,** WB5-Basic, muffled at 450 °C for min 24 h.

**11.4.2.4 Reservoir,** 100 ml.

**11.4.2.5 Connecting sleeve,** PTFE.

### 11.4.3 Procedure manual clean up: Removal of chemical interferences from fraction A

#### 11.4.3.1 General

Clean up of the (mono-ortho and indicator) PCB fraction requires two clean up columns. Only prepare these once you are ready to use then; to ensure they do not absorb moisture and de-activate.

#### 11.4.3.2 Column preparation and clean up fraction A

Column A: Plug a pasteur pipette (11.4.2.2) with silanised glass wool (11.4.2.1, 0,25 cm to 0,5 cm). Add anhydrous sodium sulphate (11.4.1.11, approximately 0,5 cm), basic silica (11.4.1.7, 3 cm to 3,5 cm), sulphuric acidic silica (11.4.1.8, 3 cm to 3,5 cm) and sodium sulphate (11.4.1.11, approximately 0,5 cm) in ascending order. Fill to a maximum of where the pipette narrows. Couple this using a suitable PTFE connecting sleeve (11.4.2.5) to a clean pasteur pipette attached to a reservoir (11.4.2.4) to be used as a solvent reservoir. Add n-hexane (11.4.1.1, 20 ml $\pm$ 2 ml) using a 25 ml measuring cylinder (6.7) to the reservoir (11.4.2.4) to condition the pipette minicolumn. Elute the n-hexane to waste.

Column B: Plug a Pasteur pipette (11.4.2.2) with silanised glass wool (11.4.2.1, 0,25 cm to 0,5 cm)). Add anhydrous sodium sulphate (11.4.1.11, approximately 0,5 cm), activated alumina (11.4.2.3, 6,5 cm to 7,5 cm) and sodium sulphate (11.4.1.11, approximately 0,5 cm) in ascending order. Couple this using a suitable PTFE connecting sleeve (11.4.2.5) to a reservoir (11.4.2.4) to be used as a solvent reservoir. Using a 25 ml measuring cylinder (6.7) add dichloromethane (11.4.1.2, 20 ml $\pm$ 2 ml) to the reservoir to condition the pipette minicolumn. Elute the dichloromethane (11.4.1.2) to waste. Add n-hexane (11.4.1.1, 20 ml $\pm$ 2 ml) to the reservoir and elute to waste. When column conditioning is complete, connect column A above column B using a suitable PTFE connecting sleeve (11.4.2.5).

NOTE Ensure that column B never goes dry.

Using a clean Pasteur pipette (11.4.2.2) quantitatively transfer the extract (11.3.4.4) from the evaporator tube (6.2) to the top of column A (11.4.3.2), washing the sloping sides of the tube/vial 3 times with n-hexane (11.4.1.1, approximately 0,5 ml) and transferring these washings to the top of column A. Once the 3 washings have been carried out, using a PTFE connecting sleeve (11.4.2.5), connect the upper column to the pipette attached to the reservoir. This should result in a stack of four connected items: a reservoir (11.4.2.4) above a clean pasteur pipette (11.4.2.2) above column A above column B (11.4.3.2). Add n-hexane (11.4.1.1, 10 ml $\pm$ 2 ml) to the reservoir and allow to elute to waste. Discard column A (Acid/base silica) and connect column B (Alumina) to the clean pasteur pipette (11.4.2.2) in its place. Using a 25 ml measuring cylinder (6.7), dispense dichloromethane/n-hexane (11.4.1.10, 20 ml $\pm$ 2 ml) into the reservoir to elute fraction A into a clean evaporator tube (6.2) and continue at (11.2.4.2).

For additional clean up especially in case of dirty complex samples (oxidized fat/oil) a small multi layer silica column might be used as described in 11.5.6 or in Annex E provided that Commission Regulation (CE) No 152/2009 [6] is obeyed.

### 11.4.4 Column preparation and clean up the planar analytes fraction B

Clean up of the PCDD/Fs and non-ortho PCBs fraction requires 2 clean up columns. Only prepare these once you are ready to use then; to ensure they do not absorb moisture and de-activate.

Column C: Plug a pasteur pipette 11.4.2.2) with silanised glass wool (11.4.2.1, 0,25 cm to 0,5 cm). Add anhydrous sodium sulphate (11.4.1.11, approximately 0,5 cm), sulphuric acidic silica (11.4.1.8, 3,5 cm to 5,5 cm) and anhydrous sodium sulphate (11.4.1.11, approximately 0,5 cm) in ascending order. Couple this using a suitable PTFE connecting sleeve (11.4.2.5) to a reservoir (11.4.2.4) to be used as a solvent reservoir. Using a 25 ml measuring cylinder (6.7) add n-hexane (11.4.1.1, 20 ml $\pm$ 2 ml) to the reservoir to condition the pipette mini-column. Elute the n-hexane (11.4.1.1) to waste.

Column D: Plug a pasteur pipette (11.4.2.2) with silanised glass wool (11.4.2.1, 0,25 cm to 0,5 cm). Add anhydrous sodium sulphate (11.4.1.11, approximately 0,5 cm), Alumina (11.4.2.3) (6,5 cm to 7,5 cm) and anhydrous sodium sulphate (11.4.1.11, approximately 0,5 cm) in ascending order. Couple this using a suitable PTFE connecting sleeve (11.4.2.5) to a reservoir (11.4.2.4) to be used as a solvent reservoir. Using a 25 ml measuring cylinder (6.7) add dichloromethane (11.4.1.2, 20 ml $\pm$ 2 ml) to the reservoir to condition the pipette mini-column. Elute the dichloromethane (11.4.1.2) to waste. Add n-hexane (11.4.1.1, 20 ml $\pm$ 2 ml) to the reservoir and elute to waste.

When column conditioning is complete, connect column C above column D using a suitable PTFE connecting sleeve (11.4.2.5). Using a clean pasteur pipette (11.4.2.2), quantitatively transfer the extract obtained in (11.3.4.5) to the top of column C, washing the vial 3 times with n-hexane (11.4.1.1, approximately 0,5 ml) and transferring these washings to the top of column C. Once the 3 washing have been carried out using a PTFE connecting sleeve (11.4.2.5), connect the upper column to the pipette attached to the reservoir. This should result in a stack of four connected items: a reservoir (11.4.2.4) above a clean pasteur pipette (11.4.2.2) above column C above column D. Using a 25 ml measuring cylinder (6.7) add hexane (11.4.1.1, 20 ml $\pm$ 2 ml) to the reservoir to elute the PCDD/Fs and non-ortho PCBs onto column D, allow the hexane to go to waste.

Discard column C (Acidic silica) and connect column D (Alumina) to the clean pasteur pipette (11.4.2.2) in its place. Using a 50 ml measuring cylinder (6.7), dispense DCM/n-hexane (11.4.1.10) 30 ml into the reservoir to elute the PCDD/Fs, and non-ortho PCBs into a evaporator tube (6.2) and continue at section 11.2.4.3

For additional clean up especially in case of dirty complex samples (oxidized fat/oil) a small multi layer silica column might be used as described in 11.5.6 or in Annex E provided that Commission Regulation (CE) No 152/2009 [6] is obeyed.

## 11.5 Module CIII: Removal of matrix, manual sample clean up and group separation

### 11.5.1 General

Use of manual large multi-layer silica column (11.5.4) for first clean up of sample (removal of lipophilic substances) after extraction. The large multi layer-silica column shall be capable of retaining interfering fat and fat-soluble matrix. The PCDD/F/PCB containing fraction elutes in a certain fraction of time depending on flow rate and amount of stationary phase of cyclohexane/toluene in volume portions of 1/1. The collected fraction is concentrated in an evaporator to near dryness and re-dissolved in suitable solvent for next clean up step (GPC (11.5.5) and/or small multi layer silica column (11.5.6)). Group separation (into fractions of dioxins and PCBs) is performed on a Florisil column (11.5.7). Finally, the extract is purified (fraction 1 = PCDD/F) and further separated into fraction 2 (= non-ortho PCB), fraction 3 (= mono-ortho PCB) and fraction 4 (= indicator PCBs) using a carbon column.

### 11.5.2 Reagents

**11.5.2.1 n-Heptane,** for dioxin and PCB analysis.

**11.5.2.2 Ethylacetate,** for dioxin and PCB analysis.

**11.5.2.3 n-Hexane,** for dioxin and PCB analysis.

**11.5.2.4 Ethylacetate/cyclohexane,** in volume portions of 1/1.

Mix 1 l ethylacetate (11.5.2.2) with 1 l cyclohexane (11.5.2.5) thoroughly. Store at room temperature in a tightly closed bottle.

**11.5.2.5 Cyclohexane,** for dioxin and PCB analysis.

**11.5.2.6 Toluene,** for dioxin and PCB analysis.

**11.5.2.7 Sulphuric acid,** $H_2SO_4$, 96 %, 1,84 specific gravity.

**11.5.2.8 Cyclohexane/toluene,** in volume portions of 1/1.

Mix 1 l cyclohexane (11.5.2.5) with 1 l toluene (11.5.2.6) thoroughly. Store at room temperature in a tightly closed bottle.

**11.5.2.9 Baked silica**

Bake silica overnight (≥ 10 h) at 600 °C.

**11.5.2.10 Deactivated silica**

Deactivate baked silica (11.5.2.9) by adding 5 g water to 95 g of the baked silica, homogenize thoroughly, equilibration at least overnight.

**11.5.2.11 NaOH pellets**

**11.5.2.12 NaOH**, 1 mol/l.

Dissolve 40,0 g NaOH pellets (11.5.2.11) in 1 l water.

**11.5.2.13 Basic silica**

Mix 30 ml of 1 mol/l NaOH (11.5.2.12, aqueous solution) to 100 g of baked silica (11.5.2.9), homogenize thoroughly, equilibration at least overnight.

**11.5.2.14 Sulphuric acidic silica**

Mix 46 g of concentrated Sulphuric acid (11.5.2.7) to 100 g of baked silica (11.5.2.9), homogenize thoroughly, equilibration at least overnight.

**11.5.2.15 Florisil**

**11.5.2.16 Baked Florisil**

Bake Florisil (11.5.2.15) over night at 600 °C, (≥ 10 h).

**11.5.2.17 Deactivated Florisil**

Deactivate baked Florisil (11.5.2.16) by adding 3 gr water to 97 g of the baked Florisil (11.5.2.16), homogenize thoroughly, equilibration at least overnight.

**11.5.2.18 Carbon,** e.g. Carbopack B.

Extract carbon with toluene using Soxhlet extractor for at least 24 h.

**11.5.2.19 Celite**

**11.5.2.20 Baked Celite**

Bake Celite (11.5.2.19) at 650 °C for ≥ 10 h.

### 11.5.3 Apparatus

All technical descriptions are examples of possible system setups and parameters and have to be scaled or adopted to the user's equipment.

**11.5.3.1 Chromatographic column, with length of 10 cm,** inner diameter approximately 1 cm.

**11.5.3.2 Chromatographic column with length of 20 cm,** inner diameter approximately 1 cm.

**11.5.3.3 Chromatographic column with length of 30 cm,** inner diameter approximately 4 cm.

**11.5.3.4 HPLC-pump**

The HPLC pump shall be capable of maintaining a flow-rate of 5,0 ml/min of ethylacetate/cyclohexane (11.5.2.4) or toluene (11.5.2.6) or n-hexane (11.5.2.3) or mixtures of toluene and n-hexane.

**11.5.3.5 Automated injection system**

The automated injection system shall be capable of performing a series of unattended injections each of 5 ml.

**11.5.3.6 GPC-column**

The GPC-column shall be capable of performing a separation as specified by EPA method 3640 A [5]. For example: length 45 cm, internal diameter 2,5 cm, stationary phase Bio Beads SX-3 (11.5.3.8) or equivalent. The dioxin/PCB containing fraction elutes in certain fraction of time depending on flow rate and amount of stationary phase. Fractionation has to be checked before use. As orientation, this column is capable of separating about 0,5 g to 1 g of fat from dioxins and PCBs (check before use).

**11.5.3.7 Fraction collector** capable of collecting fractions up to 500 ml.

**11.5.3.8 BIO Beads SX-3,** neutral, porous styrene divinylbenzene copolymer beads, with stand 5 ml/min with a back pressure of 300 psi.

### 11.5.4 Sample clean up by Large multi-layer silica column

Fill a chromatographic column with length of 30 cm (11.5.3.3), inner diameter approximately 4 cm or comparable column dimensions from bottom to top with: 5 g deactivated silica (11.5.2.10); 10 g basic silica (11.5.2.13); 5 g deactivated silica (11.5.2.10); 30 g sulphuric acidic silica (11.5.2.14); 5 g deactivated silica (11.5.2.10) and 35 g basic silica (11.5.2.13).

Pre-wash the multi-layer silica column with 150 ml of cyclohexane/toluene (11.5.2.8). Put an adequate flask of 500 ml at the outlet of the column.

Apply the evaporated residue dissolved in 50 ml of cyclohexane/toluene (11.5.2.8) to the large multi-layer silica column.

Elution of PCDD/F/PCB-containing fraction with 150 ml of cyclohexane/toluene (11.5.2.8).

The collected fraction is concentrated in an evaporator (6.1) to near dryness and re-dissolved in suitable solvent for next clean up steps GPC (11.5.5) and/or small multi-layer silica column (11.5.6).

### 11.5.5 Sample clean up by Gel permeation chromatography

Inject (11.5.3.5) the prepared sample of 2,5 g oil (9.2.2) on the GPC (11.5.3.6) in four portions of 5 ml, each containing not more than 0,75 g fat, combine the relevant fractions, or inject the silica purified extract of 10 g feed (11.5.4) into the GPC (11.5.3.6) in portions of 5 ml, each containing not more than 0,75 g fat. If more than one injection was necessary, combine the relevant fractions.

The collect(ed) GPC fraction(s) are concentrated in an evaporator (6.1) (40 °C, $N_2$) to a volume of approximately 1 ml). Continue at florisil column (11.5.7) or (optional) remove small amounts of fat / matrix by a small multi-layer silica column 11.5.6.

NOTE Equilibrate the GPC-system under the recommended operating conditions and check the GPC column performance as subscribed in EPA method 3640 A [5]. The fraction to be collected has to be determined by using a mixture of PCBs.

### 11.5.6 Small multi-layer column (optional)

Fill a chromatographic column with length of 10 cm (11.5.3.1), inner diameter approximately 1 cm or comparable column dimensions from bottom to top with: 0,3 g deactivated silica (11.5.2.10), 1,0 g basic silica (11.5.2.13), 0,3 g deactivated silica (11.5.2.10), 1 g sulphuric acidic silica (11.5.2.14) and 0,3 g deactivated silica (11.5.2.10).

Pre-wash the multi-layer silica column with 40 ml of n-heptane (11.5.2.1).

Put a flask of 25 ml at the outlet of the column.

Apply the fraction obtained at (11.5.5) dissolved in 1 ml of n-heptane, to the column. The column is eluted with 20 ml n-heptane (11.5.2.1). The purified extract can be separated in e.g. the two desired fractions by using a carbon column (A and B) (11.3.4.3) or in dioxin and PCB fraction using a Florisil column (11.5.7).

### 11.5.7 Separation of PCDD/Fs and PCBs on a Florisil column (manual column)

The Florisil-column shall be capable of separating PCDD/Fs from PCBs (indicator PCBs and dl-PCBs). Pack a chromatographic column with length of 20 cm (11.5.3.2), internal diameter 1 cm, stationary phase 6 g of deactivated Florisil (11.5.2.17). Fractionation has to be checked before use.

Add 1 ml of pre-washed eluat of the small multi-layer silica column (11.5.6) or the GPC pre-washed eluat (11.5.5) on the Florisil column (pre-eluted with 50 ml of n-heptane (11.5.2.1) containing 0,2 % of toluene (11.5.2.6)). Elution of PCB-containing fraction with 50 ml of n-heptane (11.5.2.1) containing 0,2 % of toluene (11.5.2.6) and of PCDD/F fraction afterwards with 60 ml of toluene (11.5.2.6) in adequate flasks.

The collected fractions are concentrated in an evaporator (6.1) to near dryness and re-dissolved in n-hexane (11.5.2.3) for further clean up/fractionation using carbon column (11.5.8 and 11.5.9).

### 11.5.8 Separation of PCBs (PCB fraction from Florisil column) into indicator PCBs, mono-ortho PCBs and non-ortho PCBs on an automated carbon column

The carbon-column shall be capable of separating indicator PCBs, mono-ortho PCBs and non-ortho PCBs. The clean up can be done automatically using a HPLC pump, column and a fraction collector or manually.

Pack a chromatographic column with length of 20 cm, internal diameter 1 cm (adjustable to amount of stationary phase) with a mixture of 2,0 g of carbon (11.5.2.18) and 2 g of baked Celite (11.5.2.20).

Add 1 ml eluat of PCB fraction of the Florisil column (11.5.7) on the carbon column. The PCB containing fractions elute with the following solvents and solvent mixtures. The fractionation has to be checked before use.

— Indicator PCB: n-hexane (fractionation time e.g. 18 min, flow 1,5 ml/min);

— mono-ortho PCB: n-hexane/toluene (92,5/7,5, v/v) (fractionation time e.g. 20 min, flow 1,5 ml/min);

— non-ortho PCB: toluene (fractionation time e.g. 25 min, flow 1,5 ml/min).

Elution of PCB in above mentioned solvents and solvent mixtures in adequate flasks.

To the collected fractions 100 µl TCDD-RS-VI (Annex B, B.2.20) is added and subsequently concentrated in an evaporator to near dryness and re-dissolved to an appropriate volume, e.g. 25 µl to 50 µl and transferred to a GC autosampler-vial (11.2.3.8) with a flat bottom micro insert (11.2.3.9). Continue at module D: paragraph 12, GC-HRMS.

#### 11.5.9 Clean up of PCDD/F fraction on carbon columns

The carbon column shall be capable of finally cleaning the PCDD/F fraction for GC/MS measurement. The clean up can be done manually or automated using a HPLC pump (11.5.3.4), column (11.5.3.1) and a fraction collector (11.5.3.7).

Pack a chromatographic column with length of 10 cm (11.5.3.1) internal diameter 1 cm with 0,4 g of a mixture of 0,07 g of carbon (11.5.2.18) and 0,33 g of baked Celite (11.5.2.20).

Pre-wash the carbon column with 20 ml of n-hexane.

Add 1 ml of pre-washed eluat of PCDD/F fraction of the Florisil column (11.5.7) on the carbon column and pre-wash with 2 ml of n-hexane (11.5.2.3). Put a 100 ml flask at the outlet of the column. Elute the PCDD/F fraction with 50 ml of toluene (11.5.2.6). To the collected fraction 100 µl TCDD-RS-VI (Annex B, B.2.20) is added. The collected fraction is concentrated in an evaporator to near dryness and re-dissolved in toluene (11.5.2.6) for GC/MS measurement. Continue at module D: paragraph (12) GC-HRMS.

NOTE Manual procedure can be automated as follows: Pack a chromatographic column with length of 10 cm (11.5.3.1), internal diameter 1 cm with a mixture of 0,2 g of carbon (11.5.2.18) and 0,2 g of baked Celite (11.5.2.20). Add 1 ml of pre-washed eluat (11.5.7) on the carbon column and wash with 45 ml of n-hexane (11.5.2.3) at a flow rate of 1,5 ml/min. The PCDD/F containing fraction elutes with 37,5 ml toluene (11.5.2.6) with a flow rate of 1,5 ml/min on backflush. Continue at module D: paragraph (12) GC-HRMS.

For additional clean up especially in case of dirty complex samples (oxidized fat/oil) a small multi layer silica column might be used for the PCDD/F and PCB fraction as described in Annex E provided that Commission Regulation (CE) No 152/2009 [6] is obeyed.

## 12 Module D: Gas chromatograph-high resolution mass spectrometer (GC-HRMS)

### 12.1 General

#### 12.1.1 Introduction

The gas chromatograph shall be equipped with a splitless injector or a large volume injector capable for injections up top 100 µl, see Annex A.

An example of parameters for the splitless injector are temperature of 220 °C and a split time of 2 min.

#### 12.1.2 Reagents and materials

##### 12.1.2.1 Perfluoro-kerosene (PFK)

**12.1.2.2 Gas chromatograph,** shall be capable of working with capillary columns

The use of a capillary column coated with a non-polarity stationary phase (dimensions 60 m x 0,25 mm, film thickness 0,25 µm), and the following programme is recommended. The column flow (He) is kept constant at 1,2 ml/min. The GC-column oven temperature program starts at an initial temperature of 110 °C where it is kept for 3 min. After this the temperature is ramped with 20 °C/min to a temperature of 200 °C where it is kept for 10 min. After this the temperature is ramped with 4°C/min to a final temperature of 310 °C. Finally, the GC is cooled to 110 °C. In case of separation problems with samples in the range of legal limits an additional column with different polarity might be necessary.

NOTE Alternative column and/or temperature program can be used provided that Commission Regulation (EC) No 152/2009 [6] with respect to separation of critical congeners are obeyed.

### 12.2 Procedure

NOTE In Annex D an example of selected ions to be measured is given.

#### 12.2.1 Preparation of the system

Equilibrate gas chromatographic system under the recommended operating conditions.

#### 12.2.2 Tune the HRMS system

Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10 000 (10 % valley) at m/z 304,9824 (PFK) or any other appropriate PFK reference signal within the range of m/z values defined in Tables D3-10. Monitor and record the resolution and exact m/z values of three to five reference peaks covering the mass range of the descriptor. The resolution shall be greater than or equal to 10 000, and the deviation between the exact m/z and the theoretical m/z for each exact m/z monitored shall be less than 5 ppm.

#### 12.2.3 Checking Instrument settings

Inject 2 µl of a medium calibration level, and check peak shape and retention times for all compounds of fraction ‘A’ and fraction ‘B’. Modify the SIR window if necessary for start/ending time in the MS page of the instrument method.

#### 12.2.4 Checking separation of the GC system

##### 12.2.4.1 Separation Dioxins/furans and non-ortho PCBs

On a non-polar column the valley height for the separation of the compounds $^{13}$C-1,2,3,4,7,8-HxCDF and $^{13}$C-1,2,3,6,7,8-HxCDF shall be ≤ 25 %. Requirement from EPA method 1613 [8] and EU regulations (Commission Regulation (EC) No 152/2009) [6].

##### 12.2.4.2 Separation mono-ortho PCBs and indicator PCBs

On a non-polar column the valley height for the separation of the compounds $^{13}$C-PCB-123 and $^{13}$C-PCB-118 and $^{13}$C-PCB-156 and $^{13}$C-PCB-157 shall be ≤ 25 %. Requirement only for dioxins from EPA method 1613 [6].

The co-elution is calculated as follows:

$$coelution = (\frac{x}{y}) \times 100\%$$

where

$x$ is the height of the valley to baseline and $y$ the peak height of the most intense peak.

#### 12.2.5 Checking sensitivity of the system

##### 12.2.5.1 Sensitivity dioxins/furans and non-ortho PCBs

The signal to noise of 2,3,7,8-TCDD at mass 321,8936 m/z shall be at least 15 fg for 100 fg on column. If not, appropriate action has to be taken, for example by cleaning the ion source (when a dirty ion source is present) or by retuning the instrument or by changing the analytical column (bad peak shape).

##### 12.2.5.2 Sensitivity mono-ortho PCBs and indicator PCBs

The signal to noise of PCB-157 (361,8386 m/z) shall be at least 10 fg for 500 fg on column. If not, appropriate action has to be taken, for example by cleaning the ion source (when a dirty ion source is present) or by retuning the instrument or by changing the analytical column (bad peak shape).

#### 12.2.6 Determination

Inject 2 µl of the calibration standard solutions (1 to 9, Annex B, Table B.2) for mono-ortho PCBs and indicator PCBs and an equal volume of the sample extracts fraction 'A' (obtained at CI and CII or fraction 3 and fraction 4 (obtained at CIII)), using a split less injector. Repeat these measurements for fraction 'B' (obtained at CI and CII or fraction 1 and fraction 2 (obtained at CIII)), both the calibration line and samples.

NOTE For fraction 1, 2, 3 and 4 the monitored ions (Annex D) might be reduced to the applicable ones.

Identify the individual dioxins/furans and dioxin-like PCBs and indicator PCBs peaks on basis of retention time, exact mass and ion ratio.

Determine the amount of dioxins/furans and dioxin-like PCBs and indicator PCBs by comparing the size of the sample peaks with those of the known amount of the corresponding dioxins/furans and dioxin-like PCBs and indicator PCBs peaks in the calibration standard solutions (1 to 9, Annex B, Table B.2). Calibration is based on isotope dilution principle.

## 13 Calculation and expression of results

### 13.1 General

Before processing all data, the retention times of all compounds of interest are checked and, if necessary, modified in the processing method. After processing of the data, every result is manually checked for correct integration.

Calibration by Isotope Dilution: Isotope dilution calibration is used for the dioxins, furans and dioxin-like PCBs for which labelled compounds are added to samples prior to extraction. To calibrate the analytical system by isotope dilution, inject 2 µl of calibration standards 1 through 9 (Annex B). Compute the Relative Response Factor (RRF) at each concentration.

A calibration curve encompassing the concentration range is prepared for each compound to be determined. The Response Factor (RF) (labelled to native) vs. concentration in standard .solutions is plotted or computed using a linear regression. Relative response is determined according to the procedures described below. The response of each CDD/CDF and dioxin-like PCB relative to its labelled analogue is determined using the

area under the curve relating to the responses of both the primary and secondary exact m/z values, for each calibration standard.

Linearity: If the relative response for any compound is constant (less than 20 % coefficient of variation) over the calibration range, an averaged RRF may be used for that compound. The coefficient of variation for any $^{13}C$ labelled compound shall be less than 30 %. Use at least five-point internal standard calibration (EPA method 1613 [6]), force the intercept through zero, and calculate the correlation coefficient $r^2$.

### 13.2 Calibration criteria

The sample results should fit within the range of the calibration curve. When a result exceeds the thresholds of the calibration curve the sample should be diluted and reanalysed until it fits within the calibration curve.

### 13.3 Identification and confirmation

The compounds of interest are identified on retention time, exact mass and mass ratio (SIR). The relative intensities of the detected ions, expressed as ratio, shall correspond to those mentioned in Table 1.

**Table 1 — Mass ratios (table adopted from EPA method 1613 [6])**

| Number of Chlorine Atoms | M/Z's Forming Ratio | Theoretical Ratio | QC Limit [a] Lower | Upper |
|---|---|---|---|---|
| 4 [b] | M/(M+2) | 0,77 | 0,65 | 0,89 |
| 5 | (M+2)/(M+4) | 1,55 | 1,32 | 1,78 |
| 6 | (M+2)/(M+4) | 1,24 | 1,05 | 1,43 |
| 6 [c] | M/(M+2) | 0,51 | 0,43 | 0,59 |
| 7 | (M+2)/(M+4) | 1,05 | 0,88 | 1,20 |
| 7 [d] | M/(M+2) | 0,44 | 0,37 | 0,51 |
| 8 | (M+2)/(M+4) | 0,89 | 0,76 | 1,02 |

[a] QC limits represent ± 15 % windows around the theoretical ion abundance ratios.

[b] Does not apply to $^{37}Cl_4$ -2,3,7,8-TCDD (clean up standard).

[c] Used for $^{13}C_{12}$ -HxCDF only.

[d] Used for $^{13}C_{12}$ -HpCDF only.

### 13.4 Calculation

Calculation of the response factor for native compounds:

$$RRF_{(n)} = \frac{A_x \times Q_{is}}{Q_x \times A_{is}} \quad (1)$$

Internal standards:

$$RRF_{(l)} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs}} \quad (2)$$

where

$A_x$ is the response (some of two m/z's) of native compounds;

$A_{is}$ is the response (some of two m/z's) of corresponding internal standard;

$A_{rs}$ is the response (some of two m/z's) of recovery standard;

$Q_{is}$ is the amount of internal standard pg/µl;

$Q_{rs}$ is the amount of recovery standard pg/µl;

$Q_x$ is the amount of native component pg/µl.

Consequently, the averaged relative response factor is calculated:

$$\overline{RRF_{(n)}} = \frac{1}{m} \times \sum_{i=1}^{m} RRF_i(n) \quad (3)$$

where

$m$ is the number of standards (concentration levels);

$n$ is the native component;

$i$ is the calibration level.

Consequently the averaged relative response factor is calculated for the labelled compounds

$$\overline{RRF_{(l)}} = \frac{1}{m} \times \sum_{i=1}^{m} RRF_i(l) \quad (4)$$

where

$m$ is the number of standards (concentration levels);

$l$ is the labelled compound;

$i$ is the calibration level.

NOTE For native compound 2,3,4,6,7,8-HxCDF there is no labelled analogous compound available. 2,3,4,6,7,8-HxCDF should be quantified based on internal standard $^{13}$C 1,2,3,6,7,8-HxCDF.

Calculation concentration component of interest

The content component of interest is calculated by:

$$C_x = \frac{A_x \times Q_{is}}{A_{is} \times DIV \times \overline{RRF_{(n)}}} \quad (5)$$

where

$C_x$ is the content of the component of interest in ng/kg;

$A_x$ is the response (some of two m/z values) of native compounds in sample extracts;

$A_{is}$ is the response (some of two m/z values) of corresponding labelled internal standard in sample extracts;

$Q_{is}$ is the amount of injected labelled internal standard pg/µl;

*DIV* is the calculation factor from concentration (pg/µl) to content on sample basis (ng/kg) = M/V,

where

V is final volume in µl;

M = sample intake in g.

RRF(n) is the relative response factor.

The calculated concentrations have to be corrected for 12 % moisture.

NOTE Wet samples moister content > 12 % have to be freeze dried prior to extraction.

## 13.5 Toxic Equivalent (TEQ) values

Calculated concentrations of dioxins, furans and dioxin-like PCBs (see 13.3) are recalculated to Toxic Equivalent (TEQ) values. The concentration of each congener is therefore multiplied by its Toxicity Equivalent Factor (TEF). The TEQ is calculated by:

$$TEQ(pg/g) = \sum_{i=1}^{29} (TEF)_i \times (concentration)_i \quad (6)$$

where

*TEQ(pg/g)* is Toxic Equivalent;

$(TEF)_i$ is the TEF value of compound i (see Table 2);

$(Concentration)_i$ is the calculated concentration of compound i (pg/g).

The TEQ value is calculated based on the lower, middle and upper bound concentrations.

**Table 2 — TEF values for individual compounds**

| Compound | WHO 1998 TEF | WHO 2005 TEF |
|---|---|---|
| | | |
| **chlorinated dibenzo-p-dioxins** | | |
| 2,3,7,8-TCDD | 1 | 1 |
| 1,2,3,7,8-PeCDD | 1 | 1 |
| 1,2,3,4,7,8-HxCDD | 0,1 | 0,1 |
| 1,2,3,6,7,8-HxCDD | 0,1 | 0,1 |
| 1,2,3,7,8,9-HxCDD | 0,1 | 0,1 |
| 1,2,3,4,6,7,8-HpCDD | 0,01 | 0,01 |
| OCDD | 0,000 1 | 0,000 3 |
| | | |
| **chlorinated dibenzofurans** | | |
| 2,3,7,8-TCDF | 0,1 | 0,1 |
| 1,2,3,7,8-PeCDF | 0,05 | 0,03 |
| 2,3,4,7,8-PeCDF | 0,5 | 0,3 |
| 1,2,3,4,7,8-HxCDF | 0,1 | 0,1 |
| 1,2,3,6,7,8-HxCDF | 0,1 | 0,1 |
| 1,2,3,7,8,9-HxCDF | 0,1 | 0,1 |
| 2,3,4,6,7,8-HxCDF | 0,1 | 0,1 |
| 1,2,3,4,6,7,8-HpCDF | 0,01 | 0,01 |
| 1,2,3,4,7,8,9-HpCDF | 0,01 | 0,01 |
| OCDF | 0,000 1 | 0,000 3 |
| | | |
| **non-ortho substituted dl-PCBs** | | |
| PCB 77 | 0,000 1 | 0,000 1 |
| PCB 81 | 0,000 1 | 0,000 3 |
| PCB 126 | 0,1 | 0,1 |
| PCB 169 | 0,01 | 0,03 |
| | | |
| **mono-ortho substituted dl-PCBs** | | |
| 105 | 0,000 1 | 0,0000 3 |
| 114 | 0,000 5 | 0,0000 3 |
| 118 | 0,000 1 | 0,0000 3 |
| 123 | 0,000 1 | 0,0000 3 |
| 156 | 0,000 5 | 0,0000 3 |
| 157 | 0,000 5 | 0,0000 3 |
| 167 | 0,0000 1 | 0,0000 3 |
| 189 | 0,000 1 | 0,0000 3 |
| | | |

Reference – Van den Berg et al: The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency factors for Dioxins and Dioxin-like Compounds [12]

NOTE TEF values have to be used in accordance to the current legislation.

### 13.6 Recovery

The recovery for the internal standards used is calculated by:

$$percentage\ \ recovery\ \ (\%)\ =\ \frac{A_{is}\times Q_{rs}}{Q_{is}\times A_{rs}\times RRF_{(m)}}\times 100 \qquad (7)$$

where

$A_{is}$ is the response (some of two m/z's) of the internal standard in the sample;

$A_{rs}$ is the response (some of two m/z's) of the recovery standard in the sample, see NOTE;

$Q_{is}$ is the Amount of internal standard pg/µl;

$Q_{rs}$ is the Amount of recovery standard pg/µl;

$RRF(m)$ is the relative response factor.

NOTE For the recovery of internal standards in function group 1, function group 2 and function group 3, recovery standard $^{13}$C 1,2,3,4-TCDD is used. The recovery of compounds in function group 4, function group 5 and function group 6 is calculated using recovery standard $^{13}$C 2,3,4,6,7,8- HxCDF. For the recovery calculation of compounds in fraction 'A', only $^{13}$C 1,2,3,4-TCDD is used.

## 14 Precision

An interlaboratory comparison was organized by RIKILT, Institute of Food Safety in the Netherlands in close cooperation with the CRL in Freiburg (Germany) and FERA in York (United Kingdom). This international laboratory ring trial aimed the determination of dioxins, dl-PCBs and indicator PCBs in animal feed and oil This paragraph describes the results of the interlaboratory comparison 2010. In Table 3 the overall results are described.

NOTE All results of the interlaboratory study are described in RIKILT report 2011.011 "CEN Ring trial Animal Feed" project leader W.A. Traag; http://www.rikilt.wur.nl/UK/publications/Reports/ [13].

**Table 3 — Overall results of dioxin and dl - PCBs on TEQ basis (upperbound)**

| **WHO-PCB-TEQubav** | **Sample 1 Mineral clay** | **Sample 2 Bovine compound feed** | **Sample 3 Fish oil** | **Sample 4 Fish meal** | |
|---|---|---|---|---|---|
| | | | | | |
| Year of inter-laboratory study | 2010 | 2010 | 2010 | 2010 | |
| Number of laboratories (after elimination) | 25 | 28 | 27 | 28 | |
| Number of laboratories (total) | 28 | 29 | 28 | 29 | |
| Fraction of of outliers | 0,11 | 0,03 | 0,04 | 0,03 | |
| Mean | 0,04 | 1,01 | 4,31 | 0,84 | |
| Median | 0,04 | 1,02 | 4,39 | 0,84 | |
| Minimum | 0,03 | 0,67 | 2,87 | 0,62 | |
| Maximum | 0,06 | 1,29 | 5,58 | 1,10 | |
| CV | 23,85 | 12,25 | 11,74 | 12,45 | |
| **Robust mean (Huber [14])** | **0,04** | **1,01** | **4,33** | **0,83** | |
| Robust stdv (Huber [14]) | 0,01 | 0,11 | 0,40 | 0,09 | |
| Rel robust stdv (Huber [14] | 25,65 | 10,79 | 9,19 | 10,50 | |
| Robust standard uncertainty | 0,00 | 0,02 | 0,08 | 0,02 | |
| | | | | | |
| Horrat | 1,53 | 0,56 | 0,53 | 0,57 | |
| **WHO-PCDD/F-TEQub** | **Sample 1 Mineral clay** | **Sample 2 Bovine compound feed** | **Sample 3 Fish oil** | **Sample 4 Fish meal** | **Sample 5 Standard solution** |
| | | | | | |
| Year of inter-laboratory study | 2010 | 2010 | 2010 | 2010 | 2010 |
| Number of laboratories (after elimination) | 25 | 29 | 26 | 26 | 35 |
| Number of laboratories (total) | 29 | 30 | 29 | 30 | 35 |
| Fraction of of outliers | 0,14 | 0,03 | 0,10 | 0,13 | 0 |
| Mean | 0,58 | 1,48 | 2,04 | 0,58 | 81,1 |
| Median | 0,59 | 1,50 | 2,08 | 0,60 | 79,8 |
| Minimum | 0,38 | 1,10 | 1,28 | 0,36 | 66,7 |
| Maximum | 0,78 | 2,08 | 2,47 | 0,72 | 114,9 |
| CV | 15,89 | 13,21 | 12,31 | 15,69 | 9,6 |
| **Robust mean (Huber [14])** | **0,58** | **1,48** | **2,05** | **0,59** | **79,73** |
| Robust stdv (Huber [14]) | 0,09 | 0,17 | 0,22 | 0,08 | 5,19 |
| Rel robust stdv (Huber [14]) | 15,87 | 11,79 | 10,48 | 14,11 | 6,51 |
| Robust standard uncertainty | 0,02 | 0,03 | 0,04 | 0,02 | 0,88 |
| | | | | | |
| Horrat | 1,05 | 0,60 | 1,18 | 1,36 | 0,43 |

## 15 Test report

The test report shall specify the following information:

a) information about the animal feed samples and the oil sample used for this ring trial;

b) spiking levels of all compound/matrix combinations;

c) statistical calculation on suspected values and outliers;

d) Z-score information;

e) assigned values and 95 % confidence interval;

f) calculations on coefficient of variations;

g) Horwitz coefficient of variations;

h) HORRAT Value;

i) accuracy.

# Annex A
(informative)

# Description of PTV injection system

## A.1 Large volume injection (100 µl) – GC-HRMS Calibration mixtures dioxins and non-ortho PCBs

Standard solutions shall have a 50 times lower concentration when 100 µl is injected. Here the calibration standards for the non-ortho PCBs are given. See Table A.1.

All solutions contain 0,10 pg/µl $^{13}$C labelled internal standards, 0,1 pg/µl clean up standard $^{37}$Cl-4 2,3,7,8 TCDD and 0,2 pg/µl recovery standard 1,2,3,4 $^{13}$C-TCDD and 2,3,4,6,7,8 $^{13}$C-HxCDF. Calibration mixture 1 to calibration mixture 7 contain 0,002 pg/µl, 0,005 pg/µl, 0,010 pg/µl, 0,020 pg/µl, 0,050 pg/µl, 0,100 pg/µl and 0,200 pg/µl native dioxin congeners and non-ortho PCBs respectively. Calibration mixture 8 and calibration mixture 9 contain only native non-ortho PCBs at 1,00 pg/µl and 2,00 pg/µl respectively.

**Table A.1 — Standard solutions for calibration using PTV injection**

| | **$^{12}$C standard** µl | **$^{13}$C standard** µl | **Recovery standard** µl | **Clean up standard** µl | **Add solved** | **End volume** ml | **Concentration Dioxins** pg/µl | **Concentration non-ortho PCBs** pg/µl |
|---|---|---|---|---|---|---|---|---|
| 1 | 50 DIOXNOP-1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,002 | 0,002 |
| 2 | 125 DIOXNOP-1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,005 | 0,005 |
| 3 | 250 DIOXNOP-1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,010 | 0,010 |
| 4 | 500 DIOXNOP-1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,020 | 0,020 |
| 5 | 125 DIOXNOP (B.2.24) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,050 | 0,050 |
| 6 | 250 DIOXNOP (B.2.24) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,100 | 0,100 |
| 7 | 500 DIOXNOP (B.2.24) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | 0,200 | 0,200 |
| 8 | 100 NOP-500 (B.2.23) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | | 1,000 |
| 9 | 200 NOP-500 (B.2.23) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | Toluene (10.2.1.3) | 50 | | 2,000 |

Sample preparation procedure:

The sample preparation is similar to that described in paragraph 9 (procedure) of the method. The difference is the end volume of the obtained fractions of the extract to be injected. In the case of large volume

injections, the end volume can be 0,5 ml. Instead of the 1 µl to 2 µl injected into a splitless injector, the example described is based on injection of 100 µl LV PTV (large volume PTV).

## A.2 PTV injection conditions using 100 µl injection

| | |
|---|---|
| Initial temperature: | 70 °C |
| Mode: | PTV Large Volume |
| Purge Time (min): | 1,75 |
| Ventflow (ml/min): | 100 |
| Vent Pressure (kPa): | 100 |
| Vent time (min): | 0,50 |

# Annex B
(informative)

# Description of standards and concentration of the standard solutions

## B.1 Examples of concentration of the standard solutions

CAS Registry numbers for dioxins, furans, non-ortho PCBs, mono-ortho PCBs and indicator PCBs and there labelled analogs are given in Table B.1.

**Table B.1 — Compounds and their CAS registry number** *(1 of 2)*

| COMPONENTS | CAS Registry | LABELLED ANALOGUE | CAS Registry |
|---|---|---|---|
| **Dioxins/furans** | | | |
| 2,3,7,8-TCDF | 51 207-31-9 | $^{13}$C-2,3,7,8-TCDF | 89 059-46-1 |
| 1,2,3,7,8-PeCDF | 57 117-41-6 | $^{13}$C-1,2,3,7,8-PeCDF | 109 719-77-9 |
| 2,3,4,7,8-PeCDF | 57 117-31-4 | $^{13}$C-2,3,4,7,8-PeCDF | 116 843-02-8 |
| 1,2,3,4,7,8-HxCDF | 70 648-26-9 | $^{13}$C-1,2,3,4,7,8-HxCDF | 114 423-98-2 |
| 1,2,3,6,7,8-HxCDF | 57 117-44-9 | $^{13}$C-1,2,3,6,7,8-HxCDF | 116 843-03-9 |
| 2,3,4,6,7,8-HxCDF | 60 851-34-5 | | |
| 1,2,3,7,8,9-HxCDF | 72 918-21-9 | $^{13}$C-1,2,3,7,8,9-HxCDF | 116 843-04-0 |
| 1,2,3,4,6,7,8-HpCDF | 67 562-39-2 | $^{13}$C-1,2,3,4,6,7,8-HpCDF | 109 719-84-8 |
| 1,2,3,4,7,8,9-HpCDF | 55 673-89-7 | $^{13}$C-1,2,3,4,7,8,9-HpCDF | 109 719-94-0 |
| OCDF | 39 001-02-0 | $^{13}$C-OCDF | |
| 2,3,7,8-TCDD | 1 746-01-6 | $^{13}$C-2,3,7,8-TCDD | 76 523-40-5 |
| 1,2,3,7,8-PeCDD | 40 321-76-4 | $^{13}$C-1,2,3,7,8-PeCDD | 109 719-79-1 |
| 1,2,3,4,7,8-HxCDD | 39 227-28-6 | $^{13}$C-1,2,3,4,7,8-HxCDD | 114 423-98-2 |
| 1,2,3,6,7,8-HxCDD | 57 653-85-7 | $^{13}$C-1,2,3,6,7,8-HxCDD | 116 843-03-9 |
| 1,2,3,7,8,9-HxCDD | 19 408-74-3 | $^{13}$C-1,2,3,7,8,9-HxCDD | 116 843-04-0 |
| 1,2,3,4,6,7,8-HpCDD | 35 822-46-9 | $^{13}$C-1,2,3,4,6,7,8-HpCDD | 109 719-83-7 |
| OCDD | 3 268-87-9 | $^{13}$C-OCDD | 114 423-97-1 |
| **Non-ortho PCBs** | | | |
| PCB 81 | 70 362-50-4 | $^{13}$C-PCB 81 | 208 461-24-9 |
| PCB 77 | 32 598-13-3 | $^{13}$C-PCB 77 | 105 600-23-5 |
| PCB 126 | 57 465-28-8 | $^{13}$C-PCB 126 | 208 263-65-4 |
| PCB 169 | 32 774-16-6 | $^{13}$C-PCB 169 | 208 263-70-1 |

**Table B.1** *(2 of 2)*

| COMPONENTS | CAS Registry | LABELLED ANALOGUE | CAS Registry |
|---|---|---|---|
| **Mono-ortho PCBs** | | | |
| PCB 123 | 65 510-44-3 | $^{13}$C-PCB 123 | 208 263-64-3 |
| PCB 118 | 31 508-00-6 | $^{13}$C-PCB 118 | 104 130-40-7 |
| PCB 114 | 74 472-37-0 | $^{13}$C-PCB 114 | 208 263-63-2 |
| PCB 105 | 32 598-14-4 | $^{13}$C-PCB 105 | 208 263-62-1 |
| PCB 167 | 52 663-72-6 | $^{13}$C-PCB 167 | 208 263-69-8 |
| PCB 156 | 38 380-08-4 | $^{13}$C-PCB 156 | 208 263-68-7 |
| PCB 157 | 69 782-90-7 | $^{13}$C-PCB 157 | 235 416-30-5 |
| PCB 189 | 39 635-31-9 | $^{13}$C-PCB 189 | 208 263-73-4 |
| **Indicator PCBs** | | | |
| PCB 028 | 7 012-37-5 | $^{13}$C-PCB 028 | 208 263-76-7 |
| PCB 052 | 35 693-99-3 | $^{13}$C-PCB 052 | 208 263-80-3 |
| PCB 101 | 37 680-73-2 | $^{13}$C-PCB 101 | 104 130-39-4 |
| PCB 153 | 35 065-27-1 | $^{13}$C-PCB 153 | |
| PCB 138 | 35 065-28-2 | $^{13}$C-PCB 138 | 208 263-66-5 |
| PCB 180 | 35 065-29-3 | $^{13}$C-PCB 180 | |

## B.2 Standard solutions

### B.2.1 $^{13}$C-1,2,3,4-TCDD (50 µg/ml)

Use commercially available standard $^{13}$C-1,2,3,4-TCDD (50 µg/ml) or prepare this standard solution manually by weighing the compound, dissolving and diluting in a volumetric flask.

### B.2.2 $^{13}$C-2,3,4,6,7,8-HxCDF (50 µg/ml)

Use commercially available standard $^{13}$C-2,3,4,6,7,8-HxCDF (50 µg/ml) or prepare this standard solution manually by weighing the compound, dissolving and diluting in a volumetric flask.

### B.2.3 $^{37}Cl_4$-2,3,7,8 TCDD (50 µg/ml)

Use commercially available standard $^{37}Cl_4$-2,3,7,8 TCDD (50 µg/ml) or prepare this standard solution manually by weighing the compound, dissolving and diluting in a volumetric flask.

### B.2.4 Mixture $^{13}$C-PCDD (1 µg/ml)

Use commercially available mixture $^{13}$C-PCDD (1 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.5 Mixture $^{13}$C-PCDF (1 µg/ml)

Use commercially available mixture $^{13}$C-PCDF (1 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.6 Mixture TCDD-HpCDD (5 µg/ml)

Use commercially available mixture TCDD-HpCDD (5 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.7 Mixture TCDF-HpCDF (5 µg/ml)

Use commercially available mixture TCDF-HpCDF (5 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.8 OCDD (10 µg/ml)

Use commercially available standard OCDD (10 µg/ml) or prepare this standard solution manually by weighing the compound, dissolving and diluting in a volumetric flask.

### B.2.9 OCDF (50 µg/ml)

Use commercially available standard OCDF (50 µg/ml) or prepare this standard solution manually by weighing the compound, dissolving and diluting in a volumetric flask.

### B.2.10 OCDF standard (5 µg/ml)

Dilute OCDF standard solution of 50 ug/ml with toluene to a final concentration of 5 µg/ml. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled.

### B.2.11 Mixture $^{13}$C-non-ortho PCBs (1 µg/ml)

Use commercially available mixture $^{13}$C-non-ortho PCBs (1 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.12 Mixture $^{13}$C-mono-ortho PCBs (1 µg/ml)

Use commercially available mixture $^{13}$C-mono-ortho PCBs (1 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.13 Mixture $^{13}$C-indicator PCBs (5 µg/ml)

Use commercially available mixture $^{13}$C-indicator PCBs (5 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.14 Mixture non-ortho PCBs (10 µg/ml)

Use commercially available mixture non-ortho PCBs (10 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.15 Mixture mono-ortho PCBs (10 µg/ml)

Use commercially available mixture mono-ortho PCBs (10 µg/ml) or prepare this standard solution manually by weighing the compounds, dissolving and diluting in a volumetric flask.

### B.2.16 Commercially available mixture indicator PCBs (10 µg/ml)

Use commercially available mixture indicator PCBs (10 µg/ml) or prepare this standard solution manually by weighing the compound, dissolving and diluting in a volumetric flask.

**B.2.17 Recovery standard DIOX-RS-100 (100 pg/µl $^{13}$C-1,2,3,4-TCDD and 100 pg/µl $^{13}$C-2,3,4,6,7,8-HxCDF in toluene)**

Pipet 200 µl from standard (B.2.1) and 200 µl from standard (B.2.2) in a 100 ml volumetric flask and fill with toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.18 Recovery standard DIOX-RS-1 (1,0 pg/µl $^{13}$C-1,2,3,4-TCDD, 1,0 pg/µl $^{13}$C-2,3,4,6,7,8-HxCDF in toluene)**

Dilute recovery standard DIOX-RS-100 to a final concentration of 1 pg/µl in toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.19 Recovery standard TCDD-RS-III (100 pg/µl $^{13}$C-1,2,3,4-TCDD in iso-octane)**

Pipet 200 µl from standard in a volumetric flask of 100 ml and fill with iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.20 Recovery standard TCDD-RS-VI (25 pg/µl $^{13}$C-1,2,3,4-TCDD in iso-octane)**

Dilute recovery standard TCDD-RS-III to a final concentration of 25 pg/µl in iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.21 Clean up standard CS-50 (50 pg/µl $^{37}Cl_4$-2,3,7,8-TCDD in toluene)**

Pipet 100 µl from standard in a volumetric flask of 100 ml and fill with toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.22 Clean up standard CS-1 (1,0 pg/µl $^{37}Cl_4$-2,3,7,8-TCDD in toluene)**

Dilute clean up standard CS-50 to a final concentration of 1,0 pg/µl in toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.23 Mixture non-ortho PCBs (NOP-500) (500 pg/µl in toluene)**

Pipet 50 µl from standard in a GC vial and add 950 µl and toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.24 Mixture PCDD/F and non-ortho PCBs (DIOXNOP) (20 pg/µl in toluene)**

Pipet 200 µl OCDF standard and 200 µl PCDD mixture and 200 µl from standard and 100 µl from standard and 100 µl from standard mixture non-ortho PCB into a 50 ml volumetric flask and dilute with toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.25 Mixture PCDD/F and non-ortho PCBs (DIOXNOP-1) (2,0 pg/µl in toluene)**

Dilute mixture PCDD/F and non-ortho PCBs to a final concentration of 2,0 pg/µl in toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.26 Mixture mono-ortho PCBs and indicator PCBs (MOPIP-500) (500 pg/µl in iso-octane)**

Pipet 50 µl mono-ortho PCBs mixture and 50 µl indicator PCBs mixture a GC vial and add 900 µl iso-octane Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.27 Mixture mono-ortho PCBs and indicator PCBs (MOPIP-25) (25 pg/µl in iso-octane)**

Pipet 250 µl mono-ortho PCBs mixture and 250 µl indicator PCBs mixture a volumetric flask of 100 ml and dilute with iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.28 Mixture mono-ortho PCBs and indicator PCBs (MOPIP-2.5) (2,5 pg/µl in iso-octane)**

Dilute mono-ortho PCBs and indicator PCBs (MOPIP-25) to a final concentration of 2,5 pg/µl in iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.29 Mixture mono-ortho PCBs and indicator PCBs (MOPIP-0.25) (0,25 pg/µl in iso-octane)**

Dilute mono-ortho PCBs and indicator PCBs (MOPIP-2,5) to a final concentration of 0,25 pg/µl in iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.30 Mixture $^{13}$C-PCDD/F and $^{13}$C-non-ortho PCBs ($^{13}$C-DIOXNOP) (50 pg/µl in toluene)**

Pipet 100 µl standard $^{13}$C-PCDD (B.2.4) and 100 µl standard $^{13}$C-PCDF and 100 µl non-ortho PCB mixture and add 1 700 µl toluene into a 4 ml sample vial . Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.31 Mixture $^{13}$C-PCDD/F and $^{13}$C-non-ortho PCBs ($^{13}$C-DIOXNOP-2) (0,10 pg/µl in toluene)**

Dilute $^{13}$C-DIOXNOP (50 pg/µl in toluene) to a final concentration of 0,10 pg/ul in toluene. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**B.2.32 Mixture $^{13}$C-mono-ortho PCBs and $^{13}$C-indicator PCBs ($^{13}$C-MOPIP-1) (50 pg/µl in iso-octane)**

Pipet 500 µl standard $^{13}$C-mono-ortho PCB mixture and 100 µl standard $^{13}$C-indicator PCB mixture into a volumetric flask of 10 ml. Dilute with iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

### B.2.33 Mixture $^{13}$C-mono-ortho PCBs and $^{13}$C-indicator PCBs ($^{13}$C-MOPIP-2) (2,0 pg/µl in iso-octane)

Pipet 500 µl standard $^{13}$C-mono-ortho PCB mixture and 100 µl standard $^{13}$C-indicator PCB mixture into a volumetric flask of 250 ml. Dilute with iso-octane. Store at room temperature in a tightly closed bottle. The solution is tenable under these conditions for at least 5 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

## B.3 GC-standard solutions

Standard dioxins and non-ortho PCBs mixtures

Prepare calibration mixtures according to Table B.2. Store prepared standard mixtures in a refrigerator at 4 °C (± 3 °C) in a tightly closed bottle. The solution is tenable under these conditions for at least 2 years if the weight is carefully controlled by the addition of solvent to replace any losses due to evaporation.

**Table B.2 — Calibration mixtures dioxins and non-ortho PCBs**

| | **$^{12}$C standard** µl | **$^{13}$C standard** µl | **Recovery standard** µl | **Clean up standard** µl | **Add solved** µl | **Concentration Dioxins** pg/µl | **Concentration non-ortho PCBs** pg/µl |
|---|---|---|---|---|---|---|---|
| 1 | 50 DIOXNOP -1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 650 toluene | 0,100 | 0,100 |
| 2 | 125 DIOXNOP -1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 575 toluene | 0,250 | 0,250 |
| 3 | 250 DIOXNOP -1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 450 toluene | 0,500 | 0,500 |
| 4 | 500 DIOXNOP -1 (B.2.25) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 200 toluene | 1,000 | 1,000 |
| 5 | 125 DIOXNOP (B.2.24) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 575 toluene | 2,500 | 2,500 |
| 6 | 250 DIOXNOP (B.2.24) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 450 toluene | 5,000 | 5,000 |
| 7 | 500 DIOXNOP (B.2.24) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 200 toluene | 10,00 | 10,00 |
| 8 | 100 NOP-500 (B.2.23) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 600 toluene | | 50,00 |
| 9 | 200 NOP-500 (B.2.23) | 100 $^{13}$C DIOXNOP (B.2.30) | 100 DIOX-RS-100 (B.2.17) | 100 CS-50 (B.2.21) | 500 toluene | | 100,0 |

All solutions contain 5,0 pg/µl $^{13}$C labelled internal standards, 5,0 pg/µl clean up standard $^{37}Cl_4$ 2,3,7,8-TCDD and 10,0 pg/µl recovery standard 1,2,3,4 $^{13}$C-TCDD and 2,3,4,6,7,8 $^{13}$C-HxCDF. Calibration mixture 1 to

calibration mixture 7 contain 0,100 pg/µl, 0,250 pg/µl, 0,500 pg/µl, 1,000 pg/µl, 2,500 pg/µl, 5,000 pg/µl and 10,00 pg/µl native dioxin congeners and non-ortho PCBs respectively. Calibration mixture 8 and calibration mixture 9 contain only native non-ortho PCBs at 50,00 pg/µl and 100,0 pg/µl respectively.

Preparation mono-ortho PCBs standard mixtures.

Standards mixtures of mono-ortho PCBs and indicator PCBs are prepared according to Table B.3.

**Table B.3 — Calibration mixtures mono-ortho PCBs and indicator PCBs**

| | **$^{12}$C standard** µl | **$^{13}$C standard** µl | **Recovery standard** µl | **Add solved** µl | **End volume** µl | **Concentration mono-ortho PCBs and indicator PCBs** pg/µl |
|---|---|---|---|---|---|---|
| 1 | 400 MOPIP-0.25 (B.2.29) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 510 iso-octane | 1 000 | 0,100 |
| 2 | 100 MOPIP-2.5 (B.2.28) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 810 iso-octane | 1 000 | 0,250 |
| 3 | 200 MOPIP-2.5 (B.2.28) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 710 iso-octane | 1 000 | 0,500 |
| 4 | 400 MOPIP-2.5 (B.2.28) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 510 iso-octane | 1 000 | 1,000 |
| 5 | 100 MOPIP-25 (B.2.27) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 810 iso-octane | 1 000 | 2,500 |
| 6 | 200 MOPIP-25 (B.2.27) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 710 iso-octane | 1 000 | 5,000 |
| 7 | 400 MOPIP-25 (B.2.27) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 510 iso-octane | 1 000 | 10,00 |
| 8 | 100 MOPIP-500 (B.2.26) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 810 iso-octane | 1 000 | 50,00 |
| 9 | 100 MOPIP-500 (B.2.26) | 40 $^{13}$C MOPIP-1 (B.2.32) | 50 TCDD-RS-III (B.2.19) | 710 iso-octane | 1 000 | 100,0 |

All solutions contain 2,0 pg/µl $^{13}$C-labelled internal standards, 5,0 pg/µl recovery standard 1,2,3,4 $^{13}$C TCDD and native mono-ortho PCBs and indicator PCBs at respectively 0,1 pg/µl, 025 pg/µl, 0,50 pg/µl, 1,0 pg/µl, 2,5 pg/µl, 5,0 pg/µl, 10,0 pg/µl, 50,0 pg/µl and 100,0 pg/µl.

# Annex C
(informative)

# Example of automated procedure

**Table C.1 — Automated sample clean up steps** *(1 of 2)*

| Step | Step time (min) | Cumulative (min) | Flow rate (ml/min) | Eluent | Wash | Elute | Tubing | Silica column | Alumina column | Carbon column | Collect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 5 | 10 | n-Hexane (10.2.1.2) | x | | | x | | | |
| 2 | 1 | 6 | 10 | n-Hexane (10.2.1.2) | x | | x | | | | |
| 3 | 3 | 9 | 10 | n-Hexane (10.2.1.2) | x | | | | x | | |
| 4 | 2 | 11 | 10 | n-Hexane (10.2.1.2) | x | | | | | x | |
| 5 | 25 | 36 | 10 | n-Hexane (10.2.1.2) | x | | | x | | | |
| 6 | 1,2 | 37,2 | 10 | Toluene (10.2.1.3) | x | | x | | | | |
| 7 | 4 | 41,2 | 10 | Toluene (10.2.1.3) | x | | | | | x | |
| 8 | 1,2 | 42,4 | 10 | Ethylacetate/ toluene=1+1 (11.2.2.5) | x | | x | | | | |
| 9 | 1 | 43,4 | 10 | Ethylacetate/ toluene=1+1 (11.2.2.5) | x | | | | | x | |
| 10 | 1,2 | 44,6 | 10 | Dichloromethane/ n-hexane=1+1 (11.2.2.6) | x | | x | | | | |
| 11 | 2 | 46,6 | 10 | Dichloromethane/ n-hexane=1+1 (11.2.2.6) | x | | | | | x | |

**Table C.1** *(2 of 2)*

| Step | Step time (min) | Cumulative (min) | Flow rate (ml/min) | Eluent | Wash | Elute | Tubing | Silica column | Alumina column | Carbon column | Collect |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 1,2 | 47,8 | 10 | n-Hexane (10.2.1.2) | x | | x | | | | |
| 13 | 3 | 50,8 | 10 | n-Hexane (10.2.1.2) | | | | | | x | |
| 14 | 7,4 | 58,2 | 5 | asp sample | | | | x | x | | |
| 15 | 20 | 78,2 | 10 | n-Hexane (10.2.1.2) | | x | | x | x | | |
| 16 | 1,2 | 79,4 | 10 | Dichloromethane/ n-hexane=1+1 (11.2.2.6) | x | | x | | | | |
| 17 | 12 | 91,4 | 10 | Dichloromethane/ n-hexane=1+1 (11.2.2.6) | | x | | | x | x | A-fraction |
| 18 | 1,2 | 92,6 | 10 | Ethylacetate/ toluene=1+1 (11.2.2.5) | x | | x | | | | |
| 19 | 0,4 | 93 | 10 | Ethylacetate/ toluene=1+1 (11.2.2.5) | | x | | | | x | |
| 20 | 1,2 | 94,2 | 10 | n-Hexane (10.2.1.2) | x | | x | | | | |
| 21 | 1 | 95,2 | 10 | n-Hexane (10.2.1.2) | | x | | | | x | |
| 22 | 1,2 | 96,4 | 10 | Toluene (10.2.1.3) | x | | x | | | | |
| 23 | 15 | 111,4 | 5 | Toluene (10.2.1.3) | | x | | | | x | B-fraction |
| 24 | 1 | 112,4 | 1 | n-Hexane (10.2.1.2) | x | | x | | | | |

# Annex D
(informative)

# Mass spectrometer (MS)

The ionization energy of the source is between 20 eV and 70 eV. The MS shall be capable of repetitively selectively monitoring (Single ion recording) at least 14 exact m/z's at high resolution (≥10 000) during a period of approximately one second.

For the dioxins and non-ortho PCBs the acquisition groups can for example be ordered as described in Table D.1. In Table D.2, Table D.3 and Table D.4 for the determination of the congeners of interest possible ions for monitoring are given.

For the mono-ortho PCBs and indicator PCBs the acquisition groups can for example be ordered as described in Table D.5. In Table D.6, Table D.7 and Table D.8 for the determination of the congeners of interest possible ions for monitoring are given.

These tables give an orientation for determination of PCDD/F and PCBs (dl-PCBs and ndl-PCBs) on a non-polar column. Different types of GC columns might be necessary for separation of critical congener pairs and/or confirmation and then require specific MS conditions. Different ions might be monitored to avoid disturbing effects e.g. from overlaying substances or column bleeding.

As an alternative for the measurement of indicator, a gas chromatograph-unit resolution mass spectrometer (GC-MS) may be used with respect to sensitivity and chromatographic resolution are followed.

**Table D.1 — Multi group analysis for dioxins and non-ortho PCBs; High and low masses give the mass range of the group function; Res = minimal resolution; start and end give function order in time.**

| Function group | Acquisition Type | High | Low | Res | Time (ms) | Start | End |
|---|---|---|---|---|---|---|---|
| 1. | SIR Voltage | 317 | 290 | 10 000 | 350 | A | B |
| 2. | SIR Voltage | 338 | 304 | 10 000 | 720 | B | C |
| 3. | SIR Voltage | 374 | 340 | 10 000 | 670 | C | D |
| 4. | SIR Voltage | 404 | 374 | 10 000 | 630 | D | E |
| 5. | SIR Voltage | 438 | 408 | 10 000 | 630 | E | F |
| 6. | SIR Voltage | 472 | 442 | 10 000 | 630 | F | G |

**Table D.2 — Function group 1 and function group 2; v=lock mass**

| Function group 1 (tetrachloro biphenyls) | | | | | Function group 2 (tetrachloro dioxins / furans and pentachloro biphenyls) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Mass | Time (ms) | Delay (ms) | LM | No. | Mass | Time (ms) | Delay (ms) | LM |
| 1. | 289,9224 | 60 | 20 | | 1. | 303,9016 | 40 | 20 | |
| 2. | 291,9194 | 60 | 10 | | 2. | 305,8987 | 40 | 10 | |
| 3. | 292,9825 | 50 | 10 | V | 3. | 315,9419 | 40 | 10 | |
| 4. | 301,9626 | 60 | 10 | | 4. | 316,9824 | 50 | 10 | V |
| 5. | 303,9597 | 50 | 10 | | 5. | 317,9389 | 40 | 10 | |
| | | | | | 6. | 319,8965 | 40 | 10 | |
| | | | | | 7. | 321,8936 | 40 | 10 | |
| | | | | | 8. | 323,8834 | 40 | 10 | |
| | | | | | 9. | 325,8804 | 40 | 10 | |
| | | | | | 10. | 327,8847 | 40 | 10 | |
| | | | | | 11. | 331,9368 | 40 | 10 | |
| | | | | | 12. | 333,9339 | 40 | 10 | |
| | | | | | 13. | 335,9236 | 40 | 10 | |
| | | | | | 14. | 337,9207 | 40 | 10 | |

**Table D.3 — Function group 3 and function group 4; v=lock mass**

| Function group 3 (pentachloro dioxins/furans and hexachloro biphenyls) | | | | | Function group 4 (hexachloro dioxins/furans and heptachloro biphenyls) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Mass | Time (ms) | Delay (ms) | LM | No. | Mass | Time (ms) | Delay (ms) | LM |
| 1. | 339,8597 | 40 | 20 | | 1. | 373,8208 | 60 | 20 | |
| 2. | 341,8568 | 40 | 10 | | 2. | 375,8178 | 60 | 10 | |
| 3. | 351,9000 | 40 | 10 | | 3. | 380,9760 | 50 | 10 | V |
| 4. | 353,8576 | 40 | 10 | | 4. | 383,8639 | 60 | 10 | |
| 5. | 353,8970 | 40 | 10 | | 5. | 385,8610 | 60 | 10 | |
| 6. | 355,8546 | 40 | 10 | | 6. | 389,8157 | 60 | 10 | |
| 7. | 357,8444 | 40 | 10 | | 7. | 391,8127 | 60 | 10 | |
| 8. | 359,8415 | 40 | 10 | | 8. | 401,8559 | 60 | 10 | |
| 9. | 366,9792 | 50 | 10 | V | 9. | 403,8529 | 60 | 10 | |
| 10. | 367,8949 | 40 | 10 | | 10. | | | | |
| 11. | 369,8919 | 40 | 10 | | 11. | | | | |
| 12. | 369,8846 | 40 | 10 | | 12. | | | | |
| 13. | 371,8817 | 40 | 10 | | 13. | | | | |

**Table D.4 — Function group 5 and function group 6; v=lock mass**

| Function group 5 (heptachloro dioxins/furans) | | | | | Function group 6 (octachloro dioxins/furans) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Mass | Time (ms) | Delay (ms) | LM | No. | Mass | Time (ms) | Delay (ms) | LM |
| 1. | 407,7818 | 60 | 20 | | 1. | 441,7428 | 60 | 20 | |
| 2. | 409,7788 | 60 | 10 | | 2. | 443,7399 | 60 | 10 | |
| 3. | 417,8253 | 60 | 10 | | 3. | 453,7830 | 60 | 10 | |
| 4. | 419,8220 | 60 | 10 | | 4. | 454,9728 | 50 | 10 | V |
| 5. | 423,7766 | 60 | 10 | | 5. | 455,7800 | 60 | 10 | |
| 6. | 425,7737 | 60 | 10 | | 6. | 457,7377 | 60 | 10 | |
| 7. | 430,9729 | 50 | 10 | V | 7. | 459,7348 | 60 | 10 | |
| 8. | 435,8169 | 60 | 10 | | 8. | 469,7779 | 60 | 10 | |
| 9. | 437,8140 | 60 | 10 | | 9. | 471,7750 | 60 | 10 | |

**Table D.5 —Multi group analysis for mono-ortho PCBs and indicator PCBs; High and low masses give the mass range of the group function; Res = minimal resolution; start and end give function order in time.**

| Function group | Acquisition Type | High | Low | Res | Time (ms) | Start | End |
|---|---|---|---|---|---|---|---|
| ***1.*** | SIR Voltage | 270 | 256 | 10 000 | 230 | A | B |
| ***2.*** | SIR Voltage | 304 | 290 | 10 000 | 310 | B | C |
| ***3.*** | SIR Voltage | 374 | 326 | 10 000 | 470 | C | D |
| ***4*** | SIR Voltage | 374 | 332 | 10 000 | 430 | D | E |
| ***5*** | SIR Voltage | 408 | 393 | 10 000 | 350 | E | F |

**Table D.6 — Function group 1 and function group 2; v=lock mass**

| Function group 1 (trichloro biphenyls) | | | | | Function group 2 (tetrachloro biphenyls) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Mass | Time (ms) | Delay (ms) | LM | No. | Mass | Time (ms) | Delay (ms) | LM |
| 1. | 255,9613 | 30 | 20 | | 1. | 289,9224 | 50 | 20 | |
| 2. | 257,9585 | 30 | 10 | | 2. | 291,9194 | 50 | 10 | |
| 3. | 268,0016 | 30 | 10 | | 3. | 292,9824 | 50 | 10 | V |
| 4. | 268,9824 | 50 | 10 | V | 4. | 301,9626 | 50 | 10 | |
| 5. | 269,9986 | 30 | 10 | | 5. | 303,9597 | 50 | 10 | |

**Table D.7 — Function group 3 and function group 4; v=lock mass**

| Function group 3 (penta /hexa-chloro biphenyls) | | | | | Function group 4 (hexachloro biphenyls) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No. | Mass | Time (ms) | Delay (ms) | LM | No. | Mass | Time (ms) | Delay (ms) | LM |
| 1. | 325,8804 | 40 | 20 | | 1. | 331,9368 | 50 | 20 | |
| 2. | 327,8776 | 40 | 10 | | 2. | 333,9339 | 50 | 10 | |
| 3. | 330,9792 | 50 | 10 | V | 3. | 342,9792 | 50 | 10 | V |
| 4. | 337,9207 | 40 | 10 | | 4. | 359,8415 | 50 | 10 | |
| 5. | 339,9178 | 40 | 10 | | 5. | 361,8386 | 50 | 10 | |
| 6. | 359,8415 | 40 | 10 | | 6. | 371,8817 | 50 | 10 | |
| 7. | 361,8386 | 40 | 10 | | 7. | 373,8788 | 50 | 10 | |
| 8. | 371,8817 | 40 | 10 | | | | | | |
| 9. | 373,8788 | 40 | 10 | | | | | | |

**Table D.8 — Function group 5; v=lock mass**

| Function group 5 (heptachloro biphenyls) | | | | |
|---|---|---|---|---|
| No. | Mass | Time (ms) | Delay (ms) | LM |
| 1. | 392,9760 | 50 | 20 | V |
| 2. | 393,8025 | 60 | 10 | |
| 3. | 395,7996 | 60 | 10 | |
| 4. | 405,8427 | 60 | 10 | |
| 5. | 407,8398 | 60 | 10 | |

# Annex E
(informative)

# Use of additional clean up after fractionation using a small multi-layer silica column

## E.1 Preparation of the silica mixtures

a) Bake the silica overnight (≥ 10 h) at 600 °C (silica);

b) Deactivate with 5 % of water by adding 5 g water to 95 g of the baked silica (silica/5 % water). Homogenize thoroughly, equilibration at least overnight;

c) Mix 30 ml of 1 mol/l NaOH (aqueous solution) to 100 g of baked silica (silica/NaOH). Homogenize thoroughly, equilibration at least overnight;

d) Mix 46 g of concentrated $H_2SO_4$ (96 %) to 100 g of baked silica. (silica/$H_2SO_4$), homogenize thoroughly, equilibration at least overnight.

## E.2 Preparation of the small multi-layer column

a) 10 cm chromatographic column, inner diameter ca. 1 cm;

b) filling of column bottom to top:

   1) 0,3 g silica/5 % water;

   2) 1 g silica/NaOH;

   3) 0,3 g silica/5 % water;

   4) 1 g silica/$H_2SO_4$;

   5) 0,3 g silica/5 % water.

c) pre-wash the multi-layer silica column with 40 ml of n-heptane;

d) put a flask of 25 ml at the outlet of the column.

Apply the fraction obtained at 11.2.4.2 or at 11.3.4.4 or at 11.5.8 (fraction A) or the fraction obtained at 11.2.4.3 or 11.3.4.5 or at 11.5.9 (fraction B), dissolved in 1 ml of n-heptane, to the column. The column is eluted with 20 ml n-heptane. Collect eluate and continue at section 11.2.4.3 measurement or further clean up.

# Bibliography

The method should obey "Commission Regulation (EC) No 152/2009 of 27 January 2009" laying down the methods of sampling and analysis for the official control of feed" (published in the Official Journal of the European Communities) [6]. Furthermore, for dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

[1] EN 15741, *Animal feeding stuffs — Determination of OC-pesticides and PCBs by GC/MS*

[2] EN 15742, *Animal feeding stuffs — Determination of OC-pesticides and PCBs by GC/ECD*

[3] EN ISO 6497, *Animal feeding stuffs — Sampling (ISO 6497:2002)*

[4] EPA method 3640 A, GPC-clean up

[5] Decision 2002/657/EC, Performance criteria, other requirements and procedures for analytical methods

[6] Commission Regulation (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feed

[7] Commission Regulation (EC) No 1831/2003 on additives for use in animal nutrition

[8] EPA method 1613, Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS

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