



BSI Standards Publication

Simplified method for prediction of the oral bioaccessibility of metals and metalloids in soils

National foreword

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**International
Standard**

ISO 7303

**Simplified method for prediction of
the oral bioaccessibility of metals
and metalloids in soils**

*Méthode simplifiée pour la prédiction de la bioaccessibilité orale
des métaux et des métalloïdes dans les sols*

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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.

Introduction

Soils enriched with metals and metalloids such as arsenic (As), cadmium (Cd), and lead (Pb) pose a potential threat to human health if they are directly ingested or transferred through food. Incidental ingestion of soil particles is considered an important exposure pathway of metals and metalloids, more specifically for children through outdoor hand-to-mouth activities. In the current context of managing polluted sites and soils, assessing human health risks from the ingestion of contaminated soil particles remains a challenging area, with significant uncertainties in the methods used to protect public health. In some countries, current practices of risk assessment are based on total concentrations of metals and metalloids measured in soils. However, it is known that only a fraction of these metals and metalloids, i.e. the bioavailable fraction, is assimilated by the body and is likely to induce a toxic effect. Estimating exposure based on the total concentration of metals and metalloids therefore leads to overestimating the risk and possibly misclassifying sites as being at risk. This overestimation leads to excessive measures and costs in terms of management of these sites.

Taking into account the bioavailability of metals and metalloids in soils makes it possible to better assess exposures and risks and thus optimize the management of associated sites. Bioavailability is in practice estimated by the measurement of bioaccessibility, i.e. the soluble fraction that can be released into the gastrointestinal tract and that can reach the bloodstream. Methods have been developed and validated to mimic the availability of metals and metalloids in the human gastrointestinal tract. These *in vitro* laboratory tests simulate *in vivo* results. Among these methods, ISO 17924 is a reference method for assessing the oral bioaccessibility of As, Cd and Pb. Because ISO 17924 involves high technical skills and requires numerous chemical and biological reagents to mimic biochemical conditions in the gastrointestinal tract, it can often only be used for a small number of samples. If bioaccessibility needs to be estimated on many samples, a simplified method to predict bioaccessibility is of great interest, in particular for reasons of time and cost.

It has been clearly shown that the bioaccessibility of metals and metalloids in soils critically depends on soil type, their chemical speciation, and the solid-phase distribution in soils. Consequently, a simplified single-extraction method applicable to a range of soils regardless of their pedological, physical and chemical characteristics is needed. A simplified method to study jointly both the gastric and the intestinal phases is particularly advantageous.

This document provides a rapid, simple, and reproducible approach for predicting the bioaccessibility of metals and metalloids in gastric and gastrointestinal phases. The simplified method is specifically designed for As, Cd and Pb in soil samples, as assessed using ISO 17924, and relies on mathematical equations. These equations were derived from studies on French soil samples with a wide range of physicochemical parameters (in terms of texture, pH, content of organic matter and carbonates) and metals and metalloids concentrations (described in Reference [2]).

Digestion with hydrochloric acid (HCl) solution fulfils the criteria to be satisfied by an extractant for routine analyses. It is easy for analytical laboratories to use and reproduces conditions that are close to human physiological conditions (i.e. solid/liquid ratio, reagent, temperature, pH and residence time in the stomach). It can be applied to a wide spectrum of soils (in terms of physicochemical parameters) and elements (more specifically As, Cd and Pb) in a wide concentration range. This document differs from ISO 17924 in terms of application and methodology. More specifically, the simplified test can be used as a suitable proxy to predict the bioaccessibility of metals and metalloids in a first-tier screening on several soil samples. It is intended to help users to select a few samples (approximately 10 % of samples but can be as high as 20 % to 30 % depending on the size of the starting sample, heterogeneity of total concentrations and context, e.g. high pH values, high carbonate content) in a second-tier study and as a validation approach complementary to the method defined in ISO 17924 for better assessment of human exposure.

Because the ISO 17924 test has only been validated against *in vivo* data for As, Cd and Pb, the HCl test as described in this document is only validated for approximation of the oral bioaccessibility of these elements in soils. The simplified method could potentially be used for other elements; however, because no validation exists for the other metals or metalloids, the data obtained could only be considered as a useful line of evidence in interpreting results.

Simplified method for prediction of the oral bioaccessibility of metals and metalloids in soils

1 Scope

This document deals with the assessment of human exposure to metals and metalloids from ingestion of soil. It specifies a simplified method for the prediction of the oral bioaccessibility of metals and metalloids from contaminated soils.

The method is a chemical extraction using hydrochloric acid (HCl) and can be used at least for first-tier screening for prediction of the oral bioaccessibility of arsenic (As), cadmium (Cd) and lead (Pb) in soil samples. It produces extracts that are representative of the bioaccessible concentrations measured by using the validated unified bioaccessibility method (UBM) (ISO 17924). More specifically, from the extractable concentrations of metals and metalloids by HCl, the predicted bioaccessibility in both the gastric and gastrointestinal phases is calculated using the equations and the prediction intervals provided in this document, and which have been derived from simple linear regressions of data obtained during validation studies.^[2]

NOTE The simplified method is applicable to prediction of the metals and metalloids bioaccessibility in soil with total concentrations range from 2 mg/kg to 2 600 mg/kg for As, from 0,2 mg/kg to 480 mg/kg for Cd, and from 4 mg/kg to 50 000 mg/kg for Pb.

2 Normative references

There are no normative references in this document.

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 bioaccessibility

fraction of a contaminant present in ingested soil that is solubilised in the digestive fluids and thus available for absorption

3.2 bioavailability

fraction of a contaminant present in ingested soil that reaches the systemic circulation

3.3 exposure

dose of a chemical that reaches the human body

3.4 simplified method

one-step procedure to assess the availability of metals and metalloids in soil by using a chemical extractant

4 Test principle

The principle of the method (Figure 1) is to mimic the ability of the human body to dissolve metals and metalloids without physiological fluids. The method involves the measurement of metal and metalloid release from soil in a solution of HCl 0,65 % (volume fraction, i.e. 0,078 mol/l based on NF X46-031) with a liquid/solid (L/S) ratio of 830:1 (ml/g), pH 1,2 at 37 °C and a residence time of 1 h, which is representative of the gastric compartment. However, in addition, by applying a factor to the extractable concentrations of metals and metalloids by HCl obtained using the method described in this document, it is possible to predict the bioaccessibility in both the stomach phase and the combined stomach and intestine phase that is likely to be obtained using the method described in ISO 17924.

Thus, the simplified method can be used to predict oral bioaccessibility, which can then be used to approximate bioavailability.

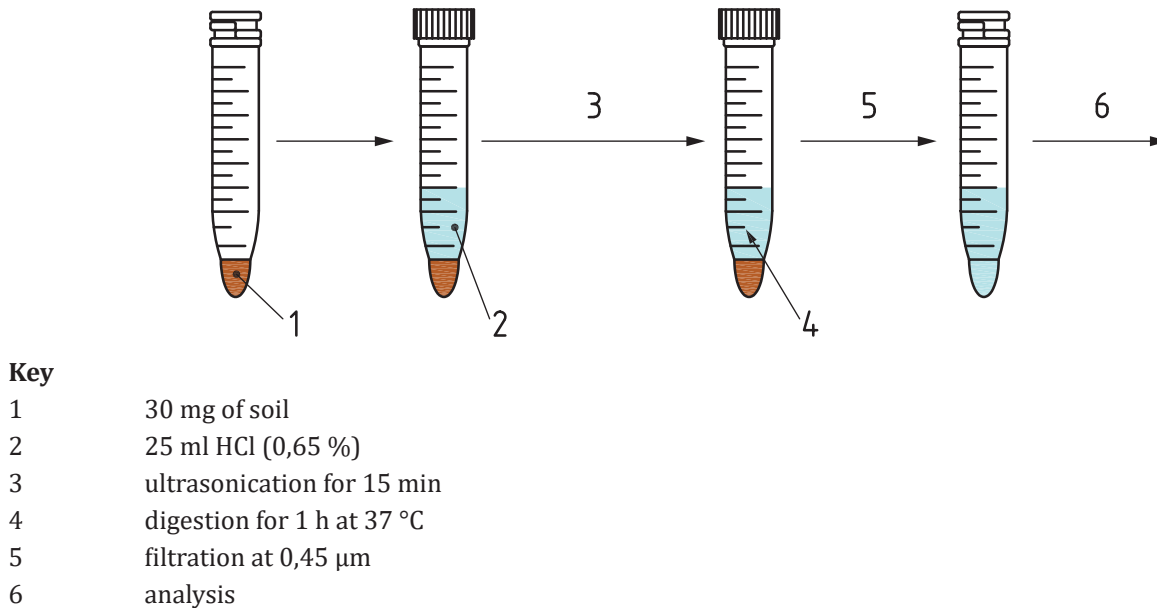


Figure 1 — Diagram of the simplified method by HCl

NOTE 1 The protocol using HCl extraction was previously developed for measuring the bioaccessible (stomach accessibility) concentrations of Pb in wipe samples of dust and soil^[4,5] and then adapted for soil samples^[2].

NOTE 2 The L/S ratio in the HCl extraction differs from the L/S in the ISO 17924 method because the simplified protocol was developed initially for wipe samples of dust. However, the 830:1 ratio was relevant and included in the range reported in the literature,^[6] i.e. between 100:1 and 5 000:1. This range was chosen to encompass a variety of conditions that can be plausible in the human stomach, and was defined to approximate the mass of soil that can inadvertently enter the mouth during normal outdoor activities and the resulting mixture that would be present in the stomach of child.

NOTE 3 Very good relationships were obtained between the predicted bioaccessibility by HCl and both the gastric and gastro-intestinal bioaccessibility measured by ISO 17924.^[2]

5 Apparatus

The apparatus specified shall be checked before use for proper operation and absence of interfering elements that can affect the results of the test. The apparatus shall also be calibrated where relevant.

All glassware and tubes shall be cleaned prior to use using a suitable acid rinsing protocol.

5.1 Analytical balance, with an accuracy of at least 0,01 mg.

5.2 Ultrasound bath.

5.3 Graphite block digestion system, water bath or incubator, capable of maintaining a temperature of $(37 \pm 2) ^\circ\text{C}$.

5.4 Volumetric flasks, suitable grades.

5.5 Polyethylene tubes (50 ml).

5.6 Pipettes.

5.7 Filtration system (0,45 μm).

5.8 Sieving equipment, with sieves of 250 μm nominal screen size.

6 Reagents

Use only reagents of recognized analytical grade.

6.1 Ultra-pure water Milli-Q or bi-distilled water.

6.2 Hydrochloric acid (HCl), $c(\text{HCl}) = 37 \%$.

6.3 Diluted hydrochloric acid, $c(\text{HCl}) = 0,65 \%$ (volume fraction).

Dissolve 6,5 ml of hydrochloric acid (37 %) in 1 l water volumetrically.

7 Procedure

7.1 Sample pre-treatment

All samples required for extractability testing shall be air-dried at a temperature below $40 ^\circ\text{C}$ and sieved to $\leq 250 \mu\text{m}$ without prior mechanical grinding as recommended in ISO 17924. Milling shall not be employed to achieve the desired particle size. Samples shall be thoroughly mixed prior to use, to ensure homogenization.

To ensure a sufficient amount of soil sample sieved to $\leq 250 \mu\text{m}$, at least 100 g of starting material shall be used (i.e. the fresh soil sampled from which the coarse elements have been removed).

NOTE Sieving to less than 250 μm allows the recovery of soil particles most characteristic of hand-sticky particles. The homogeneity of the sample will be lower with sieving compared to grinding, but sieving allows for a representative exposure.

7.2 Preparation of test samples

Two $(0,030 \pm 0,001)$ g test samples of each test soil prepared as described in [7.1](#) shall be weighed in (or placed into) uniquely labelled suitable tubes. The weighed test samples shall be stored at room temperature prior to completion of the extraction methodology. For every 10 unknown test samples, a blank sample and a reference material sample (see [8.1](#)) shall be extracted.

NOTE 10 unknown test samples, carried out in duplicate, correspond to 20 tubes.

7.3 Extraction procedure

The following procedure shall be followed:

7.3.1 Accurately add, to each polyethylene tube, via pipette 25 ml of extraction solution (i.e. diluted hydrochloric acid at 0,65 %).

7.3.2 Cap each tube and place them in the ultrasonication bath at room temperature for 15 min to mix the soil and solution.

7.3.3 Place the tubes in the graphite block or in the incubator at 37 °C for 1 h (without end-over-end rotation).

7.3.4 Filter the extracted solution (0,45 µm filter) and store the sample at < 8 °C prior to determination of the extractable contaminant concentration by an appropriate analytical method.

8 Data handling, quality control and presentation of results

8.1 General

The sufficiency of data obtained shall be evaluated in terms of analytical and testing quality.

The quality of the data to be used can be ensured by:

- setting formal data quality objectives (e.g. for accuracy, repeatability, reproducibility);
- using standard analytical and testing methods (for example ICP-OES according to EN 11885 or ICP-MS according to EN 17294-2), in this case the procedure described in this document;
- using accredited laboratories using standardized analytical methods and employing their own quality assurance procedures;
- including blanks;
- making duplicate determinations: if the deviation between the two measurements is greater than ± 10 % of the average value, an additional duplicate determination shall be carried out;
- including testing of a relevant reference material. An in-house reference material may also be used corresponding to a material developed by a laboratory for its own internal use, and for which a control chart has been established.

There are few reference soils that can be used as a relevant reference material: for example, the BGS guidance soil BGS102 available from the British Geological Survey^[9] and NIST 2710a and NIST 2711a available from the National Institute of Standards and Technology^[10]. In this document, the method performance, based on repeatability, was carried out on NIST 2710a and BGS102, but also on two other references (SS1 and SS2 from EnviroMAT™) classically used as controls for analyses on contaminated soils (i.e. total digestion values) and not specific for bioaccessibility. Method performance characteristics are presented in [Annex A](#). The mean extractable concentrations and the acceptable ranges for As, Cd and Pb for each of the reference materials are given in [Table 1](#).

Table 1 — Mean extractable concentrations of As, Cd and Pb and acceptable ranges for each reference material

Name	As		Cd		Pb	
	Mean mg/kg	Range mg/kg	Mean mg/kg	Range mg/kg	Mean mg/kg	Range mg/kg
NIST 2710a	782	687 to 857	5,77	5,24 to 6,41	3 681	3 265 to 4 340
BGS 102	3,13	2,39 to 3,56	0,25	0,22 to 0,28	26,7	22,3 to 32,2
SS1	4,78	3,88 to 5,76	2,51	2,24 to 2,96	619	509 to 712
SS2	0,92	0,57 to 1,12	0,68	0,61 to 0,76	216	188 to 240