

---

---

**Soil quality — Environmental availability of non-polar organic compounds — Determination of the potentially bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent**

*Qualité du sol — Disponibilité environnementale des composés organiques non polaires — Détermination de la fraction potentiellement biodisponible et de la fraction non biodisponible en utilisant un agent adsorbant fort ou un agent complexant*

STANDARDSISO.COM : Click to view the full PDF of ISO 16751:2020



STANDARDSISO.COM : Click to view the full PDF of ISO 16751:2020



**COPYRIGHT PROTECTED DOCUMENT**

© ISO 2020

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

	Page
<b>Foreword</b> .....	<b>iv</b>
<b>Introduction</b> .....	<b>v</b>
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Principle</b> .....	<b>2</b>
<b>5 Reagents</b> .....	<b>3</b>
<b>6 Apparatus</b> .....	<b>3</b>
<b>7 Procedure</b> .....	<b>4</b>
7.1 Sample preparation.....	4
7.2 Determining water content.....	5
7.3 Method A: Cyclodextrin.....	5
7.3.1 Extraction of the sample.....	5
7.3.2 Phase separation.....	5
7.3.3 Extraction from receiver phase.....	5
7.4 Method B: Tenax®.....	5
7.4.1 Extraction of the sample.....	5
7.4.2 Phase separation.....	6
7.4.3 Extraction from receiver phase.....	6
7.5 Measurement.....	7
7.5.1 Potential bioavailable fraction.....	7
7.5.2 Non-bioavailable fraction.....	7
7.6 Blank test.....	7
<b>8 Calculation</b> .....	<b>7</b>
8.1 Potential bioavailable fraction.....	7
8.2 Non-bioavailable fraction.....	8
<b>9 Expression of results</b> .....	<b>8</b>
<b>10 Validation</b> .....	<b>9</b>
<b>11 Test report</b> .....	<b>9</b>
<b>Annex A (informative) Preparation and regeneration of Tenax®</b> .....	<b>10</b>
<b>Annex B (informative) Illustrations</b> .....	<b>11</b>
<b>Annex C (informative) Validation study results</b> .....	<b>14</b>
<b>Bibliography</b> .....	<b>25</b>

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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](http://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](http://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 voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

## Introduction

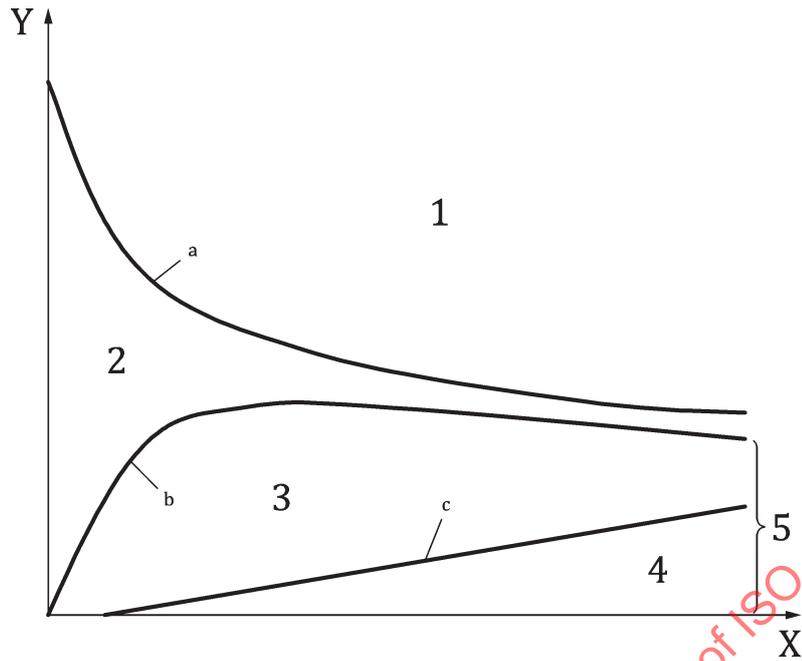
The solubility of most non-polar organic contaminants is limited and they are sorbed to the soil matrix. They may desorb and become available for organisms, which may result in an effect (toxicity, degradation or bioaccumulation). Not all sorbed (adsorbed and absorbed) contaminants will desorb and become available.

Extractions used in chemical analysis to measure the total concentration, release more contaminants from the soil than are available. It is however also possible that contaminants are so strongly bounded by the soil that they will not be released by chemical extraction. This strong sorption may also be caused by incorporation of the contaminant (or a degradation or reaction product of the contaminant) in the organic soil structure. The distribution of contaminants over sorption sites of varying sorption strength is not constant in time and contaminants will shift, with increasing contact time, to the stronger sorption sites.

[Figure 1](#) shows schematically the differentiation between:

- extractable residues that are also bioavailable (i.e. the potentially bioavailable fraction);
- residues that are extractable by harsher extraction methods but are non-bioavailable;
- residues that are neither extractable nor bioavailable.

If a degradable substance enters a soil, part of it will degrade over time (curve a). The area between curve a and c is extractable by exhaustive chemical procedures. For risk assessments, this part is considered as the “total concentration” for which values are defined in many regulations. However, only a part of this amount is bioavailable. The area between curves a and b is the bioavailable fraction and the area between curves b and c is the non-bioavailable fraction. The method described in this document enables the measurement of the potential bioavailable and the non-bioavailable fraction of a contaminant in soil.



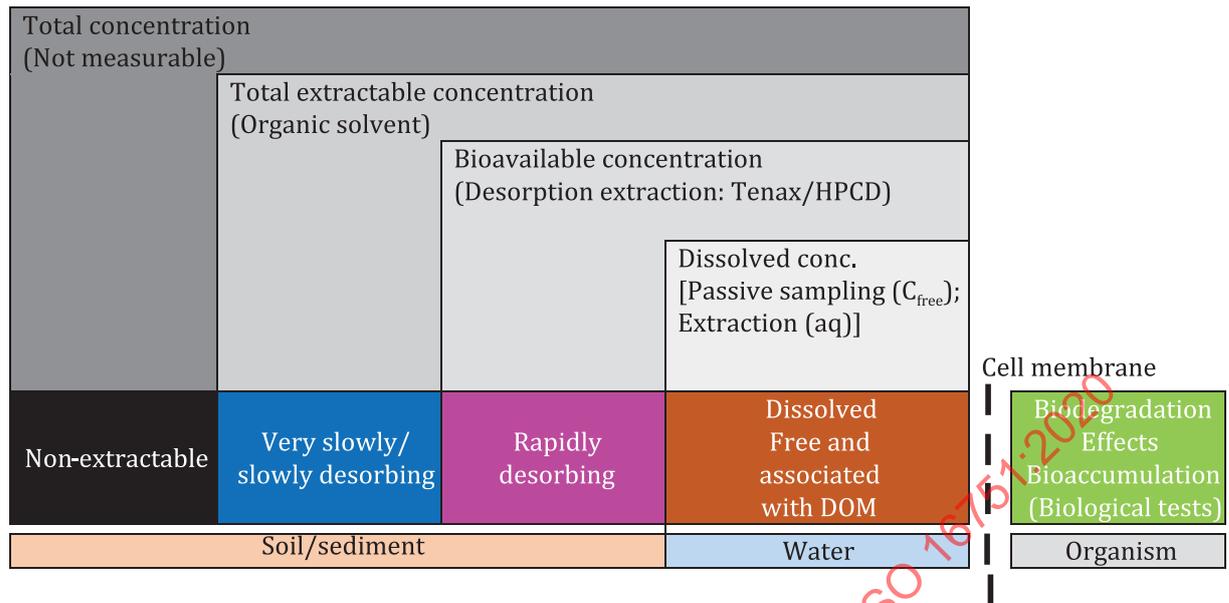
**Key**

- X time
- Y contaminant concentration
- 1 degradable
- 2 bioavailable
- 3 extractable, non-bioavailable
- 4 non-extractable: persistent residues
- 5 non-available fraction

NOTE For curves a, b and c see description in text above.

**Figure 1 — Temporal changes in extractable/bioavailable fractions, extractable/non-bioavailable fractions and non-extractable/non-bioavailable fractions of a non-polar organic contaminant (modified from [1])**

In the scientific research to bioavailability a large number of definitions and concepts are in use, which reflect the discussion in the scientific world. However, a more clear and simple approach is necessary. In regulation, organic contaminants are either bioavailable or non-bioavailable. To support decisions, both should be measurable. Therefore, this document follows the approach of Ortega-Calvo et al. (2015) [2] as illustrated in Figure 2. In this approach all defined fractions are measurable as further explained in Clause 4.



**Figure 2 — Measurement of bioavailability of organic chemicals: a simplified scheme for use in regulation<sup>[9]</sup>**

The colour boxes at the left of the biological membrane represent the distribution of pollutant molecules among four classes (non-extractable, very slowly/slowly desorbing, rapidly desorbing and water-dissolved) in soils and sediments. In the scheme in Figure 2, the bioavailable chemical is represented by the rapidly desorbing and dissolved concentrations. The chemical methods able to measure the pollutant present in each specific fraction are given in the grey boxes. The green box to the right of the cell membrane represents the processes that occur within the organism exposed to the pollutant. These biological processes can also serve as the basis for standard methods used for bioavailability measurements.

As presented in Figure 2, the bioavailable fraction can be measured using the method described in this document.

[STANDARDSISO.COM](https://standardsiso.com) : Click to view the full PDF of ISO 16751:2020

# Soil quality — Environmental availability of non-polar organic compounds — Determination of the potentially bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent

## 1 Scope

This document specifies an extraction method to determine the bioavailable (potential and environmental available) fraction and the non-bioavailable fraction of a contaminant in soil using a “receiver phase” for an organic contaminant with strong sorbing or complexing properties, for example, Tenax<sup>®1</sup>) or cyclodextrin, respectively.

NOTE 1 The bioavailable fraction is defined in ISO 17402 as environmental bioavailability.

The method is applicable for non-polar organic contaminants with an aqueous solubility of <100 mg/l. The method is applicable for soil and soil-like material including (dredged) sediments.

NOTE 2 The method is theoretically applicable to non-polar organic contaminants with an aqueous solubility of 1 000 mg/l. The method has been often applied for compounds with a much lower solubility ( $K_{ow} > 3$ ) and less for compounds with a higher solubility. The applicability is therefore defined for compounds with an aqueous solubility of <100 mg/l.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11074, *Soil quality — Vocabulary*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

ISO 18512, *Soil quality — Guidance on long and short term storage of soil samples*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11074, ISO 17402 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

1) Tenax<sup>®</sup> is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

### 3.1 potential bioavailable fraction

amount of contaminant present in the matrix that can be released from the solid phase to the aqueous phase in a well-mixed water soil mixture and in presence of a receiving phase in a period of 20 h

Note 1 to entry: In ISO 17924 the term bioaccessibility is used, which is the fraction of a substance in soil or soil-like material that is liberated in (human) gastrointestinal juices and thus available for absorption. This document does not distinguish between bioavailability and bioaccessibility and uses the general term bioavailability. The concept of bioavailability as followed in this document is described in the introduction of this document.

## 4 Principle

This method gives an estimation of the potential bioavailable and non-bioavailable fraction of organic contaminants, i.e. the amount of the contaminant in the matrix that is potentially exchangeable with the aqueous phase; specifically that, which is adsorbed/complexed by Tenax®/cyclodextrin.

The extractable and non-bioavailable fraction of the contaminant left in the sample following the action of Tenax®/cyclodextrin can be subsequently measured with an exhaustive/harsh extraction technique (designed to measure the total concentration) and in this way the non-bioavailable fraction of the contaminant is assessed.

Thus, in numerical terms, the total contaminant concentration in a sample is the sum of the bioavailable concentration (established using a strong sorbent or complexing agent) and the non-bioavailable concentration (established using a subsequent harsh extraction method performed on the residue that is left after the matrix has been extracted using a strong sorbent or complexing agent):

$$c_{\text{tot,cont}} = c_{\text{bio}} + c_{\text{non-bio}} \quad (1)$$

where

$c_{\text{tot,cont}}$  is the total contaminant concentration;

$c_{\text{bio}}$  is the bioavailable concentration;

$c_{\text{non-bio}}$  is the non-bioavailable concentration.

The soil, soil-like material or sediment sample with particle size <2 mm is extracted with water containing a “receiver phase” for the organic contaminants. This phase is either a complexing agent (cyclodextrin) or a strong adsorbent [Tenax®]. The solubility of non-polar compounds is limited and in this method the receiver phase acts as an “infinite sink”. The measured amount, which is the amount that desorbs from the soil or soil-like material during 20 h, reflects the fraction of contaminant that can have effects on biotic systems and that can become mobile.

In the following step, the contaminants adsorbed are extracted from the receiver phase and determined by appropriate analytical methods. The amount of contaminants left in the soil residue, the non-bioavailable fraction, can be measured using a subsequent harsh/exhaustive extraction designed to measure the total concentration. [Formula \(1\)](#) can then be used to determine the total contaminant concentration in the sample (if desired).

NOTE 1 ISO 13859 and ISO 13876 are examples to measure the total concentration of respectively PAH and PCB in soil and soil-like materials.

NOTE 2 [Formula \(1\)](#) shows the relationship between the “total contaminant concentration” the “bioavailable concentration” and the “non-bioavailable concentration”. If two of the concentrations are known the third concentration can be calculated through the use of [Formula \(1\)](#). For example, by measuring the “total concentration” and the “non-bioavailable concentration”, the “bioavailable concentration” can be calculated. This is allowed with homogeneous materials. If it is not known whether a material is homogeneous and the bioavailable concentration is the concentration of interest, the bioavailable concentration needs to be measured.

## 5 Reagents

Reagents used shall be of suitable grade – analytical grade or higher– if not specified otherwise. The blank value of the reagents (including water) shall be negligible compared to the lowest concentration of organic contaminants to be determined.

**5.1 Demineralized water or deionized water or water of equivalent purity** ( $5 < \text{pH} < 7,5$ ) with a conductivity of  $< 0,5$  mS/m in accordance with grade 3 specified in ISO 3696.

If biodegradation of the target compounds is to be expected, add sodium azide (5.2) to water to a final concentration of 0,2 g/l. This will minimize the biological degradation of the target compounds. If biodegradation is not to be expected, it is not necessary to add sodium azide. This is the case for some persistent target compounds, e.g. PCB.

NOTE With some soil samples it can be difficult to obtain a proper separation between the soil, aqueous phase and Tenax<sup>®</sup>. The use of 0,001 mol/l or 0,01 mol/l CaCl<sub>2</sub> (5.9) instead of water can improve this separation.

**5.2 Sodium azide**, [CAS No. 26628-22-8], NaN<sub>3</sub>.

**WARNING — Attention is drawn to the hazard deriving from the use of the sodium azide which is acutely toxic.**

**5.3 Cyclodextrin**, (hydroxypropyl- $\beta$ -cyclodextrin) of  $>97$  % purity of a Food Grade, Medicine Grade or Pharmaceutical Grade.

NOTE Analytical Grade cyclodextrin is very expensive. For this method Food Grade has shown to be fit for purpose.

**5.4 Extraction solution of cyclodextrin**, dissolve 100 mmol (=146 g) of cyclodextrin (5.3) per litre of water (5.1).

NOTE If biodegradation of the target compounds is to be expected, add sodium azide (5.2) to this solution to a final concentration of 0,2 g/l. This will minimize the biological degradation of the target compounds. If biodegradation is not to be expected, it is not necessary to add sodium azide. This is the case for some persistent target compounds, e.g. PCB

**5.5 2,6-diphenyleneoxide polymer (Tenax<sup>®</sup> TA)**, 60 mesh to 80 mesh. See Annex A for the preparation and regeneration of Tenax<sup>®</sup>.

**5.6 Petroleum ether**, [CAS No. 8032-32-4], boiling range 40 °C to 60 °C.

**5.7 Ethanol**, [CAS No. 64-17-5], C<sub>2</sub>H<sub>6</sub>O.

**5.8 Acetone**, [CAS No. 67-64-1], C<sub>3</sub>H<sub>6</sub>O.

**5.9 Calcium chloride**, [CAS no.1035-04-8], CaCl<sub>2</sub>.

**5.10 Sodium sulfate**, [CAS No. 231-820-9], Na<sub>2</sub>SO<sub>4</sub>.

## 6 Apparatus

Use the following equipment. All materials that come into contact with the sample (or reagents) shall not adsorb the contaminant of interest and shall not contaminate the sample. Glass and PTFE are suitable materials for most contaminants.

**6.1 Sieving equipment**, with 2 mm nominal screen size.

**6.2 Balance**, accuracy 0,01 g.

**6.3 Conical centrifuge tubes**, with screwing caps.

**6.4 Separation funnel**, of suitable size.

NOTE The hole in the tap of the separation funnel used for the Tenax<sup>®</sup> extraction needs to be large enough for the soil particles to leave the funnel. Otherwise no separation between Tenax<sup>®</sup> and the sample is possible.

**6.5 Shaking machine**, which limits breakdown of sample particles, e.g. an end-over-end shaker, capable of  $(20 \pm 2)$  r/min or other mild agitation method or a horizontal movement shaker, capable to have 150 r/min to 180 r/min.

**6.6 Centrifuge**, capable to centrifuge the centrifuge tubes (6.3).

**6.7 Crushing equipment**, jaw crusher or cutting device.

**6.8 Appropriate glassware and plastic ware.**

**6.9 Kuderna Danish sample concentrator.**

**6.10 Collection vessel.**

**6.11 Folded filter.**

## 7 Procedure

### 7.1 Sample preparation

The sample shall be pretreated in the laboratory in accordance with ISO 14507, but with the following restrictions:

- Intensive pretreatment like grinding may have an effect on the environmental availability of contaminants, therefore grinding is not allowed.
- In general, the test portion to be prepared shall have a grain size less than or equal to 2 mm, but on no account the material shall be ground to reach this grain size.
- Remove stones, shells and any material not representative for the sample.
- Sieve the sample using a sieve (6.1). If necessary, press the material by hand through the sieve.

Instead of using ISO 14507, also freeze-drying according ISO 16720 may be used to pretreat the sample.

Some soils (e.g. peat and some sediments) are difficult to sieve. In these cases, remove stones, shells and material not representative for the sample (e.g. plant material) by hand and process the sample without sieving.

If the sample cannot be sieved at all because of its water content, reduce the water content until the laboratory sample can be sieved. In the case of drying, the drying temperature shall not exceed 30 °C.

If a rapidly degradable fraction is to be expected, reduction of water content by air drying is not allowed. In that case the original collected sample shall be forced by hand through a 2 mm sieve or the sample can be freeze-dried.

If samples shall be stored, store them in accordance with ISO 18512 and in such a way that processes that have an effect on the bioavailability (biodegradation, change in organic matter composition) are prevented.

## 7.2 Determining water content

Determine the water content of the test portion obtained after sample preparation (7.1) as specified in ISO 11465.

## 7.3 Method A: Cyclodextrin

### 7.3.1 Extraction of the sample

Using a balance (6.2) weigh a test portion equivalent to  $(4 \pm 1)$  g of dry material and place this in an appropriate centrifuge tube (e.g. 50 ml) (6.3). Add 40 ml of the cyclodextrin extraction solution (5.4). Place the tubes on the end-over-end shaker or the horizontal shaker (6.5) in the dark for 20 h at a temperature of  $(20 \pm 2)$  °C. Use appropriate glassware and plastic ware (6.8) in order to make phase separation possible.

Use a mild agitation at a frequency that ensures that the ingredients are well mixed, and the breakdown of soil particles is limited (6.5).

### 7.3.2 Phase separation

Centrifuge the tubes during 15 min at least at 2 000 *g* to obtain phase separation (clear supernatant). Remove a portion of the supernatant, by pipetting (e.g. 10 ml) for further analysis.

The residual extraction pellet contains the non-bioavailable fraction of the contaminant. This pellet can be used for further analysis to determine the non-bioavailable fraction of the contaminant (see 7.5.2).

NOTE Depending on the sample it can be necessary to use a centrifuge with a higher *g*-value and longer centrifuge time. It is possible to get the same separation efficiency at other centrifugation conditions (shorter centrifugation time at higher centrifugation speed or extended centrifugation time at lower centrifugation speed). ISO 12782-1:2012, Annex A, provides a number of principles that need to be considered in order to ensure reproducibility of the centrifugation when deviating from the recommended centrifugation procedure.

### 7.3.3 Extraction from receiver phase

Extract the 10 ml aliquot of the complexing aqueous cyclodextrin phase (see 7.3.2) with 5 ml petroleum ether (5.6). Shake during at least 1 min. Repeat twice collecting all three aliquots of petroleum ether into the same flask.

## 7.4 Method B: Tenax®

### 7.4.1 Extraction of the sample

Using a balance (6.2) weigh a test portion equivalent to  $(4 \pm 1)$  g of dry material and place it in the separation funnel (6.4) or a centrifuge tube (6.3), both of ~100 ml. Add 70 ml water (5.1). Add  $(1,5 \pm 0,1)$  g Tenax® (5.5). Place the separation funnel on a horizontal shaking machine (6.5) in the dark for 20 h at a temperature of  $(20 \pm 2)$  °C.

1,5 g of Tenax® is not sufficient to measure the availability of mineral oil. If it is necessary to measure the availability of mineral oil, use 4 g instead of 1,5 g of Tenax®<sup>[10]</sup>.

NOTE Some soils can obstruct the separation funnel (6.4). In such cases it is preferable to use a centrifuge tube (6.3).

Use a mild agitation at a frequency that ensures that the ingredients are well mixed, and the breakdown of soil particles is limited, e.g. 150 r/min to 180 r/min.

## 7.4.2 Phase separation

### 7.4.2.1 Separation funnel

If a separation funnel is used in [7.4.1](#), then drain the aqueous phase including soil or soil-like material carefully from the separation funnel ([6.4](#)). Tenax<sup>®</sup>-polymers (beads) are hydrophobic and will float on water and Tenax<sup>®</sup> will be attached to the wall during phase separation. Remove all visible soil or soil-like material by rinsing the Tenax<sup>®</sup> with water ([5.1](#)) and drain the water (see [Annex B](#) for an example). Collect the aqueous phase including soil or soil-like material in a suitable centrifuge tube. Centrifuge the tubes during 15 min at least at 2 000 *g* to obtain the pellet with residual soil, which contains the non-bioavailable fraction. This pellet can be used for further analysis to determine the non-bioavailable fraction of the contaminant (see [7.5.2](#)).

NOTE 1 Depending on the soil it can be necessary to use a centrifuge with higher *g*-value and longer centrifuge time. It is possible to get the same separation efficiency at other centrifugation conditions (shorter centrifugation time at higher centrifugation speed or extended centrifugation time at lower centrifugation speed). ISO 12782-1:2012, Annex A, gives a number of principles that need to be considered in order to ensure reproducibility of the centrifugation, when deviating from the recommended centrifugation procedure.

NOTE 2 Some soils, especially organic soils, can contain particles that also tend to float. With these soils, losses of Tenax<sup>®</sup> can occur during phase separation. To be able to correct for these losses it is recommended to dry the remaining amount of Tenax<sup>®</sup> after extraction (see [7.4.3](#)) and to weigh the amount of dried Tenax<sup>®</sup>.

NOTE 3 In order to obtain the mass balance the residual material can be used. To obtain this residual material, the water and soil or soil-like material are centrifuged during 15 min at least at 2 000 *g* in a centrifuge ([6.6](#)) in order to obtain phase separation (clear supernatant).

### 7.4.2.2 Centrifuge tube

If a centrifuge tube is used in [7.4.1](#), then centrifuge the tube, at least at 2 000 *g* for 15 min, to obtain phase separation (i.e. a clear supernatant). Remove the Tenax<sup>®</sup> with a Pasteur pipet (see [Annex B](#) for an example) and transfer it into a collection vessel ([6.10](#)).

The residual extraction pellet contains the non-bioavailable fraction and can be used for further analysis to determine the non-bioavailable fraction of the contaminant (see [7.5.2](#)).

NOTE 1 Depending on the soil it can be necessary to use a centrifuge with higher *g*-value and longer centrifuge time. It is possible to get the same separation efficiency at other centrifugation conditions (shorter centrifugation time at higher centrifugation speed or extended centrifugation time at lower centrifugation speed). ISO 12782-1:2012, Annex A, gives a number of principles that need to be considered in order to ensure reproducibility of the centrifugation, when deviating from the recommended centrifugation procedure.

NOTE 2 Some soils, especially organic soils, can contain particles that also tend to float. With these soils losses of Tenax<sup>®</sup> could occur during phase separation. To be able to correct for these losses it is recommended to dry the remaining amount of Tenax<sup>®</sup> after extraction (see [7.4.3](#)) and to weigh the amount of dried Tenax<sup>®</sup>.

NOTE 3 In order to obtain the mass balance the residual material can be used. To obtain this residual material, the water and soil or soil-like material are centrifuged during 15 min at least at 2 000 *g* in a centrifuge ([6.6](#)) in order to obtain phase separation (clear supernatant).

## 7.4.3 Extraction from receiver phase

Extract the Tenax<sup>®</sup> in the separation funnel used in [7.4.2.1](#) or the collection vessel used in [7.4.2.2](#), using (40 ± 1) ml acetone ([5.8](#)), shake for at least 10 min. Then add 20 ml petroleum ether ([5.6](#)) in the separation funnel or collection vessel, shake again for at least 10 min. Decant the upper organic phase into a separation funnel ([6.4](#)) of 1 000 ml (see [Figure B.3](#) for an example). Repeat the extraction of the Tenax<sup>®</sup> twice with 10 ml of petroleum ether ([5.6](#)) and transfer these quantities of petroleum ether into the 500 ml separation funnel ([6.4](#)).

Remove the acetone from the organic phase by shaking twice for about 30 s with 200 ml of water and remove the aqueous phase from the separation funnel.

NOTE 1 Depending on further analysis (for example the determination of PCB or OCP) it can be necessary to remove elementary sulfur. This is done, for example, by adding 5 g of sodium sulfite to the aqueous phase and shaking this mixture during 5 min. Instead of sodium sulfite other methods can be used for removing elementary sulfur.

Take a volume,  $V_{\text{ex}}$ , necessary for further analysis of the petroleum ether phase by pipetting from the upper layer of petroleum ether.

NOTE 2 The petroleum ether extract contains residual water. A drying step using  $\text{Na}_2\text{SO}_4$  (5.10) can be necessary to enable further analysis (see 7.5.1).

If concentrating the petroleum ether phase is necessary, use a Kuderna Danish sample concentrator (6.9). Rinse the separation funnel with 10 ml petroleum ether (5.6) and transfer this amount of petroleum ether also into the sample concentrator.

## 7.5 Measurement

### 7.5.1 Potential bioavailable fraction

Measure the content of organic contaminants in the obtained petroleum ether extract (see 7.3.3 or 7.4.3) using an appropriate analysis method. Exchange solvent and concentrate the extract if necessary. Results of analysis are expressed in mass/volume in the petroleum ether extract obtained in 7.3.3 or 7.4.3.

NOTE Depending on the measurement method it can be necessary to transfer the extract to another solvent.

### 7.5.2 Non-bioavailable fraction

Apply a subsequent harsh/exhaustive extraction technique (designed to measure the total concentration) to determine the non-bioavailable fraction. Use a suitable method (if available a standard method for soil or soil-like material) to extract the organic contaminant from the residual soil (see 7.3.2 or 7.4.2) followed by analysis for quantification.

## 7.6 Blank test

Within each extraction series, perform a blank determination according to the same applied procedure.

The contribution of the blank needs to be less than 50 % of the limit of quantification. Consider correction for the contribution of the blank if necessary.

## 8 Calculation

### 8.1 Potential bioavailable fraction

Calculate the potential bioavailable fraction,  $Q_{\text{pa}}$ , expressed as  $\mu\text{g}/\text{kg d.m.}$  It is obtained by dividing the measured absolute amount in the infinite sink by the dry mass of soil extracted. It can be calculated using the following Formula (2):

$$Q_{\text{pa}} = \frac{c_{\text{ex}} \times V_{\text{ex}} \times f \times 100}{M_{\text{s}} \times W_{\text{d.m.}}} \quad (2)$$

where

$Q_{pa}$  is the potential bioavailable amount of the contaminant, calculated in micrograms per kilogram dry matter ( $\mu\text{g}/\text{kg d.m.}$ );

$c_{ex}$  is the concentration present in the obtained petroleum ether extract (see 7.3.3 or 7.4.3), in micrograms per litre ( $\mu\text{g}/\text{l}$ );

NOTE The concentration is measured in a subsequent analytical procedure. The formula does not account for volume and solvent changes and the use of for instance internal standards in this subsequent procedure. This equation does not take possible evaporation of petroleum ether into account. This can increase the concentration.

$V_{ex}$  is the volume of the petroleum ether extract, in millilitre (ml): 15 ml is used for cyclodextrin (method A) and 40 ml for Tenax<sup>®</sup> (method B);

$M_s$  is the mass of soil extracted in grams (g);

$f$  is the correction factor that accounts for the proportion of the infinite sink that is taken forward for further analysis:  $f = 4$  for cyclodextrin (method A; only 10 ml of 40 ml of the extraction solution of cyclodextrin is used) and  $f = 1$  for Tenax<sup>®</sup> (method B; All Tenax<sup>®</sup> used is extracted);

NOTE If other proportions than described in this document are used  $f$  will change accordingly.

$W_{d.m.}$  is the content of the dry matter in the field moist sample in %, determined according to ISO 11465.

Only in cases where an amount of Tenax<sup>®</sup> was lost during phase separation (see 7.4.2, NOTE 2), then correct the potential bioavailable fraction using Formula (3):

$$Q_{pa-c} = \frac{Q_{pa} \times 1,5}{m_t} \quad (3)$$

where

$Q_{pa-c}$  is the corrected potential bioavailable amount of contaminant, calculated in  $\mu\text{g}/\text{kg d.m.}$ ;

$Q_{pa}$  is the potential bioavailable amount of contaminant, calculated in  $\mu\text{g}/\text{kg d.m.}$ ;

$m_t$  is the remaining amount of Tenax<sup>®</sup>, in g;

1,5 is the originally used amount of Tenax<sup>®</sup>, in g.

## 8.2 Non-bioavailable fraction

Calculate the non-bioavailable fraction according to the method used (the volumes of solvents used and the manipulation of subsequent extracts prior to analysis).

## 9 Expression of results

Report the potential bioavailable fraction in relevant mass units: micrograms per kilogram dry matter or milligrams per kilogram dry matter ( $\mu\text{g}/\text{kg d.m.}$  or  $\text{mg}/\text{kg d.m.}$ ).

The number of decimal places reported will generally depend on the precision of the method. As a rule, the available amount or contents will be reported in two significant figures (e.g. 5,5 or 0,074).

Where both the bioavailable and non-bioavailable fractions have been determined, these can be summed to obtain a total contaminant concentration in the matrix, see [Formula \(1\)](#).

## 10 Validation

[Annex C](#) contains the results of a validation study.

## 11 Test report

The test report shall include the following information:

- a) a reference to this document, e.g. ISO 16751;
- b) a complete identification of the sample;
- c) the method that is applied (method A or B);
- d) the expression of results according to [Clause 9](#) including a statement on the dry matter content;
- e) any details not specified in this document or which are optional, as well as any factors which may have affected the results.

STANDARDSISO.COM : Click to view the full PDF of ISO 16751:2020

## Annex A (informative)

### Preparation and regeneration of Tenax®

#### A.1 Preparation of new Tenax® before use

When using new Tenax® (5.5), fine particles shall be removed first. Wash the Tenax® with water, shaking in a ratio Tenax®/water of at least 20 in volumic fraction. Repeat this procedure until no more fine particles are detected. Dry the Tenax® at 125 °C. Consecutively wash the Tenax® with ethanol (5.7) and petroleum ether (5.6). Air dry the Tenax® followed by drying at 125 °C until the Tenax® is dry.

The cleaned new Tenax® material should be checked to determine if it is free from the compounds of interest using a suitable method.

#### A.2 Regeneration of Tenax®

Tenax® can be regenerated and reused. The quality of Tenax® beads shall be checked before washing it. Beads that are full of matrix do not float in water anymore. These beads cannot be re-used and shall be disposed. The rest of the beads are suitable for re-use, after they have been washed and dried.

To regenerate the used Tenax® wash it consecutively three times with ethanol (5.7), three times with acetone (5.8) and three times with petroleum ether (5.6). Each time use 10 ml of reagents per g of Tenax®. Air dry the Tenax® followed by drying at 125 °C until the Tenax® is dry.

Pressurized extraction is also suitable to clean Tenax®. Ethanol (5.7), acetone (5.8), petroleum ether (5.6) and a mixture of acetone/petroleum ether 1:1 in volumic fraction shall be passed consecutively through the Tenax® filled extraction cells.

## Annex B (informative)

### Illustrations

#### B.1 Method B: Tenax<sup>®</sup>, using a separation funnel

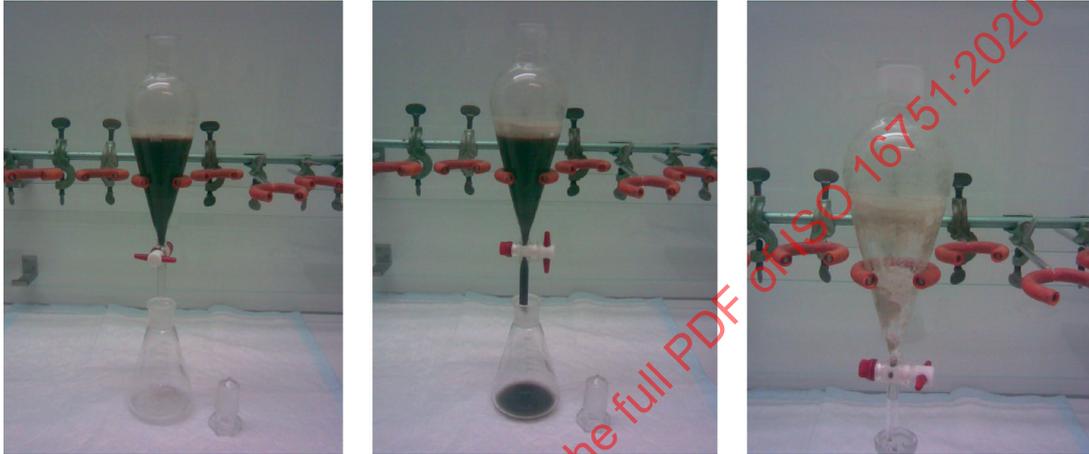


Figure B.1 — Collection of Tenax<sup>®</sup> after phase separation (see 7.4.3)



Figure B.2 — Removal of all visible soil or soil-like material by rinsing the Tenax<sup>®</sup> with water and draining the water (see 7.4.3)

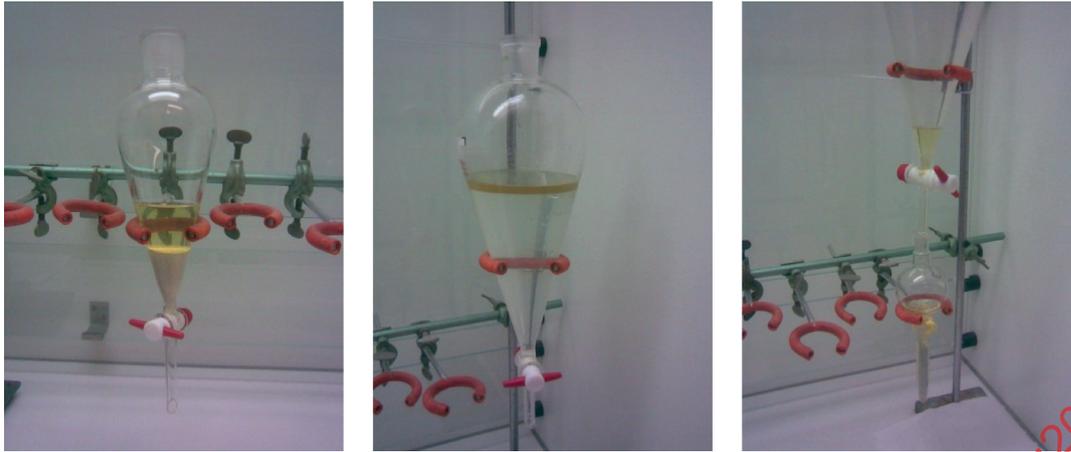


Figure B.3 — Extraction of the Tenax<sup>®</sup> and collection of the petroleum ether phase (see 7.4.4)

## B.2 Method B: Tenax<sup>®</sup>, using a centrifuge tube



Figure B.4 — Tenax<sup>®</sup> beads are floating on top of the water layer



Figure B.5 — Tenax® beads are collected using a Pasteur pipet and suction

STANDARDSISO.COM : Click to view the full PDF of ISO 16751:2020

## Annex C (informative)

### Validation study results

#### C.1 Introduction

An intra- and interlaboratory comparison on the environmental availability of non-polar organic compounds has been carried out during the development of the standard and in 2017. The extracts were measured using the method applied in the individual laboratories. The results are given in this [Annex C](#). A more detailed report<sup>[11]</sup> is available on [www.wepal.nl](http://www.wepal.nl).

#### C.2 Intralaboratory validation

In the laboratory of Rijkswaterstaat in Lelystad, The Netherlands, two samples have been analysed:

1. a freeze-dried sample, that is used as a reference sample in The Netherlands. It is a well-homogenized sample. Only 3 g of sample instead of 4 g as prescribed in ISO 16751 was used.
2. a wet sediment originating from the province of Limburg. 10 g of sample was used.

Over a period of 8 weeks, the samples have been analysed 8 times in duplicate. Repeatability ( $VC_r$  in %) and reproducibility ( $VC_R$  in %) have been calculated from the results, see [Table C.1](#) and [C.2](#). Only values above the limit of detection have been reported. The total content measured in the sample is presented in the last column of [Table C.1](#).

[Table C.1](#) and [C.2](#) show that the repeatability and reproducibility for the Tenax-method in sample 1 are comparable with values for the total analysis of organic contaminants in this sample (except DDT/DDE/DDD). They are better than values obtained in less homogenized samples. The values of the wet sediment sample in [Table C.2](#) are an example of such a less homogenized sample. In such a sample, the difference in daily subsamples can even be larger than the differences in averages of different days of analysis. The values for repeatability and reproducibility presented in [Table C.1](#) can be considered as best achievable. The values in [Table C.2](#) have to be considered as 'normal' values.

The values also show that in a homogenized sample 3 g of sample is sufficient for a proper analysis. It is not allowed to conclude that it is necessary to homogenize the samples before analysis. This may have effect on the availability.

The values for DDT/DDE and DDD are relatively high. This is caused by the instability of the chromatographic system for these compounds. The calibration of the two column GC/ECD was difficult. The values are therefore less suitable for validation. They are presented to show that also for bioavailability measurements it is important to use a proper functioning instrument.

Table C.1 — Intralaboratory performance for sample 1 (freeze dried)

	$s_r$ ( $\mu\text{g}/\text{kg dm}$ )	$C_{V,r}$ %	$S_R$ ( $\mu\text{g}/\text{kg dm}$ )	$C_{V,R}$ %	$x$ ( $\mu\text{g}/\text{kg dm}$ )	Availability (%)	Total ( $\mu\text{g}/\text{kg dm}$ )
Fenantrene	4,27	5,8	10,6	12,4	86,0	8,5	1 110
Fluoranthene	13,6	6,2	29,0	13,4	223	12,3	2 000
Pyrene	8,63	5,6	28,4	17,4	153		n.m.
benz(a)anthracene	5,28	7,0	3,77	5,2	75,2	7,5	1 100
Chrysene	8,09	6,9	5,45	4,6	117	10,9	1 190
benz(b)fluoranthene	7,60	6,4	6,24	5,3	119		n.m.
benz(k)fluoranthene	3,40	6,9	3,76	7,7	49,5	7,7	708
benz(a)pyrene	2,81	8,2	10,1	24,1	33,84	13,5	1 070
dibenz(ah)anthracene	0,52	7,5	0,50	7,1	7,00		n.m.
benzo(ghi)perylene	4,75	10,0	5,16	10,8	47,2	5,3	984
indeno(1,2,3cd)pyrene	3,49	8,0	4,11	9,2	44,5	4,5	1 090
HCB	1,32	8,8	2,67	17,6	14,2	9,4	166
PCB 28	0,13	9,8	0,28	22,6	1,19	5,5	24,1
PCB 52	0,53	8,9	1,01	17,3	5,64	18,0	34,5
PCB 101	1,14	9,9	1,80	14,0	11,9	24,8	52,7
PCB 118	0,53	9,8	0,54	9,9	5,46	18,2	33,0
PCB 153	1,24	8,3	1,82	11,6	15,3	20,9	80,2
PCB 138	1,40	8,2	2,34	13,2	17,4	24,3	78,9
PCB 180	1,28	12,4	1,90	18,7	8,69	19,4	49,2
p,p-DDD	0,56	8,8	2,83	36,6	7,25	47,7	16,7
p,p-DDE	0,62	12,9	0,47	10,2	4,78	44,6	11,8
o,p-DDD	0,43	13,0	3,99	57,7	4,75	85,1	6,14
<b>Key</b>							
$S_r$ is the repeatability standard deviation							
$VC_r$ is the relative repeatability standard deviation, in %							
$S_R$ is the reproducibility standard deviation							
$VC_R$ is the relative reproducibility standard deviation, in %							
$x$ is the mean value							

Table C.2 — Intralaboratory performance for sample 2 (wet sediment)

	$s_r$ ( $\mu\text{g}/\text{kg dm}$ )	$C_{V,r}$ %	$S_R$ ( $\mu\text{g}/\text{kg dm}$ )	$C_{V,R}$ %	$x$ ( $\mu\text{g}/\text{kg dm}$ )
Pyrene	9,56	16,0	5,73	11,1	57,0
benz(a)anthracene	5,67	25,8	1,77	12,6	16,6
benz(k)fluoranthene	3,95	24,1	1,32	11,7	12,5
benz(a)pyrene	8,28	23,6	6,17	26,2	23,1
<b>Key</b>					
$S_r$ is the repeatability standard deviation					
$VC_r$ is the relative repeatability standard deviation, in %					
$S_R$ is the reproducibility standard deviation					
$VC_R$ is the relative reproducibility standard deviation, in %					
$x$ is the mean value					

Table C.2 (continued)

	$s_r$ ( $\mu\text{g}/\text{kg dm}$ )	$C_{V,r}$ %	$S_R$ ( $\mu\text{g}/\text{kg dm}$ )	$C_{V,R}$ %	$x$ ( $\mu\text{g}/\text{kg dm}$ )
benzo(ghi)perylene	3,17	22,1	1,32	14,0	10,5
PCB 28	0,73	12,1	0,86	14,1	6,23
PCB 52	0,92	9,1	1,26	12,4	10,2
PCB 101	1,28	9,0	1,71	12,2	14,5
PCB 118	0,60	8,8	0,74	10,8	6,86
PCB 153	1,41	8,4	1,79	10,8	17,0
PCB 138	1,30	8,9	1,62	11,4	14,8
PCB 180	0,89	8,9	1,17	12,0	10,0
p,p-DDT	7,61	74,1	12,0	61,8	15,8
p,p-DDE	0,93	50,7	0,27	6,9	3,64
<b>Key</b>					
$s_r$ is the repeatability standard deviation					
$C_{V,r}$ is the relative repeatability standard deviation, in %					
$S_R$ is the reproducibility standard deviation					
$C_{V,R}$ is the relative reproducibility standard deviation, in %					
$x$ is the mean value					

### C.3 Interlaboratory validation

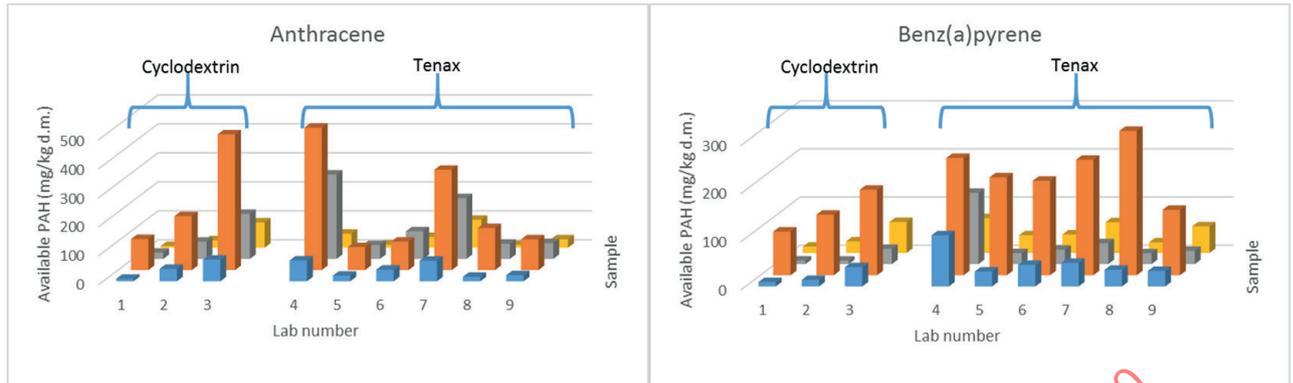
#### C.3.1 Test details

The statistical test was carried out according to ISO 5725 series. Four samples were used that are part of the WEPAL proficiency scheme and distributed to 9 participating laboratories. Each soil sample should be extracted three times according ISO 16751 and measured according the analytical procedures available in the laboratory. Four soil samples have been used with different soil properties (Table C.3) and content of organic contaminants. All materials used were homogenized and grinded materials. They were sieved over 0,5 mm and distributed in pots to contain 100 g of soil. Before sending, it was checked if the soils were homogenous distributed in the pots using previous test results and specific analysis of total concentrations in a random selection of 10 pots. Participants have submitted their results electronically through [www.wepal.nl](http://www.wepal.nl).

Table C.3 — Consensus values of general soil parameters in the analysed soils

Parameter	1	2	3	4
Clay (%)	21,2	8,82	31,0	12,9
Organic matter (LOI) (%)	3,49	3,23	6,56	3,13
PAH (sum) (mg/kg)	13,2	80,5	15,2	40
Mineral oil (mg/kg)	682	317	844	505
PCB sum (mg/kg)	0,286	0,172	0,74	0,015

Two extraction methods, cyclodextrin and Tenax, are allowed in ISO 16751 to extract the bioavailable fraction. Nine laboratories have participated, three of them applied cyclodextrin, and the other six used Tenax. These numbers are too low to establish if there is a difference between the methods, especially if the variation in the results is high as was observed in this study (Figure C.1).



**Figure C.1 — Bioavailability of Anthracene and Benz(a)pyrene. Results of cyclodextrin and Tenax extraction of participating laboratories in four samples**

[Figure C.1](#) shows the results of the measured bioavailability for two PAHs, which were analysed by all laboratories. Comparable results were obtained for other analysed parameters. The first three results in [Figure C.1](#) were analysed using cyclodextrin and the other six using Tenax. As mentioned, the variation was relatively high and results of both extraction methods are part of the same population. For the overall statistical evaluation, no differentiation was made between the two extraction methods.

### C.3.2 Results on PAHs

Not all participants have reported values for all PAHs. The PAH results with the highest number of participants and having different ring systems were evaluated. Results of the statistical evaluation for the PAHs Anthracene, Benz(a)anthracene, Benz(k)fluoranthene, Benz(a)pyrene, are presented in [Table C.4](#) to [C.6](#).

Table C.4 — PAHs available (results in mg/kg)

Sample	Element	<i>L</i>	<i>N</i>	% outliers	<i>x</i> (µg/kg dm)	<i>S<sub>R</sub></i> (µg/kg dm)	<i>S<sub>r</sub></i> (µg/kg dm)	<i>C<sub>V,R</sub></i> %	<i>C<sub>V,r</sub></i> %
1	Ant A	9	27	0,0	40,4	27,0	7,47	66,7	18,5
2	Ant A	9	26	0,0	221	172	53,2	77,9	24,1
3	Ant A	9	26	0,0	102	93,8	51,5	91,5	50,2
4	Ant A	9	26	0,0	36,5	32,2	8,56	88,3	23,5
1	BaA A	9	27	0,0	142	56,5	28,5	39,7	20,0
2	BaA A	9	26	0,0	1302	480	224	36,8	17,2
3	BaA A	9	26	0,0	126	59,5	42,1	47,2	33,5
4	BaA A	9	27	0,0	191	86,2	36,0	45,2	18,8
1	BkF A	9	27	0,0	47,6	27,2	10,0	57,2	21,0
2	BkF A	9	27	11,1	368	109	47,1	29,5	12,8
3	BkF A	9	26	22,2	43,9	20,7	5,39	47,3	12,3
4	BkF A	9	27	0,0	78,5	33,3	15,2	24,4	19,3
1	BaP A	9	27	0,0	40,0	29,1	9,12	72,7	22,8
2	BaP A	9	27	11,1	174	56,0	18,2	32,2	10,5
3	BaP A	9	26	11,1	24,0	12,9	5,25	53,7	21,8
4	BaP A	9	27	0,0	43,3	22,8	9,55	52,6	22,1

**Key**

*L* is the number of laboratories after elimination of outliers  
*n* is the number of results  
*x* is the mean value  
*S<sub>R</sub>* is the reproducibility standard deviation, in mg/kg dm  
*S<sub>r</sub>* is the repeatability standard deviation, in mg/kg dm  
*C<sub>V,R</sub>* is the relative reproducibility standard deviation, in %  
*C<sub>V,r</sub>* is the relative repeatability standard deviation, in %

Table C.5 — PAHs residue (results in mg/kg)

Sample	Element	<i>L</i>	<i>N</i>	% outliers	$\bar{x}$ ( $\mu\text{g/kg dm}$ )	$S_R$ ( $\mu\text{g/kg dm}$ )	$S_r$ ( $\mu\text{g/kg dm}$ )	$C_{V,R}$ %	$C_{V,r}$ %
1	Ant Res.	7	21	14,3	336	121	32,8	36,0	9,8
2	Ant Res.	6	18	0,0	844	416	121	49,3	14,3
3	Ant Res.	7	20	14,3	1 018	469	127	46,1	12,5
4	Ant Res.	7	21	0,0	416	221	77,4	53,1	18,6
1	BaA Res.	7	21	0,0	751	352	67,5	46,9	9,0
2	BaA Res.	7	20	0,0	4 576	2 587	469	56,5	10,3
3	BaA Res.	7	20	0,0	748	444	160	59,4	21,4
4	BaA Res.	7	20	14,3	2 737	943	244	34,5	8,9
1	BkF Res.	7	21	14,3	405	107	19,5	26,5	4,9
2	BkF Res.	7	21	0,0	2 740	1 031	226	37,6	8,2
3	BkF Res.	7	20	0,0	464	198	76,5	42,6	16,5
4	BkF Res.	7	20	0,0	1 321	619	174	46,9	13,2
1	BaP Res.	7	21	0,0	582	278	87,2	47,8	15,0
2	BaP Res.	7	21	0,0	3 998	1 232	361	30,8	9,0
3	BaP Res.	7	20	0,0	603	210	101	34,9	16,7
4	BaP Res.	7	20	28,6	2 613	1 612	117	61,7	4,5

**Key**

*L* is the number of laboratories after elimination of outliers

*n* is the number of results

$\bar{x}$  is the mean value

$S_R$  is the reproducibility standard deviation, in mg/kg dm

$S_r$  is the repeatability standard deviation, in mg/kg dm

$C_{V,R}$  is the relative reproducibility standard deviation, in %

$C_{V,r}$  is the relative repeatability standard deviation, in %