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Soil quality — Assessment of human exposure from ingestion of soil and soil material — Guidance on the application and selection of physiologically based extraction methods for the estimation of the human bioaccessibility/bioavailability of metals in soil

Qualité du sol — Évaluation de l'exposition humaine par ingestion de sol et de matériaux du sol — Lignes directrices pour l'application et la sélection de méthodes d'extraction fondées sur le point de vue physiologique pour l'estimation de la bioaccessibilité/biodisponibilité pour l'être humain de métaux dans le sol

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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.

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ISO/TS 17924 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

Introduction

When assessing soils contaminated with, for example, metals, soil ingestion, especially by children, is often considered to be the most important exposure pathway. Currently, this assessment is often carried out on the basis of the total content of the metals in question in the soil. However, several studies suggest that the availability of the metals in the gastrointestinal tract is dependent on the form of the metals present and the site-specific soil chemistry. Test methods based on *in vivo* tests with, for example, juvenile swine or mini-pigs are time consuming and expensive, and not very compatible with the decision processes connected with the assessment and clean-up of contaminated sites. At present, test methods are being developed and validated, which involve *in vitro* laboratory tests aimed at simulating *in vivo* results. This will reduce the cost and practicalities related to the use of such testing on contaminated land.

Due to the large expenditure necessary for both private landowners and public funds set aside for the remediation of contaminated land, International Standards on the assessment of contaminated soil, especially with regard to human health, are in great demand. International Standards in this complex field will support a common scientific basis for the exchange of data, development of knowledge and sound evaluation. Furthermore, International Standards will facilitate international recommendations and regulations. The aim of this Technical Specification is to describe the elements of such an *in vitro* test system, and give advice on the appropriate combination and use of these elements in the specific situation.

In human health-risk assessment, “bioavailability” is specifically used in reference to absorption into systemic circulation, consistent with the toxicological use of the term. This encompasses bioaccessibility, which again is a combined measure of the processes determining the interaction between the metal associated with the soil and the liquid in the human digestion system. Bioavailability furthermore includes the absorption of the contaminant through a physiological membrane and the metabolism in the liver. The bioavailable fraction is thus the fraction left after release into the human digestive liquid, transport across the intestinal epithelium and metabolism in the liver. A further description of these processes is given in Clause 4.

When considering bioavailability as the fraction of the chemical that is absorbed into systemic circulation, two operational definitions are important, *absolute* and *relative* bioavailability. Absolute bioavailability is the fraction of the applied dose that is absorbed and reaches the systemic circulation (and can never be greater than 100 %). Relative bioavailability represents a comparison of absorption under two different sets of conditions, for example, from a soil sample vs. food or another matrix used in a toxicity study, and can be greater than or less than 1. This factor can be used in exposure assessments for exposure by direct ingestion of soil, for instance, if the absolute bioavailability of the metal in the specific soil is suspected to differ significantly from the absolute bioavailability implicit in the toxicity value/quality criteria used.

Soil quality — Assessment of human exposure from ingestion of soil and soil material — Guidance on the application and selection of physiologically based extraction methods for the estimation of the human bioaccessibility/bioavailability of metals in soil

1 Scope

This Technical Specification deals with the assessment of human exposure from ingestion of soil and soil material.

This Technical Specification gives guidelines to be used when choosing a physiologically based test procedure for the estimation of the human bioaccessibility/bioavailability of metals from contaminated soil in connection with the evaluation of the exposure related to human oral uptake. Suggestions are made for the use of as many generic-method elements as possible, but it is important that the choice of method be based on the needs of the specific investigation. Methods that are validated for specific metals and/or contexts are highlighted.

2 Normative references

The following referenced documents are indispensable for the application 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*

3 Terms and definitions

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

3.1
absorption
process by which a body takes in substance and makes it a part of itself

3.2
bioaccessible fraction
fraction of a substance in soil or soil material that is liberated in (human) gastrointestinal juices and is thus available for absorption

3.3
bioavailable fraction
fraction of a substance present in ingested soil or soil material that reaches the systemic circulation (bloodstream)

3.4
contaminant
substance or agent present in the soil as a result of human activity

NOTE 1 There is no assumption in this definition that harm results from the presence of the contaminant.

NOTE 2 Adapted from ISO 11074:2005.

3.5
dermal contact
contact with (touching) the skin

3.6
exposure
dose of a chemical that reaches the human body

3.7
exposure pathway
route a substance takes from its source to a receptor

3.8
ingestion
act of taking substances (e.g. soil and soil material) into the body by mouth

3.9
in vitro bioaccessibility test
designation for bioaccessibility test carried out outside a living organism

3.10
no observed adverse-effect level
NOAEL
dose at which no adverse effect on a receptor can be observed

3.11
pica
eating habit where usually strange and unpalatable materials are consumed, e.g. soil material, stones

NOTE The term “pica” stems from the Latin name Pica pica for the raven bird magpie which randomly picks up any kind of material for nest construction.

3.12
provisionally tolerable weekly intake
PTWI
designation of the provisional weekly tolerable amount of a substance which can be taken in by a human body in its lifetime through the food chain without affecting human health

3.13
receptor
potentially exposed person or part of ecosystem

[ISO 11074:2005]

3.14
relative absorption fraction
RAF
ratio between the amount of a contaminant reaching systemic circulation when ingested with, for example, soil and the same amount obtained when ingested in the toxicity experiment underlying the criteria

3.15
species
different forms of a substance always arising with each other in a reaction equilibrium

3.16
tolerable daily intake value
TDI
designation of the tolerable daily amount of a substance which can be taken in by a human body in its lifetime through the food chain without affecting human health

4 Bioaccessibility/bioavailability as a concept in assessment of soils and sites with respect to human exposure

The characterisation of bioaccessibility/bioavailability is usually performed as a part of a risk and/or exposure assessment.

A risk assessment comprises the following elements:

— a hazard identification;

— a dose-response assessment;

— an exposure assessment;

— and, based on the above, a risk characterisation.

An exposure assessment is the process wherein the intensity, frequency, and duration of human exposure of a contaminant are estimated, and it comprises the following:

— source identification and characterisation;

— identification of exposure routes;

— identification of relevant receptors/target groups;

— and, based on this, the actual exposure assessment.

For the assessment of possible effects on human health, an analysis of the exposure routes is a prerequisite. Where receptors are not directly exposed to a contaminant, exposure assessment needs to consider the various ways by which indirect exposure might occur and the significance of them.

Human exposure from soil contamination may occur through different media.

Directly from the soil, the following exposure routes exist.

— Soil ingestion, both dietary and through adherence to hands and unwashed vegetables, etc.

— Dermal contact.

— Ingestion of house dust that predominantly consists of soil material.

Airborne exposure comprises the following:

— inhalation and ingestion of fugitive dust;

— inhalation of elevated outdoor concentrations;

— inhalation of vapours that have intruded into buildings.

Exposure through the food chain comprises the following:

— consumption of plants including crops, plants, wild plants and fungi;

— consumption of animals and animal products, including wild animals;

— consumption of contaminated water.

Within this Technical Specification, direct uptake of soil via ingestion and/or ingestion of fugitive dust is considered. Oral ingestion is one of the most important exposure routes for humans to soil contaminants.

Quality criteria for soil (the maximum concentration limits for soil) are usually calculated on the basis of a tolerable daily intake value (TDI) or a provisionally tolerable weekly intake (PTWI), that can be derived from the no observed adverse-effect level (NOAEL) found in human data or experimental animal data. For genotoxic carcinogens for which no lower threshold for increased risk for cancer risk is assumed, the TDI value is set at a level that corresponds to a tolerable low (negligible) cancer risk level.

For determining the TDI, data on oral toxicity are primarily considered. Often these data pertain to animal experiments where the substance is administrated to the animals mixed in the feed or in drinking water (the vehicle or transporter of the contaminant). The amount of contaminant needed to produce adverse health effects in the animal is then recorded. As an alternative, epidemiological studies relating observed human health effects to recorded exposures have been used. Most toxicological studies report the total ingested amount and only seldom indicate exact values for the bioavailability of the substances administered.

When extrapolating from such experimental conditions to other conditions, for example, the intake of contaminated soil, this approach assumes that the uptake efficiency is equal for all scenarios, i. e. that the absolute bioavailability of the contaminant is constant. The absolute, oral bioavailability can be defined as the fraction of an orally ingested contaminant that reaches systemic circulation, i. e. enters the bloodstream. The absolute oral bioavailability of a contaminant may range from close to 0 to almost 1 (i. e. 100 %) depending upon the physiochemical form of the contaminant. In this context, the use of the concept of absolute, oral bioavailability rests upon the assumption that adverse health effects are systemic and thus triggered by the contaminants reaching the bloodstream, i. e. the internal exposure, as opposed to the external exposure measured directly as the intake of contaminated medium multiplied by the concentration of the contaminant in the medium, see Figure 1.

The absolute bioavailability can be measured as the ratio between amounts in the blood of animals or man after intravenous injection (100 % bioavailability) and after oral ingestion (uptake of bioavailable fraction).

A more feasible approach is to measure the relative bioavailability or relative absorption fraction (RAF), which is the ratio between the amount of a contaminant reaching systemic circulation when ingested with, for example, soil and the same amount obtained when ingested in the toxicity experiment underlying the criteria.

It should be noted that, although most relative bioavailabilities are less than 1 and would result in increased acceptable levels, RAF values above 1 could be found that would result in a demand for a decreased acceptable level.

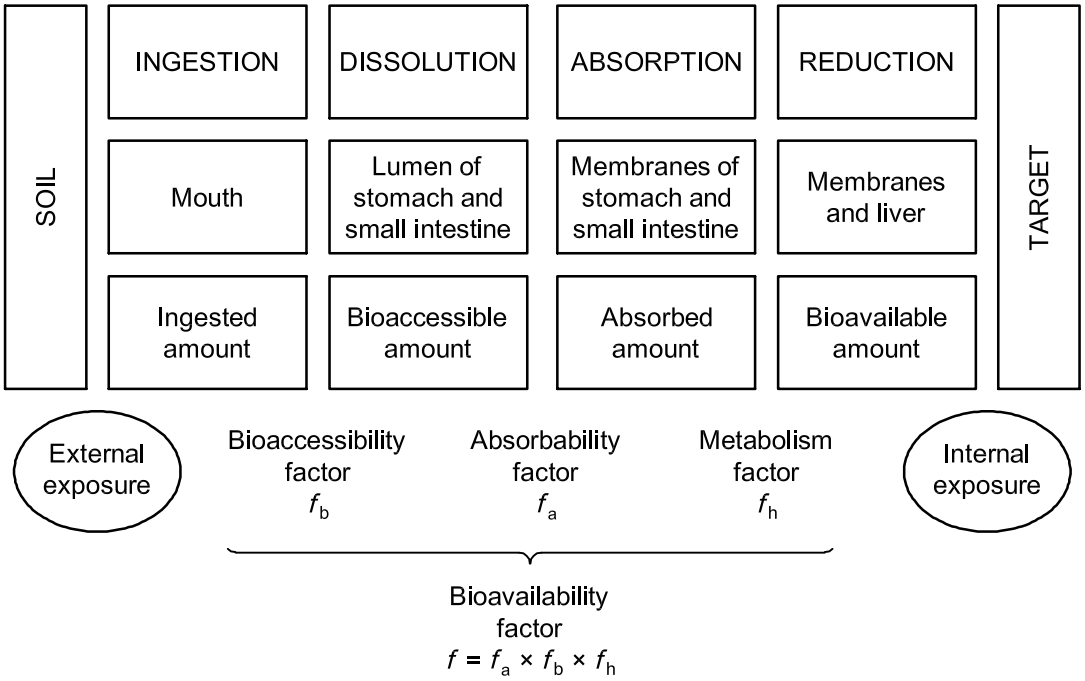

Figure 1 — Schematic presentation of oral uptake processes

5 Description of the mechanisms of human contaminant uptake

A series of compartments are involved in human bioavailability of ingested soil contaminants, as described in Clause 4.

The overall pathway leads the food and soil with contaminants from the mechanical grinding in the mouth, through a series of chemical and microbiological processes, to partial dissolution through the entire gastrointestinal tract (bioaccessibility processes). The dissolved components are transported through the membranes of the gastrointestinal epithelium (absorption) and into the bloodstream. During transport through the membranes, degradation can occur (metabolism). The blood passes through the liver before entering the systemic circulation, allowing for degradation or removal of unwanted compounds in the liver (metabolism, first-pass effect). Most of the dissolution processes are completed before the material leaves the small intestine, and it is generally accepted that most of the uptake takes place in the small intestine. To which extent uptake takes place in the stomach depends on the compound. The environment in the compartments differs and accordingly impacts the bioaccessibility process differently, see Table 1.

The pH in the stomach may vary from close to 1 under fasted conditions to as high as 5 after feeding. Residence time (1/2 time for emptying) in the stomach varies similarly from 8 min to 15 min to 30 min to 3 h for fasted and average fed conditions, respectively. Furthermore, bile release varies as well, with high releases under fed conditions. Finally, the pH in the stomach can be lower for small children than for adults.

Table 1 — Functions and conditions in the compartments involved in bioaccessibility processes

Compartment	Primary digestion functions	Main added "reagents"	pH	Residence time	Contaminant dissolution function
Mouth	Grinding Cleavage of starch	Moisture Amylase	6,5	Seconds to minutes	Grinding enhances subsequent dissolution.
Gullet	Transport	None	6,5	Seconds	None
Stomach	Cleavage of proteins and fats	Hydrochloric acid Proteases Lipases	1 - 5	8 min to 3 h	Acid dissolves labile mineral oxides, sulfides and carbonates to release metals.
Small intestine	Cleavage of oligosaccharides, proteins, fats and other constituents Solubilisation of fats	Bicarbonate Bile Proteases Lipases Oligosaccharases Phosphatases	4 - 7,5	2 h to 10 h	Organic matter is dissolved and bound contaminants released. Cationic metals are solubilised by complexation with bile acids. Some metals are precipitated by the high pH or by phosphate.

The gastrointestinal tract constitutes a complex ecosystem with aerobic and anaerobic micro-organisms. The density of micro-organisms is less in the human stomach and in the upper part of the small intestine but increases towards and in the large intestine. In human faeces, anaerobic micro-organisms dominate, whereas aerobic bacteria are found in high densities higher in the large intestine. Sulfate-reducing bacteria have been detected in the human large intestine, but, on the other hand, high concentrations of oxygen have been detected throughout the gastrointestinal tract of pigs. Overall, dominating aerobic conditions and micro-organisms would be expected in the stomach, but with increasingly anaerobic conditions from the small intestine to the large intestine.

Absorption requires that the contaminants are dissolved (free or bound to a dissolved carrier such as bile), transported to the gastrointestinal wall and, if bound to a carrier, released at the surface of the gastrointestinal membrane for absorption. The carrier mechanisms can be complexation of cationic metals by bile acids. Bile acids, proteins and other complexing agents can enhance exposure for cationic metals. Also, lipids and other soluble organic matter in the diet can add to the carrier effect of the bile.

The simple dissolution/transport/absorption processes can be complicated by chemical kinetics resulting from the sequential change in the chemical environment of the gastrointestinal tract, as well as by soil and contaminant chemistry. As an example, lead found in soil in the common contaminant anglesite ($PbSO_4$) will dissolve in the stomach and will stay in solution here at the low pH and high chloride concentration, see Figure 2. Entering the higher pH conditions in the presence of dissolved phosphate in the small intestine, the dissolved lead ions (Pb^{++}) will precipitate very quickly as lead chlorophosphate [chloropyromorphite, $Pb_5(PO_4)_3Cl$]. The phosphate can originate from digested food or from the soil. Phosphate minerals, such as hydroxyapatite, $Ca_5(PO_4)_3OH$, will dissolve in the low pH of the stomach, but dissolution will be slower and less complete at a higher pH in the stomach (as occurring after food ingestion). If stomach transit is fast (as occurring under fasting conditions), the hydroxyapatite may not dissolve in the stomach and reach the small intestine where the neutral to slightly alkaline pH will prevent further dissolution, and thus also precipitation of released lead as lead chlorophosphate. Conversely, just after transit from the stomach to the small intestine, the pH is still low and absorption of lead can take place as a result of the high dissolved lead concentration possible in acidic pH. Overall, the *de facto* dissolution of lead from soil will depend upon interacting conditions, such as soil composition, simultaneously ingested food and the feeding conditions of the human. This also means that cultural factors that affect the type of food typically consumed can have an influence on the actual uptake.

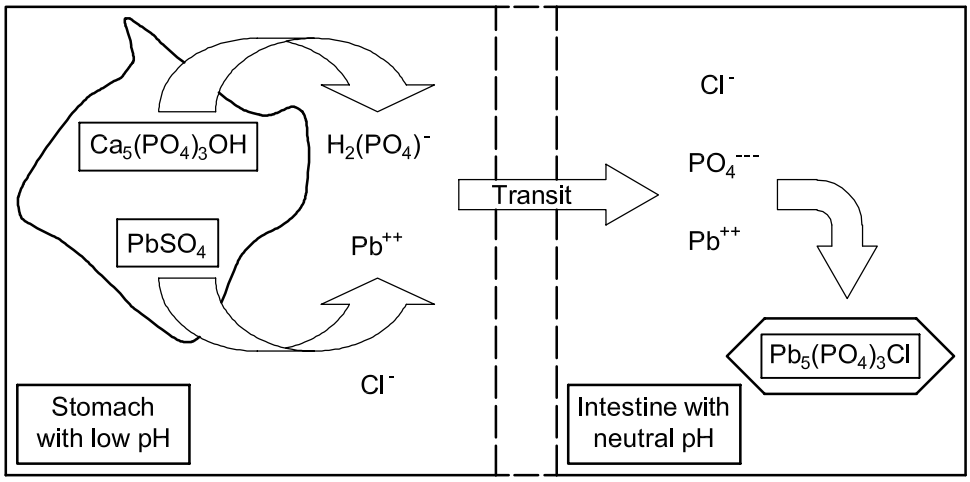

Figure 2 — Example of dissolution of a lead mineral (lead sulfate) in the stomach and subsequent precipitation in the small intestine

The absorption of dissolved contaminants predominantly occurs through the epithelium of the stomach and the small intestine (the intestinal epithelium), either through the cells (transcellular transport) or between the cells (paracellular transport). The pathway between the cells is primarily taken by polar or ionic contaminants (e.g. some metals).

Metals are absorbed by passive paracellular transport, by passive transcellular diffusion or by active transcellular transport, fitting into a transport system already present. One example is that cadmium can be absorbed by both the passive paracellular route and the passive diffusive route. Another example is lead that is probably absorbed via the calcium uptake system(s), including both active and passive transcellular transport, as well as by paracellular transport.

Metabolism of the absorbed contaminant concentrations takes place in the epithelium membranes (binding and exclusion), as well as in the liver (transformation of metals, and secretion of metals with bile). Contaminants entering systemic circulation via the lymph will be less efficiently reduced, as the liver is bypassed for this route. Finally, the contaminants are diluted when entering systemic circulation in the bloodstream.

If the sensitivity to changes in the processes of dissolution, absorption and reduction is considered to be caused by varying “vehicles” (i.e. ingestion with soil, food or in solution) and chemical forms (i.e. different metal salts ingested), it is expected that dissolution will be highly sensitive, absorption will be sensitive and reduction will be slightly sensitive (chemical form) or insensitive (vehicle). In applying the concept of relative bioavailability (see Clause 4), the most important factor to assess would thus be the bioaccessibility factor f_b (see Figure 1) followed by the absorbability factor f_a.

Estimation of the relative bioavailability factor is thus reduced to an estimation of how the two potentially rate-limiting processes of dissolution and absorption respond to variations in the vehicle and chemical form of the contaminants.

If the dissolution process is rate limiting (i.e. if dissolution is slower than absorption), changes in f_b will determine the relative bioavailability. If the absorption process is rate limiting (i.e. absorption of dissolved contaminants is too slow to be completed before transit), f_a will be “in charge” of relative bioavailability. *In vitro* tests are generally based on the measurement of bioaccessibility, and are thus based on the assumption that absorption is not rate limiting, or at least that the absorption of a compound dissolved from soil is no different from the absorption of the compound when administered in a fairly soluble form.

6 Description of metal-binding mechanisms (speciation of metals) in soil

In assessing the bioaccessibility of metals in soil, three major obstacles are encountered:

— most metals occur naturally at varying concentrations and in varying physical and chemical forms;

— chemical forms (species) of the original metal (source) may vary from the solid metal to the aqueous solution of a salt;

— chemical forms are interchangeable, depending upon the soil conditions and history.

Assessment of bioaccessibility data for metals in soil therefore needs to reflect the varying geochemical conditions. Due to their different physical-chemical properties, the mechanisms for reduced bioaccessibility differ among the metals. An example of distribution between phases and chemical forms (species) in soils is shown for copper in Figure 3. The bioaccessibility of the three solid species of copper, free metal (Cu), copper sulfide (CuS) and copper cations bound by ion-exchange mechanisms, will differ. Similarly, the absorption of the three dissolved species of copper, free copper ions, copper ions in inorganic complexes and copper in organic complexes with, for example, humic substances or organic acids, may differ, depending upon the stability of the complexes in the gastrointestinal lumen, see Clause 4.

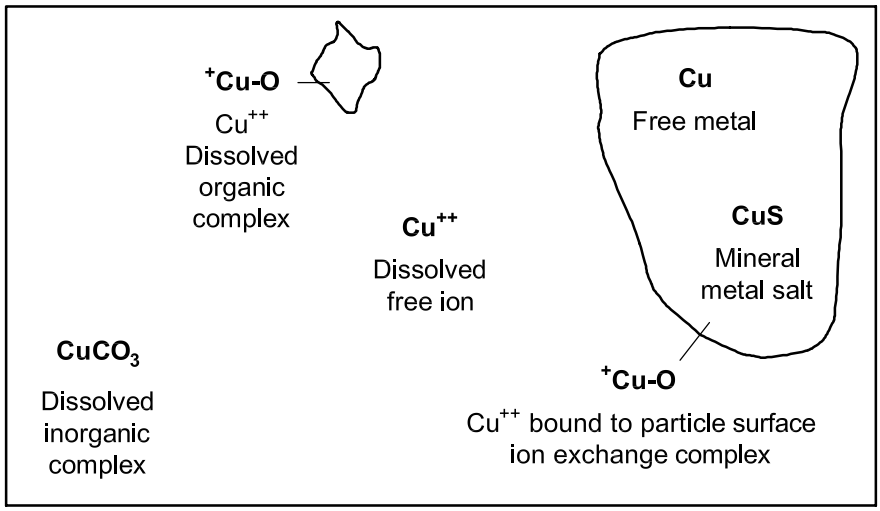

Figure 3 — Example of distribution of metals (copper) in soil

An ageing effect in soils has been observed for As(V) and Cr, which may lead to different accessibility over time. On the other hand, some heavy-metal-bearing minerals have resisted weathering and dissolution over geological time scales. Whether the aggressive chemical conditions in the human digestive tract nevertheless will cause dissolution depends upon the mineral.

7 Concept for a method to test the bioavailability through human exposure

7.1 General

If an *in vitro* test is to be used for the evaluation of the relative bioavailability, a number of conditions have to be met.

— The relative bioavailability of the metal in question can be reasonably estimated through the estimation of the relative bioaccessibility.

— The test is based on simulation of physiological conditions that influence bioaccessibility.

— A reasonable correlation can be proven between the test results and a relevant *in vivo* animal model that is relevant and validated and has a sufficient dataset.

It should be noted that *in vivo* models do not necessarily give a correct estimate of the bioavailability in humans, depending on the difference in uptake and metabolism between the animal in question and humans, and on the matrix in which the compounds are measured (e.g. faeces, blood, organs). It should also be noted that sufficient *in vivo* data do not exist for all relevant metals.

7.2 Choosing an appropriate test

When choosing an appropriate test, it is important to be aware of the questions the test results are aimed at answering, and to take the cultural setting into account and its possible influence on the actual uptake and metabolism of the compound investigated. The following set of experimental details should be considered when choosing a suitable bioaccessibility test method.

— Mixing or stirring rate

It should be taken into account that the particle size of a soil may change in the gastrointestinal tract, partly due to chewing, and partly due to the mixing that will occur primarily in the stomach. The relevant rate used in a test will also depend on the test volume used.

— Digestion time

The digestion time should be chosen depending on the situation mimicked, for example, fed or unfed state. A study of lead dissolution kinetics under simulated stomach conditions has suggested that a test time of 1 h is adequate for this compartment. The study also demonstrated higher bioaccessibility with lower pH and faster release with higher temperatures. Other studies have suggested a test time of 1,5 h to 2 h as most appropriate for the stomach simulation.

— Temperature

The temperature chosen should be similar to that of the human gut.

— Presence of food

It is generally agreed that 2 different tests should be used for the fed and unfed state, respectively. A test for the fed state will be very dependent on the actual situation evaluated and must take into account the actual sociological and cultural conditions. It has been suggested to use food consisting of protein, fat and starch in a ratio representative of the average human diet under the relevant conditions.

— Digestive pH, requirement for buffering

The development of pH during the transit of soil through the system will depend both on the type of soil investigated and on the degree and type of contamination. Carbonaceous soils may raise the pH rapidly. In general, the pH development should be assessed beforehand, based on the actual situation, and the test should then be buffered accordingly. The pH should not exceed 2 at any time in a test estimating an unfed state, and should in general stay below 2. The pH of the soil should be measured before the test and after 1 h during the testing. The pH should not drop below 1,2. Dissolution of lead minerals in the stomach depends on the acid concentration present in the test during dissolution, with low acid concentrations leading to both slower dissolution and lower final dissolved concentration. The effect of acid concentration is caused by both pH (formation of HSO_4^-) and chloride concentration (formation of soluble $PbCl^+$).

— Soil particle size

Here a differentiation should be made depending on whether it is soil ingestion or fugitive dust that is the focus of the testing, and if the aim is to estimate accidental soil ingestion or pica behaviour. Normally, soil samples are sieved so that the tested or analysed sample has a particle size of less than 2 mm. If accidental soil intake is the issue, a particle size of less than 250 µm may be more appropriate, since this is considered to be the optimum size to adhere to children's hands. The choice of relevant particle size should also depend on the soils in question, since local or regional specific conditions may exist. National methods for pretreatment of soils traditionally used may vary and may not necessarily be appropriate for the pretreatment of soils for this type of testing.

— Liquid to solid ratio (L/S-ratio)

It could be relevant to differentiate between pica behaviour and general soil ingestion. If the aim of the test is to simulate pica behaviour, an L/S-ratio of 10 could be appropriate. One should be aware that low ratios in some cases might lead to saturation of the liquid phase. Some comparisons seem to show insignificant variation in test results if the L/S-ratio is between 100 and 5 000. In non-pica situations, an L/S-ratio of 100:1 seems to be a good estimate of the conditions in a small child's gastrointestinal system. The choice of ratio is also based on practical considerations, e.g. test vessel volume.

— Addition of digestive liquids

It is recommended that the test mimic the relevant physiological conditions as much as possible. If intestinal digestion is assumed to be important, mucin should be added. Bile should be added to digestive juice for both fasting and fed conditions. Data suggest that both synthetic stomach fluid and human stomach fluid can significantly reduce toxic and soluble Cr(VI) to less toxic and more insoluble Cr(III), within the time range relevant for stomach transit. An increase in metal bioaccessibility has been reported because of added mucin, bile and pancreatin, but the effect was not seen consistently for all metals and soils tested.

— Gastric and intestinal digestion required

All studies show that it is important that the test incorporates both the gastric and the intestinal phase of the digestion

— Redox conditions

For redox-sensitive metals, this may be relevant if the test is carried out aerobically or anaerobically. Normally, the tests will be carried out under aerobic conditions, but for the redox-sensitive metals or other cases where intestinal uptake is assumed to be substantial, anaerobic conditions may be more suitable.

— Presence of solubility impacting ions such as phosphate (As, Cr and Pb)

It has been demonstrated with sequential extraction that the presence of phosphate changes the speciation of lead towards less extractable species.

— Pretreatment of sample

As stated earlier, the particle size of the soil can influence the availability of the metal and thus the result, see also under "Soil particle size" above

— Post-treatment of test solution

The treatment of the solution derived from the test prior to the final chemical analysis is very important. Filtering or centrifugation of the leachate and the conditions under which this is done is extremely important for the result, since this will decide the degree of particle-bound contamination that is subsequently included in the chemical analysis. At the moment, no definite conclusions as to the correct post-treatment can be given.

It should be noted that *in vitro* bioaccessibility tests typically do not include the effects of the microbial communities present in the *in vivo* gastrointestinal system, and do not include the influence of the active transport of contaminants out of the digestion solution.

The test, furthermore, needs to fulfil the common, basic requirements for a regulatory method. The method shall be as follows:

— simple (i.e. the number of steps and operations maintained at a minimum considering the requirements below);

— comprehensive (i.e. allow for testing of the broadest possible selection of contaminants, species and soils);

— precise (i.e. the same result is obtained when one soil is tested twice in one laboratory);

— reproducible (i.e. the same result is obtained when the same soil is tested in two different laboratories);

— interpretable (i.e. the test results can be correlated to *in vivo* bioavailability data);

— consistent (i.e. the test results should be in accordance with processes predicted from knowledge of contaminant speciation and soil chemistry).

The test should include testing of relevant, well-described reference material.

7.3 Description of applicable test methods

In vitro bioaccessibility tests range from very simple chemical extraction or leaching tests to advanced multi-step tests simulating in detail the human digestion processes. In the following, an overview is given of the tests based on physiological conditions.

Bioaccessibility tests that simulate the processes in the human gastrointestinal system, the digestion, have been developed for use in studies of drug uptake in pharmaceutical studies, of metal uptake in nutritional studies and of contaminant release from toys. In risk assessment of contaminated soils, digestion tests have been developed since the early 1990s.

A wide range of methods has been suggested for determining the availability of contaminants for soil organisms (mainly bacteria, plants and collembola) and similarly for aquatic ecosystems, including both the water and sediment phases: the ecotoxicological bioavailability. The rationale behind these methods is that only the fraction of a contaminant that is present as a free compound dissolved in the soil water is available to the soil biota. Conversely, all contaminants that can be dissolved (free and bound) in the aggressive environment of the human stomach and the small intestine will *a priori* be available for human uptake, i.e. be bioaccessible. Therefore, methods developed to measure the ecotoxicological bioavailability are generally not applicable for measuring human bioaccessibility.

Since no singular validated test exists at present, it is important to be informed of the validation stage of the test implemented, e.g. for what metals.

At present, a number of tests have been validated (that is, precision, accuracy, recovery and detection limits have been determined at reasonable levels) and correlated with *in vivo* data for a few metals in a few soils. An overview is given in Table 2. Apart from the methods shown in Table 2, further tests have been developed and compared to *in vivo* data for one or two soils.

A further description of the methods is given in Annex A. Four of the methods are currently used for routine testing:

— PBET (Physiologically Based Extraction Test method), with slight modifications;

— SBET (Solubility Based Extraction Test, developed by the Solubility/Bioavailability Research Consortium);

— OSU-IVG (Ohio State University – In Vitro Gastrointestinal method);

— DIN 19738 (Deutsches Institut für Normung);

— RIVM (National Institute for Public Health and the Environment, The Netherlands).

Table 2 — Bioaccessibility test methods correlated to *in vivo* bioavailability data for contaminated soils

Method	Segments included	Addition of food	Principle	Contaminants tested
PBET	Stomach, intestine optional	None	Simplified, based on human physiology	Lead, arsenic
SBET	Stomach, intestine optional	None	Simple buffered acid, aiming at robust worst case	Lead, arsenic
OSU-IVG	Stomach or intestine after stomach	Optional	Simplified, based on human physiology	Lead, arsenic and cadmium
RIVM	Saliva, stomach and intestine	Optional, part of fed-state version of the test for organic contaminants	Corresponding to human physiology	Lead, cadmium
DIN	Stomach and intestine, saliva optional	Optional	Corresponding to human physiology	Lead, cadmium, nickel and arsenic

7.4 Recommendations

Any test method used for measuring the bioaccessibility of soil contaminants in soil should enable quantification of the dissolution under “realistic worst case conditions”, meaning that the test should simulate the highest bioaccessibility that can be expected, without including unrealistic conditions or excessive precautions. To fulfil this, the test should be based upon the properties of the human digestion process, of the contaminants in question, and on the geochemistry and mineralogy of soils. Consequently, test specifications should include the following information.

— Both a gastric and an intestinal step.

— Sequential testing (acidic followed by alkaline) with separate release measurements in each sequence to avoid errors from dissolution followed by precipitation (all cationic metals).

— Low pH for dissolution of soil constituents, i.e. in average lower than pH 2. The pH should always be lower than 2,5 and higher than 1,0. A pH of 1,2 is recommended. Adjustment of the pH should be made using NaOH or HCl, as appropriate.

— Acidic digestion time should be at least 1 h for an estimation of the unfed state and at least 2 h for the fed state.

— Subsequent high pH for dissolution of soil constituents in the intestine. The pH in the intestinal compartment depends on the site of the intestine that is simulated. This site that should be simulated depends on the site of absorption of the compound of interest. For example, the pH in the duodenum is 5,5 to 6, in the jejunum 6 to 7. The pH should thus always be greater than 5,5 for this simulation. A pH of 6,3 is recommended for most metals. Adjustment of the pH should be done using NaOH or HCl, as appropriate.

— Alkaline digestion time should be at least 2 h.

— Additions of enzyme types found in the human gastrointestinal tract.

— Additions of bile and other chyme constituents capable of dissolving apolar contaminants and metals (concentration depending on the state simulated).

— Digestion at 37 °C.

— Optional representation of both aerobic (oxidising) and anaerobic (reducing) conditions for redox-sensitive contaminants (e.g. As and Cr).

— Mixing of the constituents should be carried out; end-over-end mixing is recommended.

— For the estimation of normal hand-to-mouth behaviour, an L/S-ratio of 1:100 is recommended, while 1:10 is suggested for the estimation of pica behaviour.

— Pretreatment and post-treatment procedures should be related to the situation studied. Based on the available experience, sieving or crushing to obtain a particle size of < 250 µm is recommended after oven or air drying at < 40 °C. Separation by centrifugation is recommended at 3 000 min^{-1} for 5 min.

It should be noted that test solutions can be sensitive to conservation and storage, even when employing traditionally accepted methods. Therefore, the preservation and storage method used for test solutions should be validated with, at the least, recovery documentation after spiking of test solutions from the testing of representative soil samples and including all metals, where bioaccessibility is reported.

NOTE As an example, preservation of test solutions obtained with a bioaccessibility test method, employing a slightly alkaline intestinal step with concentrated nitric acid, resulted in low recoveries of lead from some soil samples due to co-precipitation with humic substances at the resulting very low pH.

7.5 Use and interpretation of *in vitro* tests for risk assessment

When deciding whether or not to make use of physiologically based extraction methods for the estimation of actual bioavailability, the following considerations should be made:

— Is the site considered to be at risk using conventional methods (comparison of total concentrations to the appropriate quality criteria)? If not, it can reasonably be expected that including bioavailability considerations will not alter this conclusion.

— If the aswer is yes, does the available data imply that the metals in question are likely to be less bioavailable in soil? This could be information on type of contamination source, type of soil/ soil material.

— Is soil ingestion considered to be a major pathway of exposure? If not, studies on oral bioavailability of the contaminants from soil would not be meaningful.

— Would the result of the site-specific risk assessment be sufficiently sensitive to the suspected variation in bioavailability?

— Would the decisions regarding remediation at the site be impacted, or would the collection of the sufficient new data be over costly, in relation to the expected change in remedial costs?

— Is a sufficiently validated method available for the contaminants and context in question, taking into account the relevant cultural setting?

If all the above questions can be answered with a yes, the use of *in vitro* tests is justifiable. In choosing the appropriate tests the information given previously in Clause 7 should be taken into account. Furthermore, it should be evaluated how and where samples should be taken, and also how large an area or volume a sample can represent, based on the knowledge of the uniformity of the contamination.

Using the chosen test, the relative bioaccessibility is determined as the ratio between the results obtained for the soil in question to the results obtained in the test applying the contaminant in a way consistent with the toxicity evaluation that is the basis for the soil quality criteria used in the risk assessment. This relative bioaccessibility or relative absorption fraction (RAF) can then be multiplied with the total contaminant concentration in the soil to obtain the bioaccessible soil concentration. This concentration can then be used in the risk assessment, instead of the total concentration, when evaluating the soil ingestion pathway.

8 Data handling, quality control and presentation of results

The confidence that can be attached to any judgements made, for example, through comparison with the requirements of a published standard, or a site-specific risk assessment, is no greater than the confidence there is in the representativity of the data.

Before any assessment can be made about risk to humans, the sufficiency of data to be used shall be evaluated. The data shall be sufficient in terms of

— type,

— quantity, and

— analytical/ testing quality.

Before testing it is essential to

— define the objectives of the test,

— establish a testing strategy, taking into account the guidance in this Technical Specification and other relevant International Standards, and

— set data quality objectives consistent with the assessment procedure to be used.

It is essential to have sufficient data. Care shall be taken in deciding what statistical expression(s) of the data is (are) to be used in the assessment, as this may affect the choice of procedure.

The quality of the data to be used can be assured by

— setting formal data quality objectives (e.g. for accuracy, reproducibility),

— using standard analytical and testing methods, such as those listed in this Technical Specification, or, where International Standard methods are not available, those published by national standardization or equivalent bodies,

— using accredited laboratories that use standardized methods,

— using laboratories that take part in relevant proficiency-testing schemes,

— the commissioning agent employing its own quality assurance procedures,

— including blanks,

— making duplicate testing, and

— including testing of a relevant reference material.

It should be emphasised here that comparison of bioaccessibility data from different laboratories might be severely impeded if different methods are used for analysing total concentrations of the soil contaminants. Whereas it is generally accepted that analytical methods for organic contaminants in soils should aim at including the full and total amount of the contaminant, methods are accepted for metals that include only parts of the soil metal contents, for example, nitric acid destruction prior to quantification with atomic absorption spectrometry (AAS) or inductively coupled plasma (ICP) methods. It is therefore recommended always to report the mass fraction of “total” metals, the mass fraction of bioaccessible metals (both in mg/kg, w_d) in addition to the percentage bioaccessibility. The impact of using different methods for analysing the “total” mass fraction of metals on percentage bioaccessibilities should be considered.

Reports presenting the results of assessments will often be scrutinised by regulators and other interested parties, including the general public. It is important, therefore, that such reports are of a high technical standard but also take account of the diverse and often non-technical readership. Use should therefore be made of tabular summaries, graphical and other means to present the data in ways that will make the data as easy as is practicable to assimilate and assess.

Annex A
(informative)

Human bioaccessibility testing

Table A.1 — Summary of selected methods for human bioaccessibility testing

Method	Resolution in compartment and time	Oxygen access in test	General test parameters	Mouth and esophagus	Stomach	Small intestine
PBET	Stomach and intestine determined separately and with time resolution	No, argon purge	L/S [a]	—	100	100
			Solution	—	Hydrochloric acid, pepsin, citrate, malate, acetic acid, pH = 1,3	Sodium bicarbonate, bile, salts, pancreatin, pH = 7,0
			Time	—	1 h	3 h
			Temperature	—	37 °C	37 °C
SBET [b]	Stomach only	No	L/S	—	100	—
			Solution	—	Glycine buffer, pH = 1,5, 2,0 and 2,5	—
			Time	—	1 h	—
			Temperature	—	37 °C	—
Mass-balance, MB	Stomach and intestine determined separately	Yes	L/S	160	2 000	4 400
			Solution	Mucin, urea, phosphate buffer, sodium, calcium and potassium chloride, pH = 5,5	Hydrochloric acid, pepsin, sodium chloride, pH not specified	Sodium bicarbonate, pH not specified
			Time	5 s	2 h	2 h
			Temperature	ambient	37 °C	37 °C
DIN 19738	None	Yes	L/S	15	50	100
			Solution	Mucin, amylase, urea, uric acid, phosphate and bicarbonate buffers, calcium, potassium and sodium chloride, sodium sulfate, sodium thiocyanate, pH = 6,4	Hydrochloric acid, phosphate buffer, sodium and potassium chloride, pepsin, mucin, whole milk powder, pH = 2	Potassium, calcium and magnesium chloride, bicarbonate buffer, trypsin, pancreatin, bile, urea, pH = 7,5
			Time	30 min	2 h	6 h
			Temperature	37 °C	37 °C	37 °C

Table A.1 (*continued*)

Method	Resolution in compartment and time	Oxygen access in test	General test parameters	Mouth and esophagus	Stomach	Small intestine
RIVM	None	Yes	L/S	15	37,5	97,5
			Solution	Mucin, amylase, urea, uric acid, phosphate buffer, sodium hydroxide, potassium and sodium chloride, sodium sulfate, sodium thiocyanate, pH = 6,5	Hydrochloric acid, phosphate buffer, calcium, ammonium, sodium and potassium chloride, glucose, glucoronic acid, urea, glucosamine, serum albumine, pepsin, mucin, pH = 1,07 ± 0,07 (pH of a mixture of saliva and gastric juice is 1,2 to 1,3, allowed up to pH 1,5 ± 0,5)	Hydrochloric acid, potassium, sodium, calcium and magnesium chloride, phosphate and bicarbonate buffers, serum albumine, lipase, pancreatin, bile, urea, pH = 8,0 ± 0,2 (pH of a mixture of saliva, gastric juice and intestinal juices is 5,5 to 6,5)
			Time	5 min	2 h	2 h
			Temperature	37 °C	37 °C	37 °C
OSU-IVG	Stomach and intestine determined separately	Yes	L/S	—	150	150
			Solution	—	Hydrochloric acid, pepsin, sodium chloride, pH = 1,8 ± 0,1	Sodium carbonate, pancreatin, bile, pH = 6,1 ± 0,1
			Time	—	1 h	2 h
			Temperature	—	37 °C	37 °C
SHIME	None	Yes	L/S	—	2,5	4,0
			Solution	—	Baby food, cream, pectin, mucin, starch, cellobiose, proteose, peptone, pH = 1,8	Sodium carbonate, pancreatin, bile, pH = 6,5
			Time	—	3 h	5 h
			Temperature	—	37 °C	37 °C
TIM	Stomach and intestine determined separately and with time resolution	Yes	L/S	5	25	46
			Solution	Not specified, pH = 5	Not specified in detail, lipase, pepsin, pH = 5,0 to 2,0	Not specified in detail, bile, phosphate, pancreatin pH = 6,5 to 7,2
			Time	5 min	0,5 + 1 + 1,5 h	6 h
			Temperature	37 °C	37 °C	37 °C
JMOE. Japan	Stomach	Yes	L/S	—	33,3	—
			Solution	—	HCl, pH not specified	—
			Time	—	2 h	—
			Temperature	—	25 °C	—

[a] L/S = liquid to solid ratio.

[b] Developed as a simplified version of the PBET method by SBRC after the Solubility/Bioavailability Research Consortium.

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