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Water quality — Determination of polychlorinated naphthalenes (PCN) — Method using gas chromatography (GC) and mass spectrometry (MS)

Qualité de l'eau — Détermination des naphtalènes polychlorés (PCN) — Méthode par chromatographie en phase gazeuse (CG) et spectrométrie de masse (SM)

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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

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

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Water quality — Determination of polychlorinated naphthalenes (PCN) — Method using gas chromatography (GC) and mass spectrometry (MS)

WARNING — Persons using this Technical Specification should be familiar with normal laboratory practice. This Technical Specification does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

Attention is drawn to any relevant national safety regulations. A number of PCN congeners have dioxin-like properties and are toxic chemicals. All work with PCNs requires the utmost care; the national safety measures which correspond to those for toxic substances shall be strictly followed.

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

1 Scope

This Technical Specification specifies a method for the determination of polychlorinated naphthalenes (PCNs), where "poly" means "mono" to "octa", in waters and waste waters [containing less than 2 g/l solid particulate material (SPM)] using high resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS).

NOTE 1 The congeners analysed by this method are listed in Table 1.

The working range of the method is 20 pg/l to 8 ng/l. The method is optimized for PCNs, but can be modified to include other coplanar compounds such as polychlorinated dioxins and furans (PCDDs/PCDFs) and dioxin-like tetra- to heptachlorinated biphenyls (dlPCBs). This method can be used to determine PCNs in other matrices (e.g. biota, sediments, air); however, additional clean-up steps and techniques can be necessary for samples with high organic loadings. Low resolution mass spectrometry (LRMS) and mass spectrometry–mass spectrometry (MS–MS) can be used.

NOTE 2 LRMS and MS–MS conditions are summarized in Annex A.

Both LRMS and MS–MS can be less selective than HRMS and there is a possibility of bias due to interfering compounds if these techniques are used.

The detection limits and quantification levels in this method are dependent on the level of interferences as well as instrumental limitations.

NOTE 3 The minimum levels (ML) in Table 4 are the levels at which the PCNs can typically be determined with no interferences present.

This method is performance based. The analyst is permitted to modify the method, e.g. to overcome interferences, provided that all performance criteria in this method are met.

NOTE 4 The requirements for establishing method validation or equivalency are given in Clause 9.

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.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8466 (all parts), *Water Quality — Calibration and evaluation of analytical methods and estimation of performance characteristics*

3 Terms, definitions, and abbreviated terms

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

3.1 Terms and definitions

3.1.1
analyte
substance to be determined

EXAMPLE A polychlorinated naphthalene (PCN) congener tested for by the method specified in this Technical Specification.

Table 1 — PCNs determined by this method

PCN No. (Reference[4])	Chlorine substitution	CAS Registry No.
Total MonoCNs	Mono congener total	
2	2-MonoCN	91–58–7
Total DiCNs	Di congener total	
6	1,5-DiCN	1825–30–5
Total TriCNs	Tri congener total	
13	1,2,3-TriCN	50402–52–3
Total TetraCNs	Tetra congener total	
27	1,2,3,4-TetraCN	20020–02–4
28	1,2,3,5-TetraCN	53555–63–8
36	1,2,5,6-TetraCN	67922–22–9
42	1,3,5,7-TetraCN	53555–64–9
46	1,4,5,8-TetraCN	3432–57–3
48	2,3,6,7-TetraCN	34588–40–4
Total PentaCNs	Penta congener total	
49	1,2,3,4,5-PentaCN	67922–25–2
50	1,2,3,4,6-PentaCN	67922–26–3
52/60	1,2,3,5,7-/	53555–65–0/
	1,2,4,6,7-PentaCN	150224–17–2
53	1,2,3,5,8-PentaCN	150224–24–1
54	1,2,3,6,7-PentaCN	150224–16–1
Total HexaCNs	Hexa congener total	
63	1,2,3,4,5,6-HexaCN	58877–88–6
64/68	1,2,3,4,5,7-/	67922–27–4/
	1,2,3,5,6,8-HexaCN	103426–95–5

Table 1 *(continued)*

PCN No. (Reference[4])	Chlorine substitution	CAS Registry No.
66/67	1,2,3,4,6,7-/	103426–96–6
	1,2,3,5,6,7-HexaCN	103426–97–7
69	1,2,3,5,7,8-HexaCN	103426–94–4
70	1,2,3,6,7,8-HexaCN	17062–87–2
71/72	1,2,4,5,6,8-/	90948–28–0
	1,2,4,5,7,8-HexaCN	103426–92–2
Total HeptaCNs	Hepta congener total	
73	1,2,3,4,5,6,7-HeptaCN	58863–14–2
74	1,2,3,4,5,6,8-HeptaCN	58863–15–3
75 (OctaCN)	1,2,3,4,5,6,7,8-OctaCN	2234–13–1
Note: PCN numbering nomenclature is detailed in Reference[4]. The CAS Registry Number is a unique numerical identifier assigned by Chemical Abstracts Service (CAS) to every chemical substance described in the open scientific literature.		

3.1.2
calibration standard
solution prepared from a secondary standard or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration

[SOURCE: ISO 17858:2007, 3.1.2 — modified]

3.1.3
calibration verification standard
VER
midpoint calibration standard that is used to verify calibration

[SOURCE: ISO 17858:2007, 3.1.3]

3.1.4
congener
member of the same kind, class or group

[SOURCE: ISO 17858:2007, 3.1.5]

EXAMPLE Any one of the 75 individual PCNs.

3.1.5
critical pair
pair of isomers that must be separated to a predefined degree (e.g. 50 % valley) to ensure chromatographic separation meets minimum quality criteria

[SOURCE: ISO 17858:2007, 3.1.6, modified — "50 %" replaces "25 %".]

3.1.6
dioxin-like isomer
PCN for which a relative potency to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been calculated see Table 2

Table 2 — Examples of relative potencies[16]

Compound	REP
1,3,5,7CN(42)	0,000 01
1,2,5,6CN(36)	0,000 01

Table 2 *(continued)*

Compound	REP
1,2,3,5CN(28)	0,000 001
1,2,3,4CN(27)	0,000 01
2,3,6,7CN(48)	0,001
1,4,5,8CN(46)	0,000 000 1
1,2,3,5,7CN/1,2,4,6,7CN(52/60)	0,000 1
1,2,3,4,6CN(50)	0,000 1
1,2,3,6,7CN(54)	0,000 1
1,2,3,5,8CN(53)	0,000 01
1,2,3,4,5CN(49)	0,000 001
1,2,3,4,6,7CN/1,2,3,5,6,7CN(66/67)	0,01
1,2,3,4,5,7CN/1,2,3,5,6,8CN(64/68)	0,001
1,2,3,5,7,8CN(69)	0,001
1,2,4,5,6,8CN/1,2,4,5,7,8CN(71/72)	0,001
1,2,3,4,5,6CN(63)	0,001
1,2,3,6,7,8CN(70)	0,01
1,2,3,4,5,6,7CN(73)	0,01
1,2,3,4,5,6,8CN(74)	0,01
1,2,3,4,5,6,7,8CN(75)	0,1

3.1.7
homologue group
complete group of isomers

EXAMPLE Tetrachloronaphthalenes.

[SOURCE: ISO 17858:2007, 3.1.8 — modified]

3.1.8
isotope dilution
method using labelled (usually ^{13}C) internal standards to correct for losses during sample preparation and analysis

[SOURCE: ISO 17858:2007, 3.1.9, modified — "^{13}C" replaces "$^{13}C_{12}$".]

3.1.9
method blank
aliquot of reagent water free of analytes treated exactly as a sample through the complete analytical procedure including extraction, clean-up, identification and quantification including all relevant reagents and materials

[SOURCE: ISO 17858:2007, 3.1.11, modified — "free of analytes" replaces "that is".]

3.1.10
recovery standard
$^{13}C_{10}$-labelled PCN added before injection into the GC, to monitor variability of instrument response, and determine recovery of surrogate/internal standards

Note 1 to entry: An alternate compound with similar properties can be used if a labelled PCN standard is not available.

3.1.11
solid particulate material
SPM
suspended solids
non dissolved particle matter present in the sample

3.1.12
toxic equivalent factor
TEF
relative toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)

[SOURCE: ISO 17858:2007, definition 3.1.17]

3.1.13
toxic equivalent quantity
TEQ
sum of toxic equivalents of each individual congener

[SOURCE: ISO 17858:2007, 3.1.18]

3.1.14
surrogate standard
$^{13}C_{10}$-labelled PCN added to the sample prior to analysis and used to correct for losses of the PCN analytes during sample extraction or clean-up

Note 1 to entry: Surrogate standards have the same chemical formula and structure as the analyte of interest.

3.1.15
internal standard
$^{13}C_{10}$-labelled PCN or analogue added to the sample prior to analysis and used to correct for losses of the PCN analytes during sample extraction or clean-up

Note 1 to entry: Internal standards do not have the same structure as the analyte of interest but can or may not have the same chemical formula.

3.2 Abbreviated terms

AR	analytical reagent
CRM	certified reference material
GC–MS	gas chromatography–mass spectrometry
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
HRGC	high resolution gas chromatography
HRMS	high resolution mass spectrometry
IPR	initial precision and recovery
LRMS	low-resolution mass spectrometry
MDL	method detection limit
ML	minimum level
PAR	precision and recovery
PCB	polychlorinated biphenyl
PCDD/PCDF	polychlorinated dibenzo-*p*-dioxin/dibenzofuran
PCN	polychlorinated naphthalene
PFK	perfluorokerosene
PLE	pressurized liquid extractor
SIM	selected ion monitoring
SMS	spiked matrix samples
SPE	solid-phase extraction
SPM	solid particulate material
TEF	toxic equivalent factor
TEQ	toxic equivalent quantity
VER	calibration verification standard

4 Principle

4.1 Extraction

4.1.1 Stable isotopically labelled analogues of PCNs (diluted in a suitable solvent such as 2-propanone) are spiked into a ~1 l aqueous sample. Sample size can be adjusted in order to meet required detection limits and data quality objectives. Where available, a minimum of one labelled standard per homologue group should be used and the sample extracted using the procedures as specified in 4.1.2 or 4.1.3.

4.1.2 Samples containing no visible particles are extracted using liquid/liquid extraction or by solid phase extraction (SPE) cartridge or disk. The extract is concentrated for clean-up.

4.1.3 Samples containing visible particles are vacuum filtered through a glass fibre filter. The filter is extracted in a Soxhlet extractor or a pressurized liquid extractor (PLE). The filtrate is extracted in a separating funnel. The extract is concentrated and combined with the Soxhlet extract prior to clean-up. Alternatively, the sample is vacuum filtered through a solid phase extraction (SPE) disk or cartridge. The disk is eluted with suitable solvent mixtures or extracted in a Soxhlet or a PLE, and the extract is concentrated for clean-up.

NOTE Other solvents and extraction techniques can be substituted, provided that all the performance criteria are met.

4.2 Clean-up

After extraction, sample extracts are cleaned to remove interfering components. Sample clean-up procedures may include washes with acid or base, gel permeation, silica, Florisil[1)] and activated carbon chromatography. Due to the large number of potential interfering compounds, efforts should be taken to ensure unique identification and accurate quantification of as many PCN congeners as possible.

4.3 Identification and quantification

An individual PCN is identified by comparing the GC retention time and ion abundance ratio of two exact masses monitored (see Table 3) with the corresponding retention time of a labelled internal standard (isotope dilution) and the theoretical or acquired ion abundance ratio of the two exact masses. The isomers and congeners for which there are no labelled analogues (internal standard method) are identified when retention times or relative retention times and ion abundance ratios agree within predefined limits.

NOTE Resolution of greater than or equal to 10 000 is recommended. High resolution gas chromatography–high resolution mass spectrometry (HRGC–HRMS) at a resolution of greater than or equal to 10 000 is at present required to achieve adequate sensitivity and selectivity, and to allow the use of some ^{13}C labelled standards. Resolutions of less than 10 000 can be used for specific analytes groups (PCBs, PCNs) where the matrix and potential interferences such as chlordane and related compounds are well characterized.

Table 3 — Congener function groups and ions

Function group	Quantitation ions	Compound	Dwell	Delay	Theoretical isotopic ratio	Acceptable range
	m/z		ms	ms		
0	162,0236[a], 164,0208	MonoCNs	50	10	0,33	0,17 to 0,48
	195,9847[a], 197,9818	DiCNs	50	10	0,65	0,50 to 0,80
	180,9888	PFK Lock Mass	30	10		
1	229,9457[a], 231,9428	TriCNs	50	10	1,02	0,87 to 1,17
	265,9038[a], 263,9067	TetraCNs	50	10	1,30	1,11 to 1,5
	275,9373[a], 273,9402	$^{13}C_{10}$-TetraCNs	25	10	1,30	1,11 to 1,5
	268,9824	PFK Lock Mass	30	10		

[a] Most abundant ion.

[b] Injection standard.

NOTE When the availability of ^{13}C-labeled PCN standards is limited, ^{13}C-labeled PCB standards can be used as injection standards

1) Florisil is the trade name of a product supplied by US Silica Co. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Table 3 *(continued)*

Function group	Quantitation ions	Compound	Dwell	Delay	Theoretical isotopic ratio	Acceptable range
	m/z		ms	ms		
2	299,8648[a], 297,8677	PentaCNs	50	10	1,62	1,38 to 1,86
	309,8983[a], 307,9013	$^{13}C_{10}$-PentaCNs	25	10	1,62	1,38 to 1,86
	292,9824[b]	PFK Lock Mass	30	10		
3	333,8258[a], 335,8229	HexaCNs	50	10	1,23	1,01 to 1,45
	343,8594[a] 345,8564	$^{13}C_{10}$-HexaCNs	25	10	1,23	1,01 to 1,45
	337,9207[a],335,9236	$^{13}C_{12}$-PentaCB [b]	25	10	1,62	1,38 to 1,86
	342,9792	PFK Lock Mass	30	10		
4	367,7868[a], 369,7839	HeptaCNs	50	10	1,02	0,87 to 1,17
	377,8204[a], 379,8174	$^{13}C_{10}$HeptaCNs	50	10	1,15	0,98 to 1,32
	380,9760	PFK Lock Mass	30	10		
5	403,7449[a], 401,7479	OctaCN	50	10	1,15	0,98 to 1,32
	413,7785[a], 411,7814	$^{13}C_{10}$-OctaCN	25	10	1,15	0,98 to 1,32
	405,8428[a],407,8398	$^{13}C_{12}$-HeptaCB [b]	25	10	1,02	0,87 to 1,17
	392,9760	PFK Lock Mass	30	10		

[a] Most abundant ion.

[b] Injection standard.

NOTE When the availability of ^{13}C-labeled PCN standards is limited, ^{13}C-labeled PCB standards can be used as injection standards

4.4 Quality

The quality of the analysis is ensured through reproducible calibration and testing of the extraction, clean-up, and GC–MS systems. Interferences, biases and limitations should be determined and identified for each target analyte through intercalibration (round-robin) studies, certified reference materials (CRMs) and spiked matrix samples (SMSs). A series of quality control (QC) samples (CRM, SMS) should be analysed with each set of samples and monitored through control charting or other quality review procedures.

5 Contamination and interferences

5.1 Reagents. Solvents, reagents, laboratory-ware, and other sample processing hardware can yield artefacts or elevated baselines causing misinterpretation of chromatograms. Check reagents for potential interfering compounds and clean and check laboratory-ware to ensure that analytes of interest are not present. Specific selection of reagents and purification may be required. When a clean reference matrix that simulates the sample matrix under test is not available, use reagent water (6.6) or a matrix that most closely resembles the sample.

5.2 Clean laboratory-ware, to meet the method blank requirements of this method (9.4).

An example of a cleaning procedure follows.

Dismantle laboratory-ware with removable parts, particularly separating funnels with fluoropolymer stopcocks, prior to detergent washing. Rinse laboratory-ware with solvent and wash with a detergent solution as soon after use as is practical. Sonication of laboratory-ware containing a detergent solution for approximately 30 s may aid in cleaning.

After detergent washing, rinse laboratory-ware immediately with hot tap water. The tap water rinse shall be followed by solvent rinse or soak, using a suitable solvent (6.3) to remove contaminants. For known contaminated laboratory-ware, use toluene as a final rinse or soak.

Number each piece of re-usable laboratory-ware or minimally identify each set of specific type of laboratory-ware (e.g. Soxhlet extractors, round-bottomed flasks) to associate that specific laboratory-ware with the processing of a particular sample or set of samples. This practice assists the laboratory in tracking possible sources of contamination for individual samples, identifying laboratory-ware associated with highly contaminated samples that may require extra cleaning, and determining when laboratory-ware shall be discarded.

IMPORTANT — Proper cleaning of laboratory-ware is extremely important, because laboratory-ware can contaminate the samples, but can also remove the analytes of interest by surface adsorption if the surface is activated during the cleaning procedure. Glassware can be checked for contamination by analysing solvent rinses.

Demonstrate that all materials used in the analysis are free from interferences by running reference matrix method blanks initially and with each sample batch (to a maximum of 20 samples); (see 9.4, 14.5).

The reference matrix shall simulate, as closely as possible, the sample matrix under test. Ideally, the reference matrix shall not contain analytes in detectable amounts, but shall contain matrix compounds and potential interferents in the concentrations expected to be found in the samples to be analysed.

NOTE Interferences co-extracted from samples can vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds, including PCBs of higher degrees of ^{37}Cl substitution, dibenzofurans of lower degrees of ^{37}Cl substitution, chlordane and related compounds and labelled dibenzo-*p*-dioxins can be present at concentrations orders of magnitude higher than the PCNs being analysed. Because the levels of PCNs are measured by this method are typically lower than these compounds, the elimination of interferences is essential. The example clean-ups given in Clause 13 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PCNs at the levels shown in Table 4.

Table 4 — Suggested quantification relationships

PCN	Quantification reference	Minimum level[a]	Minimum level[a]
		Waters (pg/l)	Extract (pg/µl)
Total MonoCNs	$^{13}C_{10}$-PCN 42	20	1,0
2-MonoCN (2)	$^{13}C_{10}$-PCN 42	20	1,0
Total DiCNs	$^{13}C_{10}$-PCN 42	20	1,0
1,5-DiCN (6)	$^{13}C_{10}$-PCN 42	20	1,0
Total TriCNs	$^{13}C_{10}$-PCN 42	20	1,0
1,2,3-TriCN (13)	$^{13}C_{10}$-PCN 42	20	1,0
Total TetraCNs	Mean of $^{13}C_{10}$-PCN 27/42	20	1,0
1,2,3,4-TetraCN (27)	$^{13}C_{10}$-PCN 27	20	1,0
1,2,3,5-TetraCN (28)	Mean of $^{13}C_{10}$-PCN 27/42	20	1,0
1,2,5,6-TetraCN (36)	Mean of $^{13}C_{10}$-PCN 27/42	20	1,0
1,3,5,7-TetraCN (42)	$^{13}C_{10}$-PCN 42	20	1,0
1,4,5,8-TetraCN (46)	Mean of $^{13}C_{10}$-PCN 27/42	20	1,0
2,3,6,7-TetraCN (48)	Mean of $^{13}C_{10}$-PCN 27/42	20	1,0

[a] The minimum level ML for each analyte is defined as the level for which the entire analytical system shall give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specific sample masses/volumes and clean-up procedures have been used. i.e. based on 1 l of sample.

NOTE Minimum levels are given for guidance only. Mean refers to mean recovery of both internal standards.

Table 4 *(continued)*

PCN	Quantification reference	Minimum level[a]	Minimum level[a]
		Waters (pg/l)	Extract (pg/µl)
Total PentaCNs	$^{13}C_{10}$-PCN 52	20	1,0
1,2,3,4,5-PentaCN (49)	$^{13}C_{10}$-PCN 52	20	1,0
1,2,3,4,6-PentaCN (50)	$^{13}C_{10}$-PCN 52	20	1,0
1,2,3,5,7-PentaCN (52)/ 1,2,4,6,7-PentaCN (60)	$^{13}C_{10}$-PCN 52	20	1,0
1,2,3,5,8-PentaCN (53)	$^{13}C_{10}$-PCN 52	20	1,0
1,2,3,6,7-PentaCN (54)	$^{13}C_{10}$-PCN 52	20	1,0
Total HexaCNs	$^{13}C_{10}$-PCN 64	20	1,0
1,2,3,4,5,6-HexaCN (63)	$^{13}C_{10}$-PCN 64	20	1,0
1,2,3,4,5,7-HexaCN (64)/ 1,2,3,5,6,8-HexaCN (68)	$^{13}C_{10}$-PCN 64	20	1,0
1,2,3,4,6,7-HexaCN (66)/ 1,2,3,5,6,7-HexaCN (67)	$^{13}C_{10}$-PCN 64	20	1,0
1,2,3,5,7,8-HexaCN (69)	$^{13}C_{10}$-PCN 64	20	1,0
1,2,3,6,7,8-HexaCN (70)	$^{13}C_{10}$-PCN 64	20	1,0
1,2,4,5,6,8-HexaCN (71)/ 1,2,4,5,7,8-HexaCN (72)	$^{13}C_{10}$-PCN 64	20	1,0
Total HeptaCNs	$^{13}C_{10}$-PCN 75	20	1,0
1,2,3,4,5,6,7-HeptaCN (73)	$^{13}C_{10}$-PCN 75	20	1,0
1,2,3,4,5,6,8-HeptaCN (74)	$^{13}C_{10}$-PCN 75	20	1,0
1,2,3,4,5,6,7,8-OctaCN (75)	$^{13}C_{10}$-PCN 75	20	1,0

[a] The minimum level ML for each analyte is defined as the level for which the entire analytical system shall give a recognizable signal and acceptable calibration point. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specific sample masses/volumes and clean-up procedures have been used. i.e. based on 1 l of sample.

NOTE Minimum levels are given for guidance only. Mean refers to mean recovery of both internal standards.

6 Reagents and standards

If not stated otherwise, use reagent grade chemicals.

6.1 pH adjustment and back-extraction

6.1.1 Water, H_2O according to grade 3 in ISO 3696.

6.1.2 Potassium hydroxide solution, dissolve 20 g of potassium hydroxide (KOH) in 100 ml of water.

6.1.3 Sulfuric acid, $\rho(H_2SO_4)$ = 1,84 mg/l.

6.1.4 Hydrochloric acid, c(HCl) = 6 mol/l.

6.1.5 Sodium chloride solution, dissolve 5 g of sodium chloride (NaCl) in 100 ml of water.

6.1.6 Sodium thiosulfate, $Na_2S_2O_3$.

6.2 Reagents for drying and evaporation

6.2.1 Sodium sulfate, Na_2SO_4, granular, anhydrous, baked at 300 °C for 24 h minimum, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw cap that prevents moisture from entering.

If, after heating, the sodium sulfate develops a noticeable greyish cast (due to the presence of carbon in the crystal matrix), discard that batch of reagent as it is not suitable for use. Rinse with about 20 ml of dichloromethane (6.3.6) per gram of Na_2SO_4 or extract with dichloromethane (6.3.6) if background contamination is detected.

6.2.2 Prepurified nitrogen, N_2, volume fraction 99,999 %.

6.3 Solvents for extraction and clean-up, in glass, pesticide quality, free of interferences.

6.3.1 2-Propanone (Acetone), C_3H_6O.

6.3.2 Toluene, C_7H_8.

6.3.3 Cyclohexane, C_6H_{12}.

6.3.4 Hexane, C_6H_{14}.

6.3.5 Methanol, CH_3OH.

6.3.6 Dichloromethane, CH_2Cl_2.

6.3.7 Diethyl ether, $C_4H_{10}O$.

6.3.8 Ethanol, C_2H_6O.

6.3.9 Nonane, C_9H_{20}, distilled.

6.4 Gel permeation chromatography (GPC) calibration

6.4.1 GPC calibration solution, containing 300 mg/ml of corn oil, 15 mg/ml of *bis*(2-ethylhexyl) phthalate ($C_{24}H_{38}O_4$), 1,4 mg/ml of pentachlorophenol (C_6Cl_5OH), 0,1 mg/ml of perylene, ($C_{20}H_{12}$), and 0,5 mg/ml of sulfur (S).

6.5 Adsorbents for sample clean-up

6.5.1 Silica, 70 µm to 230 µm.

Prepare each type of silica at least every 2 weeks.

6.5.1.1 Activated silica

Silica (6.5.1)baked at 180 °C for a minimum of 1 h, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw cap that prevents moisture from entering.

6.5.1.2 Acid silica

To prepare 30 % mass fraction acid silica, thoroughly mix 44,0 g of sulfuric acid (6.1.3) with 100 g of activated silica (6.5.1.1) in a clean container. Break up aggregates with a stirring rod until a uniform

mixture is obtained. Store in a bottle with a fluoropolymer-lined screw cap. Prepare 22 % acid silica and 44 % acid silica in a similar manner using 29 g and 80 g of sulfuric acid, respectively.

6.5.1.3 Basic silica

Thoroughly mix 30 g of 1 mol/l potassium hydroxide solution (6.1.2) with 100 g of activated silica in a clean container. Break up aggregates with a stirring rod until a uniform mixture is obtained. Store in a bottle with a fluoropolymer-lined screw cap.

6.5.1.4 **Potassium silicate**, 36 % mass fraction.

Dissolve 56 g of high purity potassium hydroxide (6.1.2) in 300 ml of methanol (6.3.5) in a 750 ml flat-bottom flask. Add 100 g of silica (6.5.1) and a stirring bar, and stir on a hotplate at 60 °C to 70 °C for 1 h to 2 h. Decant the liquid and rinse the potassium silicate twice with 100 ml portions of methanol, followed by a single rinse with 100 ml of dichloromethane (6.3.6). Spread the potassium silicate on solvent-rinsed aluminium foil and dry for 2 h to 4 h in a hood. Activate overnight at 200 °C to 250 °C. Store in a bottle with a fluoropolymer-lined screw cap.

6.5.1.5 Diatomaceous earth

6.5.2 Carbon (graphitized)

6.5.2.1 Activated carbon

Thoroughly mix 9,0 g of graphitized carbon (6.5.2) packing material and 41,0 g of diatomaceous earth (6.5.1.4) to produce a mass fraction of 18 % of the mixture. Activate the mixture at 130 °C for a minimum of 6 h. Store in a desiccator.

6.5.3 Florisil[2], 70 µm to 250 µm. Activate in an oven above 130 °C for a minimum of 24 h. Use as soon as possible after removal from oven.

Activity of Florisil[2] may be dependent on relative humidity.

Prepare freshly for each use.

6.5.4 Silver nitrate–silica, (10 % mass fraction) for elimination of organosulfur and organohalogen compounds, made of silver nitrate ($AgNO_3$) analytical reagent (AR) grade or equivalent and activated silica (6.5.1.1).

Dissolve 10 g of silver nitrate in 40 ml water, add in portions 90 g activated silica and shake until the mixture is homogeneous. Allow to stand for 30 min. Transfer the mixture to a drying oven pre-heated to 70 °C and heat from 70 °C to 125 °C over a 2 h period. Activate at 125 °C for at least 10 h. Store the mixture in a brown glass bottle.

Prepare freshly for each use.

6.6 Blank reference matrices

Matrices in which PCNs and interfering compounds are not detected by this method, e.g. reagent water, bottled water purchased locally, HPLC grade water or water prepared by passage through activated carbon.

2) Florisil is the trade name of a product supplied by US Silica Co. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.7 Standard solutions

Purchase standard solutions as final working or calibration solutions or mixtures with certification indicating their purity, concentration, and authenticity. Alternatively, prepare standard solutions from materials of known purity and composition.

NOTE 1 If the chemical purity is 98 % mass fraction or greater, the mass can be used without correction to compute the concentration of analytes in the standard.

When not being used, store standards in the dark in sealed ampoules or screw-capped vials with fluoropolymer lined caps. Check the concentrations regularly so that solvent loss by evaporation can be detected. If solvent loss has occurred, replace the solution.

NOTE 2 Standard preparation (6.7 to 6.13) and Tables 3 and 4 give examples of a standard scheme that are acceptable. Other concentrations and spiking schemes can be used provided the performance criteria of the method are met.

Check stock standard solutions for signs of degradation prior to the preparation of calibration or performance test standards.

Use certified reference standards and solutions to determine the accuracy of calibration standards, if available.

6.8 Precision and recovery stock solution

The precision and recovery (PAR) stock solution should contain PCNs at the concentrations shown in Table 5. When diluted to the final concentration, the solution is referred to as the PAR standard solution (6.12). If possible, obtain this solution from an alternate supplier. This enables an ongoing verification and validation of the calibration (6.11) and labelled spiking solutions (6.9).

Table 5 — Suggested concentration of PCNs in stock and spiking solutions

PCNs	Labelled compound stock solution	PAR stock solution	Recovery standard spiking solution
	ng/ml	ng/ml	ng/ml
2 MonoCN (2)	—	10	—
1,5C DiN (6)	—	10	—
1,2,3 TriCN (13)	—	10	—
1,3,5,7 TetraCN (42)	—	10	—
1,2,5,6 TetraCN (36)	—	10	—
1,2,3,5 TetraCN (28)	—	10	—
1,2,3,4 TetraCN (27)	—	10	—
2,3,6,7 TetraCN (48)	—	10	—
1,4,5,8 TetraCN (46)	—	10	—
1,2,3,5,7 PentaCN (52)/1,2,4,6,7 PentaCN (60)	—	10	—
1,2,3,4,6 PentaCN (50)	—	10	—
1,2,3,6,7 PentaCN (54)	—	10	—
1,2,3,5,8 PentaCN (53)	—	10	—
1,2,3,4,5 PentaCN (49)	—	10	—

[a] Injection standard.

NOTE When the availability of 13C-labeled PCN standards is limited, 13C-labeled PCB standards can be used as injection standards.

Table 5 *(continued)*

PCNs	Labelled compound stock solution	PAR stock solution	Recovery standard spiking solution
	ng/ml	ng/ml	ng/ml
1,2,3,4,6,7 HexaCN/1,2,3,5,6,7 HexaCN (66/67)	—	10	—
1,2,3,4,5,7 HexaCN/1,2,3,5,6,8 HexaCN (64/68)	—	10	—
1,2,3,5,7,8 HexaCN (69)	—	10	—
1,2,4,5,6,8 HexaCN/1,2,4,5,7,8 HexaCN (71/72)	—	10	—
1,2,3,4,5,6 HexaCN (63)	—	10	—
1,2,3,6,7,8 HexaCN (70)	—	10	—
1,2,3,4,5,6,7 HeptaCN (73)	—	10	—
1,2,3,4,5,6,8 HeptaCN (74)	—	10	—
Octa CN (75)	—	10	—
$^{13}C_{10}$-1,2,3,4 TetraCN (27)	100	—	—
$^{13}C_{10}$-1,3,5,7 TetraCN (42)	100	—	—
$^{13}C_{10}$-1,2,3,5,7 PentaCN (52)	100	—	—
$^{13}C_{10}$-1,2,3,4,5,7 HexaCN (64)	100	—	—
$^{13}C_{10}$-Octa CN (75)	100	—	—
$^{13}C_{10}$- 2,3,4,4',5-pentaCB (114)[a]	—	—	100
$^{13}C_{10}$-2,3,3',4,4',5,5'-heptaCB (189) [a]	—	—	100

[a] Injection standard.

NOTE When the availability of 13C-labeled PCN standards is limited, 13C-labeled PCB standards can be used as injection standards.

6.9 Surrogate spiking solution

Prepare the surrogate spiking solution to contain the labelled analytes in nonane at the concentrations shown in Table 5.

Dilute a sufficient volume of the labelled compound solution with 2-propanone (6.3.6) to prepare a diluted spiking solution.

Each sample requires 1,0 ml of the diluted solution, but no more solution should be prepared than can be used within 1 d.

6.10 Recovery standard(s)

Prepare the recovery standard solution to contain $^{13}C_{10}$-labelled compounds(s), or alternate compound(s) with similar properties, in nonane at the concentration shown in Table 5 for the specific groups of compounds analysed.

6.11 Calibration standards

Combine the solutions in 6.8 to 6.10 to produce at least five calibration solutions shown in Table 6 in nonane (6.3.9).

These solutions permit the relative response (labelled to native) and response factor to be measured as a function of concentration. A minimum of five solutions over the calibration range of the method should

be used. Each concentration step should range between 3 times and 10 times the lower concentrated standard.

Use the mid-point standard for calibration verification.

Table 6 — Suggested calibration standard concentrations

PCN congener	CS1	CS2	CS3	CS4	CS5
	µg/l	µg/l	µg/l	µg/l	µg/l
2-MonoCN (2)	1	5	20	100	400
1,5C-DiCN (6)	1	5	20	100	400
1,2,3-TriCN (13)	1	5	20	100	400
1,3,5,7-TetraCN (42)	1	5	20	100	400
1,2,5,6-TetraCN (36)	1	5	20	100	400
1,2,3,5-TetraCN (28)	1	5	20	100	400
1,2,3,4-TetraCN (27)	1	5	20	100	400
2,3,6,7-TetraCN (48)	1	5	20	100	400
1,4,5,8-TetraCN (46)	1	5	20	100	400
1,2,3,5,7-PentaCN (52)/1,2,4,6,7-PentaCN (60)	2	10	40	200	800
1,2,3,4,6-PentaCN (50)	1	5	20	100	400
1,2,3,6,7-PentaCN (54)	1	5	20	100	400
1,2,3,5,8-PentaCN(53)	1	5	20	100	400
1,2,3,4,5-PentaCN(49)	1	5	20	100	400
1,2,3,4,6,7-HexaCN/1,2,3,5,6,7-HexaCN (66/67)	2	10	40	200	800
1,2,3,4,5,7-HexaCN/1,2,3,5,6,8-HexaCN (64/68)	2	10	40	200	800
1,2,3,5,7,8-HexaCN (69)	1	5	20	100	400
1,2,4,5,6,8-HexaCN/1,2,4,5,7,8-HexaCN (71/72)	2	10	40	200	800
1,2,3,4,5,6-HexaCN (63)	1	5	20	100	400
1,2,3,6,7,8-HexaCN (70)	1	5	20	100	400
1,2,3,4,5,6,7-HeptaCN (73)	1	5	20	100	400
1,2,3,4,5,6,8-HeptaCN (74)	1	5	20	100	400
Octa CN (75)	1	5	20	100	400
$^{13}C_{10}$-1,2,3,4-TetraCN (27)	50	50	50	50	50
$^{13}C_{10}$-1,3,5,7-TetraCN (42)	50	50	50	50	50
$^{13}C_{10}$-1,2,3,5,7-PentaCN (52)	50	50	50	50	50
$^{13}C_{10}$-1,2,3,4,5,7-HexaCN (64)	50	50	50	50	50
$^{13}C_{10}$-octa CN (75)	50	50	50	50	50
$^{13}C_{10}$- PCB 114	50	50	50	50	50
$^{13}C_{10}$-PCB189	50	50	50	50	50

6.12 Precision and recovery standard solution

Use this standard solution for determination of initial and ongoing PAR. For each sample matrix, dilute the required amount the PAR stock solution (6.8) to 2,0 ml with 2-propanone (6.3.1). Use an amount that is representative of the levels being determined in the samples being analysed or at the regulatory limit that the samples under test are governed by.

6.13 GC retention time window-defining solution and column performance

Use a standard or reference compound, e.g. PCN technical mixture that contains the first and last eluting PCN for each group to define the beginning and ending retention time windows for the PCNs under test. Demonstrate the ability to isolate all congeners from interferences either by use of separate GC columns or the splitting of extracts using sample preparation procedures (e.g. carbon column chromatography to separate planar compounds from non-planar ones). This standard shall contain at least the compounds listed in Table 2. The congener pair: 1,2,3,4,5,6,7-CN (73)/1,2,3,4,5,6,8-CN (74) shall be resolved with <50 % valley(see 10.5).

7 Apparatus and materials

7.1 Sampling equipment for discrete sampling

7.1.1 Sample bottles, made from amber glass, 1 l, with screw cap for liquid samples (waters, sludges and similar materials containing 2g/l SPM or less).

If amber bottles are not available, protect samples from light.

Bottle caps may be lined with either fluoropolymer or metal foil.

7.2 Equipment for sample preparation

7.2.1 Oven, capable of maintaining temperatures within 5 % of the target temperature and range from 100 °C to 600 °C.

7.2.2 Desiccator.

7.2.3 Balances, consisting of an analytical type capable of weighing 0,1 mg and a top-loading type capable of weighing 10 mg.

7.2.4 pH meter, with combination glass electrode.

7.2.5 pH paper, wide range (pH 1 to pH 14).

7.2.6 Graduated cylinder, 1 l capacity.

7.2.7 Beakers, 250 ml to 500 ml.

7.2.8 Spatulas, made of stainless steel.

7.3 Extraction apparatus

7.3.1 Liquid/liquid extraction-separating funnels, 250 ml, 500 ml, and 2 000 ml, with glass or fluoropolymer stopcocks.

7.3.2 Solid-phase extraction equipment, consisting of 7.3.2.1 to 7.3.2.5.

7.3.2.1 Filtration apparatus, 1 l, including glass funnel, glass frit support, clamp, adapter, stopper, filtration flask, and vacuum tubing.

For waste water samples, the apparatus shall accept 47 mm, 90 mm or 144 mm extraction disks. For drinking water or other samples containing low suspended solids, smaller disks may be used.

7.3.2.2 Vacuum source, capable of maintaining 90 kPa equipped with shut-off valve and vacuum gauge.

7.3.2.3 Glass-fibre filter, 1 µm pore size, to fit filtration apparatus in 7.3.2.1.

7.3.2.4 Solid-phase extraction media.

7.3.2.4.1 Solid-phase extraction disk, containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix, to fit the filtration apparatus (7.3.2.1).

7.3.2.4.2 Solid-phase extraction cartridge, containing octadecyl (C_{18}) bonded silica uniformly enmeshed in an inert matrix with sufficient packing material to trap all analytes under test.

7.3.2.5 Glass Petri dishes, appropriate to filter size.

7.3.3 Soxhlet extractor, for filters and SPE disks, consisting of 7.3.3.1 to 7.3.3.3.

7.3.3.1 Soxhlet, 50 mm ID, 200 ml capacity with 500 ml round-bottomed flask.

7.3.3.2 Thimble, 43 × 123 to fit Soxhlet.

7.3.3.3 Hemispherical heating mantle, to fit 500 ml round-bottomed flask.

7.3.4 Heated pressurized liquid extractor, for extraction of filters, SPE disks and other solid materials.

7.4 Filtration apparatus

7.4.1 Glass wool, extracted by Soxhlet for 3 h minimum or PLE using toluene.

7.4.2 Glass funnel, of 125 ml to 250 ml.

7.4.3 Glass-fibre filter paper, to fit the glass funnel (7.4.2).

7.4.4 Drying column, 15 mm to 20 mm ID quartz chromatographic column equipped with coarse-glass frit or glass-wool plug.

7.4.5 Buchner funnel.

7.4.6 Glass-fibre filter paper, capable of fitting Buchner funnel in 7.4.5.

7.4.7 Filtration flasks, of 1,5 l to 2,0 l with side arm.

7.4.8 Pressure filtration apparatus.

7.5 Clean-up apparatus

7.5.1 Automated gel permeation chromatograph, consisting of 7.5.1.1 to 7.5.1.4.

7.5.1.1 Column, 600 mm to 700 mm long × 25 mm ID, packed with 70 g of SX-3 Bio-beads[3)].

3) SX-3 Bio-beads is the trade name of a product supplied by Bio-Rad. This information is given for the convenience

7.5.1.2 **Syringe**, 10 ml, with Luer fitting.

7.5.1.3 **Syringe filter holder**, stainless steel, and glass-fibre or fluoropolymer filters.

7.5.1.4 **UV detector**, 254 nm, preparative or semi-preparative flow cell.

7.5.2 **Disposable pipettes**. Either disposable Pasteur pipettes, 150 mm long × 5 mm ID or disposable serological pipettes, 10 ml (6 mm ID).

7.5.3 **Glass chromatographic columns**, of the following sizes:

7.5.3.1 150 mm to 280 mm long × 6 mm ID, with coarse-glass frit or glass-wool plug and 250 ml reservoir;

7.5.3.2 280 mm long × 6 mm ID, with 300 ml reservoir and glass or fluoropolymer stopcock;

7.5.3.3 300 mm long × 11 mm ID, with 300 ml reservoir and glass or fluoropolymer stopcock;

7.5.3.4 200 mm long × 15 mm ID, with coarse-glass frit or glass-wool plug and 250 ml reservoir.

7.6 Concentration apparatus

7.6.1 **Rotary evaporator**, equipped with a variable temperature water bath and **round-bottomed flask**, 100 ml and 500 ml or larger, with ground-glass fitting compatible with the rotary evaporator.

7.6.2 **Kuderna-Danish (K-D) concentrator**, consisting of 7.6.2.1 to 7.6.2.4.

7.6.2.1 **Concentrator tube**, 10 ml, graduated with calibration verified. Use a ground-glass stopper (size 19/22 joint) to prevent evaporation of extracts.

7.6.2.2 **Evaporation flask**, 500 ml, attached to concentrator tube with springs.

7.6.2.3 **Snyder column**, three ball macro and two ball micro.

7.6.2.4 **Boiling chips**.

7.6.3 **Nitrogen blowdown apparatus**, equipped with heater controlled in the range of 30 °C to 60 °C, installed in a fume hood.

7.6.4 **Sample vials**, of the types specified in 7.6.4.1 to 7.6.4.2.

7.6.4.1 **Amber glass**, 2 ml to 5 ml, with fluoropolymer-lined screw cap.

7.6.4.2 **Glass**, 0,3 ml, conical, with fluoropolymer-lined screw or crimp cap.

7.7 Other equipment

7.7.1 **Gas chromatograph**, with a splitless or on-column injection port for capillary column, multi-level temperature programme with isothermal hold. The chromatograph shall meet all of the performance

specifications given in Clause 15 with a GC column for the determination of PCN congeners listed in Table 1.

A column or series of columns that is able to resolve the PCN congeners as listed in Table 4. Suggested columns include: 60 m × 0,25 mm ID; 0,25 µm 5 % phenyl, 94 % methyl, 1 % vinyl silicone bonded-phase fused-silica capillary column or any column that show equivalent or better separation (see Table 7 for examples).

Table 7 — Typical GC columns and temperature programmes

Column type	Temperature programme	Injector
DB-5 30 m 0,25 mm ID, 0,25 µm film thickness	160 °C (1,0 min) 4 °C/min to 280 °C	280 °C
DB-1701 30 m 0,25 mm ID, 0,25 µm film thickness	160 °C (1,0 min) 4 °C/min to 280 °C	280 °C
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	90 °C (1,0 min) 15 °C/min to 180 °C, (2 min) 4 °C/min to 280 °C, hold 20 min	250 °C
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	80 °C (0,5 min) 15 °C/min to 160 °C, 3 °C/min to 265 °C, 5 °C/min to 280 °C hold 5 min	260 °C
DB-5 60 m, 0,25 mm ID, 0,25 µm film thickness	100 °C (1 min) 40 °C/min to 200 °C, 3 °C/min to 235 °C, 6 °C/min to 300 °C hold 12 min	270 °C
Rtx-Dioxin-2 40 m, 0,18 mm ID, 0,18 µm film thickness	110 °C (1 min) 25 °C/min to 200 °C (3 min), 2.5 °C/min to 235 °C (3 min), 3 °C/min to 267 °C, 10 °C/min to 300 °C	250 °C

NOTE Table 7 lists specific examples that can be used for PCN analysis. Any GC columns that meet the criteria outlined in this standard can be used.

7.7.2 Mass spectrometer, 28 eV to 80 eV electron impact ionization, capable of repetitively selectively monitoring at least 12 exact masses at high resolution (≥10 000) during a period of approximately 1 s, and shall meet all of the performance specifications in Clause 10.

The mass spectrometer (MS) shall be interfaced to the GC such that the end of the capillary column does not intercept the electron or ion beam and is controlled with a data system, capable of collecting, recording, and storing MS data.

8 Sample collection, preservation, storage and holding times

8.1 General

Collect samples in amber glass containers (7.1.1) in accordance with ISO 5667-1 and ISO 5667-3.

Maintain aqueous samples in the dark at ≤4 °C from the time of collection until receipt at the laboratory. If residual chlorine is present in aqueous samples, add 80 mg of sodium thiosulfate ($Na_2S_2O_3$) (6.1.6) per litre of water. If sample pH is greater than 9, adjust to pH 7 with sulfuric acid (6.1.3) or lower if SPE is used.

8.2 Storage times

Store samples and sample extracts in the dark until analysed.

NOTE 1 If stored in the dark at <–10 °C, cleaned sample extracts can be stored indefinitely.

NOTE 2 There are no demonstrated maximum holding times associated with PCNs in aqueous sample matrices. If stored in the dark at 2 °C to 8 °C and preserved in accordance with 8.1 and 8.2 (if required), aqueous samples can be stored for up to one year.

9 Quality assurance and quality control

9.1 General

Each laboratory that uses this method shall implement a formal quality assurance programme. The minimum requirements of this programme consists of an initial demonstration of laboratory capability, analysis of samples spiked with labelled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance shall be compared to established performance criteria to determine whether the results of analyses meet the performance characteristics of the method.

As part of the method validation procedure, the analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability should be established as described in Annex B.

In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst shall be permitted certain options to improve separations, enhance sensitivity and selectivity or lower the costs of measurements. These options include alternate extraction, concentration, clean-up procedures, and changes in columns and detectors. Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, shall not be allowed. These techniques can be used for screening purposes if false negatives are not observed. If an analytical technique other than the techniques specified in this method is used, that technique shall have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

Each time a modification is made to this method, the analyst shall repeat the method validation procedure (see Annex B). If the detection limit of the method is affected by the change, the laboratory shall demonstrate that the MDL is lower than one-third of any regulatory compliance level in force or one-third the ML in this method, whichever is higher. If calibration is affected by the change, the analyst shall recalibrate the instrument in accordance with Clause 10.

The laboratory shall maintain historical records of modifications made to this method.

Analyses of method blanks shall demonstrate freedom from contamination (5.2). The procedures and criteria for analysis of a method blank are specified in 9.4 and 14.5.

The analyst shall spike all samples with surrogates and internal standards to monitor method performance. See 9.2.

When results of these spikes indicate atypical method performance for samples, dilute, reclean or re-analyse the samples to bring method performance within acceptable limits. Procedures for dilution are specified in 16.4.2.

The laboratory shall, on an ongoing basis, demonstrate through calibration verification that the analytical system is in control. These procedures are specified in 14.3.

The laboratory shall maintain records to define the quality of data that is generated.

9.2 Spiking

Spike all samples with surrogate standards [diluted surrogate spiking solution (6.9)] to assess method performance on the sample matrix.

Analyse each sample in accordance with the procedures given in Clauses 11 to 16.

Determine the percentage recovery of the labelled surrogate standards using the internal standard method (16.3).

If the surrogate standards exceed the range 25 % to 150 %, method performance shall be considered to be unacceptable for that compound in that sample. To overcome such difficulties, samples shall be diluted and/or addition sample cleanup performed and re-analysed.

9.3 Recovery of labelled compounds assessment

Assess recovery of labelled compounds from samples and maintain records.

After the analysis of five samples of a given matrix type for which the labelled compounds pass the tests in Annex B, compute the average percentage recovery (R) and the standard deviation of the percentage recovery (s_{rec}) for the labelled compounds only. Express the assessment as a percentage recovery interval from $R - 2s_{rec}$ to $R + 2s_{rec}$ for each matrix. For example, if R = 90 % and s_{rec} = 10 % for five analyses, the recovery interval is expressed as 70 % to 110 %.

Update the accuracy assessment for each labelled compound in each matrix on a regular basis (e.g. after each 5 to 10 new measurements) until at least 30 measurements have been accumulated.

9.4 Method blanks

Analyse reference matrix method blanks to demonstrate freedom from contamination (5.2).

Prepare, extract, clean-up and concentrate a method blank with each sample batch (samples of the same matrix to a maximum of 20 samples). The matrix for the method blank shall be similar to sample matrix for the batch, e.g. an aliquot of reagent water blank (6.6). Analyse the blank immediately prior to analysis of the samples to demonstrate freedom from contamination.

If any PCNs are found in the blank at greater than the minimum level (Table 4) or one-third of any regulatory compliance level in force, whichever is greater, or if any potentially interfering compound is found in the blank at the minimum level for each level of chlorination given in Table 4, halt analysis of samples until the blank associated with the sample batch shows no evidence of contamination at this level. Associate all samples with a method blank that does not significantly impact results before the results for those samples may be reported for regulatory compliance purposes. Results that are less than 5 times the blank shall be flagged in the test report.

9.5 QC check sample

Analyse at least one QC check sample (B.2) regularly to ensure the accuracy of calibration standards and the overall reliability of the analytical process.

Analyse the QC check sample at least quarterly. This is not required if a QC check sample (e.g. CRM or blank spiked with external standards) is used with each set. If this method is used less frequently than once every 4 months, the QC check sample shall be run in duplicate to confirm original IPR results. If IPR limits cannot be met, repeat the complete IPR procedure.

10 Calibration

10.1 Operating conditions

GC operating conditions are dependent on GC column type. Examples of suggested conditions are shown in Table 7.

Optimize GC conditions for compound separation and sensitivity on selected column phase and to ensure reqirements of 6.13 (critical pair resolution) are met. Once optimized, use the same GC conditions for the analysis of all standards, blanks, IPR tests and samples.

10.2 Mass spectrometer resolution

Obtain a selected ion monitoring (SIM) of each analyte in Table 6 at the two exact masses chosen (examples shown in Table 3) and at ≥10 000 resolving power by injecting an authentic standard of PCNs either singly or as part of a mixture in which there is no interference between closely eluted components.

Excessive perfluorokerosene (PFK) (or any other reference substance) can cause noise problems and contamination of the ion source, necessitating increased frequency of source cleaning. After tuning, reduce PFK levels into the ion source to levels as low as possible.

Using a PFK (or any other suitable reference material) with a molecular leak or heated inlet, tune the instrument to meet the minimum required resolving power of 10 000 (10 % valley) at mass 292,9825 or 330,9792 (PFK) or any other reference signal in the analytical mass range. For each descriptor (ion monitored; for examples see Table 3), monitor and record the resolution and exact masses of three to five reference peaks covering the mass range of the descriptor. The resolution shall be ≥10 000.

10.3 Ion abundance ratios, minimum levels, signal-to-noise ratios, and absolute retention times

Choose an injection volume consistent with the capability of the HRGC–HRMS instrument and dimension of the GC column used. An injection volume of 1 µl is typically used for 0,25 mm ID columns. Smaller injection volumes — 1 µl to 0,5 µl are used for narrow bore — 0,18 mm and 0,10 mm ID columns. Injection volumes should be identical for standards and sample extracts.

NOTE 1 Large volume injection can be used.

Inject an aliquot of the CS1 calibration solution (Table 6) using the GC conditions given in 10.1.

Measure the SIM areas for each analyte, and compute the ion abundance ratios at the exact masses specified in Table 3. Compare the computed ratio to the theoretical ratio given in Table 3.

A signal to noise ratio (S/N) of at least 5 shall be obtained for all analytes in the calibration solution CS1 (S/N of 10 in other calibration solutions).

Make sure that all analytes are within the QC limits in Table 3 for their respective ion abundance ratios. Minimum levels in Table 4 should be obtained for all analytes.

Otherwise adjust the mass spectrometer and repeat this test until the limits specified are obtained. If the adjustment alters the resolution of the mass spectrometer, verify the resolution (10.2) prior to repeat of the test.

Additional masses may be monitored in each descriptor, and the masses may be divided among more than the descriptors listed in Table 3, provided that the laboratory is able to monitor the masses of all the PCNs that may elute from the GC in a given retention-time window.

Operate the mass spectrometer in a mass-drift correction mode, using PFK or any other suitable reference compound to provide lock masses. Monitor each lock mass and ensure that it does not vary by more than ±20 % throughout its respective retention time window.

NOTE 2 The lock-mass for each group of masses is shown in Table 3. Variations of the intensity of the lock mass by more than 20 % indicate the presence of coeluting interferences that can significantly reduce the sensitivity of the mass spectrometer. Reinjection of another aliquot of the sample extract usually does not resolve the problem. Additional clean-up or dilution of the extract can be required to remove the interferences.

10.4 Retention time

Analyse the window-defining mixtures (6.13) using the optimized temperature programme in 10.1.

10.5 Column resolution performance check

Analyse the isomer specificity test standards (6.13) using the procedure in Clause 14 and the optimized conditions for sample analysis (10.1).

Compute the percentage valley between the GC peaks of each critical pair on their respective columns.

Verify that the height of the valley between PCN 73 and 74 isomers is less than 50 %. If the valley exceeds the maximum level; adjust the analytical conditions and repeat the test or replace the GC column and recalibrate (10.2 to 10.8).

10.6 Calibration by isotope dilution

Isotope dilution calibration is used for the PCNs for which labelled compounds are available and added to samples prior to extraction. For compounds without labelled internal standards, internal standard calibration is used. The reference compound (label or internal standard) for each PCN compound is shown in Table 4.

Prepare a calibration curve encompassing the concentration range for each compound to be determined. Plot the relative response (RR), f_{RR} (labelled to native) versus concentration in standard solutions or compute using a linear regression. Determine the relative response factor for each PCN according to the procedures described below. Employ at least five calibration points.

Determine the response of each PCN relative to its labelled analogue using the area responses of both the primary and secondary exact masses specified in Table 3, for each calibration standard, as in Formula (1):

$$fRR = \frac{\left(A_{1,n} + A_{2,n}\right)\rho_1}{\left(A_{1,1} + A_{2,1}\right)\rho_n} \quad (1)$$

where

f_{RR} is the relative response factor for each PCN quantified by isotope dilution;

$A_{1,n}, A_{2,n}$ are the areas of the primary and secondary masses for the PCN;

$A_{1,1}, A_{2,1}$ are the areas of the primary and secondary masses for the labelled compound;

ρ_1 is the concentration of the labelled compound in the calibration standard (Table 6), in picograms per microlitre, pg/µl;

ρ_n is the concentration of the native compound in the calibration standard (Table 6), in picograms per microlitre, pg/µl.

To calibrate the analytical system by isotope dilution, inject a volume of calibration standards CS1 to CS5 (6.11 and Table 6) identical to the volume chosen in 10.3, using the procedure in Clause 14 and the conditions in 10.1 and Table 4. Compute the RR at each concentration.

If the relative response for any compound is constant (less than 20 % coefficient of variation) over the five-point calibration range, an averaged relative response may be used for that compound; otherwise, use the complete calibration curve for that compound over the five-point calibration range.

10.7 Calibration by internal standard

The internal standard method is applied to determination of PCNs for which no labelled standards are available or have been added to the sample (9.2). See Table 4 for internal standard for each PCN congener determined by this method.

Calibration requires the determination of response factors (RFs), f_{resp}, defined by Formula (2):

$$f_{resp} = \frac{\left(A_{1,s} + A_{2,s}\right)\rho_{is}}{\left(A_{1,is} + A_{2,is}\right)\rho_{s}} \quad (2)$$

where

f_{resp} is the response factor for PCN quantified by internal standardization;

$A_{1,s}, A_{2,s}$ are the areas of the primary and secondary masses for the PCN;

$A_{1,is}, A_{2,is}$ are the areas of the primary and secondary masses for the internal standard;

ρ_{is} is the concentration of the internal standard (Table 6) in picograms per microlitre, pg/µl;

ρ_{s} is the concentration of the compound in the calibration standard (Table 6) in picograms per microlitre, pg/µl.

To calibrate the analytical system by internal standard, inject a volume of calibration standards CS1 to CS5 (6.11 and Table 6) identical to the volume chosen in 10.3, using the procedure in Clause 14 and the conditions in 10.1 and Table 3. Compute the RF at each concentration.

If the RF for any compound is constant (less than 20 % coefficient of variation) over the five-point calibration range, an averaged response factor may be used for that compound; otherwise, use the complete calibration curve for that compound over the five-point range.

10.8 Combined calibration

10.8.1 General

By using calibration solutions (6.11 and Table 6) containing the PCNs and labelled compounds and the surrogate or internal standards, use a single set of analyses to produce calibration curves for the isotope dilution and internal standard methods. Verify these curves each shift or analytical run (14.3) by analysing the calibration verification standard (VER – CS3, Table 6). Recalibration is required if any of the calibration verification criteria (14.3) cannot be met.

10.8.2 Data storage

Collect, record and store MS data on standard storage media.

Media should be retrievable and in a common format so that data can be restored and transferred between instruments.

10.8.3 Data acquisition

Collect the signal at each mass repetitively throughout the monitoring period and store on a mass storage device.

10.8.4 Response factors and multipoint calibrations

Use the data system to record and maintain lists of response factors (response ratios for isotope dilution) and multipoint calibration curves in accordance with ISO 8466 (all parts). Use computations of coefficients of variation to test calibration linearity. Statistics on initial performance (Annex B) should be computed and maintained, either on the instrument data system, on a separate computer system or hardcopy that is available for review.

11 Sample preparation

11.1 General

Sample preparation involves modifying the physical form of the sample so that the PCNs can be quantitatively extracted and the bulk matrix removed. In general, the samples shall be in a liquid form or in the form of finely divided solids in order for efficient extraction to take place. Samples should be as homogeneous as possible in order to obtain the most accurate results possible.

For samples known or expected to contain high levels of PCNs or other matrix co-extractables, use an appropriate sample size that is representative of the entire sample. Sample size, internal standard concentrations, and spikes should be adjusted to bring the levels into the linear range of the method.

For all samples, process the blank and PAR through the same steps as the sample to check for contamination and losses in the preparation processes.

For samples that contain particles, determine the concentration of SPM using the procedure in 11.2.

Prepare aqueous samples visibly absent of particles in accordance with 11.3 and extract directly using the separating funnel or SPE techniques in 12.1 or 12.2, respectively.

Prepare aqueous samples containing visible particles and containing 2 g/l of solid particulate material or less using the procedure in 11.3. After preparation, extract the sample directly using the SPE technique in 12.2 or filter in accordance with 11.3.3. After filtration, extract the particles and filter using the procedures specified in 12.3 and extract the filtrate using the procedure specified in 12.1 or 12.2.

NOTE PCNs can be bound to suspended particles and therefore the preparation of aqueous samples is dependent on the suspended solids content of the sample.

11.2 Determination of solid particulate material

Desiccate and weigh a glass fibre filter paper (7.4.3) to three significant figures.

Filter (10,0 ± 0,02) ml of well-mixed sample through the filter media.

Dry the filter for a minimum of 12 h at (110 ± 5) °C and cool in a desiccator for a minimum of 1 h.

Calculate the amount of solid particulate material as in Formula (3):

$$w_{\mathrm{SPM}} = (m_{\mathrm{s}} - m_{\mathrm{f}}) / V \tag{3}$$

where

w_{SPM} is the amount of suspended sediment, expressed as g/l;

V is the volume of sample filtered in millitres, ml;

m_{s} is the mass of sample after drying, in grams, g;

m_{f} is the mass of filter, in grams, g.

11.3 Preparation of aqueous samples containing 2 g/l of solid particulate material or less

11.3.1 General

Prepare aqueous samples visibly absent of particles as follows and extract directly using the separating funnel or SPE techniques in 12.1 or 12.2, respectively. Prepare aqueous samples containing visible

particles and 2 g/l SPM or less using the procedure in 11.3.2 and extract using either the SPE technique in 12.2 or further prepare using the filtration procedure in 11.3.3. After the filtration procedure, continue with extraction of the filter and particles (12.3) and extraction of the filtrate (12.1 or 12.2). If a SPE procedure is used, proceed with extraction of the filter and disk after the sample has passed though the disk.

11.3.2 Preparation of sample and QC aliquots

Mark the original level of the sample on the sample bottle for reference. Weigh the sample plus bottle to ±1 g.

Spike 1,0 ml of the surrogate spiking solution (6.9) into the sample bottle. Cap the bottle and mix the sample by careful shaking. Allow the sample to equilibrate for at least 30 min, with occasional shaking.

For each sample or sample batch (to a maximum of 20 samples) to be extracted in an analytical run, place one 1,0 l aliquot of reagent water in a clean sample bottle or flask.

Spike 1,0 ml of the diluted labelled compound spiking solution (6.9) into the reagent water aliquot. Use this as the method blank.

If SPE is to be used, add 5 ml of methanol (6.3.1) to the sample, cap and shake the sample to mix thoroughly, and proceed to 12.2 for extraction. If SPE is not to be used, and the sample is visibly absent particles, proceed to 12.1 for extraction. If SPE is not to be used and the sample contains visible particles, proceed to 11.3.3 for filtration of particles.

11.3.3 Filtration of particles

Assemble a Buchner funnel (7.4.5) on top of a clean filtration flask. Apply vacuum to the flask, and pour the entire contents of the sample bottle through a glass-fibre filter (7.4.6) in the Buchner funnel, swirling the sample remaining in the bottle to suspend any particles.

Rinse the sample bottle twice with approximately 5 ml portions of reagent water to transfer any remaining particles on to the filter.

Rinse any particles off the sides of the Buchner funnel with two 5 ml portions of reagent water.

Weigh the empty sample bottle to ±1 g. Determine the mass of the sample by difference. Save the bottle for further use.

Extract the filtrate using the separating funnel procedure in 12.1 or SPE procedure in 12.2.

Extract the filter containing the particles using the Soxhlet procedure or PLE in 12.3.

12 Extraction

12.1 Separating funnel extraction of filtrates and of aqueous samples that are visibly absent of particles

Pour the spiked sample or filtrate into a 2 l separating funnel. Rinse the bottle or flask twice with 5 ml of reagent water and add these rinsings to the separating funnel.

Add 60 ml of dichloromethane (6.3.6) to the empty sample bottle, seal, and shake 60 s to rinse the inner surface.

NOTE 1 Other solvents can be used provided that all of the performance criteria of the method are met.

Transfer the solvent to the separating funnel, and extract the sample by shaking the funnel for 2 min with periodic venting. Allow the organic layer to separate from the aqueous phase for a minimum of 10 min. If an emulsion forms and is more than one-third the volume of the solvent layer, employ mechanical techniques to complete the phase separation (see Note 2). Drain the dichloromethane

extract through a solvent-rinsed glass funnel approximately one-half full of granular anhydrous sodium sulfate (6.2.1) supported on clean glass-fibre paper into a solvent-rinsed concentration device (12.4).

NOTE 2 The optimum technique depends upon the sample, but can include stirring, filtration through glass wool, use of phase separation paper, centrifugation, use of an ultrasonic bath with ice, addition of NaCl, or other physical methods. Alternatively, solid-phase or other extraction techniques can be used to prevent emulsion formation.

Any alternative technique is acceptable so long as the requirements of Clause 9 are met.

Extract the water sample two more times with 60 ml portions of dichloromethane (6.3.6). Drain each portion through sodium sulfate into the concentrator. After the third extraction, rinse the separating funnel with at least 20 ml of dichloromethane (6.3.6), and drain these rinsings through sodium sulfate into the concentrator.

Concentrate the extract using one of the macro-concentration procedures in 12.4.

If the extract has colour or is visually dirty, adjust the final volume of the concentrated extract to approximately 10 ml with hexane (6.3.4), transfer to a 250 ml separating funnel, and back-extract using the procedure in 13.2. If the extract is from the aqueous filtrate (11.3), set aside the concentration apparatus for addition of the Soxhlet extract from the particles (12.3).

12.2 Solid phase extraction (SPE) of samples containing less than 2 g/l suspended particulate matter

12.2.1 Disk/cartridge preparation

Place an SPE disk on the base of the filter holder and wet with toluene. While holding a glass fibre filter above the SPE disk with tweezers, wet the filter with toluene and lay the filter on the SPE disk, making sure that air is not trapped between the filter and disk. Clamp the filter and SPE disk between the 1 l glass reservoir or manifold and the vacuum filtration flask.

Rinse the sides of the filtration flask with approximately 15 ml of toluene using a squeeze bottle or syringe. Apply vacuum momentarily until a few drops appear at the drip tip. Release the vacuum and allow the filter/disk to soak for approximately 1 min. Apply vacuum and draw all of the toluene through the filter/disk. Repeat the wash step with approximately 15 ml of 2-propanone and allow the filter/disk to air dry.

Re-wet the filter/disk with approximately 15 ml of methanol, allowing the filter/disk to soak for approximately 1 min. Pull the methanol through the filter/disk using the vacuum, but retain a layer of methanol approximately 1 mm thick on the filter. Do not allow the disk to go dry from this point until the end of the extraction.

Rinse the filter/disk with two 50 ml portions of reagent water by adding the water to the reservoir and pulling most through, leaving a layer of water on the surface of the filter.

Prepare the SPE cartridge in a similar manner as above using toluene and methanol to rinse the cartridge.

12.2.2 Sample extraction

Pour the spiked sample (9.5), blank (9.4), or IPR aliquot into the reservoir and turn on the vacuum to begin the extraction. Adjust the vacuum to complete the extraction in not less than 10 min.

NOTE For samples containing a high concentration of SPM such as pulp mill effluents, filtration times can be 8 h or longer. PCNs and other dioxin-like compounds are strongly hydrophobic. Neglecting the analytes adsorbed on the particulate fraction for samples containing >2 g/l can result in a significant bias.

Before the entire sample has been pulled through the filter/disk or cartridge, rinse the sample bottle with approximately 50 ml of reagent water to remove any solids, and pour into the reservoir. Pull

through the filter/disk or cartridge. Use additional reagent water rinses until all visible solids are removed.

Allow the filter/disk or cartridge to dry by pulling air through the disk for at least 10 min, then remove the filter and disk and place in a glass Petri dish (7.3.2.5). Extract the filter and disk in accordance with 12.3. Filters/disks and cartridges may also be extracted directly *in situ* using ethanol/toluene. A 30 % volume fraction to 70 % volume fraction ethanol and toluene mixture is allowed to soak the particles and SPE disk for 30 min after which the disk is eluted with the solvent in the reservoir. This step is repeated 2 more times.

Concentrate the extract using one of the macro-concentration procedures in 12.4.

12.3 Soxhlet or PLE extraction of filters or disks

NOTE PLE extraction can be used in place of Soxhlet extraction.

Charge a clean extraction thimble (7.3.3.2) with 5,0 g of 70 µm to 230 µm of silica (6.5.1.1).

Place the thimble in a clean extractor. Place 30 ml to 40 ml of toluene in the receiver and 200 ml to 250 ml of toluene in the flask.

Pre-extract the glassware by heating the flask until the toluene is boiling. When properly adjusted, 1 to 2 drops per second of toluene fall from the condenser tip into the receiver. Extract the apparatus for a minimum of 3 h.

After pre-extraction, cool and dismantle the apparatus and allow the thimble/silica to dry taking precautions to avoid contamination.

Load the filter or disk into the thimble.

Re-assemble the pre-extracted Soxhlet apparatus, and add a fresh charge of toluene to the receiver and reflux flask. Apply power to the heating mantle to begin refluxing. Adjust the reflux rate to match the rate of percolation through the silica bed until water removal lessens the restriction to toluene flow. Frequently check the apparatus for foaming during the first 2 h of extraction. If foaming occurs, reduce the reflux rate until foaming subsides.

Reflux the sample for at least 16 h or 100 cycles. Cool and dismantle the apparatus.

Remove the distillation flask and add any toluene in the receiver to the extract in the flask.

Concentrate the extract using one of the macro-concentration procedures in 12.4.

12.4 Macro-concentration

12.4.1 General

Concentrate extracts in toluene using a rotary evaporator or a heating mantle. Concentrate extracts in dichloromethane (6.3.6) or hexane (6.3.4) using a rotary evaporator, heating mantle, or Kuderna-Danish apparatus.

12.4.2 Rotary evaporation

Assemble the rotary evaporator in accordance with the manufacturer's instructions, and warm the water bath to 35 °C. Between samples, rinse three 2 ml to 3 ml aliquots of solvent down the feed tube into a waste beaker.

Attach a round-bottomed flask containing the sample extract to the rotary evaporator. Slowly apply vacuum to the system, and begin rotating the sample flask.

Lower the flask into the water bath, and adjust the speed of rotation and the temperature as required to complete concentration in 15 min to 20 min. At the proper rate of concentration, the flow of solvent into the receiving flask is steady, but no bumping or visible boiling of the extract occurs.

NOTE If the rate of concentration is too fast, bumping analyte loss can occur.

When the liquid in the concentration flask has reached an apparent volume of approximately 2 ml, remove the flask from the water bath and stop the rotation. Slowly and carefully admit air into the system. Be sure not to open the valve so quickly that the sample is blown out of the flask. Rinse the feed tube with approximately 2 ml of solvent.

Proceed to micro-concentration and solvent exchange (12.5).

12.4.3 Heating mantle

Add one or two clean boiling chips to a round-bottomed flask, and attach a three-ball macro Snyder column. Pre-wet the column by adding approximately 1 ml of solvent through the top. Place the round-bottomed flask in a heating mantle, and apply heat as required to complete the concentration in 15 min to 20 min.

NOTE At the proper rate of distillation, the balls of the column actively chatter, but the chambers do not flood.

When the liquid has reached an apparent volume of approximately 10 ml, remove the round-bottomed flask from the heating mantle and allow the solvent to drain and cool for at least 10 min. Remove the Snyder column and rinse the glass joint into the receiver with two 5 ml portions of solvent.

Proceed to micro-concentration and solvent exchange (12.5) or back-extraction (13.2) (if required).

12.4.4 Kuderna-Danish (K-D)

Concentrate the extracts in separate 500 ml K-D flasks equipped with 10 ml concentrator tubes. The K-D technique is used for solvents such as dichloromethane (6.3.6) and hexane (6.3.4). Toluene is difficult to concentrate using the K-D technique unless a water bath fed by a steam generator is used.

Add one to two clean boiling chips to the receiver. Attach a three ball macro Snyder column. Pre-wet the column by adding approximately 1 ml of solvent through the top. Place the K-D apparatus in a hot water bath so that the entire lower rounded surface of the flask is bathed with steam.

Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 min to 20 min.

NOTE At the proper rate of distillation, the balls of the column actively chatter, but the chambers do not flood.

When the liquid has reached an apparent volume of 1 ml, remove the K-D apparatus from the bath and allow the solvent to drain and cool for at least 10 min. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 ml to 2 ml of solvent. A 5 ml syringe is recommended for this operation.

Remove the three-ball Snyder column, add a fresh boiling chip, and attach a two-ball micro Snyder column to the concentrator tube. Pre-wet the column by adding approximately 0,5 ml of solvent through the top. Place the apparatus in the hot water bath.

Adjust the vertical position and the water temperature as required to complete the concentration in 5 min to 10 min. At the proper rate of distillation, the balls of the column actively chatter, but the chambers do not flood.

When the liquid reaches an apparent volume of 0,5 ml, remove the apparatus from the water bath and allow to drain and cool for at least 10 min.

Proceed to micro-concentration and solvent exchange (12.5) or back-extraction (13.2) (if required).

12.5 Micro-concentration and solvent exchange

Extracts to be subjected to GPC clean-up are exchanged into dichloromethane (6.3.6). Extracts to be cleaned up using silica, carbon, or Florisil[4] are exchanged into hexane (6.3.4).

Transfer the vial containing the sample extract to a nitrogen blowdown device. Adjust the flow of nitrogen so that the surface of the solvent is just visibly disturbed.

NOTE A large vortex in the solvent can cause analyte loss.

Lower the vial into a 35 °C water bath and continue concentrating.

When the volume of the liquid is approximately 100 µl, add 2 ml to 3 ml of the desired solvent [dichloromethane (6.3.6) for GPC, or hexane (6.3.4) for the other clean-ups] and continue concentration to approximately 100 µl. Repeat the addition of solvent and concentrate once more.

If the extract is to be cleaned up by GPC, adjust the volume of the extract to 5,0 ml with dichloromethane (6.3.6). Proceed with GPC clean-up (13.2).

If the extract is to be cleaned up by column chromatography [silica, carbon, or Florisil[4]], bring the final volume to 1,0 ml with hexane (6.3.4). Proceed with column clean-ups (13.3 to 13.6).

If the extract is to be concentrated for injection into the GC–MS (14), quantitatively transfer the extract to a conical vial of suitable volume for final concentration, rinsing the larger vial with hexane (6.3.4) and adding the rinse to the conical vial. Reduce the volume to approximately 100 µl. Add 10 µl of nonane to the vial, and evaporate the solvent to the level of the nonane (10 µl). Seal the vial and store in the dark at room temperature until ready for GC–MS analysis. If GC–MS analysis is not performed on the same day, store the vial in the dark at a suitable temperature to avoid losses by evaporation.

13 Extract clean-up

13.1 General

Exact masses of packing materials and volumes or mixtures of elution solvents for each clean-up stage shall be determined and confirmed (see B.1) before routine sample analysis can commence.

The analyst may use any of the example procedures in the following or any suitable alternative procedures. Before using a clean-up procedure, the analyst shall demonstrate that the requirements of 9.2 can be met using the clean-up procedure. In addition the isomer pattern and congener profile of a well-characterized sample extract containing all PCN (e.g. PCN technical mixture) shall not be altered by the applied clean-up procedure.

Gel permeation chromatography (13.2) removes high molecular mass interferences that cause GC column performance to degrade, for this reason it may be used for water extracts that are expected to contain high molecular mass organic compounds (e.g. polymeric materials, humic acids).

Acid, neutral, and basic silica (6.5.1.3) and Florisil[4] (6.5.3) may be used to remove non-polar and polar interferences.

Carbon (6.5.2.1) may be used to remove non-polar, non-planar interferences.

Silver nitrate–silica (6.5.4) may be used to remove sulfur compounds and some organohalogen compounds.

4) Florisil is the trade name of a product supplied by US Silica Co. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

13.2 Back-extraction with acid and base

Transfer the extract to a 250 ml separating funnel. Rinse the concentration vessel with small portions of hexane (6.3.1), adjust the hexane volume in the separating funnel to 10 ml to 20 ml. Partition the extract against 50 ml of sulfuric acid (6.1.3). Shake for 2 min with periodic venting into a hood. Remove and discard the aqueous layer. Repeat the acid washing until no colour is visible in the aqueous layer, to a maximum of four washings.

Partition the extract against 50 ml of sodium chloride solution (6.1.5) in the same way as with acid. Discard the aqueous layer.

Proceed as follows if additional base washing is required.

Partition the extract against 50 ml of potassium hydroxide solution (6.1.2) in the same way as with acid. Repeat the base washing until no colour is visible in the aqueous layer, to a maximum of four washings.

NOTE Stronger potassium hydroxide solutions can be employed for back-extraction, provided that the laboratory meets the specifications for internal standard compound recovery and demonstrates acceptable performance using the procedure in 9.2.

Repeat the partitioning against sodium chloride solution and discard the aqueous layer.

Proceed as follows if only acid washing is required.

Pour each extract through a drying column containing 7 cm to 10 cm of granular anhydrous sodium sulfate (6.2.1). Rinse the separating funnel with 30 ml to 50 ml of solvent, and pour through the drying column. Collect each extract in a round-bottomed flask. Re-concentrate the sample and QC aliquots in accordance with 12.4 and 12.5 and clean-up the samples and QC aliquots in accordance with Clause 13.

13.3 Gel permeation chromatography (GPC)

13.3.1 Column packing

Place 70 g to 75 g of SX-3 Bio-beads[5)] in a 400 ml to 500 ml beaker.

Cover the beads with dichloromethane (6.3.6) and allow to swell overnight (a minimum of 12 h).

Transfer the swelled beads to the column and pump dichloromethane (6.3.6) through the column, from bottom to top, at 4,5 ml/min to 5,5 ml/min prior to connecting the column to the detector.

After purging the column with dichloromethane (6.3.6) for 1 h to 2 h, adjust the column head pressure to 7 Pa to 10 Pa and purge for 4 h to 5 h to remove air. Maintain a head pressure of 7 Pa to 10 Pa. Connect the column to the detector.

13.3.2 Column calibration

Load 5 ml of the calibration solution (6.4.1) into the sample loop.

Inject the calibration solution and record the signal from the detector. The elution pattern is corn oil, *bis*(2-ethyl hexyl) phthalate, pentachlorophenol, perylene, and sulfur , the calibration solution(6.4.1).

Set the “dump time” to allow >85 % removal of the corn oil and >85 % collection of the phthalate.

Set the “collect time” to the peak minimum between perylene and sulfur.

Verify the calibration with the calibration solution after every 20 extracts. Calibration is verified if the recovery of the pentachlorophenol is greater than 85 %. If calibration is not verified, the system shall

5) SX-3 Bio-beads is the trade name of a product supplied by Bio-Rad. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

be recalibrated using the calibration solution, and the previous 20 samples shall be re-extracted and cleaned up using the calibrated GPC system.

13.3.3 Extract clean-up

It is a requirement of GPC that the column shall not be overloaded. If the extract is known or expected to contain more than 0,5 g of high molecular mass material, split the extract into aliquots for GPC, and combine the aliquots after elution from the column.

NOTE The column specified in this method is designed to handle a maximum of 0,5 g of high molecular mass material in a 5 ml extract. The residue content of the extract can be obtained gravimetrically by evaporating the solvent from a 50 µl aliquot.

Filter the extract or load through the filter holder to remove the particles. Load the 5,0 ml extract on to the column.

Elute the extract using the calibration data determined in 13.3.2. Collect the eluate in a clean 400 ml to 500 ml beaker.

Rinse the sample loading tube thoroughly with dichloromethane (6.3.6) between extracts to prepare for the next sample.

If a particularly dirty extract is encountered, run a 5,0 ml dichloromethane (6.3.6) blank through the system to check for carry over.

Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC-MS

13.4 Silica clean-up

Place a glass-wool plug in a 15 mm ID chromatography column (7.5.3.4). Pack the column bottom to top with: 1 g of activated silica (6.5.1.1), 4 g of basic silica (6.5.1.3), 1 g of silica (6.5.1.1), 8 g of acid silica (6.5.1.2), 2 g of activated silica, and 4 g of granular anhydrous sodium sulfate (6.2.1). Tap the column to settle the adsorbents.

NOTE 1 The silver nitrate/silica packing (13.6) can be placed at the bottom of the silica column (13.3) and run in tandem.

Pre-elute the column with 50 ml to 100 ml of hexane (6.3.4). Close the stopcock when the hexane is within 1 mm of the sodium sulfate (6.2.1). Discard the eluate. Check the column for channelling. If channelling is present, discard the column and prepare another.

Apply the concentrated extract to the column. Open the stopcock until the extract is within 1 mm of the sodium sulfate.

Rinse the receiver twice with 1 ml portions of hexane (6.3.4), and apply separately to the column. Elute the PCNs with 100 ml hexane (6.3.4), and collect the eluate.

Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC-MS.

For extracts of samples known to contain large quantities of other organic compounds, it may be advisable to increase the capacity of the silica column. This may be accomplished by increasing the strengths of the acid and basic silicas. The acid silica (6.5.1.2) may be increased in strength to as much as 44 % mass fraction (7,9 g of sulfuric acid added to 10 g of silica). The basic silica (6.5.1.3) may be increased in strength to as much as 33 % mass fraction (50 ml in NaOH added to 100 g silica), or the potassium silicate (6.5.1.4) may be used.

NOTE 2 The use of stronger acid silica (44 % mass fraction) can lead to charring of organic compounds in some extracts. The charred material can retain some of the analytes and lead to lower recoveries of PCNs. Increasing the strengths of the acid and basic silica can also require different volumes of hexane (6.3.4) than those specified above to elute the analytes off the column.

After modification, verify the performance of the method by the procedure in 9.2.

13.5 Carbon column

Cut both ends from a 10 ml disposable serological pipette to produce a 10 cm column. Anneal both ends and flare both ends if desired. Insert a glass-wool plug at one end, and pack the column with 0,35 g to 0,55 g of carbon (6.5.2.1) to form an adsorbent bed approximately 2 cm long. Insert a glass-wool plug on top of the bed to hold the adsorbent in place.

Pre-elute the column with 5 ml of toluene followed by 2 ml of dichloromethane (6.3.6): methanol: toluene (volume fraction 15:4:1), 1 ml of dichloromethane (6.3.6): cyclohexane (6.3.3) (volume fraction 1:1), and 5 ml of hexane (6.3.4). If the flow rate of eluate exceeds 0,5 ml/min, discard the column.

When the solvent is within 1 mm of the column packing, apply the sample extract to the column. Rinse the sample container twice with 1 ml portions of hexane (6.3.4) and apply separately to the column. Apply 2 ml of hexane (6.3.4) to complete the transfer.

Elute the interfering compounds with two 3 ml portions of hexane (6.3.4), 2 ml of dichloromethane (6.3.1): cyclohexane (6.3.3) (volume fraction 1:1), and 2 ml of dichloromethane (6.3.6): methanol: toluene (volume fraction 15:4:1). Discard the eluate.

Invert the column, and elute the PCNs with 30 ml of toluene. If carbon particles are present in the eluate, filter through glass-fibre filter paper.

Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC-MS.

13.6 Florisil[6] clean-up

Place a glasswool plug into a column (7.5.3). Pack with 1,5 g (approximately 2 ml) of Florisil[6] (6.5.3) top with approximately 1 ml of sodium sulfate (6.2.1) and a glass wool plug.

Pre-elute the activated Florisil[6] column (6.5.3) with 100 ml of hexane (6.3.4) and discard the solvents.

When the solvent is within 1 mm of the packing, apply the sample extract [in hexane (6.3.4)] to the column. Rinse the sample container twice with 1 ml portions of hexane (6.3.4) and apply to the column.

Elute with 150 ml of hexane to remove potentially interfering compounds like the mono- and di-*ortho* PCBs. This fraction can be collected for analysis if desired.

Elute and collect PCNs with 100 ml of 6 % volume fraction diethyl ether: hexane (6.3.4).

Concentrate the eluate in accordance with 12.4 and 12.5 for further clean-up or injection into the GC-MS.

13.7 Silver nitrate–silica column

Fill a chromatography column to about 5 mm with sodium sulfate (6.2.1) and add 2 g of silver nitrate–silica (6.5.3). Top with a 5 mm layer of sodium sulfate.

Rinse the column with 50 ml of hexane (6.3.4) and add the extract [approximately 3 ml in hexane (6.3.4)] to the column. Rinse the extract container three times with hexane (6.3.4) and add to the column ensuring that the column does not run dry.

Elute and collect the PCNs with 35 ml of hexane (6.3.4).

Concentrate the eluate in accordance with 12.4 to 12.5 for further clean-up or for injection into the GC–MS.

NOTE The silver nitrate–silica packing can be placed at the bottom of the silica column (13.3) and run in tandem.

6) Florisil is the trade name of a product supplied by US Silica Co. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

14 HRGC–HRMS analysis

14.1 General

Establish the operating conditions given in 10.1. Add 10 µl of the appropriate recovery standard solution (6.10) to the sample. If an extract is to be re-analysed and evaporation has occurred, use solvent to bring the extract back to its previous volume (e.g. 9 µl).

Inject a volume of the concentrated extract containing the recovery standard using on-column or splitless injection. The volume injected shall be identical to the volume used for calibration (see Clause 10). Start MS data collection after the solvent peak elutes. Stop data collection not less than 30 s after the last target PCN has eluted. Repeat this procedure for the remaining samples and standards and laboratory performance samples.

At the beginning of batch of samples (up to 20), verify GC–MS system performance and calibration for all PCNs and labelled compounds. For these tests, use analysis of the CS3 VER (6.11 and Table 6) and the isomer specificity test standards (6.13) to verify all performance criteria. Perform adjustment or recalibration (see Clause 10) until all performance criteria are met. Only after all performance criteria are met may samples, blanks, and PARs be analysed.

14.2 MS resolution

A static resolving power of ≥10 000 (10 % valley definition) or the designated value shall be demonstrated at the appropriate mass before any analysis is performed. Implement corrective actions whenever the resolving power does not meet the requirement.

14.3 Calibration verification

Inject the VER standard (CS3 Table 6) using the procedure specified in Clause 10.

The mass abundance ratios for all PCNs shall be within 20 % of the theoretical shown in Table 3; otherwise, adjust the mass spectrometer until the mass abundance ratios fall within the limits specified and repeat the verification test. If the adjustment alters the resolution of the mass spectrometer, verify the resolution (10.2) prior to repeating the verification test.

The peaks representing each PCN and labelled compound in the low level standard (CS1) shall be present with S/N of at least 5 (10.3); otherwise, adjust the mass spectrometer and repeat the verification test.

Compute the concentration of each PCN compound by isotope dilution (16.1) for those compounds that have labelled analogues (Table 4) and internal standard method (16.2) for those that do not. Compute the concentration of the labelled compounds by the internal standard method (16.2). These concentrations are computed based on the calibration data in Clause 10.

For each compound, confirm that the result of the VER analysis is within 20 % of the nominal concentration shown in Table 6. If all compounds are within this limit, calibration has been verified and analysis of standards and sample extracts shall proceed. If, however, any compound fails its respective limit, the measurement system is not performing properly for that compound. In this event, prepare a fresh calibration standard or correct the problem causing the failure and repeat the resolution (14.2) and verification (14.3) tests, or recalibrate (see Clause 10).

14.4 GC resolution

Inject the isomer specificity standards (6.13) on their respective columns to meet required conditions.

14.5 Blank

Make sure that the results of the analysis of the blank meet the specifications in 9.4 before sample analyses proceed.

15 Qualitative determination

A PCN or labelled compound is identified as being present in a standard, blank or sample when all of the criteria below are met. If the criteria are not met, the PCN has not been identified and the results shall not be reported for regulatory compliance purposes. If interferences preclude identification, extract a new aliquot of sample or clean up further, and re-analyse. If the interference cannot be removed, flag the data to indicate results are maximum concentrations.

The signals for the two exact masses in Table 3 shall be present and shall maximize within ±2 s.

The signal to noise ratio (S/N) for the GC peak at each exact mass shall be greater than or equal to 3 for each PCN detected in a sample extract, and greater than or equal to 10 for all PCNs in the calibration standard (10.3 and 14.3).

The ratio of the integrated areas of the two exact masses specified in Table 3 shall be within 20 % of the theoretical shown in Table 3, or within ±10 % of the ratio in the midpoint (CS3) calibration or calibration verification, whichever is most recent.

The retention time of a native PCN shall be within a time window of ±3 s based on the retention time of the corresponding $^{13}C_{10}$–labelled congener in the sample. If there is no labelled analogue, the PCN congener shall be within ±3 s of the relative retention time of the corresponding unlabelled analogue.

At present, there is no chromatographic column available that is able to separate all PCN congeners. Even multi-analysis of the sample extract on different columns of different nature (polarity) may not separate all PCN isomers. However, in practice, the contribution of non-toxic congeners to the total TEQ from a single column analysis may be of the same order as the precision of the test (10 % to 20 %). Single column data may therefore be reported by this method if it can be documented that results are not significantly biased (>20 %); however, in cases where a regulatory limit is exceeded by 20 % or less, a confirmatory analysis should be performed on a second column.

16 Quantitative determination

16.1 Isotope dilution quantification

By adding a known amount of a labelled compound to every sample prior to extraction, correction for recovery of the PCNs can be made because the PCNs and their labelled analogues exhibit similar effects upon extraction, concentration, and gas chromatography. Use RR values in conjunction with the initial calibration data described in 10.6 to determine concentrations directly, so long as labelled compound spiking levels are constant, using Formula (4):

$$m_{ex} = \frac{(A_{1,n} + A_{2,n})m_l}{(A_{1,l} + A_{2,l})fRR} \quad (4)$$

where

m_{ex} is the amount of the PCNs in the extract, in picograms, pg;

$A_{1,n}, A_{2,n}$ are the areas of the primary and secondary masses for the native compound;

$A_{1,l}, A_{2,l}$ are the areas of the primary and secondary masses for the labelled compound;

m_l is the amount of the labelled compound in the calibration standard (see Table 6), in picograms, pg;

fRR is the relative response as defined in 10.6.

Quantify coelutions of PCNs 66/67, 64/68 and 71/72 as single results; correct using the recovery of $^{13}C_{10}$-labelled PCN 64.

If any of the $^{13}C_{10}$-labelled standards are used as recovery standards (i.e. not added before extraction of the sample), do not use them to quantify the corresponding native. Quantify those native congeners using the response of a labelled analogue of the same homologue series.

Quantify any peaks representing PCNs without corresponding labelled compounds using an average of the response factors from all PCN isomers at the same level of chlorination.

16.2 Internal standard quantification

Compute the amounts of those native congeners referred to in 16.1 and the ^{13}C-labelled analogues in the extract using the response factors determined from the initial calibration data (10.6) and Formula (5):

$$m_{ex} = \frac{(A_{1,s} + A_{2,s})m_{is}}{(A_{1,is} + A_{2,is})f_{resp}} \quad (5)$$

where

m_{ex} is the amount of the PCNs in the extract, in picograms, pg;

$A_{1,s}$ and $A_{2,s}$ are the areas of the primary and secondary masses for the PCNs;

$A_{1,is}$ and $A_{2,is}$ are the areas of the primary and secondary masses for the internal standard;

m_{is} is the amount of the internal standard (Table 6) in picograms, pg;

f_{resp} is the response factor as defined in 10.7.

16.3 Determination of labelled compound recovery

Compute the percentage recovery, R, of the $^{13}C_{10}$-labelled compounds using Formula (6):

$$R = \frac{\left(A_{1,l} + A_{2,l}\right)m_{is}}{\left(A_{1,is} + A_{2,is}\right)f_{RR}m_1} \times 100 \quad (6)$$

where

R is the recovery of the labelled internal standard as a percentage %;

$A_{1,l}$, $A_{2,n}$ are the areas of the primary and secondary masses for the labelled PCN;

$A_{1,is}$, $A_{2,is}$ are the areas of the primary and secondary masses for the internal standard;

m_1 is the amount of the labelled compound added to the sample (Table 5), in nanograms, ng;

m_{is} is the amount of the recovery standard added to the sample (Table 5), in nanograms, ng;

f_{RR} is the relative response factor.

If recovery is outside the range 25 % to 150 %, data congeners associated with this internal standard shall be flagged as out of control.

16.4 Concentration in sample

16.4.1 General

Compute the concentration of a PCN in the aqueous phase of the sample using the concentration of the compound in the extract and the volume of water extracted (11.3) following Formula (7):

$$\rho_{aq} = \frac{m_{ex}}{V_s} \quad (7)$$

where

ρ_{aq} is the concentration in aqueous phase in picograms per litre, pg/l;

m_{ex} is the amount of the compound in the extract in picograms, pg;

V_s is the sample volume in litres, l.

16.4.2 Treatment of samples exceeding calibration range

If the SIM area at either quantification mass for any compound exceeds the calibration range of the system, extract a smaller sample aliquot. Sample extracts may be diluted by a factor of 10 and reanalysed. If a dilution of greater than 50× is required, the sample should be diluted and or internal standards or surrogates should be adjusted to compensate.

Some samples may contain high levels (>10 µg/l) of the compounds of interest, interfering compounds, or polymeric materials. Some extracts do not concentrate to 10 µl; others may overload the GC column or mass spectrometer. In cases where the extract does not concentrate to 10 µl after all clean-up procedures have been exhausted, analyse a smaller aliquot of the sample or diluted extract.

If a smaller sample size is not representative of the entire sample, dilute the sample extract by a factor of 10, adjust the concentration of the instrument internal standard to 100 pg/µl in the extract, and analyse an aliquot of this diluted extract by the internal standard method.

16.5 Results and reporting

Report results to two significant figures for each PCN and labelled compounds found in all standards, blanks, and samples. For standards (VER, IPR) and samples, report results at or above the minimum level (ML), see Table 4. Report results below the minimum level as not detected or as required by the regulatory authority. For blanks, report results that are above one-third the ML. Calculate detection limits based on a signal to noise ratio of 3:1 as in 16.3.

Report sample results in picograms per litre, pg/l. TEQ concentrations may be calculated as given in Annex C.

If the mass of a congener or congeners is below the detection limit, then two TEQ concentrations should be reported per sample:

a) with the concentration of those congener(s) below the lower detection limit being taken as equal to the detection limit multiplied by 1, 0,5 or any value indicated in applicable regulations;

b) with the concentration of those congener(s) taken as zero.

For samples that have been diluted, report results for PCNs at the least dilute level at which the areas of the quantification masses are within the calibration range.