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BSI Standards Publication

Soil quality — Bioaccessibility of organic and inorganic pollutants from contaminated soil and soil-like materials

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National foreword

This British Standard is the UK implementation of ISO 8259:2024.

Bioaccessibility is dealt with in both this standard and BS ISO 17924:2018, incorporating corrigendum June 2022, *Soil quality – Assessment of human exposure from ingestion of soil and soil material – Procedure for the application estimation of human bioaccesibility/bioavailability of metals in soil*. Both standards have been validated against *in vivo* tests. They will, however, yield method specific results which, because the procedures differ, are likely to differ. The specifier needs to make a judgement about which method to use based, for example, on expert advice, experience, regulator preferences and laboratory convenience. BS ISO 17924, but not BS ISO 8259, can be used in conjunction with BS ISO 7303:2025, *Soil quality – Simplified method for oral bioaccessibilty of metal(loids) in soils materials*. It has been validated for the prediction of the results likely to be obtained using BS ISO 17924.

The UK participation in its preparation was entrusted to Technical Committee EH/4, Soil quality.

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

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International Standard

ISO 8259

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Soil quality — Bioaccessibility of organic and inorganic pollutants from contaminated soil and soil-like materials

Qualité du sol — Bioaccessibilité des polluants organiques et inorganiques provenant d'un sol ou de matériaux de type sol pollués

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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).

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

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Introduction

The concept of bioavailability is used during risk assessment of contaminants and contaminated land. It is used during human health risk assessment by application of in vitro methods designed to assess the bioaccessible fraction of a chemical such as the one described in this document.

ISO 17402 provides a general overview of the definitions and concept of bioavailability and of available methods to assess the bioavailability for several exposure pathways. ISO 15800 provides guidance on the soil and site characterization necessary for the evaluation of human exposure to substances present in soil including bioaccessibility and bioavailability. ISO 17924 describes a method to assess the bioaccessible fraction of metals in a contaminated soil after soil ingestion. This document describes a test procedure for the estimation of human bioaccessibility after oral uptake of both metals and non-volatile organic contaminants from soil.

To evaluate the health effects of the oral ingestion of contaminated soil or soil-like materials by humans, it is necessary to consider the total concentration of a contaminant in soil, the quantity of soil ingested, the dissolution of the contaminants from soil in the gastrointestinal tract as well as their absorption through membranes. Simulation of human absorption and bioavailability of contaminants can be determined in animal tests. For ethical reasons, and to reduce the amount of work and time needed, in vitro methods have been developed determining the quantity of contaminants that can be dissolved from the contaminated soil and soil-like materials by the digestive juices in the digestive tract.

Contaminants bound to soil and soil-like materials are generally only dissolved to a partial extent by the digestive juices in the digestive tract. The degree of dissolution depends on the type of contaminant, the characteristics of the soil and soil-like materials and the components of the digestive juices in the digestive tract. The dissolved contaminants can be absorbed into the organism in the gastrointestinal tract. Contaminants that remain bound are largely excreted in an unchanged state. The quantity actually resorbed is always less than or, at most, equal to the dissolved quantity.

Digestive juices are complex mixtures of electrolytes, enzymes and digestive aids. The composition of the digestive juices varies depending on their type, characteristics and quantity, and depends on exogenous and endogenous factors. The type of food consumed is a significant exogenous factor in this regard.

The presence of food has an impact on the dissolution process of contaminants from soil particles ingested by humans to their gastrointestinal juices. Therefore, it is relevant to add a food surrogate in an in vitro test, to mimic the true situation in vivo. Food surrogates like milk powder are acting as emulsifiers because the absorption of lipophilic substances depends significantly on the presence of lipids, for example from food, which causes the secretion of bile salts, as well as the formation of micelles resulting in higher bioaccessibilities for hydrophobic organic contaminants.[56,61] In addition, they can also have an impact on inorganic contaminants. The use of food additives in in vitro testing simulates dissolution in children after a standard meal (e.g. baby food) to achieve “realistic worst case” estimates for risk assessment especially for organic contaminants[34].

This document standardizes a test system to assess the bioaccessible fraction of pollutants in contaminated soils and soil-like materials with the aid of artificial digestive juices. The composition of the used artificial digestive juices corresponds approximately to the average composition of natural digestive juices of humans in the case of stimulated secretion, as occurs during the intake of foodstuffs. The treatment duration of the samples with gastric juice is 2 h and corresponds to the average residence times of foodstuffs in the stomach. In contrast, the treatment duration with intestinal juice is 3 h and based on the residence time of foodstuff components in the upper section of the small intestine, which is the main site of absorption.[61] To simulate food consumption and, hence, take the influence of foodstuff into account, milk powder is added to the test system as food surrogate. Soil and soil-like sample material that has not been crushed or ground is generally used after sieving at ≤ 2 mm simulating soil ingestion by eating without extensive mastication (e.g. by young children).

This method has been validated in vivo for arsenic, lead, cadmium, chromium, nickel and mercury by animal testing.[40] Polycyclic aromatic hydrocarbons (PAHs) including Benzo[a]pyrene (BaP) were also used in in vivo animal testing. An interlaboratory trial was carried out using arsenic, lead, cadmium and antimony as well as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and trinitrotoluene.[61]

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Several in vitro methods have been developed for the estimation of contaminant bioaccessibility. There are simplified approaches simulating the human gastrointestinal physiology by separate gastric and/or intestinal tests or by using gastric and intestinal phases subsequently. The test method described in ISO 17924 uses both a separate gastric phase as well as combined gastric and intestinal phases to assess the bioaccessibility of contaminants. The methodology used in this document is based exclusively on a combined gastric and intestinal phase. In contrast to ISO 17924, milk powder is used as food surrogate in the method described in this document. In addition, the method described in this document is not limited to inorganic contaminants but is also applicable to organic contaminants like PAHs or PCBs. The methods of both standards are in vivo validated and comparisons of both methodologies have been published.[42,43,46,47]

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Soil quality — Bioaccessibility of organic and inorganic pollutants from contaminated soil and soil-like materials

1 Scope

This document specifies a method for testing the bioaccessibility of substances from contaminated soil and soil-like materials. The method is not applicable for volatile contaminants. Furthermore, the method is only applicable if suitable analytical methods for extraction and detection of substances and/or elements from complex digestion assays are available.

NOTE During the in vivo validation with minipigs, the PAHs naphthalene, acenaphthylene, acenaphthene and fluorene were not evaluated due to their volatility.[40] However, the results of the overall recovery indicate if volatilisation has occurred during the test.

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/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

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

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

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
contaminated soil and soil-like material
<assessment of human bioaccessibility> soil and soil-like material containing *contaminants* (3.2) that can pose health risks if ingested by humans

3.2
contaminant
substance or agent present in the soil as a result of human activity

Note 1 to entry: There is no assumption in this definition that harms results from the presence of the contaminant.

3.3
total content
analyte concentration in a sample that is measured using a largely exhaustive extraction method, e.g. after aqua regia digestion (heavy metals) or solvent extraction (organic components)

3.4
duplicate determination
carrying out the method twice to determine the *bioaccessibility* (3.6), each time with a new subsample

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3.5
bioavailability
fraction of a substance present in ingested soil or soil-like materials that reaches the systemic circulation (blood stream)

3.6
bioaccessibility
fraction of a substance in soil or soil-like material that is liberated in (human) gastrointestinal juices and thus available for absorption

Note 1 to entry: Bioaccessibility is expressed and calculated as percentage transfer of a substance from the solid sample into the liquid phase (the gastrointestinal phase solution) of the in vitro test system specified by this document, where the reference quantity is the conventional *total content* (3.3) of the solid sample as analysed after digestion or extraction.

3.7
solid phase after centrifugation
solid phase remaining after centrifugation at the end of simulated digestion

Note 1 to entry: The solid phase after centrifugation contains the non-bioaccessible content and/or fraction.

3.8
mass balance
relationship between input and output of a specified substance in a defined system

Note 1 to entry: In this context, mass balance is the calculation of the contents of a *contaminant* (3.2) in the gastrointestinal phase (supernatant) and in the pellet after centrifugation as well as the calculation of their sum.

3.9
overall recovery
quality assurance measure whereby the sum of the content in the gastrointestinal phase (supernatant) and the *solid phase after centrifugation* (3.7) is compared with the *total content* (3.3), taking into account *sample inhomogeneity* (3.10)

3.10
sample inhomogeneity
inhomogeneous distribution of the analyte in a sample that results in subsamples with different analyte concentrations, described by the standard deviation of a replicate determination

4 Principle of the test

4.1 Human digestion

Digestion starts in the mouth, where, in addition to mechanical size reduction, an α-amylase contained in the spittle initiates the hydrolysis of starches. However, experience shows that separate consideration of this partial process is not essential with regard to the release of contaminants.[40] Food is denatured in the stomach by hydrochloric acid. At the same time, digestion of proteins by peptide hydrolases (pepsins) begins. In addition, lipases that originate from the duodenum initiate the digestion of fats. Maximum secretion of hydrochloric acid is achieved after 1 h. The hydrochloric acid is buffered to a pH value of between 3 and 4 by food components.[61] The pH value only falls into a range of 1 to 2 again when digestion has progressed. Depending on the characteristics of the food, it is transferred to the duodenum after a residence time of between just a few minutes and several hours. Here, the hydrochloric acid is first buffered to a pH value of 4 by hydrogen carbonate from the duodenum juice, pancreatic juice and bile. The pH value then increases more slowly to between 6 and 7,5. The pancreatic juice contains a range of peptide hydrolases (trypsin, chymotrypsin etc.), α-amylases for lysis of carbohydrates, and lipases for lysis of triglycerides into mono- or diacylglycerides and fatty acids. Lipids can be digested by lipases only in emulsified form. Already in the stomach, fats are mechanically suspended by the movements of the stomach. As a result of the influx of bile, which contains bile acids and lecithins as natural surfactants, strong emulsification of fats and the products of fat decomposition (di- and monoglycerides and fatty acids) occurs. In the small intestine (jejunum and ileum), further digestive enzymes are also present, but these do not differ significantly from the enzymes

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in the pancreatic juice in their effect. In total, the body produces an average of 0,5 l to 1,5 l of spittle, 2 l to 3 l of gastric juice, 0,5 l to 1,5 l of pancreatic juice, 0,8 l to 1 l of liver bile, which is concentrated by a factor of approximately 7 in the gall bladder, and 2 l to 3 l of intestinal juice from the small intestine in 24 h. The small intestine is the main organ for the absorption of food and drink components and pollutants. Here, approximately 8 l to 9 l of water is resorbed with approximately 70 g of electrolytes, 300 g of carbohydrates, 100 g of proteins and 70 g of fats every day. In addition, a large majority of the bile acids is recovered as no more than 4 g to 6 g is available to the body while several times this amount is required for digestion.

4.2 General test description

To assess the bioaccessibility of contaminants in soil or soil-like materials after oral ingestion, an in vitro test is described using two artificial digestive juices (artificial gastric juice and artificial intestinal juice) simulating the digestion in the human gastrointestinal tract under close to realistic physiological conditions, i.e. at an elevated temperature (37 °C), with continuous agitation and at a pH value that is typical for gastric or intestinal juices. A schematic workflow of the test is presented in Figure 1.

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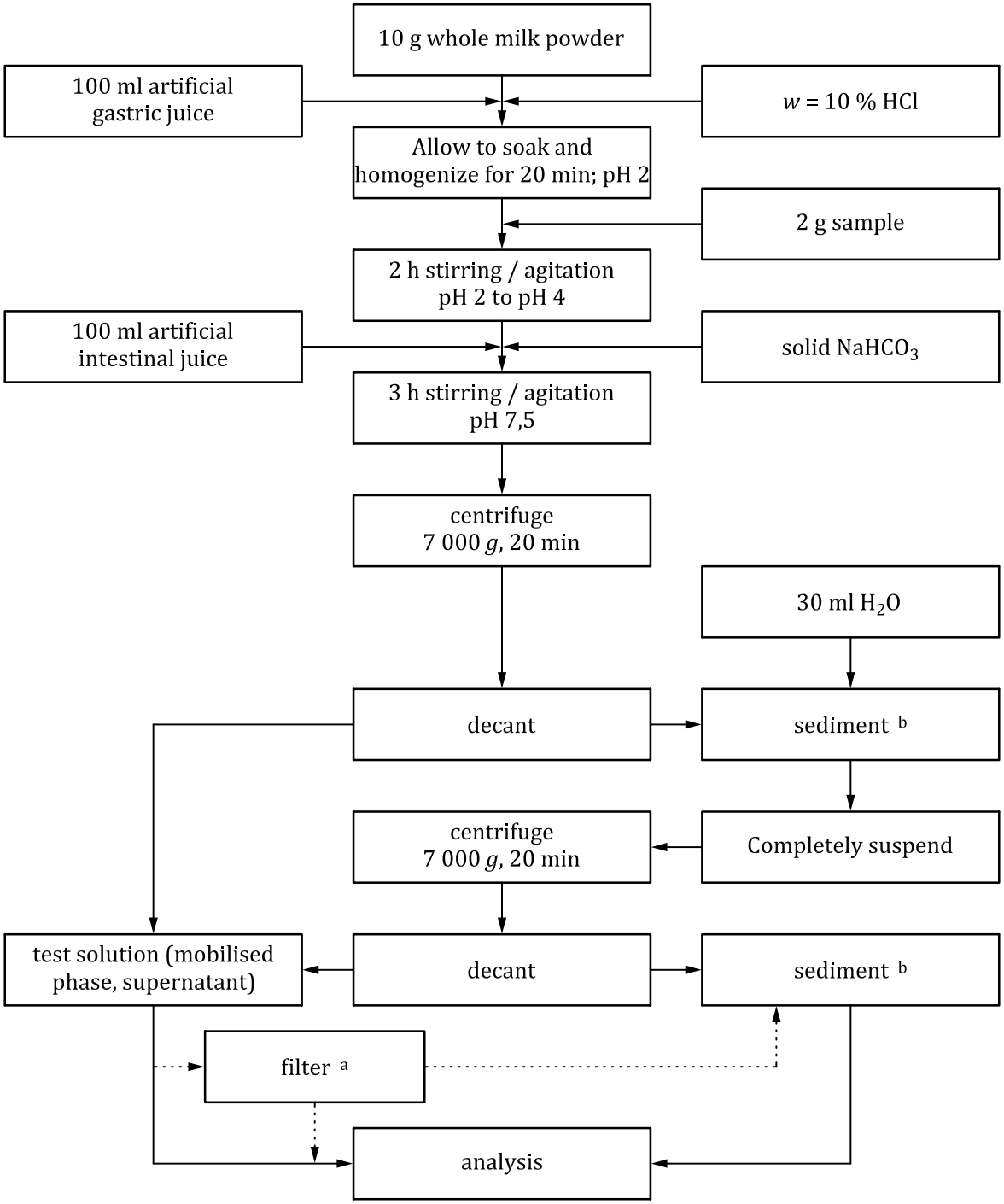

[a] If necessary when particles floating on the surface of the test solution.

[b] Quality assurance measure; if necessary, incl. filtration residue.

Figure 1 — Schematic diagram of test process

4.3 Applicability

The test to assess the bioaccessibility of contaminants in soil or soil-like materials can be used for scientific research as well as for risk assessment of contaminated sites. Concerning the risk assessment, it is presupposed that the national legal context is considered when and how bioavailability estimates can, or should, contribute to risk assessment. Usually, bioaccessibility data are used during a detailed and site-specific risk assessment (see ISO 15800) and in vitro tests are carried out if national threshold values based

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