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Contamination

Human Bioaccessibility of Heavy Metals and PAH from Soil

Christian Grøn and Lizzi Andersen
DHI - Water and Environment

Danish Environmental Protection Agency

Danish Ministry of the Environment

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Preface

The present literature review on bioaccessibility of PAH and heavy metals from contaminated soils was prepared for the Danish Environmental Protection Agency (DEPA) in 2002 - 2003 by DHI Water & Environment (DHI).

The contributors at DHI were:

Lizzi Andersen
Christian Grøn

A steering committee followed the project with participants from the DEPA, the Danish Veterinary and Food Administration (VFD) and the Danish National Environmental Research Institutions (NERI):

Irene Edelgaard, DEPA (chairman)
Poul Bo Larsen, DEPA
Ole Ladefoged, VFD
John Jensen, NERI

A reference group was invited to discuss the conclusions and recommendations of the review:

Cathy Rompelberg, National Institute of Public Health and the Environment, the Netherlands
Barry Smith, British Geological Survey, United Kingdom
Michael Ruby, Exponent Environmental Group, United States

Sammenfatning og konklusioner

De danske kvalitetskriterier og afskæringskriterier for forurenede jord er udarbejdet for at sikre, at mennesker ikke udsættes for sundhedsskader, primært ved indtagelse af jorden. I dyreforsøg til fastlæggelse af stoffernes giftighed benyttes i reglen letopløselige salte i vand eller i foder, mens stofferne i jord efter al sandsynlighed er langt mindre tilgængelige for optag i mennesket (reduceret human biotilgængelighed).

Biotilgængeligheden af jordforureninger afhænger først og fremmest af mave-tarmsystemets mulighed for at opløse forureningerne (den humane bioopløselighed) og af tarmvæggens mulighed for at optage dem (absorptionen). Jordforureningers bioopløselighed afhænger af forureningers egenskaber, af jorden og af forholdene i mave-tarmsystemet.

Derfor er gennemført en indsamling, opsummering og vurdering af den tilgængelige viden om human bioopløselighed (bioaccessibility) af 7 tungmetaller og 7 PAH fra forurenede jord.

En række metoder er til rådighed til test af jordforureningers bioopløselighed. Testresultaterne er ikke ens med forskellige metoder, og kvaliteten af testmetoderne er ikke veldokumenteret. Udfra en gennemgang af tilgængelige metoder er udpeget 4 metoder, der alle kan udvælges som udgangspunkt for udvikling af en fremtidig standardmetode. Endvidere er identificeret de metodeparametre og de kvalitetsparametre, en metode skal omfatte/honorere.

Data om jordforureningers bioopløselighed peger på, at reduceret tilgængelighed er sandsynlig for cadmium, bly og krom (som Cr(III)), mulig for arsen og krom (som Cr(VI)), og måske mulig for kobber, nikkel, zink og PAH forbindelser.

En mindre påvirkning af mennesket som følge af reduceret bioopløselighed af jordforureninger forudsætter, at opløsning er bestemmende for menneskets optag af stofferne. En mindre påvirkning af mennesker er sandsynliggjort ved dyrestudier af optag for arsen, cadmium, bly og PAH forbindelser.

Jordforureningers humane biotilgængelighed har været inddraget i risikovurdering en række tilfælde i USA, Canada og Storbritannien, hvor oprydningsskravene er blevet betydeligt reduceret. Højere forureningskoncentrationer er fundet forsvarlige og derefter accepteret.

Det anbefales, at reduceret human biotilgængelighed (bioavailability) tages i betragtning ved individuel risikovurdering af grunde med forurenede jord. Derimod tillader datamaterialet ikke en generel regulering af jordkvalitetskriterier og afskæringskriterier udfra human biotilgængelighed, idet effekten efter alle oplysninger at dømme vil være forskellig fra grund til grund.

Der er præsenteret to modeller (en kortsigtet national og en langsigtet international) for implementering af test for human bioopløselighed i dansk praksis for undersøgelse og oprydning af forurenede grunde. Forudsætningerne for de 2 modeller er beskrevet.

Summary and conclusions

Most soil quality criteria and cleanup levels for soil contaminants are based upon oral exposure and effect studies with soluble, highly bioavailable contaminant forms ingested with water or with food. When ingested with soil, metals and PAH are likely to be less available than in the toxicity studies underlying the soil quality criteria and cleanup levels.

Bioavailability of soil contaminants for humans depends primarily upon the ability of the stomach and the small intestine to dissolve the contaminant (bioaccessibility) and upon the ability of the intestinal membranes to absorb the contaminant. Bioaccessibility of the soil contaminants depends upon the contaminant chemistry, the soil properties and the chemical conditions in the gastrointestinal system.

Therefore, the current knowledge on bioaccessibility of 7 metals and 7 PAH has been reviewed.

A number of different *in vitro* test methods are available to measure bioaccessibility of soil contaminants. The results are not generally comparable between methods, and data on the quality of the bioaccessibility test methods are limited. Based upon reported bioaccessibility test methods, candidates for one common test method are proposed and requirements for method parameters and performance are given.

The overall picture from available data on bioaccessibility of soil contaminants is, that reduced soil bioaccessibility is very likely for cadmium, lead and chromium (III) (uptake in small intestine), likely for arsenic and chromium (VI), and possible for copper, nickel, zinc and PAH.

Bioaccessibility will impact human exposure if dissolution of the soil contaminants is rate limiting compared to absorption and this is suggested to be the case by studies of lead, arsenic and PAH. Reduced bioavailability has been reported for *in vivo* uptake studies with animals for at the least arsenic, cadmium, lead and PAH.

Reduced bioaccessibility and/or bioavailability has been taken into consideration in site specific regulation of cleanup levels for contaminated sites in the US, Canada and UK (regional specific), in particular for mine waste and ore processing sites.

The general conclusion is that regulation of soil quality criteria and cleanup levels based upon reduced bioavailability/bioaccessibility of the contaminants is recommended after site specific risk assessment. Conversely, the data available at present does not allow for general regulation of soil quality criteria and cleanup levels for specific contaminants, soil types or sources.

A short term and a long term model for implementation of bioaccessibility in risk assessment of contaminated sites is suggested, and the requirement for their implementation is described.

1 Introduction

1.1 Background

Contaminants in Danish soils are regulated according to sets of criteria for maximum contaminant levels (MCLs) before interventions are required. The MCLs are based upon evaluations of acute and chronic toxicity of the contaminants to humans without considering consistently differences in human uptake imposed by different exposure types (*e.g.*: orally from solution, with food or with soil). During the past 10 years, evidence has emerged that the oral uptake of contaminants may differ widely with the exposure type. In order to evaluate the need for including this new information in MCL setting or enforcement, the DEPA has initiated the present review of the information currently available on variations in human uptake with oral exposure type.

1.2 Scope

The scope of the review is to compile and extract information on bioaccessibility of soil contaminants for human oral exposure emphasising data on bioaccessibility and, if possible, their correlation with soil types, description of processes underlying bioaccessibility variations and finally presentation and evaluation of methods for testing bioaccessibility. Topics with lack of knowledge in order to utilise bioaccessibility data in risk assessment of human, oral exposure should be identified.

The contaminants selected for the review were selected heavy metals and polycyclic aromatic hydrocarbons (PAH):

- As, Cd, Cr, Cu, Ni, Pb and Zn
- fluoranthene, benzo(b+j+k)fluoranthenes, benzo(a)pyrene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene

These contaminants all have MCLs given as soil quality criteria and cleanup levels in Denmark /1/.

1.3 Methods

The information for the review was retrieved as a literature search in the databases STNEasy (chemical and environmental literature) and MEDLINE (medical literature), as well as with the web search engines Yahoo and Scirus. The web sites for the Environmental Protection Agency of the US (US EPA) and the US National Technical Information Service (NTIS) were searched as well.

Search terms were combinations of (? indicates truncation):

bioaccessibility, human uptake, human bioavailability, human absorption, oral bioavailability, systemic bioavailability (OR)
AND

PAH?, polycyclic aromatic hydrocarbon?, fluoranthene,
benzo(b+j+k)fluoranthenes, benzo(a)pyrene, dibenz(a,h)anthracene
indeno(1,2,3-cd)pyrene, metals, As, Cd, Cr, Cu, Ni, Pb, Zn (OR)
AND
soil

Search for test methods was further done with the terms:

oral, drug?, in vitro, bioaccessibility, test (AND)
oral, drug?, test?, dissolution (AND)

Cross references were retrieved subsequently and key author names were searched for additional references. Furthermore, the available information on the standardisation of bioaccessibility tests within the International Organization for Standardization (ISO) was included.

The references were filed in a Reference Manager system containing currently just over to 200 references.

Finally, the report was discussed at a workshop held in Copenhagen on March 17, 2003 and afterwards revised to reflect the conclusions at the workshop and additional material subsequently supplied by the workshop participants.

During the literature survey, a number of recent international reviews and textbooks on the topic, as well as a few basic textbooks were identified /2-17/. Text without references cited in this review are based upon these international reviews and textbooks.

2 Human risk assessment for soil contaminants

The highest concentrations of contaminants that are acceptable in soils are generally based upon estimates of human exposure (how large amounts of the contaminant can impact the human via the sum of exposure routes) and of the toxicity of the contaminants to humans.

Thus quality criteria for soil (the maximum concentration limits for soil) are calculated on the basis of a tolerable daily intake value (TDI) or a provisional, tolerable weekly intake (PTWI), that can be derived from the no observed adverse effect level (the NOAEL) found in human data or experimental animal data. For genotoxic carcinogens for which no lower threshold for increased risk for cancer is assumed, the TDI value is set at a level that corresponds to a tolerable low (negligible) cancer risk level. In Denmark, the TDI is set to a dose comparable to an excessive risk of 10^{-6} *i.e.*: a calculated hypothetical risk of one extra cancer outcome among 1 million people in a lifetime.

In calculating the tolerable soil exposure estimates, the impact of other sources is taken into account by allocating the total tolerable amount to different exposure routes, *e.g.*: food, drinking water and soil. The allocation is given as the allocation factor (f_a) which is the fraction of TDI that is allowed from soil exposure.

Oral ingestion is one of the most important exposure routes for humans to soil contaminants /18/, and the Danish MCLs are in most cases developed based upon oral uptake by children /19/. The MCL for soil ingestion is obtained by dividing the TDI (corrected for allocation) with the estimated daily soil exposure (EDE).

$$\text{MCL (mg contaminant/kg soil)} = \frac{\text{TDI (mg contaminant/person/day)} \times f_a}{\text{EDE (kg soil/person/day)}}$$

For determining the TDI, data on oral toxicity are primarily considered. Often these data pertain to animal experiments where the substance is administered to the animals mixed in the feed or in drinking water (the vehicle or transporter of the contaminant). As an alternative, epidemiological studies relating observed human health effects to recorded exposures have been used¹. The amount of contaminant needed to produce adverse health effects in the animal is then recorded. Most toxicological studies report the total ingested amount only and do seldom indicate exact values for the bioavailability of the substances administered.

When extrapolating from such experimental conditions to other conditions *e.g.*: to intake of contaminated soil, this approach requires, that the uptake

¹ An example is that the US toxicity value for arsenic was developed from epidemiological data on exposure in drinking water and it should be noted, that water soluble arsenic ingested with drinking water is nearly completely absorbed (*i.e.*: 80-90%) /82/.

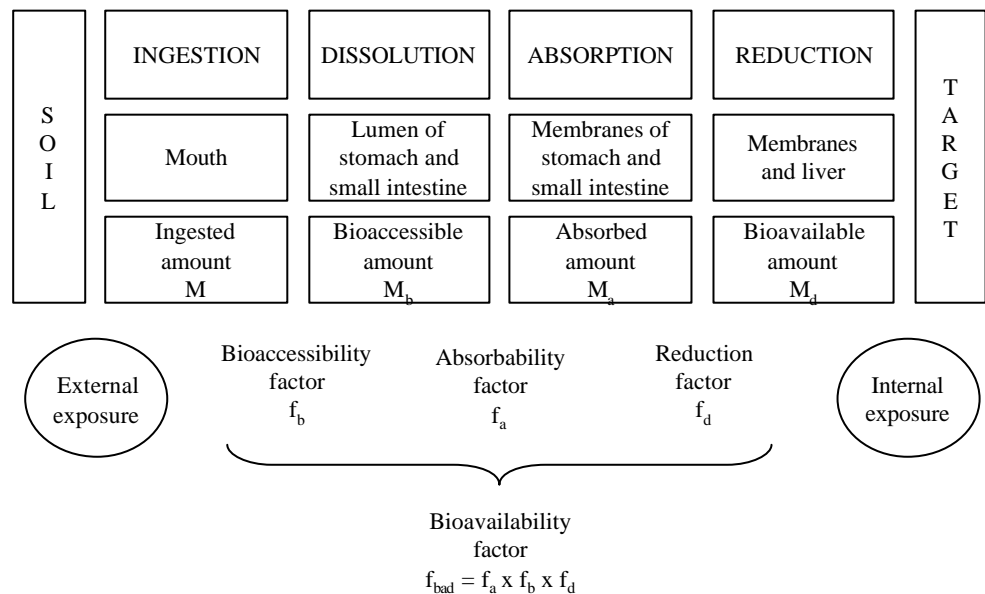
efficiency is equal for all scenarios, *i.e.*: that the absolute bioavailability, AB, of the contaminant is constant. The absolute, oral bioavailability can be defined as:

$$AB = \text{internal dose/external (administered) dose}$$

In words, the absolute, oral bioavailability is the fraction of an orally ingested contaminant that reaches systemic circulation, *i.e.*: enters the blood stream. 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 blood stream, *i.e.*: the internal exposure as opposed to the external exposure measured directly as intake of contaminated medium multiplied by the concentration of the contaminant in the medium, figure 2.1.

The absolute bioavailability can be measured as the ratio between amounts in the blood of laboratory animals after intravenous injection (100% uptake) and after oral ingestion (uptake of bioavailable fraction). Alternative and less direct approaches are at hand as *e.g.*: measuring the absorbed amount of orally ingested contaminant as the amount that is excreted with urine, see chapter 5.

Figure 2.1 Schematic presentation of oral uptake processes



A more feasible approach is to measure the relative bioavailability or relative absorption fraction (RAF). RAF is obtained as:

$$RAF = \text{amount taken up from new matrix/amount taken up from the matrix used in the toxicity study}$$

In words, the relative bioavailability is the ratio between the amount of a contaminant reaching systemic circulation when ingested with *e.g.*: soil and the same amount obtained when ingested in the toxicity experiment.

If the relative bioavailability of a contaminant deviates from 1 (~100%) when ingested in soil as compared to ingestion in the toxicity experiments behind

the TDI, a correction of the MCL to account for this can be argued for. If a reliable and safe RAF value can be found and agreed upon, this would then result in a proportional change in the MCL:

$$MCL_{\text{true}} = MCL/RAF$$

For substances where the critical toxic effect is not systemic toxicity but local toxicity (*i.e.*: local irritation), the toxic effect is considered to be dependent of the concentration in the gastrointestinal tract, and the MCL will be dependent of bioaccessibility rather than the bioavailability.

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

In the US and Canada, the RAF values have been used to increase cleanup levels after risk assessment on a case by case basis, see chapter 9 for examples. In summary: adjustment of cleanup levels based upon bioavailability studies has been reported from the US for arsenic, lead, mercury, PAH, PCB's and dioxins /8;9/ and from Canada for lead and nickel /20/. In Denmark, specific considerations regarding bioavailability have only been made for few substances, *e.g.*: nickel, when calculating the MCL.

The US EPA allows for using the concept of relative bioavailability in risk assessment /21/, but does not give guidance to the practical implementation, see chapter 9. According to the recent reviews /8;9/, several state regulatory agencies have issued guidance documents. Adjustment of the bioavailability is an option in the US EPA model for risk assessment of lead uptake in children /22/. *In vivo* data for relative bioavailability are in most cases required to allow the adjustment of lead bioavailability. This reflects the general attitude in the US EPA: that bioavailability based adjustments of maximum contaminant levels or cleanup levels should be based upon *in vivo* studies with experimental animals resembling humans, *e.g.*: with immature swine /20/. Still, the US EPA is moving towards accepting "validated" *in vitro* tests for lead and is chairing a meeting on this subject in April 2003.

As set of general factors to be considered deciding whether to include bioavailability studies at a site has been suggested /4/:

- + Limited number of critical contaminants
- + Contaminant levels exceeding but close to MCLs or cleanup goals
- + Form of contaminant likely to exhibit low RAF
- + High probability of public and regulatory acceptance of RAF based MCL adjustment
- + Large soil volumes affected
- + Costly cleanup technologies required
- + Adequate cleanup technologies not available
- + Risk of environmental deterioration due to required cleanup
- + Old, weathered contamination (not unambiguous)

- Demand for fast intervention
- Contaminant species likely to yield high RAF
- Soil characteristics likely to yield high RAF

Table 2.1 Summary of the toxicological data behind the Danish soil MCL, compiled from /19;24/ by Poul Bo Larsen, DEPA

Compound	Species	Toxicological response	Toxicological parameter established	Toxicological basis	Bioavailability incorporated
As	inorganic As-species	circulatory diseases, acute toxicity	both chronic and acute endpoints	25% of TDI ² -value (chronic toxicity) allocated to soil exposure NOAEL ³ of 0,01 mg/kg for acute exposure	the MCL is based on readily absorbed As, <i>i.e.</i> : the water soluble As content
Cd	inorganic Cd-species	adverse effects on kidneys, nephrotoxicity	PTWI ⁴	10% of PTWI results in MCL-value of 5 mg/kg The MCL of 0,5 mg/kg is set to protect from uptake in plants for consumption	the MCL specifically addresses bioavailability in plants
Cr	Cr(VI)	carcinogenic by inhalation, sensitising, acute toxicity	no specific NOAEL/LOAEL ⁵	overall assessment of data	no specific considerations
Cr	Cr(III + VI)	-	-	the MCL of 500 mg/kg set on an administrative basis	no specific considerations
Cu	inorganic Cu-species	irritation of the gastrointestinal tract, adverse effects on the liver	the provisional TDI of 0,5 mg/kg bw ⁶ /d ⁷ for chronic exposure also used for acute exposure	the MCL is set to prevent acute effects from soil ingestion	the MCL is to protect against water soluble Cu
Ni	inorganic Ni-species	increased reactivity in Ni-allergic persons	LOAEL with respect to worsening of allergic reactions	human clinical study	a RAF ⁸ of 30 is used specifically for soil
Pb	inorganic Pb-species	neurotoxic response, neurobehavioural disturbances in children, impaired IQ	NOAEL with respect to increase in blood lead level	epidemiological/clinical studies	the overall bioavailability for lead from different sources is considered no specific factor is used for lead in soil.

² Tolerable daily intake

³ No observed adverse effect level

⁴ Provisional tolerable weekly intake

⁵ Lowest observed adverse effect level

⁶ Body weight

⁷ Day

⁸ Relative bioavailability factor

Compound	Species	Toxicological response	Toxicological parameter established	Toxicological basis	Bioavailability incorporated
Zn	inorganic Zn-species	acute toxicity, irritation of gastrointestinal tract	-	comparable dose tolerated from drinkingwater	the MCL is based on water soluble Zn.
PAH	total PAH ⁹ benzo(a)pyrene dibenz(a,h)anthracene	carcinogenic effects	cancer potency estimates	epidemiological data, experimental animal data	the MCL set based on an overall assessment for protection of skincancer by dermal contact Intended to reflect a 10 ⁻⁶ risk level for PAH in soil

⁹ Fluoranthene, benzo(b+j+k) fluoranthene, benzo(a) pyrene, dibenz(a,h) anthracene and indeno(1,2,3-cd) pyrene

In other words: if an increase in MCLs is likely to result from a bioavailability study, and if the costs of cleanup are sufficiently large, a bioavailability study is worth considering. It should be noted that site specific RAF data are generally required in the US/8/.

In Europe, the emphasis in risk assessment is to develop *in vitro* tests for bioavailability of soil contaminants /14/. The rationale behind this is that *in vitro* tests:

- are faster, less costly and more reproducible than *in vivo* tests
- yield a conservative estimate of internal exposure

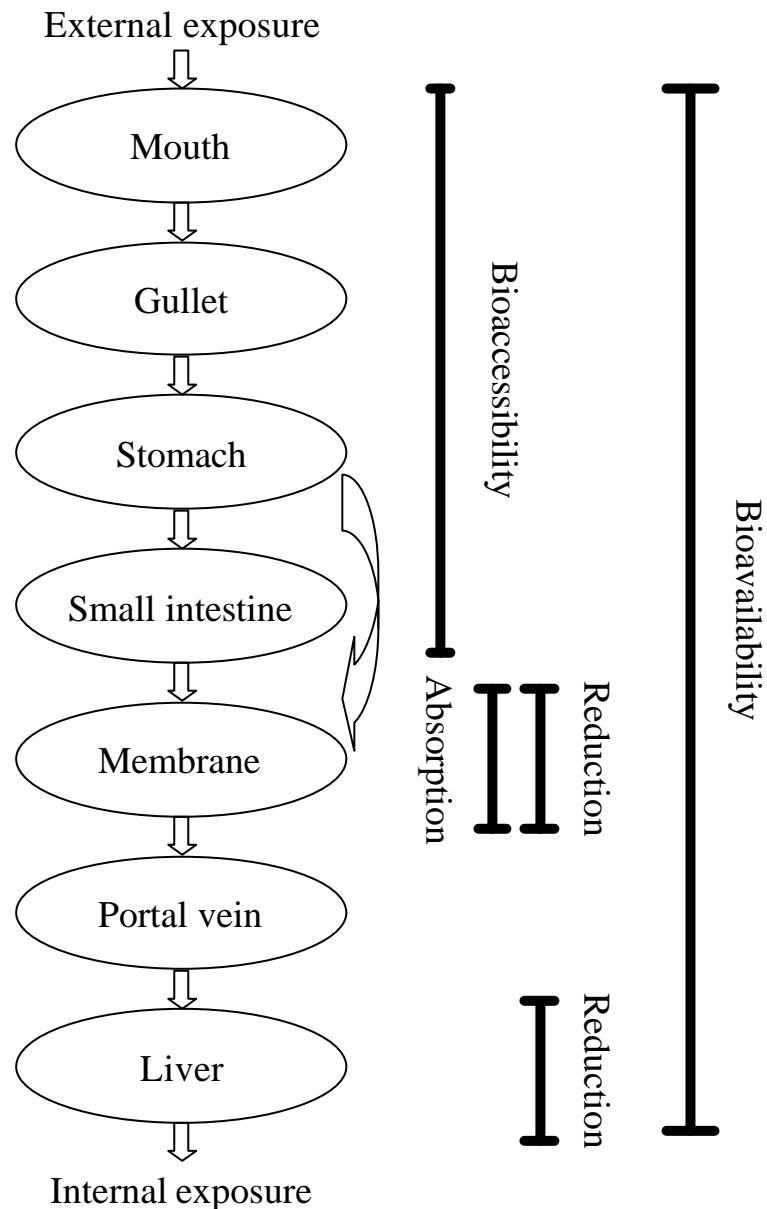
In Denmark, bioavailability of soil contaminants is currently not part of the risk assessment at contaminated sites /1;23/ and has only been addressed in the MCL setting to the degree allowed for by the limited data available /19;24/.

A summary of the toxicological data behind the Danish soil MCL values is given in table 2.1, and the Danish MCLs are summarised in table 4.3.

3 Physiology of human contaminant uptake

A series of compartments are involved in human uptake of ingested soil contaminants, figure 3.1.

Figure 3.1 Compartments involved in human uptake of contaminants



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 blood stream. During transport through the membranes, degradation can occur (reduction). The blood passes the liver before entering the systemic circulation allowing for degradation or removal of unwanted compounds in the liver (reduction, first pass effect). It should be noted that in medical and toxicological literature, the term absorption often pertains to absorption into the systemic blood stream, *i.e.*: absorption includes the process of first-pass metabolism.

Most of the dissolution processes are completed before the material is leaving the small intestine, and it is generally accepted that most of the uptake takes place in the small intestine /25/. To which extent uptake takes place in the stomach is currently not clear. The environment in the compartments differs and accordingly impacts the bioaccessibility process differently, table 3.1.

Table 3.1 Functions and conditions in the compartments involved in bioaccessibility processes, combined from /2;8;14;25/

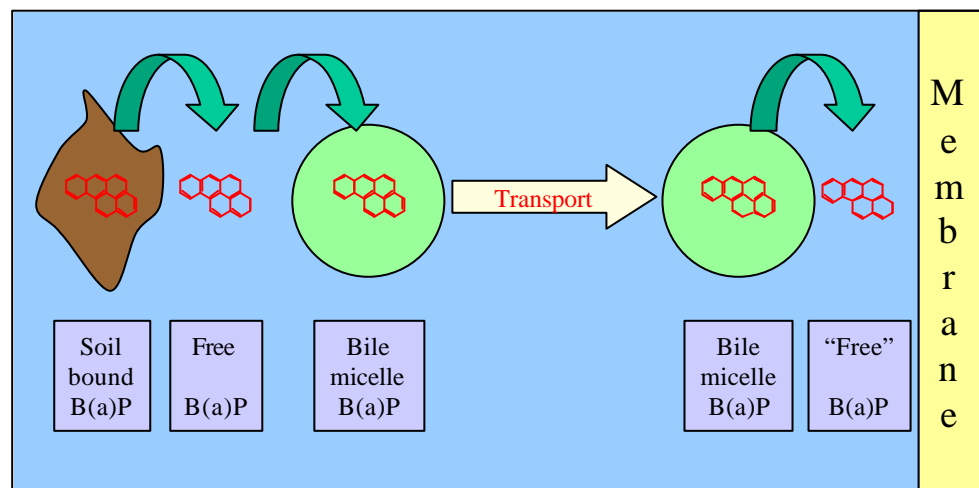
Compartment	Primary digestion functions	Main added "reagents"	pH	Residence time	Contaminant dissolution function
Mouth	grinding cleavage of starch	moisture amylase	6,5	seconds to minutes	grinding enhances subsequent dissolution
Gullet	transport	none	6,5	seconds	none
Stomach	cleavage of proteins and fats	hydrochloric acid proteases lipases	1-5	8 minutes to 3 hours	acid dissolves labile mineral oxides, sulfides and carbonates to release metals and adsorbed organic compounds
Small intestine	cleavage of oligosaccharides, proteins, fats and other constituents solubilisation of fats	bicarbonate bile proteases lipases oligosaccharases phosphatases	4-7,5	3-10 timer	organic matter is dissolved and bound contaminants released apolar organic contaminants are solubilised by bile cationic metals are solubilised by complexation with bile acids some metals are precipitated by the high pH or by phosphate

The pH in the stomach may vary from close to 1 under fasted conditions to as high as 5 after feeding. Residence time ($\frac{1}{2}$ -time for emptying) in the stomach varies similarly from 8-15 minutes to $\frac{1}{2}$ -3 hours for fasted and fed conditions, respectively. Furthermore, bile release varies as well with high releases under fed conditions. Finally, the pH in the stomach is lower with small children than with adults.

It should be noted, that the gastrointestinal tract constitute a complex ecosystem with aerobic and anaerobic microorganisms /26/. The density of microorganisms is less in the human stomach and in the upper part of the small intestine but increasing towards and in the large intestine. In human faeces, anaerobic microorganisms dominate, whereas aerobic bacteria are found in high densities higher in the large intestine /27/. Sulphate reducing bacteria have been detected in the human large intestine /28/ but on the other hand, high concentrations of oxygen have been detected throughout the gastrointestinal tract of pigs /29/. Overall, dominating aerobic conditions and microorganisms would be expected in the stomach, but with increasingly anaerobic conditions from the small intestine to the large intestine.

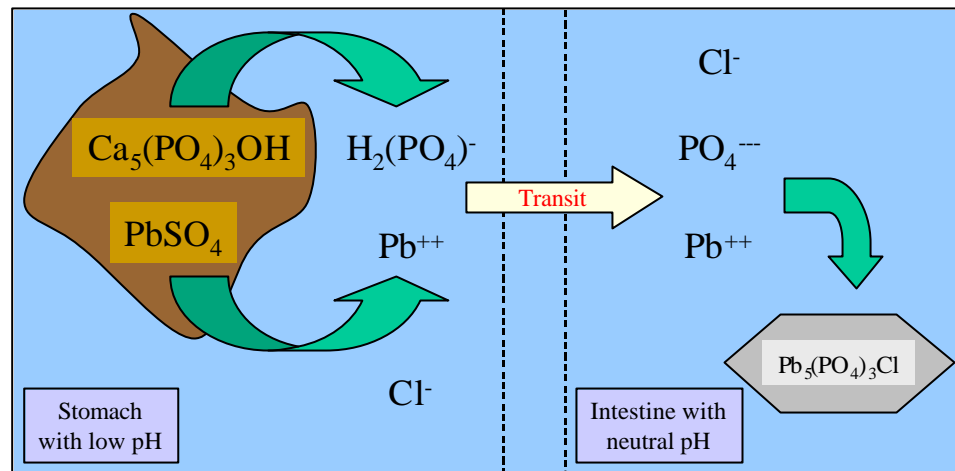
Absorption requires that the contaminants are dissolved (free or bound to a dissolved carrier such as bile), transported to the gastrointestinal wall and, if bound to a carrier, released at the surface of the gastrointestinal membrane for absorption, figure 3.2. The carrier mechanisms can be dissolution of apolar contaminants in bile micelles or complexation of cationic metals by bile acids. For apolar contaminants such as many PAH, the carrier will counteract the low water solubility and thus enhance exposure of the membrane to freely dissolved contaminants. Likewise, 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.

Figure 3.2 Dissolution and transport of an apolar contaminant in the gastrointestinal lumen, benzo(a)pyrene (B(a)P) as example



Unfortunately, the simple dissolution – transport –absorption processes can be complicated by 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 as the common contaminant anglesite ($PbSO_4$) will dissolve in the stomach and will stay in solution at the low pH and high chloride concentration here, figure 3.3.

Figure 3.3 Dissolution of a lead mineral in the stomach and subsequent precipitation in the small intestine, lead sulphate as example

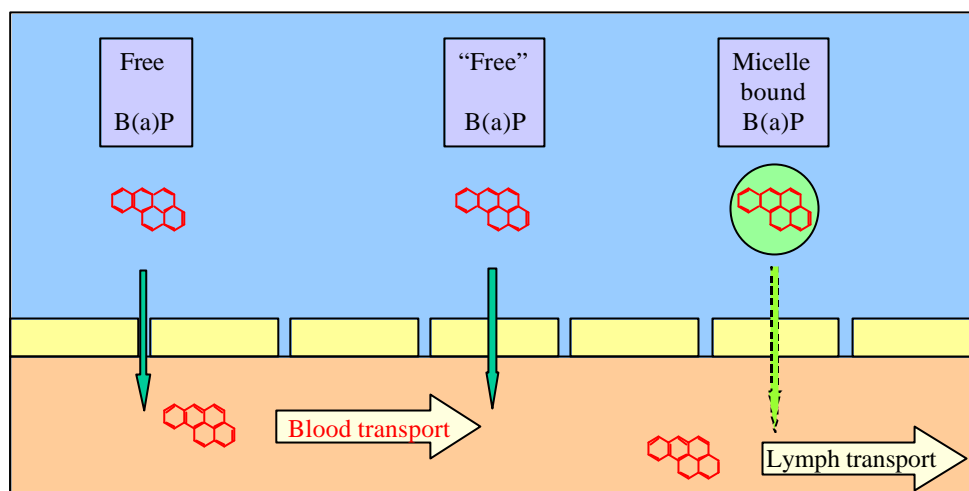


Entering the higher pH in the presence of dissolved phosphate in the small intestine, the dissolved lead ions (Pb^{++}) will precipitate very quickly as chloroleadphosphate (chloropyromorphite, $\text{Pb}_3(\text{PO}_4)_3\text{Cl}$) /30/. The phosphate can originate from digested food or from the soil. Phosphate minerals, such as hydroxyapatite, $\text{Ca}_5(\text{PO}_4)_3\text{OH}$, will dissolve in the low pH of the stomach, but dissolution will be slower and less complete at 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 chloroleadphosphate. Conversely, just after transit from the stomach to the small intestine, the pH is still low and absorption of lead can take place driven by 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 feeding condition of the human.

The absorption of dissolved contaminants is 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), figure 3.4. The pathway through the cells is primarily taken by apolar contaminants (*e.g.*: PAH) that can easily pass the lipid phase of the membranes, whereas the pathway between the cells is primarily taken by polar or ionic contaminants (*e.g.*: some metals).

The transport of apolar organic contaminants through the cells is by passive diffusion. Active transport across the membrane requires that the contaminant "fits" into a transport system already present (*e.g.*: the monosaccharide transport system) and this has not been demonstrated for PAH. In addition, it has been suggested /2/ that absorption of apolar contaminants can occur by the fatty acid route with the contaminants entering the organism through the lymph system and not through the blood stream. In principle, this pathway is based upon transport of micelles of lipids, bile and contaminants towards the membrane, diffusion across the cell membrane, re-incorporation of the contaminants in mixed micelles with lipids followed by secretion of these into the lymphatic circulation /31/. This pathway has not been supported for PAH.

Figure 3.4 Intestinal absorption of an apolar contaminant, benzo(a)pyrene (B(a)P) as example



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 /32/. 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 /33/.

Reduction and transformation of the absorbed contaminant concentrations takes place in the epithelium membranes (binding and exclusion) and cells (degradation and transformation of organic contaminants), as well as in the liver (degradation and transformation of organic contaminants, transformation of metals, and secretion of metals and PAH 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 blood stream.

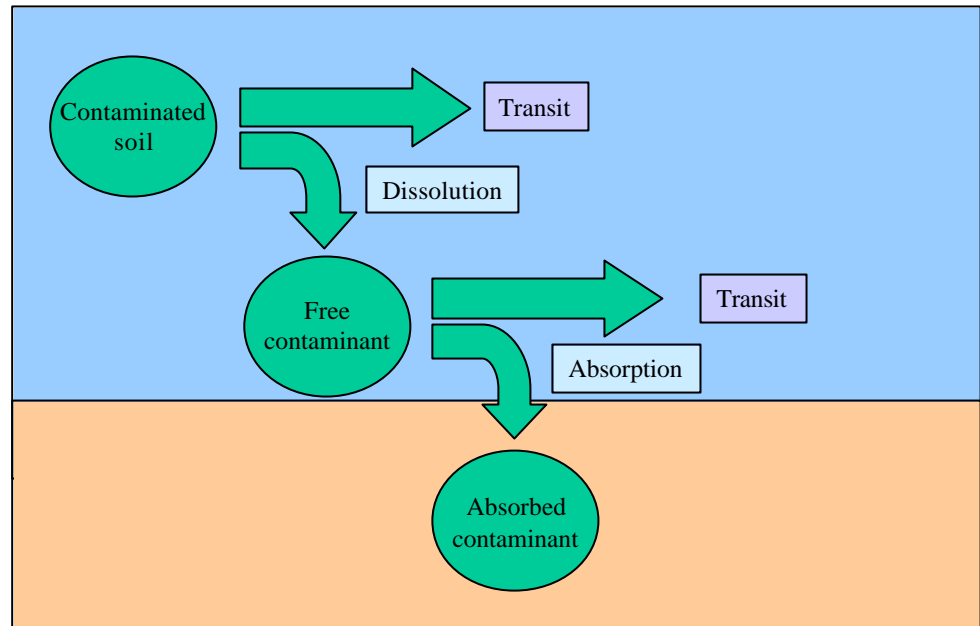
If we consider the sensitivity of the processes of dissolution, absorption and reduction to changes caused by varying “vehicles” (*i.e.*: ingestion with soil, food or in solution) and chemical forms (*i.e.*: different metal salts ingested), we would expect dissolution to be highly sensitive, absorption to be sensitive and reduction to be slightly sensitive (chemical form) or insensitive (vehicle). In applying the concept of relative bioavailability (chapter 2), the most important factor to assess would thus be the bioaccessibility factor f_b (figure 2.1) followed by the absorbability factor f_a .

Estimation of the relative bioavailability factor thus reduces to an estimation of how the two potentially rate limiting processes of dissolution and absorption responds to variations in vehicle and chemical form of the contaminants, figure 3.5.

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, see also chapter 8 for elaboration of the relationship between bioaccessibility and bioavailability in the soil contaminant context.

Figure 3.5 Dissolution and absorption as rate limiting processes of human uptake of contaminants, modified from /34/



3.1 Implications of human contaminant uptake physiology for design of bioaccessibility tests

A test for bioaccessibility of contaminants in soil should be designed to simulate a realistic worst case scenario based upon the description of the human digestion and uptake processes, *i.e.*: it should enable estimation of the highest bioaccessibility likely to occur. Consequently, test design should include:

- low pH for dissolution of soil constituents, lower than 2
- acidic digestion time, at the least 3 hours
- subsequent high pH for dissolution of soil constituents, higher than 7
- alkaline digestion time, at the least 10 hours
- additions of enzyme types found in the human gastrointestinal tract
- additions of bile and other chyme constituents capable of dissolving apolar contaminants and metals
- digestion at 37°C
- optional representation of both aerobic (oxidising) and anaerobic (reducing) conditions for redox sensitive contaminants (*e.g.*: As and Cr)

4 Soil contaminants

The human uptake is highly dependent upon the chemical conditions encountered during digestion (chapter 3) but also upon the matrix and chemical form (speciation) of the contaminants. The specific physical-chemical properties and potential interactions with soil constituents of each contaminant are controlling the processes of dissolution and transport of the contaminants in the gastrointestinal lumen (*i.e.*: the bioaccessibility processes).

Structures (PAH only) and selected physical-chemical data for the project contaminants:

- As, Pb, Cd, Cr, Cu, Ni and Zn
- fluoranthene, benzo(b+j+k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene

are given in figure 4.1 and in tables 4.1-4.2.

Figure 4.1 Structures of PAH

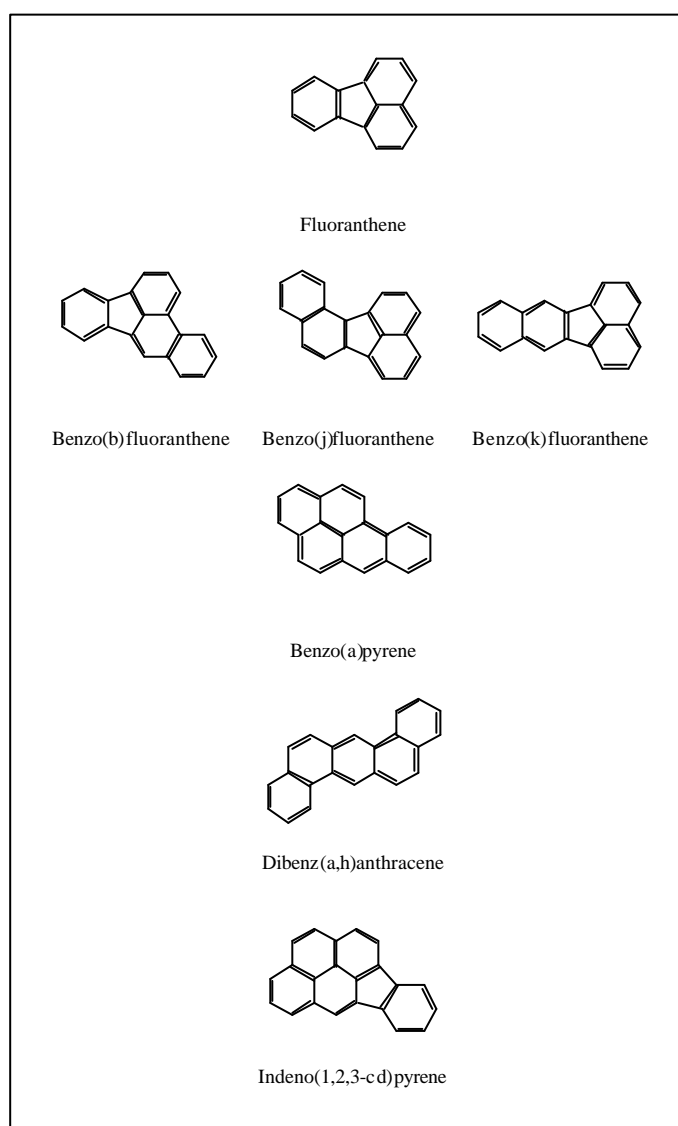


Table 4.1 Physical-chemical data of PAH /35/

Property	Fluoran- thene	Benzo (b+j+k) fluoran- thene	Benzo(a) pyrene	Dibenz (a,h)an- thracene	Indeno (1,2,3- cd)pyrene
Molecular weight g/mol	202,3	252,3	252,3	278,4	276,3
Melting point °C	111	166-217	175	270	163
Boiling point °C	375	480-481	496	524	536
Vapor pressure 10 ⁻⁶ Pa	1.300	0,013-0,5	0,73	0,013 ¹⁰	0,017 ¹⁰
Water solubility µg/L	210	0,8-3	3,8	0,5	0,19 ¹⁰
Partitio- ning coefficient log (K _{ow})	5,2	6,4-6,8	6,5	6,5	7,7

The selected PAH are solids at room temperature with high boiling points, low vapor pressures, low water solubilities and high affinity for an organic phase (high log (K_{ow})).

Table 4.2 Physical-chemical data of metals /3;5-7;9;13;35/

	Arsenic	Cad- mium	Chro- mium	Copper	Lead	Nickel	Zinc
	As	Cd	Cr	Cu	Pb	Ni	Zn
Atomic weight g/mole	74,9	112,4	52,0	63,5	207,2	58,7	65,4
Aqueous species I	H ₃ AsO ₃ H ₂ AsO ₃ ⁻	Cd ⁺⁺	Cr ⁺⁺⁺	Cu ⁺⁺	Pb ⁺⁺	Ni ⁺⁺	Zn ⁺⁺
Oxida- tion state	III	II	III	II	II	II	II
Aqueous species II	H ₂ AsO ₄ ⁻ HAsO ₄ ⁻	None	HCrO ₄ ⁻ CrO ₄ ⁻	None	None	None	None
Oxida- tion state	V		VI	-	-	-	-

Arsenic is strictly speaking a metalloid and not a metal but for simplicity, the term metals is used for all the inorganic elements covered by this review.

For reference, the Danish soil quality criteria are given in table 4.3. The soil quality criteria are enforced as limits for sensitive use (gardening, day care institutions etc) of the contaminated area, the cleanup levels require intervention, whereas the ecotoxicological soil quality criteria generally are not

¹⁰ Reference: /117/

enforced. For simplicity, these criteria and levels are referred to as MCLs in this review.

Table 4.3 Danish soil quality criteria and cleanup levels, from /1;36/

Compound	Soil quality criterium (mg/kg dw ¹¹)	Ecotoxicological soil quality criterium (mg/kg dw)	Cleanup level (mg/kg dw)
Arsenic	20	10	20
Cadmium	0,5	0,3	5
Chromium (VI)	20	2	-
Chromium (III + VI)	500	50	1.000
Copper	500	30	500
Lead	40 ¹²	50	400
Nickel	30	10	30
Zink	500	100	1.000
Total PAH ¹³	1,5	1,0	15
Benzo(a)pyrene	0,1	0,1	1
Dibenz(a,h)anthracene	0,1	-	1

4.1 Speciation of PAH in soil

An example of distribution between phases and chemical forms (species) in soils is shown for benzo(a)pyrene in figure 4.2.

Due to their physical-chemical properties, the PAH will primarily be absorbed into the organic matter of the soil, with smaller amounts adsorbed to inorganic soil particle surfaces and adsorbed to dissolved organic matter (dissolved organic “complex”) and a very minor fraction present as free, dissolved PAH. In soils contaminated with separate phases of *e.g.*: petroleum products, PAH may also be present dissolved in the separate phase.

The fraction absorbed into the soil organic matter becomes less desorbable with time, a phenomenon called aging. In very recently contaminated soils, PAH will consequently be more bioaccessible as compared to soils with the same PAH composition and concentration that has aged for years after contamination, even though the PAH are still present. The molecular mechanism behind aging is still debated (*e.g.*: /37/) and a more detailed discussion is beyond the scope of this review. Still, it should be noted that as low as 10% bioavailability has been measured (as mutagenic activity) for benzo(a)pyrene in soil /38/, suggesting a significant effect of aging. Bioavailability reductions varying from 5% to 50% has been measured (as biodegradation) for 16 different soils /39/, suggesting large differences in the magnitude of the aging effect among different soils. Certainly, it has been suggested /40/ /41/ that the effects of aging should be considered in risk assessment of soils contaminated with compounds that age.

The bioaccessibility of the two solid species of benzo(a)pyrene: absorbed, possibly aged in organic matter and adsorbed to mineral surfaces will differ. Likewise, the bioaccessibility of separate phase benzo(a)pyrene will differ

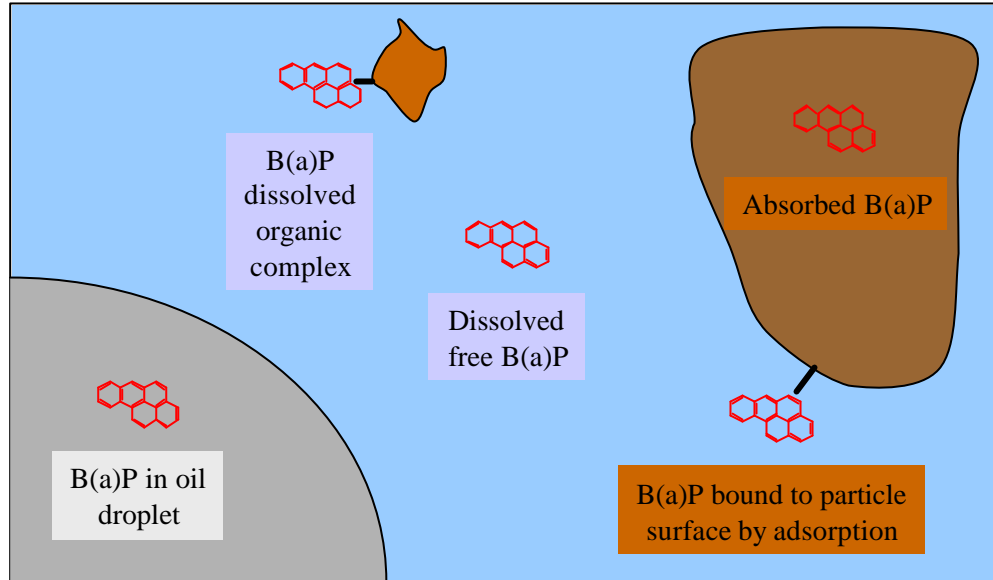
¹¹ dw: dry weight

¹² Tetraethyl- and tetramethyllead 4 mg/kg dw

¹³ Fluoranthene, benzo(b+j+k) fluoranthene, benzo(a) pyrene, dibenz(a,h) anthracene and indeno(1,2,3-cd)pyrene

from the accessibility of the solid species. The absorption of the two dissolved species: free benzo(a)pyrene and bound to dissolved organic compounds such as humic substances may differ, depending upon the stability of the organic “complex” in the gastrointestinal lumen, see chapter 3.

Figure 4.2 Distribution of PAH in soil , benzo(a)pyrene as example



The primary mechanism for reduced bioaccessibility of PAH from soil will thus be low solubility and absorption into soil organic matter. The most important factors for release from the soil will be dissolution (“detergent aided” by bile) and release from the soil organic matter. Dissolution of soil organic matter can increase accessibility by increasing the capacity for forming dissolved organic complexes.

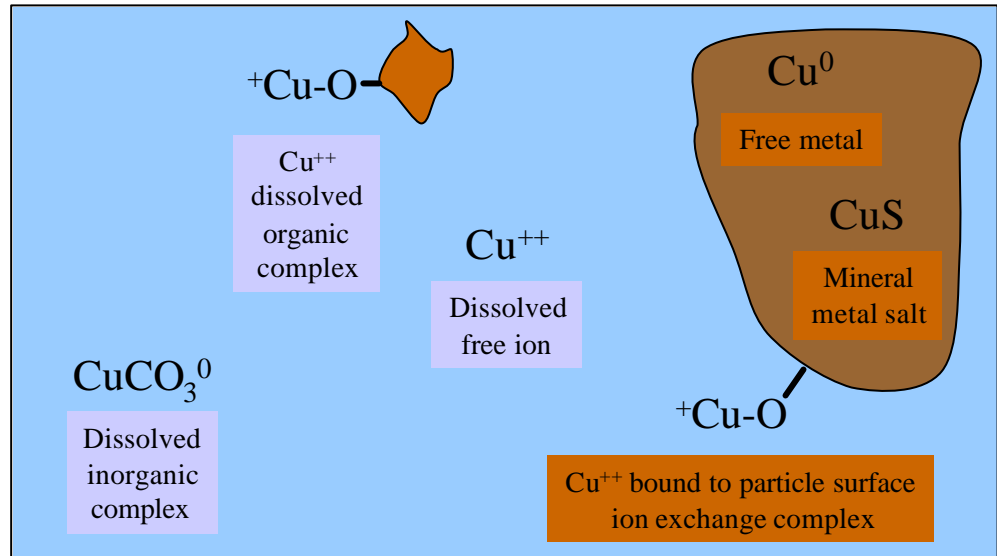
4.2 Speciation of metals in soil

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

- most metals occur naturally at varying concentrations and in varying chemical forms
- chemical form (species) of the original metal (source) may vary from solid metal to 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. An example of distribution between phases and chemical forms (species) in soils is shown for copper in figure 4.3. The bioaccessibility of the three solid species of copper: free metal (Cu^0), 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 *e.g.*: humic substances or organic acids, may differ, depending upon the stability of the complexes in the gastrointestinal lumen, see chapter 3.

Figure 4.3 Distribution of metals in soil , copper as example



An aging effect (compare section 4.1) in soils has been observed for As(V) /42/ and Cr /43/, see below and chapter 7.

It is important to remember that 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.

Due to their different physical-chemical properties, the mechanisms for reduced bioaccessibility of metals differ among the metals. Summaries of relevant physical-chemical properties are compiled below from review papers and textbooks on metals /3;5-7;9;13;35/. The summaries emphasise the chemical conditions relevant to soils and to the human digestion only, *e.g.*: pH between 1 and 8.

4.2.1 Arsenic

In fully oxidised water such as most soil pore waters, the primary aqueous species will be arsenates (oxidation state V), and in reduced waters arsenites (oxidation state III), table 4.2. For the arsenates, the mono-anionic dihydrogen arsenate will dominate at low pH (below pH 6,9), and for the arsenites, the non-ionic trihydrogen arsenite will dominate below pH 9,2.

Arsenic salts with low solubility include calcium, iron and manganese arsenates. The direct effect of pH on water solubility of arsenic salts will be limited.

Arsenic is found in all rocks and soils at low concentrations (typical 2 mg/kg, median from Danish soil quality monitoring: 3,3 mg/kg dw /44/) and in a variety of species, with arsenopyrite (FeAsS) as the most common. Frequent anthropogenic (Man made) sources are metal mining and smelting (arsenic trioxide, As₂O₃), and tanneries, pesticides and wood preservatives (arsenate, H₂AsO₄⁻). In soils, arsenic may be found associated with iron sulphides and iron oxyhydroxides, and arsenate – iron oxide associations are more stable than for arsenite /45/. Organic acids and other anions such as phosphates

compete with arsenate and arsenite for iron oxyhydroxide sites /45;46/. Organic arsenic may be formed in soils.

For As(V), aging has been observed and the effect can be explained by formation of mixed minerals with iron oxyhydroxides (inner sphere surface complexes) at low pH (pH < 6) within a period of less than 3 months (see also chapter 7)/42/.

Overall, the primary mechanism for reduced availability of arsenic in soil will be minerals of low solubility and adsorption to iron oxyhydroxides, and the most important factor for release from the soil will be (acidic) dissolution of the arsenic minerals and the iron oxyhydroxides. Reduction of arsenate to less efficiently sorbed arsenite and displacement by other anions such as organic acids and phosphate may be important release mechanisms as well.

4.2.2 Cadmium

Cadmium occurs in natural waters in oxidation state +II as the cation Cd^{++} and complexes of Cd^{++} : cadmium hydroxide (CdOH^+) and carbonate (CdCO_3^0) at high pH; cadmium sulphate (CdSO_4^0) and chloride (CdCl^+) at lower pH and depending upon the cadmium concentration.

Cadmium salts with low solubility includes cadmium phosphate ($\text{Cd}_3(\text{PO}_4)_2$), sulphide (CdS), hydroxide ($\text{Cd}(\text{OH})_2$) and carbonate (CdCO_3). The solubilities of these cadmium salts will increase with decreasing pH.

Cadmium is found in all soils at low concentrations (typical 0,1-0,4 mg/kg, median from Danish soil quality monitoring: 0,16 mg/kg dw /44/) and in a variety of species, mainly as sulphide and carbonate, but also as phosphate. Frequent anthropogenic sources are metal (zinc) mining and smelting due to co-occurrence of cadmium sulphide with the important zinc ore: zinc sulphide. Phosphate fertilisers, atmospheric deposition and sewage sludges are other important sources of cadmium in soils. Metal plating industries, PVC stabilisers, batteries and pigments are potential cadmium sources. In soils, cadmium will also be found bound to the cation exchange sites (clay minerals, iron oxyhydroxides and calcium carbonate minerals).

Overall, the primary mechanism for reduced availability of cadmium in soil will be minerals and salts of low solubility and cation exchange, and the most important factor for release will be (acidic) exclusion from and/or dissolution of the cation exchange complexes, as well as acidic dissolution of cadmium minerals and salts.

4.2.3 Chromium

In fully oxidised water such as most pore waters, the stable aqueous species will be chromates (oxidation state VI), and in reduced waters chromium cations (oxidation state III). For the chromates, the mono-anionic hydrogen chromate will dominate below pH 6,5, and for chromium cations, complexes of the trivalent cation with hydroxide will dominate above pH 4. The conversion between the two oxidation states depend upon the presence of catalysts, the redox conditions and the pH and both oxidation states can thus be present in soils depending on these environmental factors and on the oxidation state of the chromium source, see also chapter 7. The extent of conversion between the two redox states ("chromium cycling") is disputed

and for practical purposes, Cr(III) can be considered stable, whereas Cr(VI) can convert slowly to Cr(III) in natural systems.

Chromium salts with limited solubility include hydroxides of Cr(III) and calcium, lead, zinc and barium salts of Cr(VI). The solubility of Cr(III) hydroxides will increase with decreasing pH.

Chromium is found in all soils at varying concentrations (typical 10-50 mg/kg, median from Danish soil quality monitoring: 9,9 mg/kg dw /44/) depending upon the soil parent material and from natural sources mainly as Cr(III), whereas occurrence of Cr(VI) is almost exclusively the result of human activities. Frequent anthropogenic sources include mining and smelting (primarily chromite, $\text{FeO}\cdot\text{Cr}_2\text{O}_3$), metal plating and corrosion control, wood treatment, leather tanning and pigments. In soils, Cr(III) will mainly be found as precipitated hydroxides and possibly bound to the cation exchange sites (clay minerals and iron oxyhydroxides), whereas Cr(VI) will be found bound to iron oxyhydroxides.

For Cr(VI), an aging effect has been observed and can be explained by conversion of more soluble Cr(VI) to Cr(III) cations that are bound to the soil in cation exchange complexes or as hydroxides (see also chapter 7) /43/. For Cr(III), an aging effect has been observed that can be explained by slow (50 days) transformation of comparatively bioaccessible Cr^{+++} bound in particle surface ion exchange complexes to less bioaccessible $\text{Cr}(\text{OH})_3$.

Overall, Cr(III) will have reduced bioavailability in soils as the low solubility salt $\text{Cr}(\text{OH})_3$, whereas binding to iron oxyhydroxides to some degree will be a factor for Cr(VI). The most important factor for release will be (acidic) dissolution of $\text{Cr}(\text{OH})_3$.

4.2.4 Copper

In natural waters, the most important Cu species will be Cu^{++} (in hydrated form), the hydroxy complexes (e.g.: CuOH^+ and $\text{Cu}(\text{OH})_2^0$) and the carbonate complexes (CuCO_3^0 and CuHCO_3^+). Organic complexes will also be present.

Copper salts with limited solubilities include hydroxides, carbonates and sulphides. Particularly insoluble is copper hydroxide ($\text{Cu}(\text{OH})_2$) and overall, copper has a very limited solubility at pH above 7-8. The solubility of copper hydroxide will increase with decreasing pH.

Copper is found in all soils at varying concentrations (typical 30 mg/kg, median from Danish soil quality monitoring: 0,9 mg/kg dw /44/). The natural occurrences are dominated by copper sulphides (including mixed sulphides with iron) and hydroxycarbonates (e.g.: malachite $\text{CuCO}_3 \cdot \text{Cu}(\text{OH})_2$). Frequent anthropogenic sources include waste incineration slags (tenerite, CuO), manure from live stock treated with growth promoters, wood preservatives, metal industry and electrical industry. In soils, copper will primarily be found as hydroxides, carbonates and sulphides, and also as bound to the cation exchange sites of soil organic matter and iron oxyhydroxides. Metallic copper (Cu^0) is a frequent soil contaminant.

Overall, reduced bioavailability of copper in soils can be attributed to occurrence of metallic copper, low solubility salts and cation exchange

complexes. The most important factors for release will be (acidic) dissolution of carbonates and hydroxides, (acidic) exclusion from or dissolution of iron oxyhydroxide complexes and (alkaline) dissolution of organic complexes.

4.2.5 Lead

In natural waters, the most important Pb species will be Pb^{++} and the carbonate complex $PbCO_3^0$. Sulphate, chloride and organic complexes will also be present. At low pH, $PbSO_4^0$ will dominate and at high pH, $PbCO_3^0$. Most lead salts are insoluble with lead nitrate and to some degree lead chloride as the important exceptions. Lead phosphates (e.g.: $Pb_3(PO_4)_2$ and $PbHPO_4$) as well as mixed lead chloride phosphate ($Pb_5(PO_4)_3Cl$) are very sparingly soluble, but lead sulphate, carbonate, hydroxide and sulphide exhibit limited solubility as well. Lead also forms insoluble salts with some organic acids. Lead carbonate and hydroxide typically found in alkaline soils will exhibit increasing solubility with decreasing pH, whereas the lead sulphate and phosphates found in acidic soils will respond less to acidification.

Lead is found in all soils at varying concentrations (typical 5-30 mg/kg, median from Danish soil quality monitoring: 11 mg/kg dw /44/). The natural occurrences are dominated by lead sulphate, sulphide and carbonate. Frequent anthropogenic sources include metal mining, smelting and processing, traffic (leaded gasoline), incineration processes, disposed lead acid batteries (in particular metal scrap sites), paints and waste. Although organic lead compounds are released from traffic and with gasoline spills, their transformation to inorganic lead compounds are believed to be fast and their significance in soils therefore low. In soils, lead phosphates dominate, followed by carbonates and hydroxides. Complex formation with cation exchange sites may be important, in particular with iron oxyhydroxides and organic matter. Lead is considered the least mobile of the heavy metals in soils.

Overall, reduced bioavailability of lead in soils can be attributed to occurrence of low solubility salts and maybe to cation exchange complexes. The most important factor for release will be (acidic) dissolution of lead carbonate and hydroxide, but phosphates present will counteract release.

4.2.6 Nickel

In natural waters, the most important nickel species will be the Ni^{++} cation and complexes with carbonate ($NiCO_3^0$) and with organic compounds. Hydrogencarbonat complexes ($NiHCO_3^+$) complexes and at high pH also hydroxy complexes may be present.

Nickel salts with limited solubility includes nickel phosphate ($Ni_3(PO_4)_2$), nickel hydroxide ($Ni(OH)_2$) and sulphide (NiS) and to some degree nickel carbonate ($NiCO_3$), whereas most other nickel salts are soluble in water. The nickel salts are more water soluble than most of the other cationic heavy metals. Solubilities of these salts will increase with decreasing pH.

Nickel is found in all soils at varying concentrations (typical 5-15 mg/kg, median from Danish soil quality monitoring: 4,0 mg/kg dw /44/). The natural occurrences are mostly mixed ore sulphides with iron or copper, but nickel is also present in elevated concentrations in pyrite (FeS_2). Frequent anthropogenic sources include mining and melting, metal processing and nickel plating, as well as nickel-cadmium batteries. In soils, nickel bound to

cation exchange sites in organic matter, iron oxyhydroxides and clay minerals will dominate.

Overall, reduced bioavailability will mainly be caused by cation exchange but the presence of low solubility salts may be of importance for alkaline soils. Release will primarily be (acidic) exclusion from and dissolution of ion exchange complexes.

4.2.7 Zinc

In natural waters, zinc will mainly be present as the cation Zn^{++} but complexes with carbonates ($ZnCO_3^0$ and $ZnHCO_3^+$) and hydroxide (*e.g.*: $ZnOH^+$) may be present as well. The importance of organic complexes is not well described.

Zinc salts with limited solubility include zinc phosphate ($Zn_3(PO_4)_2$), zinc sulphide (ZnS), zinc hydroxide ($Zn(OH)_2$) and zinc carbonate ($ZnCO_3$). Zinc phosphate, hydroxide and carbonate will exhibit increasing solubilities with decreasing pH.

Zinc is found in all soils at varying concentrations (typical 10-300 mg/kg, median from Danish soil quality monitoring: 19 mg/kg dw /44/). The natural occurrences are mainly zinc sulfide (ZnS). Frequent anthropogenic sources include mining and smelting, metal processing and plating, fertilisers and sludges. In soils, zinc will primarily be present bound to cation exchange sites of iron oxyhydroxides, clay minerals and organic matter.

Reduced bioavailability will mainly be by ion exchange and to some degree by the presence of low solubility salts for alkaline soils. Release will be by (acidic) exclusion from and dissolution of ion exchange complexes.

4.3 Implications of speciation for design of bioaccessibility tests

A test for bioaccessibility of contaminants in soil should be designed to simulate a realistic worst case scenario, *i.e.*: it should enable estimation of the highest bioaccessibility likely to occur. Consequently, test design should include:

- low pH dissolution of iron oxyhydroxides and/or disruption of cation exchange complexes (all metals)
- high pH and enzymatic dissolution of soil organic matter (PAH)
- “detergent” aided dissolution (PAH)
- complex binder aided dissolution (all cationic metals, high chloride important for lead)
- presence of solubility impacting ions such as phosphate (As, Cr and Pb)
- sequential testing (acidic followed by alkaline) with separate release measurements in each sequence to avoid errors from dissolution followed by precipitation (all cationic metals)
- aerobic and anaerobic conditions where pertinent (As and Cr)

5 Quantification of bioavailability

The relative bioavailability factors required for adjusting MCLs for variations in contaminant bioavailability from soils, see chapter 2, can be obtained at different levels:

- characterisation of source and site chemistry
- *in vivo* tests
- *in vitro* tests

Each approach has benefits and disadvantages, and each has a separate role in the implementation of bioavailability in risk assessment. It should be noted that bioavailability may be taken into account in the toxicity studies behind the MCLs.

5.1 Characterisation of source and site

A characterisation of the source and the site with respect to the chemistry of the contaminants and the soil is mandatory in advance of deciding in favour of a bioavailability study, see also chapter 2. The main objective for this characterisation is to evaluate, whether bioavailability is likely to be reduced with the current contaminant and soil chemistry. The evaluation should at the least include (originally elaborated for metal contaminated soils):

- species of contaminants at source (*e.g.*: is the original contamination metallic copper scrap of limited bioavailability or more bioavailable copper sulphate solution?)
- number and concentrations of contaminants (*e.g.*: do we face many contaminants at high concentrations or a few with concentrations close to nominal MCL?)
- soil geochemistry and its potential for contaminant (im)mobilisation (*e.g.*: do we deal with a highly organic soil with large potential for reduction of Cr(VI) to the less toxic and less bioaccessible Cr(III) or a sandy soil without this potential?)
- species and vehicle comparison between site and the toxicity studies behind the nominal MCL (*e.g.*: was the toxicity or epidemiological study made with an aqueous solution of lead nitrate compared to the insoluble lead phosphate in the soil at the site?)

Very high contaminant concentrations suggest that even with very low contaminant bioavailabilities, the safe, revised MCLs will not approach actual concentrations.

In this phase, geochemical modelling (with *e.g.*: MINEQL or MINTEQ) can assist in identifying probable metal species in soil water /10;35/.

Access to previous bioavailability data for the same site type (source, soil and age) can assist the evaluation.

Due to the complexity of the soil matrix, the chemical characterisation alone is not considered sufficient to allow for quantitative bioavailability adjustments /9/.

5.2 *In vivo* tests

The ultimate bioavailability test is measurements in humans, followed by animal experiments and then by *in vitro* tests.

In vivo tests are generally considered the best bioavailability tests available, as the animal uptake measured in these tests is believed to resemble the conditions applied during toxicity testing. Oral *in vivo* tests generally include both dissolution (bioaccessibility), absorption and reduction. Absorption of soil contaminants is not covered by the present review, but an overall understanding of the techniques used for *in vivo* bioavailability studies is useful as a reference for subsequent chapters on bioaccessibility and in particular on the correlation between bioavailability and bioaccessibility, chapters 7 and 8.

Different approaches have been taken for *in vivo* bioavailability tests /4;14;47/:

- intestinal perfusion
- excretion measurements
- blood kinetics
- target tissue measurements

In the intestinal perfusion techniques, a section of the intestine of an experimental animal is separated, the contaminated matrix for testing is introduced in the intestine, and the concentration of contaminant is subsequently measured in the matrix after passing the section. The absorbed fraction is the fraction of contaminant that disappeared during intestinal passage. Strictly speaking, the intestinal perfusion techniques are not real *in vivo* techniques, as the intestinal section is separated from the animal to varying degrees in different versions of these techniques. Pros et contras are:

- + dissolution and transport close to real conditions
- + removal by absorption close to real conditions
- transport over the epithelium membrane during absorption is not included
- reduction in membrane cells and liver not included
- metabolites formed in the intestine are not considered
- removal by degradation (in lumen and at membrane surface) measured as available
- costly
- only experimental animals available for contaminants

In excretion measurements, experimental animals are fed the contaminated matrix and the excreted (faeces) fraction measured. The non-excreted or retained fraction of contaminant is the bioavailable fraction. Pros et contras are:

- + dissolution and transport close to real conditions
- + removal by absorption close to real conditions

- the transport over the epithelium membrane during absorption is not included
- reduction in membrane cells and liver not included
- metabolites formed in the intestine not considered
- removal by degradation (in lumen and at membrane surface) measured as available
- excretion with bile is measured as non-available
- time consuming and costly
- only experimental animals available for contaminants

Distinguishing the initial excretion of unabsorbed contaminant with faeces and the re-excretion of contaminant occurring later may refine the mass balance technique. Further refinements include measurements of urinary excretion and blood concentrations. Also, urinary excretion alone has been used to give a lower boundary for bioavailability of contaminants that are not metabolised /5/.

An experimental approach combining the perfusion and excretion techniques is the *in situ* test. Here, the full gastrointestinal system of the experimental animal is used for digestion and uptake while the animal is anaesthetised but still alive. This technique exhibits the pro et contra of the perfusion and excretion techniques but is more comprehensive and consistent with true *in vivo* conditions.

In blood kinetic studies (traditional bioavailability studies), the contaminated matrix is ingested and approximately the same amount is injected intravenously. The blood concentration of contaminant is measured over time and the bioavailability is calculated as the ratio between the area under the concentration curves for oral administration and for intravenous injection. Pros et contra are:

- + dissolution and transport under to real conditions
- + removal by absorption under to real conditions
- + removal by degradation (in lumen and at membrane surface) under real conditions
- + the transport over the epithelium membrane during absorption included
- + reduction in membrane cells and liver included
- metabolites not considered, unless specifically analysed for
- demands sensitive analytical methods due to limited amount of blood available
- demands larger experimental animals than rodents or many experimental animals
- very costly
- only experimental animals available for toxic contaminants

In target tissue measurements, the contaminated matrix is ingested and after due delay, the resulting concentration is measured in the target tissue, such as the liver if liver cancer is the effect driving the MCL. Pros et contra are:

- + dissolution and transport close to real conditions
- + removal by absorption close to real conditions
- + removal by degradation (in lumen and at membrane surface) close to real conditions

- + the transport over the epithelium membrane during absorption included
- + reduction in membrane cells and liver included
- + distribution and potential tissue accumulation included
- metabolites not considered, unless specifically analysed for
- demands identification of target tissue
- demands specific target tissue(s) without general effects
- very costly
- only experimental animals available for contaminants

Interpretation of liver concentrations as estimates of overall bioavailability has been suggested based upon the assumption that the liver reflects the overall systemic level of the contaminant /5/. Use of this method is valid only for contaminants where the liver is the major organ for distribution and metabolism and this should be verified in advance.

All *in vivo* methods for bioavailability measurements are subject to large variability, as are all biological systems. Conversely, all the methods address the overall bioavailability including both bioaccessibility and absorption, see chapters 3 and 4, but reduction is included in the blood kinetic and target tissue approaches only.

Epidemiological studies where exposure and health effects are recorded and correlated for large population groups are rarely available for MCL derivation, compare the US TDI for arsenic, see chapter 2.

5.3 *In vitro* tests

Bioavailability tests *in vitro* are based upon two different approaches /4;14;47/:

- bioaccessibility or dissolution tests
- absorption tests

Test simulating the dissolution processes of the contaminants from the matrix, *i.e.*: the bioaccessibility, are addressed in chapter 6. The common *in vitro* tests for absorption are using:

- membrane chambers
- everted sacs
- cell culture chambers

In the membrane chamber technique, a sheet of intestinal epithelium (the mucosa) is set up as a membrane between two chambers. One chamber is filled with a solution of the contaminant, the other with a medium that receives the contaminant transported over the membrane. After incubation, the resulting concentration of contaminant is measured in the receiving medium. The pros et contras are:

- + absorption close to real conditions
- + removal by degradation (at membrane surface) close to real conditions
- + the transport over the epithelium membrane during absorption included
- + fast and simple
- + interspecies comparisons possible
- includes only absorption and excludes matrix effects

- metabolites not considered
- reduction in liver not included
- effect of blood supply and lymph drain not included

In the everted sac technique, a small piece of intestinal epithelium is taken out and everted to a small sac “inside out”, *i.e.*: with the inner part of the epithelium facing the outside of the sac. The sac is filled with a medium that receives the contaminant, closed and situated in a solution of the contaminant. After incubation, the resulting concentration of contaminant is measured in the receiving medium. The pros et contras are

- + absorption close to real conditions
- + removal by degradation (at membrane surface) close to real conditions
- + the transport over the epithelium membrane during absorption included
- + fast and simple
- + interspecies comparisons possible
- includes only absorption and excludes matrix effects
- metabolites not considered
- reduction in liver not included
- demands sensitive analytical methods due to limited amount of receiving medium available
- effect of blood supply and lymph drain not included

Both methods using sheets of intestinal epithelium is impaired if fresh intestine is not used, and both require highly skilled staff and well developed techniques.

In cell culture techniques, intestinal epithelium cells (*e.g.*: Caco-2 cultured from a human colon carcinoma) are cultured to form a cell monolayer on a filter support. The monolayer is polarised, *i.e.*: exhibits the physiological features of *in vivo* epithelium cells with an upper and an under side, and it tolerates artificial soil digests after slight dilution. The filter with the cell culture is set up as a membrane between two chambers. One chamber is filled with a solution of the contaminant, the other with a medium that receives the contaminant transported over the membrane. After incubation, the resulting concentration of contaminant is measured in the receiving medium and in the cells. The pros et contras are:

- + absorption close to real conditions
- + removal by degradation (at membrane surface) close to real conditions
- + the transport over the epithelium membrane during absorption included
- + can be used with soil digests
- + cell cultures more reproducible than most biological tests
- + fast and simple
- includes only absorption, unless combined with bioaccessibility pre-test
- metabolites not considered
- reduction in liver not included
- comparability between original intestinal epithelium and the cultured cells can be disputed
- currently available only for research purposes and not for routine testing

Preparation of filters coated with monolayers of original intestinal membrane cells has not yet been successful.

The absorption tests are designed to address the absorption step and consequently, most of the techniques cover only one of the two main processes susceptible to matrix and speciation variations, see chapter 3. An exception is the cell culture method that can be used with digests of contaminants from soil and thus may include matrix effects upon the absorption process. The absorption tests can though be useful to elucidate differences in uptake among different species of a contaminant.

6 Quantification of bioaccessibility

A test for bioaccessibility of soil contaminants in soil must 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 precaution. To fulfil this, test must be based upon the properties of the human digestion process, of the contaminants in question and the geochemistry of soils, see chapters 3 and 4.

If the test shall be used for practical risk assessment, it furthermore needs to fulfil the common, basic requirements for a regulatory method. The method must be:

- simple (*i.e.*: the number of steps and operations maintained at the minimum considering the below requirements)
- 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 at 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 *in vitro* bioaccessibility tests range from very simple chemical extraction or leaching tests to advanced multistep tests simulating in detail the human digestion processes.

6.1 Chemical extraction tests

Simple chemical extraction tests are used to evaluate the leaching risks associated with solid wastes and contaminated soils /48/. Examples of leaching tests are the European Norm EN 12457 and the US EPA methods 1311 (TCLP) and 1312 (SPLP) /49-51/, see table 6.1 for principles. The toxicity characteristic leaching procedure (TCLP) is designed to simulate the leaching by slightly acidic organic acids in a waste deposit, whereas the synthetic precipitation leaching procedure (SPLC) is intended to simulate leaching by slightly acidic precipitation.

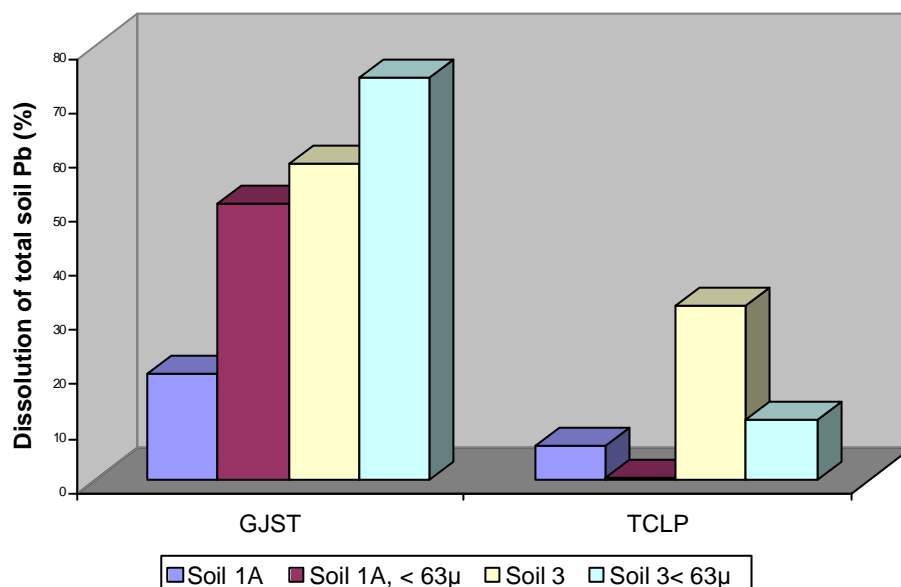
Evidently, the leaching tests do not fulfil the requirements set up for bioaccessibility tests. Accordingly, lack of correspondence has been reported for lead in soils between TCLP testing and bioaccessibility testing /9;52/, see figure 6.1. Testing for bioaccessibility with the simple stomach simulating test GJST, see table 6.3, evidently released a higher fraction of soil lead than the TCLP leaching test. Furthermore, the ratios between the dissolution data for the two tests were varying for different soils and even for different soil particle fractions within the same soil, indicating qualitative differences (*i.e.*: different mechanisms simulated) in addition to the expected quantitative differences

(i.e.: different yields of dissolution). The same pattern was reported for Cu and Zn /53/.

Table 6.1 Principles of selected simple chemical extraction methods (leaching tests) for soils and wastes

Sequence	Conditions	EN 12457-1	EN 12457-3	EPA 1311 (TCLP)	EPA 1312 (SPLP)
First	L/S ¹⁴	2	2	20	20
	Solution	water	water	acetic acid pH < 5	sulfuric and nitric acid pH < 4,2-5
	Time	24 hours	6 hours	18 hours	18 hours
	Temperature	20 ± 5 °C	20 ± 5 °C	23 ± 2 °C	23 ± 2 °C
Second	L/S	-	8	-	-
	Solution	-	water	-	-
	Time	-	18 hours	-	-
	Temperature	-	20 ± 5 °C	-	-

Figure 6.1 Dissolution of soil lead with a leaching procedure (TCLP) and with a simple test simulating stomach conditions (GJST), data from /52/

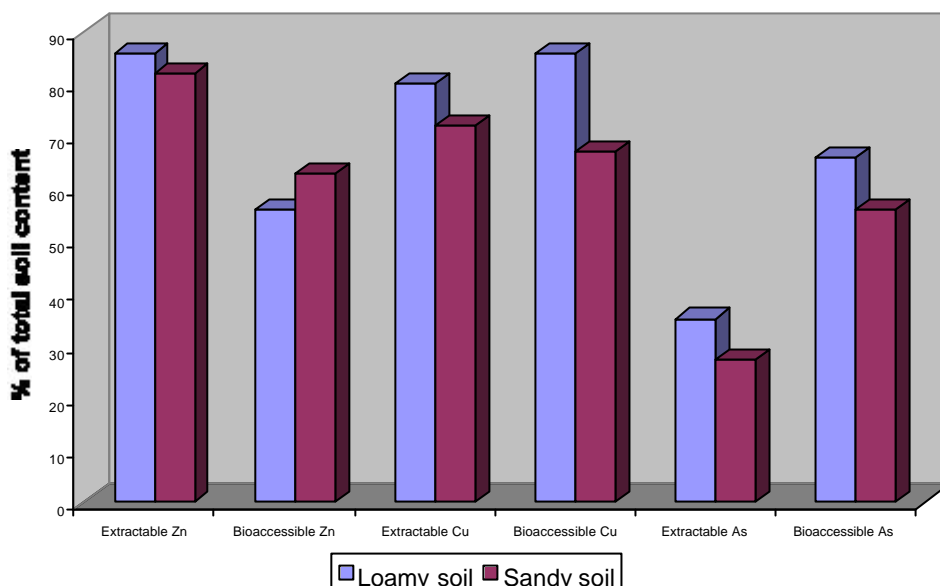


Data from a preliminary comparison (52 soils, 11 metals) of simple chemical extraction (dilute nitric acid, pH = 1,07) with bioaccessibility testing according to the RIVM method (see table 6.3) (unpublished DHI data) demonstrates the lack of correspondence between the two test types for zinc, copper and arsenic, figure 6.2. For zinc, the bioaccessible fraction was lower than the extractable, for arsenic higher and for copper comparable. Furthermore, the ratios between extractable and bioaccessible fractions were not the same for the two soils.

Overall, simple chemical extraction can not at present be recommended as a test for bioaccessibility of soil contaminants.

¹⁴ L/S: liquid to solid ratio, volume by weight

Figure 6.2 Comparison of simple chemical extraction of heavy metals with bioaccessibility test (RIVM method) for a sandy and a loamy soil from two sites contaminated with wood preservatives, unpublished DHI data



Sequential chemical extraction schemes (*e.g.*: /54/) are used for speciation of metals in soils or waste and also for evaluation of potential leaching to the groundwater. From the dissolution profile obtained after treatment with reagents of increasing solubilising effect, metal such as arsenic can be characterised as surface sorbed, bound in iron oxyhydroxides or bound in insoluble salts /55/. An attempt to correlate lead, copper and zinc sequentially extracted fractions in mine waste contaminated soils with bioaccessibility measured with the PBET method (see table 6.3) did not succeed /56/.

Similarly to the sequential extraction schemes used for metals, methods have been suggested for partial extraction of organic contaminants as an estimate of bioavailability or bioaccessibility (*e.g.*: /57-59/. The methods are based upon partial extraction with an organic solvent or an extraction method that can dissolve only those contaminants that are not firmly bound in the soil. The partial extraction can then be correlated to bioaccessibility or bioavailability and used as a fast surrogate for bioavailability or bioaccessibility testing. The approach has mostly been used as a test for bioavailability of organic contaminants to the soil biota, but correlation to human bioaccessibility has been attempted for pesticides from soil without convincing results /59/.

The interpretation of sequential dissolution tests in terms of metal speciation is debated and the use of partial extraction for organic contaminants is not ready for routine use, in spite of the first promising results with combinations of aqueous and organic solvent extractions /60/. For both metals and organic contaminants, the sequential or partial chemical extraction methods do not satisfy the requirements stated for bioaccessibility methods and are therefore not recommended for this purpose. Still, sequential or partial extraction methods may be of use in evaluating whether a contaminated soil is a candidate for risk assessment based upon bioavailability, *i.e.*: by answering the question, is the form of the contaminant likely to exhibit low RAF in soil, see chapter 2.

6.2 Digestion tests

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 1990's.

6.2.1 Product methods

Release of metals from art materials, *e.g.*: toys, textiles and paints, can according to be tested by simple extraction with pH = 1,2 hydrochloric acid at liquid to solid ratio, L/S = 50 and 37 °C for 1 hour /61/. The method resembles the European Standard for Safety of Toys, Migration of Certain Elements /62/. In addition to the simple extraction with hydrochloric acid, the European Standard allows for additional extraction with an organic solvent. The methods are intended to give an estimate of the bioavailability of metals after ingestion. It has recently been suggested to include extraction simulating the conditions in the human gastrointestinal system in order to improve the correspondence with *in vivo* bioavailability data /63;64/.

Product methods are currently not satisfying the requirements stated for soil contaminant bioaccessibility test methods.

6.2.2 Methods for pharmaceuticals

Dissolution tests form an integrated part of the development of drugs. The standard method is described in the U.S. Pharmacopeia (USP) and involves one step dissolution from a rotating container (paddle or basket) in simple media adapted to the drug and drug formulation in question/65;66/.

Several more advanced dissolution tests simulating the processes in the gastrointestinal system have been proposed, *e.g.*: /25;34/, see table 6.2. The test principles are acidic dissolution with added synthetic tenside (enhances wettability and prevents adsorption to equipment surfaces) simulating dissolution in the empty (fasted state) stomach and long life milk simulating stomach dissolution in the fed state. Neutral dissolution with added synthetic chyme (bile salts and phospholipids) are used to simulate dissolution in the upper parts of the small intestine with slightly lower pH and higher buffer and bile concentrations for the fed state. For lipophilic drugs (compare PAH), it has been clearly demonstrated that the use of high buffer/high bile concentrations simulating fed state dissolution in the small intestine results in higher dissolution of the compounds /34/.

For drugs formulated in lipid solutions or emulsions (compare PAH in separate oil phase), an advanced test with addition of lipases (enzymes hydrolysing lipids) and continuous titration (pH stat) maintaining constant pH has been suggested /67/.

Currently, a diversity of methods (*e.g.*: varying pH, buffer and bile concentrations simulating fasting and fed conditions differently) has been proposed as illustrated in a recent review presenting 9 different methods all intending to simulate drug dissolution in the small intestine /67/.

It should be emphasised that the purpose of drug dissolution tests is to verify sufficient drug dissolution (*e.g.*: 85%) in the correct compartment (stomach,

small intestine or large intestine). Furthermore, drug dissolution tests are generally used in development of drugs and drug formulations and followed by *in vivo* tests of bioavailability. An alternative use of the dissolution tests is in quality control during production. Therefore, drug dissolution tests with a specified method and with a specified, good precision are required to produce statements like:

- the drug is more than XX% dissolved
- 2 drugs are equally well dissolved
- the drug is preferentially dissolved in the stomach, not in the small intestine
- the drug is dissolved more than XX% within YY hours

Table 6.2 Principles of selected drug dissolution test methods for solid formulations /25;34/

Sequence	Conditions	Stomach, fasted state	Stomach, fed conditions	Small intestine, fasted state	Small intestine, fed state
First	L/S ¹⁵	depends upon dose ¹⁶	depends upon dose	depends upon dose ¹⁷	depends upon dose
	Solution	hydrochloric acid, sodium chloride and sodium lauryl sulphate pH = 1,0 - 1,3	long life milch, 3,5% fat	phosphate buffer pH = 6,5, lechitin, taurocholate, potassium chloride	acetate buffer pH = 5,0, lechitin, taurocholate, potassium chloride
	Time	15 minutes	15 minutes	15 minutes	15 minutes
	Temperature	37 °C ¹⁸	37 °C	37 °C	37 °C
Second	L/S	-		-	-
	Solution	-		-	-
	Time	-		-	-
	Temperature	-		-	-

Drug dissolution tests generally are done with the formulation in question, *i.e.*: without disturbing matrices such as soil. Among the lessons learned from drug dissolution experiments with bearings for bioaccessibility tests are /25;34;68/:

- dissolution declines with increasing particle size
- solubility limitation of uptake is important at compound solubilities below 100 mg/L (*i.e.*: for most organic contaminants considered here)
- dissolution data will change with the test details applied
- high precision tests can be performed

The typical data form for a drug dissolution test is presented schematically in figure 6.3. The data interpretation would be that the tested drug was poorly

¹⁵ L/S: liquid to solid ratio in L/kg

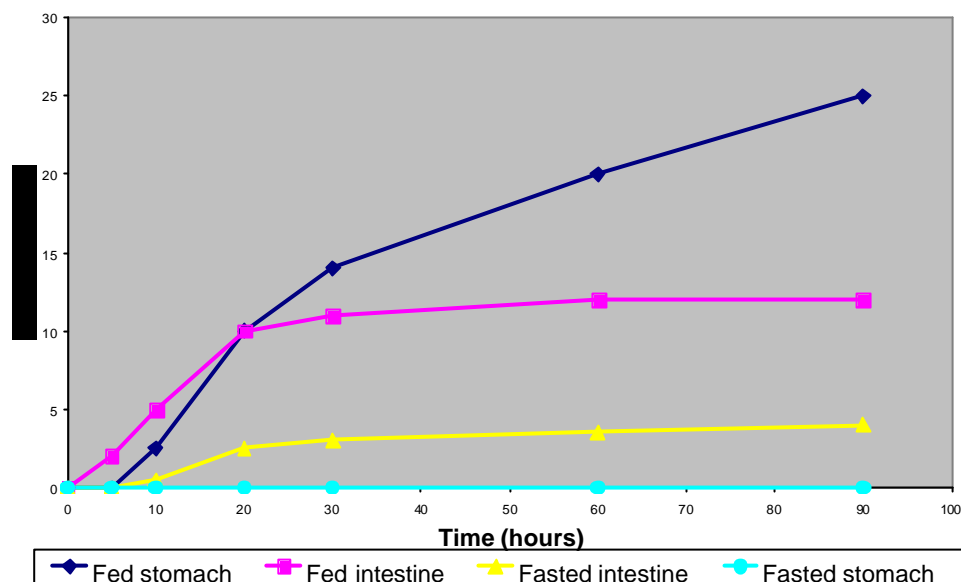
¹⁶ With 300 mL solution per dose and a dose of maximum 500 mg, L/S = 600

¹⁷ With 500 mL solution per dose and a dose of maximum 500 mg, L/S = 1.000

¹⁸ Anticipated from context, not specified in reference

water soluble, not dissolved in the stomach in the absence of food, dissolved to some degree in the small intestine but reached a solubility limitation here, probably caused by insufficient concentration of solubilising bile constituents.

Figure 6.3 Typical data presentation from drug dissolution test, rearranged from /69/



Since 1997, the US Food and Drug Administration (US FDA) has endorsed use of *in vitro* dissolution tests as a surrogate for *in vivo* uptake investigations in bioequivalence studies (*i.e.*: testing whether two products have similar uptake properties) /70/. A guideline for establishing *in vitro in vivo* correlations (IVIVC) has been released for drugs where dissolution is the limiting step for uptake. The approach includes:

- do the *in vitro* test and generate dissolution time profiles for at the least two drug formulations
- do *in vivo* test and generate uptake profiles (blood concentrations) with the same time intervals and the same drugs
- extract a linear correlation between *in vitro* and *in vivo* data
- test the correlation by predicting *in vivo* time profiles from *in vitro* dissolution data for another formulation and comparing to *in vivo* profiles measured for the same formulation
- apply the correlation in future tests of new formulations

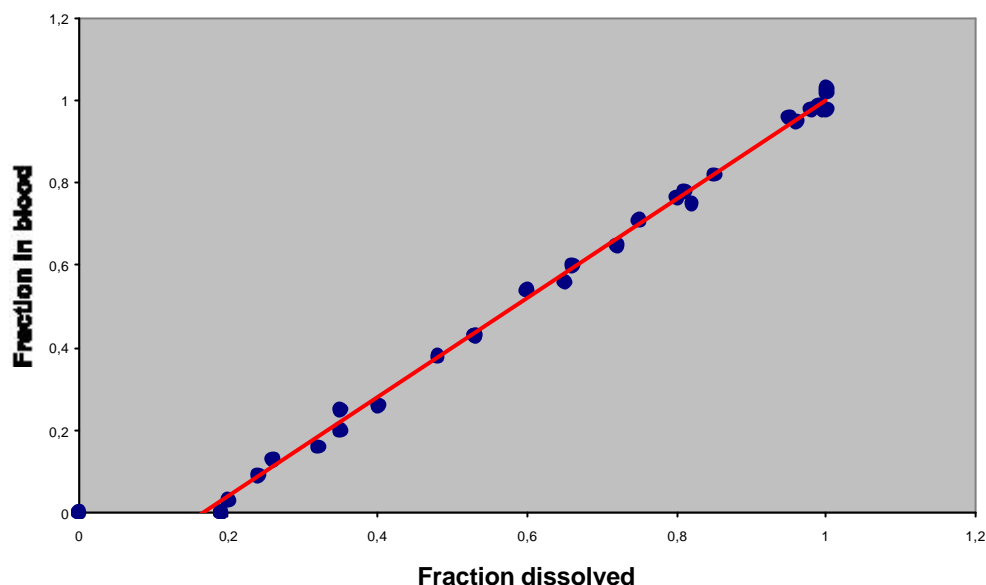
In the hypothetical example of a linear relationship presented in figure 6.4, please note that that the relationship is not a simple 1:1 relationship, *i.e.*: the line is not through (0,0) with slope 1. In other words, if 50% of a drug is dissolved in the test, the resulting total uptake might be 20%.

It should also be noted, that the actual IVIVC depends upon the details of the employed test method, *e.g.*: fasted or fed state simulation of small intestine dissolution /71/.

The logical next step in development of dissolution tests for predicting resulting *in vivo* blood concentrations is the use of models to predict uptake directly from dissolution tests data and thus circumventing the need for *in*

vivo uptake studies. This development has started (e.g.: /72/) but evidently, the modelling tools have not yet been developed to yield satisfying predictions.

Figure 6.4 Hypothetical relationship between in vitro dissolution test data and in vivo blood concentration data (rearranged from /70/)



In routine pharmaceutical applications, the different dissolution tests for different uptake compartments are used separately, e.g.: one test for fed state small intestine dissolution, one test for fed state stomach dissolution etc, see table 6.2. Multicompartmental tests have been suggested that simulate the sequential dissolution of drugs passing from the stomach through the small intestine /73;74/. A multicompartmental model has been used to demonstrate how a clay mineral impacts dissolution of drugs in the stomach and the upper sections of the small intestine /73/.

6.2.3 Contaminated soil methods

A wide range of methods has been suggested for determining the availability of contaminants, in particular organic 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 free compound dissolved in the soil water is available to the soil biota, compare figure 4.2. 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: be bioaccessible. Therefore, methods developed to measure the ecotoxicological bioavailability are generally not applicable for measuring human bioaccessibility.

A survey of methods for in vitro testing of bioaccessibility from soils is presented in table 6.3.

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, *e.g.*: nitric acid destruction prior to quantification with atomic absorption spectroscopy (AAS) or inductively coupled plasma (ICP) methods /17/. It is therefore recommended always to report the concentration of “total” metals, the concentration of bioaccessible metals (both in mg/kg dw) in addition to the percentage bioaccessibility. In the present report, the impact of using different methods for analysing “total” metal concentrations on percentage bioaccessibilities quoted in this report is not considered.

The PBET method was based upon a method developed for estimation of iron bioaccessibility for nutritional research /75/. This method is used in a modified version entitled SBET by the British Geological Survey (BGS) excluding the intestinal digestion step /76/ and by others /43;77-79/. Now, the BGS uses the simple SBET for lead bioaccessibility and PBET for arsenic and other contaminants /80/.

Presently, the PBET method is used in a simplified version featuring extraction with glycine buffer at pH = 1,5 and L/S = 100 for 1 hour /7/. This version of the method, called SBRC after the Solubility/Bioavailability Research Consortium, can be extended with a step simulating small intestine digestion: titration to pH = 7,0, addition of bile and pancreatin and digestion for 4 hours. The SBRC does not exclude oxygen during testing.

The PBET method has been modified including features from the Digestive tract model and RIVM methods for bioaccessibility testing of polychlorinated dibenzodioxins and –furans /89/. A simpler version including features from the Digestive tract method has been used for bioaccessibility testing of PAH in soil /90/. Furthermore, a modified version of the PBET method has been used for determination of pesticide bioaccessibility in soils /59/.

The RIVM method was based upon a method developed by Rotard /91/ for bioaccessibility testing of organic contaminants from mine waste and was used in a slightly modified version by Oomen /2/. The DIN method was also derived from the Rotard method.

The TIM model was based upon a dynamic model developed by Minekus simulating the gastrointestinal system for general research purposes /74/.

Several of the methods presented in table 6.3 have been used to produce bioaccessibility data as presented in chapter 7.

Table 6.3 Summary of methods for bioaccessibility testing

Reference	Method	PBET	Mass-balance	Digestive tract model	DIN 19738	RIVM	GJST	PREP	IVG	SHIME	TIM
	Conditions	/81;82/	/83/	/84/	/85/	/86/	/52/	/87/	/88/	/76/	/76/
Target contaminants		metals	metals	metals and organic contaminants	metals and organic contaminants	metals and organic contaminants	metals	metals	metals	metals	metals
Target matrices		soils, mine wastes	soils	soils	contaminated soils, wastes, sediments	soils	soils	soils	soils	soils	soils
Resolution in compartment and time		stomach and intestine determined separately and with time resolution	stomach and intestine separately	none	none	none	none	none	stomach and intestine separately	none	stomach and intestine determined separately and with time resolution
Oxygen access in test		no, argon purge	yes	yes	yes	yes	yes	yes	no, argon purge	yes	yes
Mouth and esophagus	L/S	-	160	-	15 ¹⁹	15	-	-	-	-	5
	Solution	-	mucin, urea, phosphate buffer, sodium, calcium and potassium chloride pH = 5,5	-	mucin, amylase, urea, uric acid, phosphate and bicarbonate buffers, calcium, potassium and sodium chloride, sodium sulphate, sodium thiocyanate pH = 6,4	mucin, amylase, urea, uric acid, phosphate buffer, sodium hydroxide, potassium and sodium chloride, sodium sulphate, sodium thiocyanate pH = 6,5	-	-	-	-	not specified pH = 5,0
	Time	-	5 seconds	-	30 minutes	5 minutes	-	-	-	-	5 minutes
	Temperature	-	ambient	-	37 °C	37 °C	-	-	-	-	37 °C

¹⁹ Mouth and esophagus is optional

	Method	PBET	Mass-balance	Digestive tract model	DIN 19738	RIVM	GJST	PREP	IVG	SHIME	TIM
Stomach	L/S	100	2.000	105-120	50	37,5	22,2	30	150	2,5	25
	Solution	hydrochloric acid, pepsin, citrate, malate, acetic acid pH = 1,3 ²⁰	hydrochloric acid, pepsin, sodium chloride pH not specified	hydrochloric acid, sodium chloride, pepsin, mucin ⁸ , whole milk powder pH = 2,0	hydrochloric acid, phosphate buffer, sodium and potassium chloride, pepsin, mucin, whole-milk powder ²¹ pH = 2	hydrochloric acid, phosphate buffer, calcium, ammonium, sodium and potassium chloride, glucose, glucuronic acid, urea, glucosamine, serum albumine, pepsin, mucin pH = 1,07 ± 0,07	acetic acid, hydrochloric acid pH = 6 → 2	baby food, hydrochloric acid, phosphate buffer pH = 2,0	hydrochloric acid, sodium chloride, pepsin, dough pH = 1,8	baby food, cream, pectin, mucin, starch, cellobiose, protease, peptone pH = 4,0	not specified in detail, lipase, pepsin pH = 5,0 → 2,0
	Time	1 hour	2 hours	2 hours	2 hours	2 hours	2,67 hours	1 hour	1 hour	3 hours	1,5 hour
	Temperature	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C	37 °C
Small intestine	L/S	100	4.400	120	100	97,5	-	40	150	4,0	46
	Solution	sodium bicarbonate, bile salts, pancreatin pH = 7,0	sodium bicarbonate pH not specified	sodium bicarbonate, trypsin, pancreatin, bile pH = 7,0	potassium, calcium and magnesium chloride, bicarbonate buffer, trypsin, pancreatin, bile, urea pH = 7,5	hydrochloric acid, potassium, sodium, calcium and magnesium chloride, phosphate and bicarbonate buffers, serum albumine, lipase, pancreatin, bile, urea pH = 8,0 ± 0,2	-	sodium carbonate, metallothionein pH = 6,9 ± 0,1	sodium bicarbonate, pancreatin, bile pH = 5,5	sodium bicarbonate, bile, pancreatin pH = 6,5	not specified in detail, bile, phosphate, pancreatin pH = 6,5 → 7,2
	Time	3 hours	2 hours	6 hours	6 hours	2 hours	-	1,5 hours	1 hour	5 hours	6 hours
	Temperature	37 °C	37 °C	37 °C	37 °C	37 °C	-	37 °C	37 °C	37 °C	37 °C

²⁰ Other pH values optional, pH maintained by stepwise acid additions

²¹ Optional

In development of the published bioaccessibility test methods, a range of important experimental details was identified for specification in a suitable bioaccessibility test method (first record listed is referenced, most findings published by several authors):

- mixing or stirring rate /87/
- digestion time /87/
- presence of food /87/
- use of milk powder as food substitute /85;92/
- digestion pH, requirement for buffering /82/
- soil particle size /93/
- liquid to solid ratio, L/S > 100 /94/
- presence of organic acids in digestion fluid /95/
- bile amount added /2/
- mucine added /84/
- gastric and intestinal digestion required /84/
- chloride added/52/

A few additional, important points should be made with respect to test method conditions to support the listing above.

In vitro bioaccessibility tests do not include the effects of the microbial communities present in the *in vivo* gastrointestinal system, and do not include the effects of active transport of contaminants from the digestion solution /17/.

It has been demonstrated with sequential extraction that the presence of phosphate changes the speciation of lead towards less extractable species /96/.

A study of lead dissolution kinetics under simulated stomach conditions has suggested that a test time of 1 hour is adequate for this compartment /97/. Fast release of lead from contaminated soils (66) and wastes (19) was reported simulating gastric conditions over time /97/, with more than 30% of total lead dissolved within 10 minutes for most samples. The study also demonstrated higher bioaccessibility with lower pH and faster release with higher temperatures. Other studies have suggested a test time of 1,5-2 hours as most appropriate for the stomach simulation /81/.

Dissolution of lead minerals in the stomach depend on the acid concentration (figure 6.5) present in the test during dissolution, with low acid concentrations leading to both slower dissolution and lower final dissolved concentration /93/. An average decrease in stomach bioaccessibility of 57% was observed with 7 soils impacted by mine wastes when raising the test pH from 1,3 to 2,5 /82/. The effect of acid concentration is caused by both pH (formation of HSO_4^-) and chloride concentration (formation of soluble PbCl^+) /81/. Arsenic bioaccessibility from 2 soils was lower by 8 - 25% in the PBET test with pH = 2.5 than with pH = 1,3 but the effect was less than for *e.g.* lead /82/. The pH and dissolution time ranges studied here are comparable to the conditions found in the human stomach (see table 3.1).

Recent 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 to stomach transit /98/.

Figure 6.5 Dissolution profiles for selected lead bearing minerals and a mine waste contaminated soil simulating gastric dissolution, redrawn and modified after /93/.

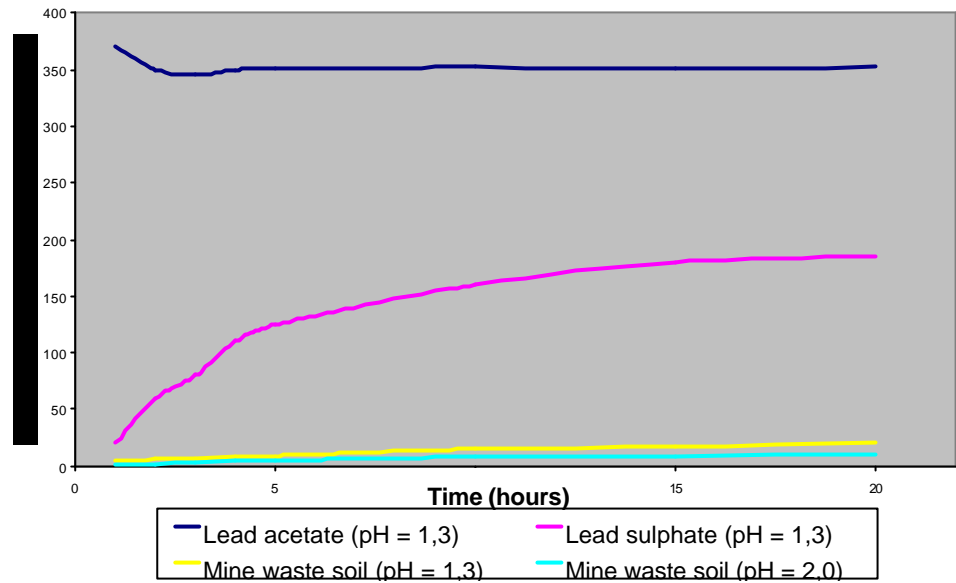
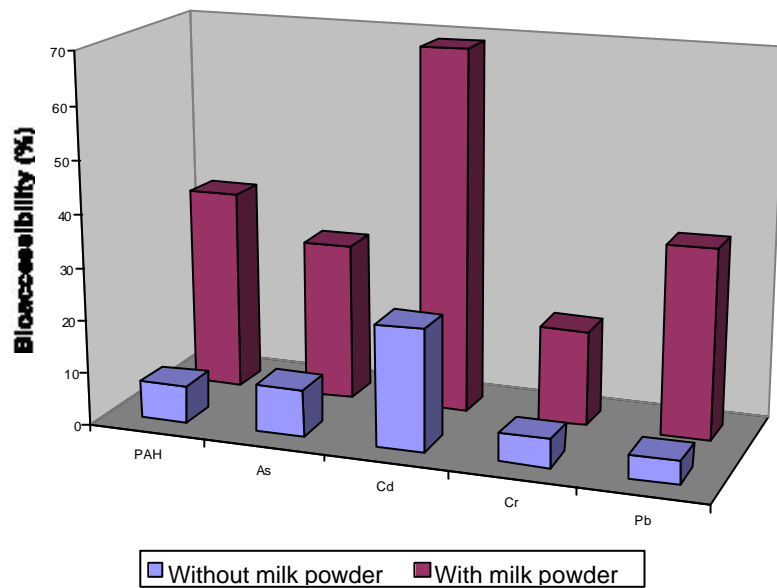


Figure 6.6 Bioaccessibility of contaminants from 14-29 soils with the Digestive tract model with and without added milk powder, data from /92/.



The general effect of adding milk powder to the test system is to enhance dissolution of soil contaminants, as seen for the Digestive tract model in figure 6.6. The effect was also observed for arsenic, cadmium and lead using the DIN test in a method comparison /76/.

The increase in PAH bioaccessibility from soil upon addition of milk powder and of mucine to the test system has been reported several times, *e.g.*: /84/, /99;100/. Also, an increase in metal bioaccessibility has been reported upon factor 6 increase of the concentrations of added mucin, bile and pancreatin in the DIN method /92/, but the effect was not seen consistently for all metals and soils tested, see also figure 6.7

6.2.4 Comparisons of contaminated soil methods

An interlaboratory comparison of generally applied bioaccessibility test methods demonstrated considerable within laboratory variation (As: 31% mean coefficient of variation, CV, Cd: 34% CV and Pb: 71% CV) and very large between laboratory variation (table 6.4) /76/. Please, note that part of the between laboratory variation may be caused by different pretreatment methods applied prior to bioaccessibility testing. One method is omitted from the evaluation (SHIME), as the method evidently employs a too high pH (4,0) in the gastric digestion step and consequently yields to low results.

Differences in pH of the acidic gastric digestion step are given as the most likely explanation of the poor correspondence obtained with the different methods in the interlaboratory comparison, even with the remaining data sets /76/. In particular, the SBET method without a neutral digestion step simulating small intestinal processes gave higher results for the cationic metals (Cd, Pb, compare chapter 4) than did the other methods. The lower results obtained with the combined gastric-intestinal methods are probably caused by precipitation of metals dissolved in the acidic gastric step after neutralisation in the subsequent neutral to alkaline intestinal step

Table 6.4 Interlaboratory method validation data for bioaccessibility testing, % bioaccessibility from /76/

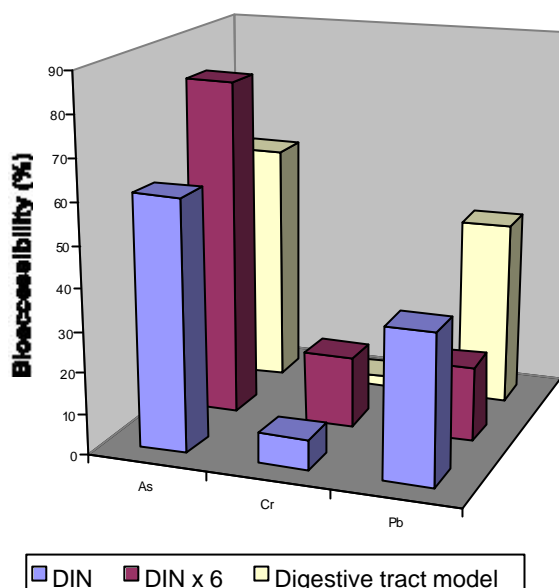
Soil	Arsenic	Cadmium	Lead
Flanders	30-50	38-92	13-91
Oker 11	11-19	51-92	4-56
Montana 2711	41-59	40-99	11-90

A method comparison has been performed for bioaccessibility of polychlorinated dibenzodioxins and -furans from mine waste with methods similar to the RIVM and the Digestive tract approaches (see table 6.3) /101/. Again, the data demonstrated reasonable precision employing one method but large variations when different methods were used. The main methods differences were use of a mouth and esophagus simulating step, the L/S ratio and the composition of the digestion fluids. Furthermore, an increased bioaccessibility is demonstrated with methods including addition of milk or oil in the digestive steps.

Comparison of a method resembling the DIN method, the same method with 6 times higher concentrations of mucine, bile and pancreatine and the Digestive tract model demonstrated that measured bioaccessibility depend entirely upon the specific version of the method used, figure 6.7 /92/. Furthermore, the different versions resulted in higher concentrations for one contaminant and lower concentrations of another.

Comparison of methods resembling the RIVM, the GJST and the Digestive tract methods demonstrated that lead mobilisation occurs in the stomach step, but lead is probably demobilised in a subsequent intestinal step due to the higher pH here /102/. The presence of phosphate in the intestinal step may increase the demobilisation as lead phosphate is sparingly water soluble, see chapter 4 and section 6.2.3.

Figure 6.7 Bioaccessibility of selected soil contaminants from an urban soil obtained with three different test methods, data from /92/.



A comparison of the PBET method with the simpler IVG version demonstrated that bioaccessibility of arsenic from 13 soils impacted by mine wastes was higher with the IVG method. The difference was probably caused by the addition of dough and the higher pepsin concentration used in the IVG method /88/, see table 6.5.

Table 6.5 Bioaccessibility of As obtained with 2 different methods (PBET and IVG) for 13 soils impacted by mine waste, % bioaccessibility from /88/

	PBET	IVG
Stomach	12%	17%
Stomach and intestine	8,3%	15%

6.3 Bioaccessibility test performance requirements

Analytical methods for use in environmental regulation must fulfil minimum requirements with respect to analytical quality or performance. The requirements are generally established in terms of the analytical detection limit (the lowest values that can be detected), accuracy (recovery of true value, “trueness”) and precision (the scatter of replicate data). In Denmark, minimum performance is defined for a number of environmental matrices and analytical parameters /103/, but no requirements have yet been put forward for soil contaminants. The lowest quality accepted is Quality Class 3 that requires:

- precision as total relative standard deviation (coefficient of variation, CV) better than 7%, within laboratory variation including day to day variation
- accuracy better than 95-105% recovery of true value for internal quality control samples, within laboratory “error” or bias
- accuracy better than 70-130% recovery of true values for interlaboratory comparisons (external quality control), between laboratory variation

For a test where the result depends upon the precise test conditions, see above in this chapter, no true value can be established. In such cases, the median or mean value obtained by several laboratories with an accepted method is designated the “true value”.

Generally, the analytical limit of detection should be lower than 1/10 the maximum contaminant concentration to be controlled. As no maximum limits are currently established for soil bioaccessibility of contaminants, it is suggested to set the limit of detection requirement to 1/10 of the cleanup level.

Table 6.6 gives estimated method detection limits for metals with the RIVM test based upon instrument detection limits reported by the Danish commercial laboratory Eurofins A/S for standard analysis (ICP-AES) and for the most sensitive method (ICP-MS). The estimated test method detection limits should be considered lower limits of detection limits, as variability from the test is not included. Also, the test method detection limits will depend upon the test conditions and the analytical set up and performance. For PAH, estimates can not be established similarly, as the volumes of digest available do not correspond to current standard PAH analytical requirements.

Table 6.6 Examples of bioaccessibility method detection limits estimated for the RIVM test from Eurofins A/S instrument detection limits

	Estimated method detection limits (mg/kg dw)			
	For 10% bioaccessibility		For 90% bioaccessibility	
	ICP-AES	ICP-MS	ICP-AES	ICP-MS
As	8	0,8	0,9	0,09
Cd	1	0,1	0,1	0,01
Cr	1	0,3	0,1	0,04
Cu	2	0,3	0,2	0,04
Pb	3	0,3	0,4	0,04
Ni	3	0,8	0,4	0,09
Zn	2	2	0,2	0,2

For the PBET method, a limit of detection of 1,5 mg bioaccessible As/kg dw and a within laboratory precision (between series) of 16% (arsenic) has been reported /104/.

A method detection limit corresponding to 5 mg/kg dw bioaccessible lead has been reported /81/ and a detection limit for 20% bioaccessible As of 5 mg/kg dw has been given /107/. For several studies, the coefficient of variation reported is of the same magnitude as the bioaccessibility measured suggesting that the reported values were in reality below method limits of detection.

Within laboratory variation can be estimated for several of the test methods for soil contaminant bioaccessibility described, table 6.7. Overall, fine precision (<7% CV) has been attained for test simulating stomach bioaccessibility of metals from soils, whereas tests simulating stomach **and** intestine have been used with less precision due to the extra complicating step. Furthermore, precision seems to be worse for lead (precipitating in the intestinal step), and for organic contaminants not as good as for metals such as arsenic. Finally, it should be considered whether the general requirement for a 7% total CV is attainable and necessary analysing a heterogeneous matrix such as soil.

No interlaboratory comparisons have been published with one accepted method used and allowing for evaluation of the accuracy of that method and at the participating laboratories. The interlaboratory comparisons published with more than one method employed, see above in this chapter, clearly demonstrate that the requirement for a maximum variation of $\pm 30\%$ from a designated true value is not fulfilled.

Table 6.7 Selected precision data reported for test methods for soil contaminant bioaccessibility

Method	Precision (CV)	Comments	Reference
PBET	2-6% (n = 3)	lead, stomach, probably within day variation	/81;82/
PBET	36-70% (n = 3)	lead, stomach and intestine, probably within day variation	/81;82/
PBET ²²	6,2 – 15 (n = 3)	individual dioxins, stomach and intestine, probably within day variation	/89/
Mass-balance	15% (n = 4)	lead, stomach, probably within day variation	/105/
Mass-balance	24% (n = 4)	lead, stomach and intestine, probably within day variation	/105/
Mass balance	8% (n = 4)	arsenic, stomach, probably within day variation	/105/
Mass-balance	21% (n = 4)	arsenic, stomach and intestine, probably within day variation	/105/
DIN	<1 – 38% (n = 3-4)	arsenic, stomach and intestine, probably within day variation	/99/
DIN	<1 – 33% (n = 3-4)	lead, stomach and intestine, probably within day variation	/99/
DIN ²³	2,3 – 31% (n = 3)	individual dioxins, stomach and intestine, probably within day variation	/92/
RIVM	7-10% (n = 4-5)	PCB, stomach and intestine, probably within day variation	/106/

²² Modified for use with organic contaminants

²³ Modified version with increased concentrations of digestion constituents

6.4 Implications of method studies for bioaccessibility testing

A number of bioaccessibility test methods have been developed and details to be specified in a test method has been listed, see section 6.2.3.

The conclusions from the method comparisons and studies are that:

- the data obtained with different methods may vary at the least one order of magnitude
- the variability of bioaccessibility data obtained with one method is larger than generally accepted for test methods
- a test method for bioaccessibility from soils that does not simulate all important solubilising processes will be biased

It is debatable whether one method suitable for all contaminants (metals and organic compounds) can be found. It has been suggested by the project reference group to use a simple stomach test (SBET, PBET stomach step or SBRC) for Cd, Cu, Pb and Zn, a combined stomach and small intestine test (full PBET, DIN or RIVM) for As and Cr, and a full stomach and small intestine test with bile and food (milk) added for organic contaminants.

Test method validation data corresponding to what is generally required for methods to be used for regulatory purposes have not been published. The interlaboratory comparison data required to evaluate a test method have not been published.

Realistic test performance requirements should be not higher than Quality Class 3 and method detection limits of 1/10 of MCLs to be enforced, see section 6.3.

7 Bioaccessibility data

During the last 10 years, bioaccessibility data have been published for both the metals and PAH from soil. The data are presented in the appendix and summarised in the last part of this chapter, and specific studies are briefly described below, in particular where relationship between bioaccessibility and *soil or contaminant properties* can be deduced.

It should be noted that in this review, the term “mine waste” is used when the source of soil contamination is all types of mining and ore processing activities.

Also, data have not been discussed separately for sources and source impacted soils, *e.g.*: an incineration slag and a slag contaminated soil. As a comment to this, most reported bioaccessibilities of cadmium and chromium from soils are below 100%, see appendix, whereas test of slag from steel industries for stomach bioaccessibility with a method resembling the GJST method demonstrated 100% bioaccessibility of chromium (total and Cr(VI)) and cadmium /108/.

In a few cases, general soil properties are measured and a sufficient number of soils tested for bioaccessibility to allow for correlation (native soil studies). Also, a few mechanistic studies have been performed using addition of soluble metal salts to different soils and measurements of the resulting speciation and bioavailability (spiking studies). Native soil studies are closer to the diversity of real contaminated soils but in such studies, bioaccessibility differences caused by different source mineralogy and speciation might overrule the effects of soil properties. Spiking studies are easier to interpret, but the simplicity and artefacts arising from this should be borne in mind during interpretation.

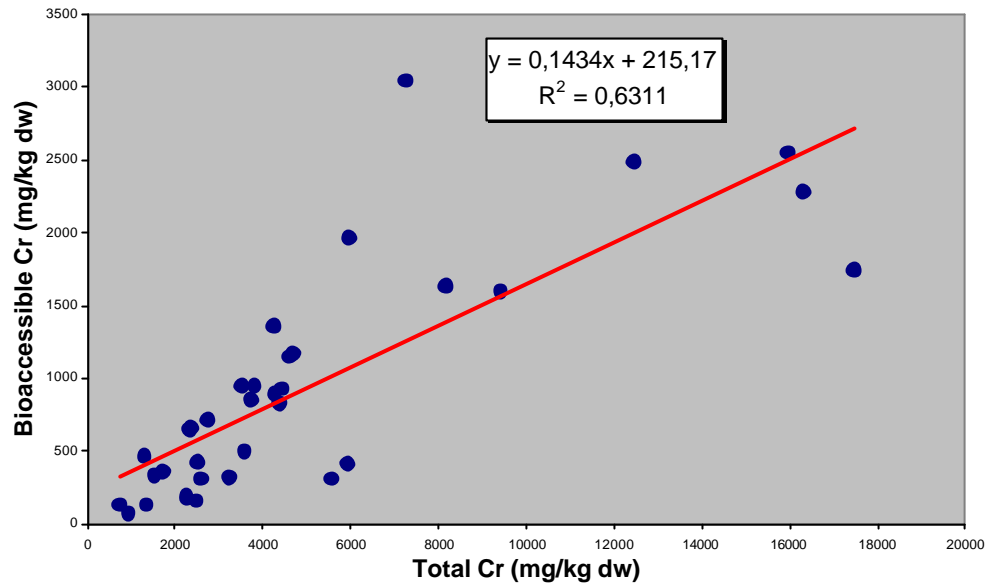
7.1 Heavy metal bioaccessibility

That soil total concentrations does not necessarily indicate bioaccessible concentrations can be seen from figure 7.1 presenting data from a study with CrCl_3 added to 35 different soils covering typical soils encountered at US Department of Defence contaminated sites /77/. For the same total Cr concentration of approximately 6.000 mg/kg dw, the measured bioaccessible concentrations were in the range 300-2.000 mg/kg dw. Different *soil properties* resulting in variations in Cr *speciation* were responsible for the varying bioaccessibility. A similar lack of correlation between bioaccessibility and total concentrations were observed in a study of arsenic, cadmium, chromium, lead and nickel in 22 soils /99/.

In a spiking study with 36 soils, it was demonstrated that soils with *high contents of iron oxyhydroxides* (5 g/kg dw) and *low pH* reduced bioaccessibility of added arsenate, As(V), whereas the remaining soil properties did not impact the bioaccessibility (*e.g.*: CEC) or was interpreted to have an indirect effect of local pH variations (carbonate) /42/.

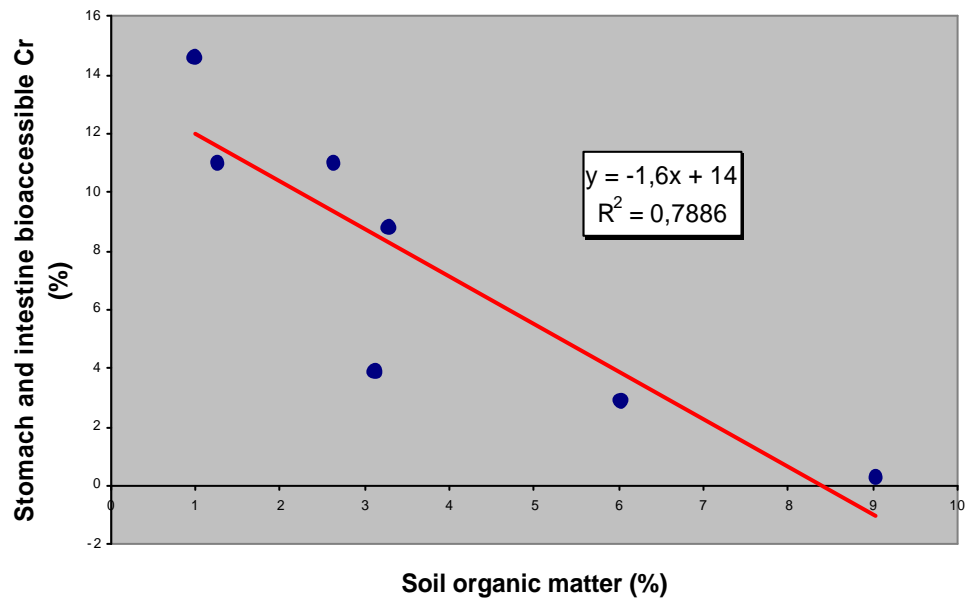
In a study of arsenic bioaccessibility with the PBET method covering 110 US soil samples, bioaccessibilities in the range 10-60% were found with the *arsenic mineralogy* and the *soil particle size* as the major determinands of bioaccessibility (109/, as referred in 12/).

Figure 7.1 Bioaccessible Cr concentrations against total Cr concentrations for 35 soils spiked with CrCl_3 , data from 77/



Preliminary indication has been presented of a higher bioaccessibility of Cr(III) and Cr(VI) added to soils as soluble salts after cold storage (2-3 °C) as compared to storage at higher *temperatures* (21-25 °C) 110/.

Figure 7.2 Correlation between stomach and intestine bioaccessible chromium and soil organic matter 95/



With data on 7 soil samples from one site contaminated with construction waste /95/, no general correlation could be observed between bioaccessible chromium or lead (stomach and stomach/intestine) and soil properties such as *cation exchange capacity (CEC)*, *soil organic matter (SOM)*, *soil pH* or even with *total soil concentrations* of the same metals. Only stomach/intestine bioaccessible chromium did exhibit a linear correlation with *soil organic matter (SOM)*, figure 7.2. The correlation is not strong but can result from SOM mediated reduction of soluble, anionic Cr(VI) as chromate to less soluble, cationic Cr(III). For this particular study, it should be noted that the variability of results for the same soil was larger than generally accepted, for several samples in the range of 100%.

The effect of *SOM* on Cr bioaccessibility and the underlying mechanism was supported by a detailed study on the effects of aging on chromium bioaccessibility and speciation /43/, see section 4.2. It was demonstrated that Cr(III) was less bioaccessible from soils with high (> 5,5) pH and high SOM, whereas Cr(VI) was less bioaccessible from acidic soils with high contents of iron oxyhydroxides. Cr(VI) was transformed to the less bioaccessible Cr(III) in all soils, but most with high SOM. Further studies demonstrated that Cr(III) found as Cr(OH)₃, mostly in alkaline and calcareous soils, is far less bioaccessible than Cr(III) found as Cr⁺⁺⁺ bound to the clay cation exchange sites /77/. Furthermore, these studies suggest that Cr(III) bound to SOM has limited bioaccessibility. Conversely Cr(III) can be adsorbed to clay minerals by ion exchange yielding high concentrations, but clay mineral bound Cr(III) is bioaccessible due to the ready desorption by acid.

Table 7.1 Bioaccessibility (GJST) of Lead and selected soil properties from two soils from one site (1A and 1 C) and one soil from a different site (2), data from /52/

	Soil 1A	Soil 1C	Soil 2
Sources	municipal waste incinerator bottom ash, de-icing salt, urban snow, industrial waste		municipal waste incinerator bottom ash, metal industry waste
Speciation	lead carbonate, lead oxide lead on silicates and iron oxyhydroxides lead in mixed mineral with tin, carbonate and oxide		lead carbonate, lead oxide lead on silicates and iron oxyhydroxides lead in mixed mineral with tin, carbonate and oxide
Soil organic carbon (%)	5,8	8,4	1,2
Lead concentration (mg/kg dw)	920	6.800	2.200
Lead in particle fraction < 125 µm (% of total lead)	20	29	70
Bioaccessibility (%)	19	20	58
Bioaccessibility of lead in < 125 µm fraction (range, % of total lead)	27-51	46-56	49-74

For lead added to 11 different soils as soluble lead salt, stomach bioaccessibility decreased with increasing *cation exchange capacity* /111/ as would be expected for the cation lead with high affinity for cation exchange.

Soil particle size have been demonstrated to impact lead bioaccessibility (the GJST test, /52/), but the study did not demonstrate whether this was due to different speciation of lead in small particles or to an impact by the soil particle size directly. Bioaccessibility of lead from small particles (< 63 µm) up to 5 times higher than from large particles (1-2 mm) was reported, see figure 6.1.

The effect of *soil organic matter* and *soil particle size* on lead bioaccessibility can be demonstrated from this study, table 7.1. The lead bioaccessibility with the simple stomach simulating test GJST was 2½ times higher for the soil with low SOM (measured as organic carbon) and a high content of lead in small particles, /52/. High SOM can prevent dissolution of soil bound lead by the gastric acid, whereas small particle have a larger surface and consequently exhibits faster dissolution. No other soil (inorganic carbon, texture), site (source type) or speciation (mineral distribution of lead) properties could explain the large difference in bioaccessibility. Conversely, higher bioaccessibility was reported with the PBET test for soils with higher SOM /82/. The suggested explanation here was that the presence of soil organic matter during weathering of mine waste minerals causes binding of the released lead by cation exchange followed by release in the acidic stomach simulation, whereas the lead minerals formed in the absence of SOM are less acid soluble.

The bioaccessibility of lead in soil vary with the *mineralogy of the lead*, as can be seen from table 7.2.

Table 7.2 Lead bioaccessibility from mine waste obtained with the PBET test, data from /81/

Soil	Dominating mineral species	Bioaccessibility (% of total)
Mine waste 1	lead sulphate (59%) lead sulphide (26%)	stomach 4,2% intestine 0,7%
Mine waste 2	lead iron oxides (29%) lead iron sulphates (24%) lead manganese oxides (31%)	stomach 0,5% intestine <0,2%
Mine waste 3	lead phosphate (37%) lead iron sulphates (39%)	stomach 5,6% intestine 0,1%
Mine waste 4	lead phosphate (39%) lead manganese oxides (26%) lead oxide and hydroxide (17%) lead silicates (11%)	stomach 2,1% intestine 0,6%
Soluble lead salt	lead acetate	stomach 76% intestine 34%
Soluble lead salt and mine waste	lead acetate	stomach 68% intestine 6,4%

The lead iron oxide dominated soil exhibited lower bioaccessibility than the soils dominated by lead sulphate, sulphide and oxide. Furthermore, even for the soluble lead acetate, the presence of mine waste decreased bioaccessibility in the stomach simulating test and even more including the small intestine simulating test. Finally, table 7.2 clearly demonstrates that lead was

precipitated and thus exhibited reduced bioavailability upon transit from the stomach to the small intestine.

Also, very soluble lead *minerals* as lead acetate, which is frequently used for *in vivo* bioavailability studies, dissolves much faster than the less soluble lead sulphate and than soil lead /82/, see figure 6.5.

Furthermore, the dissolution of soil lead minerals depends not alone upon the mineral present, but also upon the presence of “*coatings*” with other minerals /93/. Such coatings and also more soluble lead minerals (carbonates and iron oxyhydroxides) are more likely to be formed in soils and wastes with lower pH /82/. Lead sulphate constituted 59% of the lead in a soil tested for dissolution simulating stomach conditions (figure 6.5), but the dissolution rate was much slower for the soil than for the pure mineral and again, the final dissolved concentration was considerably lower.

In a study of As, Cd, Cr, Ni and Pb bioaccessibility with the DIN method /99/, no correlation could be found to *soil organic matter*.

A study including 7 German soils demonstrated correspondence between the bioaccessibilities of Cd, Cr, Ni and Pb and their distribution in fractions obtained by sequential extraction, see chapter 6 /99/. These data further support that the *soil mineralogy* interacts with bioaccessibility but a direct interpretation is not straight forward.

7.2 PAH bioaccessibility

The number of studies of PAH bioaccessibility is limited and includes only two methods (DIN and Digestive tract model) performed at one institution, see appendix. Additionally, a modification of the PBET test including the intestinal step only has been applied for PAH from soil /90/ but data were not available at the time of that publication and subsequent publication of data has not been found.

A study of PAH bioaccessibility from 4 coal mine waste and waste contaminated soils did not exhibit the expected lower bioaccessibility neither with higher *soil organic matter* content, nor generally with *increasing PAH partitioning coefficient* /99/. Still, one soil (Lothringen 2, see chapter 8) did exhibit the decreasing bioaccessibility with increasing size, ring number and partitioning coefficient that would be expected if reduced bioaccessibility was caused by absorption in soil organic matter only.

Similarly, a study of PAH bioaccessibility with 5 waste contaminated soils from one German site showed overall bioaccessibility of total (11) PAH of 11-15%. The mean bioaccessibilities for the 11 individual PAH were all in the same range, except for phenanthrene that exhibited a mean bioaccessibility of just above 20% /100/.

A validation study of the RIVM test for bioaccessibility of benzo(a)pyrene concluded that considerable variation (14-50%) in bioaccessibility was observed for additions of different amounts of the contaminant (higher *contaminant concentration*) to the same soil /11/. A larger bioaccessibility was seen from spiked sandy and silty soils (*texture effect*), as compared to loamy soils.

A few bioaccessibility studies of other organic contaminants from soil turned up in the literature survey (not specifically sought for) and their data are quoted below for perspective, table 7.3.

For the polychlorinated dibenzodioxins and –furans, no correlation between bioaccessibility from soil contaminated via industrial emissions to the air and congener *partitioning coefficients* was observed /89/, but the range of bioaccessibilities were the same as the ranges reported for bioavailabilities to rodents. Still, for dioxins from copper ore processing, a correlation between bioaccessibility and congener partitioning coefficient was observed /101/.

Table 7.3 Bioaccessibilities of organic contaminants other than PAH from soil with partitioning coefficients from /112/

Compound group	Partitio-ning coeffi-cients log (K_{ow})	Soils and sources	Test	Bioaccessi-bility	References
Dioxins ²⁴	6-12	soil with air emissions as source	PBET ²⁵	stomach and intestine 20-34%	/89/
Dioxins ²⁶	6-12	slag from copper ore processing	Digestive tract model	stomach and intestine 44-52%	/101/
PCB ²⁷	4-8	spiked artificial soil	RIVM	stomach and intestine 34-40%	/106/
PCB ²⁸	4-8	soils	Digestive tract model	stomach and intestine 32-83%	/84/
Lindane	4	spiked artificial soil	RIVM	stomach and intestine 57%	/106/
Pesticides ²⁹	3-6	spiked soil	PBET	stomach or stomach and intestine 2-44%	/59/

7.3 Overall contaminant bioaccessibility

Summaries of the reported bioaccessibilities of the 7 heavy metals and the 7 PAH are given in table 7.4, and all data can be found in appendix.

It should be noted that the typical intervals in table 7.4 are overall range estimates that should not be used for setting general bioaccessibility values. Data are compared across source types, species and methods and this allows for identifying major differences only. Also, a high variability of measured bioaccessibilities for same contaminant and same type of source, soil and test method precludes generic use of the reported values, see appendix.

²⁴ 7 polychlorinated dibenzodioxins and 10 polychlorinated dibenzofurans included

²⁵ Modified for use with organic contaminants

²⁶ 5 polychlorinated dibenzodioxins and 5 polychlorinated dibenzofurans included

²⁷ 4 polychlorinated biphenyl congeners included

²⁸ 6 polychlorinated biphenyl congeners included

²⁹ 6 pesticides included: diazinon, malation, chlorpyrifos, *trans*-chlordane, *cis*-chlordane and p,p'-DDT

As an overall conclusion it can be stated that all studied metals and PAH might exhibit bioaccessibility well below 100% in a particular contaminated soil, but cadmium, lead and chromium (Cr(III)) are most likely to do so.

No generic correlation between contaminant bioaccessibility and compound, soil or source properties can currently be deduced from the data. Properties of importance for one contaminant are summarised in above sections but in most cases, reported data can be found contradicting an emerging generic statement.

Access to an increased amount of bioaccessibility data for different sources and soils but with one method will enable more reliable generic statements on the relation between sources, soil characteristics and bioaccessibility. Likewise, more bioaccessibility data for different compounds and species but with one method will enable a better understanding of the contaminant properties determining bioaccessibility.

Table 7.4 Summary of reported bioaccessibility intervals, see appendix for details

Compound	Species	Bioaccessibility (%)		Comment
		Stomach	Stomach and intestine	
Arsenic	generally not specified	10-50	10-50	-
Cadmium	generally not specified	50-100	10-80	data material small
Chromium	Cr(III)	1-20	1-20	data material small, Cr(VI) data may be biased by reduction in tests
	Cr(VI)	20-100	20-100	
Copper	generally not specified	(10-90)	(10-90)	data material insufficient
Nickel	generally not specified	(10-90)	(10-90)	data material insufficient
Lead	generally not specified	10-90	0,1-10	-
Zink	generally not specified	(5-50)	(5-50)	data material insufficient
PAH	does not apply	(10-90)	(10-90)	data material insufficient

Still, a few overall trends with respect to differences in bioaccessibility with source can be stated, but should be taken with the same reservations as the intervals of table 7.4. Bioaccessibility of arsenic and lead seems to be higher when diffuse sources, urban activities, waste or wood preservation (arsenic only) are the sources, as compared to mine wastes as sources. Furthermore, bioaccessibility from gastric conditions is higher or much higher than from intestinal conditions for cadmium and lead, respectively.

8 Bioaccessibility – bioavailability correlations

Studies of bioavailability (*in vivo* studies with experimental animals) and bioaccessibility (*in vitro* dissolution studies simulating the gastrointestinal tract) on the same soils are few.

8.1 Heavy metal correlations

Studies of bioavailability are available for lead and to some degree for arsenic and were reviewed recently /113/. From these studies, a few points should be made regarding bioavailability, although this topic is not part of the current review.

The US EPA presupposes a 50% absolute bioavailability of lead from water or food, a 30% absolute bioavailability of lead in soil and thus a relative bioavailability of soil lead of 60% (referred from /113/, see chapter 9). The general picture for the reviewed bioavailability studies is given in table 8.1. It has been suggested that a range of arsenic bioavailability from soils of 8-30% is more appropriate /12/. Assuming 100% bioavailability of soluble As species, this would also be the range for the relative arsenic bioavailability from soils to expect.

Table 8.1 Main features of relative bioavailability of lead and arsenic, concluded from /113/

	Lead	Arsenic
Soils	30-80%	0-50%
Mine wastes	10-30%	20-50%

A study of bioavailability to monkeys of arsenic from five soils gave relative bioavailabilities in the range 11-25% /114/.

A frequently quoted study of lead uptake in adult humans from mine waste contaminated soil demonstrated 26% absolute bioavailability of ingested lead for fasted individuals and only 1,4% bioavailability in fed individuals /115/.

It should be noted that most soils tested are in effect impacted by mine wastes or other metal industry activities. Basically, the 60% relative bioavailability assumed by the US EPA for lead resembles measured relative bioavailability for most soils, whereas a lower relative bioavailability can be expected for mine wastes. For arsenic, fewer data are available but a relative bioavailability below 50% is to be expected.

Recent studies of arsenic, lead and benzo(a)pyrene in soils gave relative bioavailabilities in minipigs of 7-58% (6 soils), 22-72% (6 soils) and 14-39% (4 soils), respectively /99/.

Correspondence has been demonstrated between lead bioaccessibility measured with the PBET method (*in vitro*) and the dissolution process

occurring in the rabbit stomach and to some degree also in the small intestine (*in vivo*), table 8.2.

Table 8.2 Amounts of lead in rabbit stomach and intestinal fluid compared to concentrations in PBET test solutions after dissolution of comparable amounts of lead containing mine waste, data from /81/

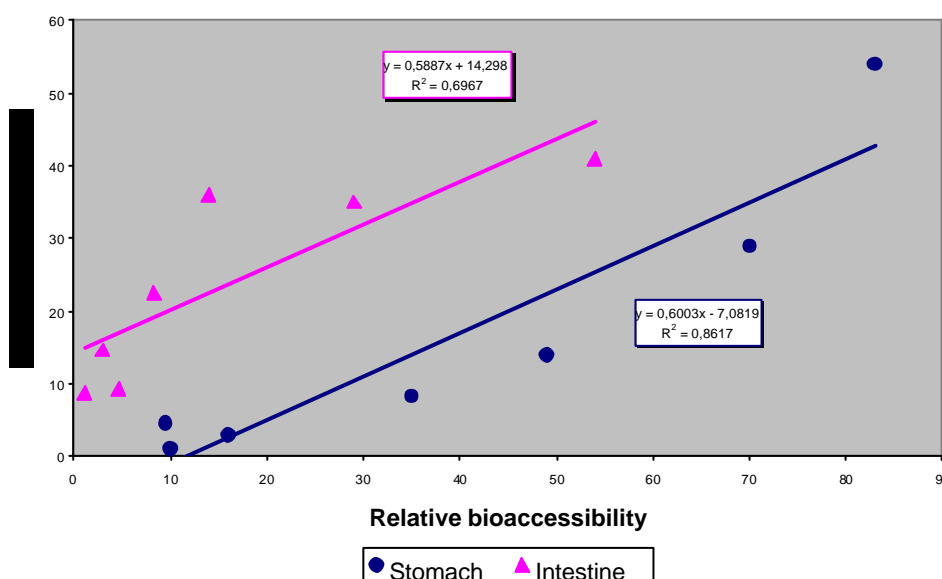
	Rabbit	PBET
Stomach	0,62 mg	0,59 mg
Small intestine	0,25 mg	0,11 mg

A comparison of relative bioaccessibility with relative bioavailability in experimental animals (rats for lead, rabbits and monkeys for arsenic) from 7 soils impacted by mine waste suggests a linear relationship, figure 8.1. Similar results were obtained for arsenic (2 soils and a house dust) /82/ and for 15 soils contaminated primarily with mine wastes /88/. In the later study, the mean bioaccessibility of As obtained with the IVG test method (17% in stomach simulation) was statistically indistinguishable from the bioavailability obtained in pig dosing studies (21%). In the first study /82/, data obtained with pH = 2,5 in the PBET stomach bioaccessibility test were best correlated to bioavailability and were suggested for future use.

Please, note from figure 8.1 that the relative bioaccessibility from the intestine simulation was lower than the relative bioavailability. This suggests that the precipitation of lead observed after transit from the stomach to the intestinal compartment may not result in reduced uptake. In other words, the test simulating intestinal bioaccessibility produced to low results.

Additional data correlating lead bioavailability to bioaccessibility obtained with the stomach part of the PBET test (the SBRC test) demonstrated fine linear correspondence but the same higher bioavailability than bioaccessibility /7/.

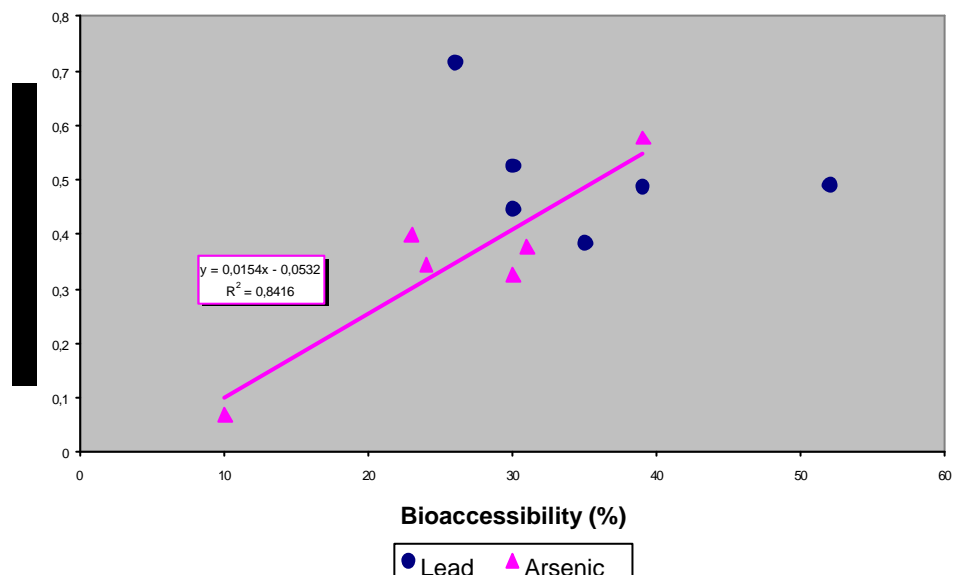
Figure 8.1 Relative bioavailability of lead against relative bioaccessibility (PBET, pH = 1,3) for 7 soils, data from /82/



Relative bioavailability in minipigs and bioaccessibility according to the DIN test was measured for arsenic and lead from 6 soils, figure 8.2 /99/. A reasonable correspondence is seen for As, whereas the correspondence is poor for Pb. The poor correspondence for lead is probably caused by a very low bioavailability of lead in the minipig system (3% for soluble lead, 1-2% for soil lead) as compared to the much higher values for arsenic (50% for soluble arsenic, 3-30% for soil arsenic). The quite narrow span of bioavailabilities measured for lead as compared to that for arsenic provide an additional explanation.

It should be emphasised here that the slope and the intercept calculated for the linear regression of As in figure 8.2 should be interpreted with caution, because the regression line may be “tilted” by just one value (data point with 10% bioaccessibility). Still, the regression coefficient (R^2) of 0,84 demonstrates that a linear relationship between bioaccessibility and bioavailability does explain approximately 84% of the data variability. It is therefore justified to deduce a linear relationship from the data, in spite of the “tilting” effect of one data point.

Figure 8.2 Relative bioavailability (minipigs) against bioaccessibility (DIN test) for arsenic and lead from soil /99/.



8.2 PAH correlations

Bioaccessibilities were obtained for 12 PAH from 4 soils after the DIN method and worst case estimates of bioavailabilities in minipigs were obtained by calculating the fractions of PAH that were not secreted with faeces (the retained fraction, % retention) /99/. The resulting data for retention of PAH overestimate bioavailability as PAH degraded or adsorbed to membranes in the gastrointestinal system are included as bioavailable, see chapter 5. Correlation between measured bioaccessibility and retention is poor (figure 8.3) with all PAH and all soils included. Still for one soil (Lothringen 2), a linear correlation is obviously obtained. This soil is the same where the bioaccessibility pattern suggested reduced PAH bioaccessibility caused by simple absorption into soil organic matter (see chapter 7).

Figure 8.3 Retention as worst case estimate of bioavailability in minipigs against bioaccessibility according to the DIN test for 12 PAH from 4 soils, data from /99/

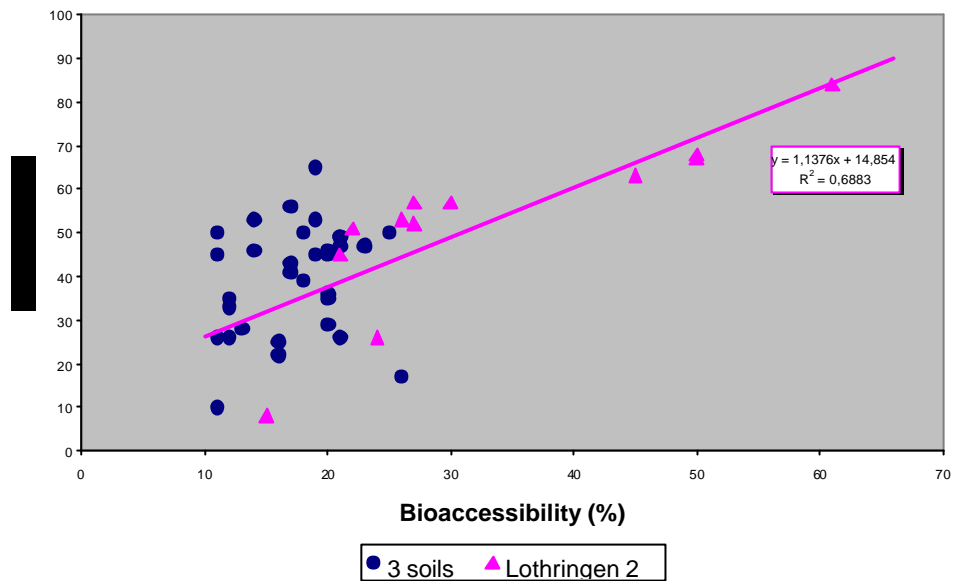
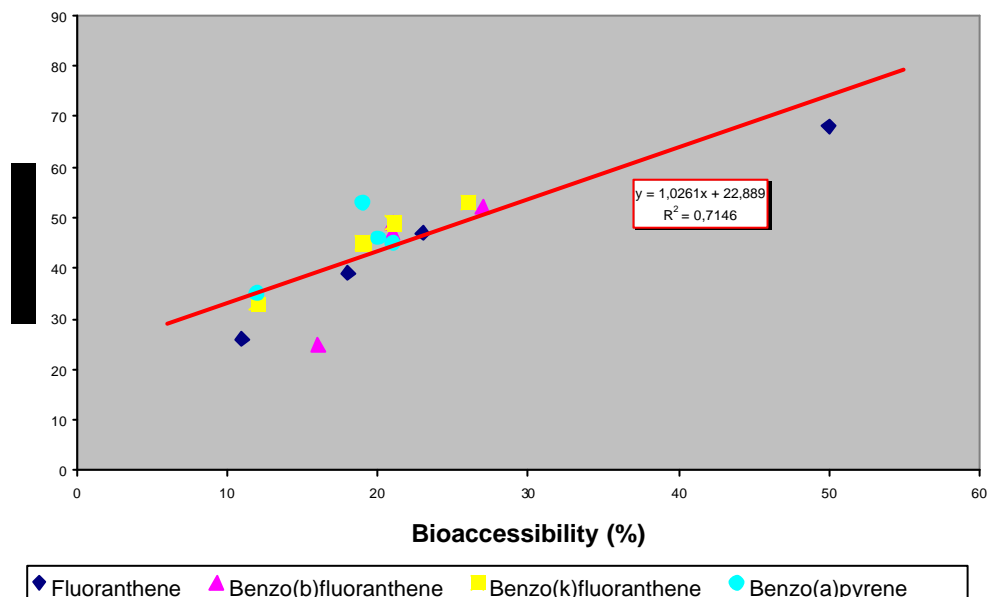


Figure 8.4 Retention as worst case estimate of bioavailability in minipigs against bioaccessibility according to the DIN test for 4 selected PAH from 4 soils, data from /99/.



If the correlation between retained fraction and bioaccessibility of this study is considered for one PAH at a time for the 4 soils, a better correlation is obtained. In effect, a reasonable correlation is obtained for 3 of the 7 PAH of this review (fluoranthene, benzo(b)fluoranthene and benzo(k)fluoranthene), an indication of linear correlation for one PAH (benzo(a)pyrene) and no correlation for 2 PAH (dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene), figure 8.4 (recall comment on linear regression given for figure 8.2). One PAH (benzo(j)fluoranthene) was not analysed for in this study.

That the estimated bioavailabilities are higher than the obtained bioaccessibilities suggests that retention indeed overestimates bioavailability. This interpretation is supported by the fact that the amount of PAH eliminated by the primary pathway (metabolites in the urine) is much lower than the amount retained, *e.g.*: 2,4-13,3% pyrene metabolites excreted with urine, 35-53% pyrene retained in the gastrointestinal system. Alternatively, the bioaccessibility test conditions are insufficient to dissolve all PAH that are made available in the minipig gastrointestinal system.

8.3 Overall bioaccessibility – bioavailability correlations

For lead, arsenic and PAH, correspondence or linear correlation between bioaccessibility and bioavailability has been reported but the limited data available clearly demonstrate, that the correspondence or correlation is not obtained with all soils and tests. For the remaining metals, no corresponding bioaccessibility and bioavailability data have been found.

The correlation between bioaccessibility and bioavailability to be expected depend upon the interactions between dissolution and absorption. Four scenarios can be set, compare chapter 3:

- fast dissolution and fast absorption, equilibrium
- slow dissolution and fast absorption, dissolution rate limiting
- fast dissolution and slow absorption, absorption rate limiting
- no dissolution or no absorption

In the equilibrium scenario, bioaccessibility and bioavailability will both be 100%. If the contaminant is present as two species, one dissolving fast and one dissolving very slowly or not at all, the bioaccessibility and bioavailability can be below 100% and still equal.

In the scenario with a rate limiting dissolution step, bioaccessibility will be below 100% and will be equal to bioavailability if all conditions of importance for the dissolution processes in the bioaccessibility test correspond exactly to the conditions in the gastrointestinal tract of the experimental animal. A complete correspondence (reagents and their concentrations, reaction times etc.) is probably not attainable and the system may furthermore be highly susceptible to impacts from the soil matrices. Still, if all processes of importance to the dissolution are possible in the test, a linear correlation can be expected between bioaccessibility and bioavailability, see also section 6.2.2 for the approaches taken in drug dissolution testing. In this scenario, measured bioaccessibilities may be higher or lower than bioavailabilities, whereas the relative bioaccessibilities should equal relative bioavailabilities.

In the scenario with rate limiting absorption, no correlation between bioaccessibility and bioavailability is to be expected.

In the no dissolution/no absorption scenario, bioaccessibility can predict bioavailability of contaminants that are not dissolved, whereas bioaccessibility will not be able to predict bioavailability in cases with no absorption.

It should be emphasised here, that the use of experimental animals for both toxicity studies and for bioavailability studies does complicate the interpretations. Interspecies differences in the gastrointestinal system, metabolism, distribution and excretion, as well as in sensitivity will be present. One ap-

proach to solving this would be to use tests for bioavailability or bioaccessibility in rabbits, if toxicity data were derived from rabbit studies, and to use tests simulating human conditions, if toxicity data were of epidemiological origin.

Conversely, we would intuitively prefer to use tests that simulate the human system for correcting estimates of human toxicity. As an operational and practicable approach, this is suggested for the future work.

9 Application of bioavailability in risk assessment, site examples

Recently, adjustment of intervention levels has been implemented based upon specific risk assessment including bioavailability studies of metals as soil contaminants. A number of examples are presented below, but the listing should by no means be considered to be complete.

Conversely, the use of site specific bioavailability data for polychlorinated dibenzodioxins and –furans in risk assessment of contaminated soils in the US has been opposed by the regulatory community. The reason for this is probably a high level of concern regarding these compounds with both high acute toxicity and severe long term effects (carcinogenesis) /8/.

The US EPA integrated exposure uptake biokinetic model, IEUBK, for estimating lead exposure from contaminated soils uses a default oral bioavailability of 30% from soil and 50% from water (*i.e.*: 60% relative bioavailability of soil lead) /22/. After bioavailability studies, both higher (35-40%) and lower (12-19%) site specific absolute bioavailabilities from soils have been used in risk assessment based upon IEUBK /9/.

Overall, 7 of 10 US EPA regions have no guidelines for implementation, 1 region has limited guidelines and 2 officially allows for cleanup level adjustments based upon bioavailability if backed by scientific data /16/. Still, 4-5 EPA regions have accepted use of bioavailability based adjustments, and one has rejected to do so.

The general trend in the US is towards accepting bioavailability as one tool in a “weight of evidence” approach, where results obtained with several, each in their own right imperfect, tools are combined to provide sufficient basis for decisions on land use, remediation goals etc /16/.

In the UK, bioaccessibility data are used to an increasing extent, in particular for lands with naturally elevated concentrations of metals /80/. For areas with the same, proven history and geology, semi-generic use has been made of bioaccessibility data for adjusting MCLs.

In the NL, bioaccessibility data has been used for site specific risk assessment, and considerable efforts are done to expand the data set and to use the data in exposure modelling (for Pb) /116/.

9.1 Rodney Street, Port Colborne, Ontario, Canada

Risk assessment of widespread soil contamination with heavy metals, in particular nickel and lead, caused by a nickel ore processing industry was performed /20/. Basic data are summarised in table 9.1. The combined use of total exposure calculations, health effect compilations and bioaccessibility estimates yielded significant increase in the standard health criterion specifying the level for intervention or further assessment.

Table 9.1 Summary of soil contamination with nickel, Rodney Street, from /20/

Mean concentration (mg/kg dw)	Max concentration (mg/kg dw)	Bioaccessibility (%)	Human health criterion (mg/kg dw)	Revised intervention level (mg/kg dw)
2.500	17.000	19	310	8.000

9.2 Coal tar distillation plant, US

A combination of literature data and site specific data on bioavailability produced an estimate of oral PAH bioavailability from coal tar contaminated soils of 25-30% /8/. This estimate was used in the final risk assessment of the site.

9.3 Gas manufacturing plant, California, US

To support risk assessment of the site, oral bioavailability (rats), dermal bioavailability, ecotoxicity (earthworms and Microtox) and leaching (SPLP, see chapter 5) was evaluated /8/. The obtained site specific oral bioavailability of PAH of 33% and its use in combination with site specific dermal bioavailability values yielded a factor 5 increase in cleanup levels. It is not clear from the reference, whether the increased cleanup levels were enforced.

9.4 Metal and PAH contaminated sites, US

Three reviews summarise the use of bioavailability data in risk assessment of contaminated soils up to Summer 2000 /9;4;16/. Table 9.2 summarises data from these reviews. A number of sites with enforced reductions in cleanup level but with data missing are not included in the table.

Table 9.2 Summary of sites with bioavailability based risk assessment of contaminated soils, from /9;4;16/

Contaminant	Bioavailability test type	Number of sites	Relative bioavailability employed (%)	Enforced cleanup level (mg/kg)
Arsenic	<i>in vivo</i> , swine or monkey	3	<2 18 42	>1.800 250 100
	<i>in vitro</i> and speciation	2	10 25	60 68
	literature	1	80	230
Cadmium	<i>in vivo</i> , rat, and speciation	1	33	100
Lead	<i>in vivo</i>	4	40	925
			24	1.200
			30	650
			58	800
PAH	<i>in vivo</i> , mouse	1	18	increased
	literature	2	29	increased

In one case (Crego Park, Michigan, US) with industrial waste contaminated soil, a combination of *in vitro* bioaccessibility studies and speciation demonstrated a 10% bioaccessibility of As. Consequently, a factor 10 increase in the cleanup level from 6,8 to 68 mg/kg dw was enforced /9/, /4/.

In Massachusetts and Michigan, US, oral relative bioavailability factors of 0,5-0,91 has been used for some PAH and petroleum hydrocarbons, and of 0,5 for As and Cd /16/.

Overall, the current experience with site specific use of bioavailability and bioaccessibility test as part of risk assessment is limited but a more widespread use in the future is expected.

9.5 A hypothetical application example, As in soil

In a typical site study, 6-7 samples will be taken for bioaccessibility testing, 1 of which in triplicate, totally 8-9 samples for testing /107/. A control soil sample with known content and bioaccessibility of As and a solution of water soluble As are included in the test series. All samples should be analysed for total arsenic after digestion.

In a hypothetical case, total arsenic in the site samples was 50 mg/kg dw \pm 3 mg/kg dw (mean \pm standard deviation). The test results were 20% \pm 5% bioaccessibility in the stomach test and 18% \pm 4% in the stomach + intestine test for soil As from the site. The relative standard deviation for the triplicate test was 10%, 13% and 15% for total As, stomach test and stomach + intestine test, respectively. The total As result in the control soil was 53 mg/kg dw (mean of previous data 48 mg As/kg dw \pm 4,2 mg/kg dw) with measured bioaccessibilities of 50% and 48% for stomach and stomach + intestine tests, respectively (mean of previous data 45% \pm 8,7% and 49% \pm 9,5%, respectively). The bioaccessibility measured for the As solution was 103%.

As the soil quality criterion (MCL) is derived from toxicity data obtained with readily absorbed As (compare table 2.1), the generic MCL (20 mg/kg dw) can be adjusted using the site specific relative bioaccessibility. The relative bioaccessibility factor, RAcF, for this site can be estimated to 20% based upon similar bioaccessibilities recorded for stomach and for stomach + intestine tests and the ~100% bioaccessibility of the soluble As in the tests done here. The results from analysis and test of the control sample support the validity of the obtained data. The bioaccessible amounts are well above the estimated test method detection limits (compare table 6.6). The variation obtained for the triplicate test of one sample from the site was low and does not indicate an excessive soil inhomogeneity that could disqualify the results.

The revised soil quality criterion, MCL_{rev} , for this site would then be calculated as:

$$MCL_{rev} = MCL/RAcF = 20 \text{ mg/kg dw}/0,2 = 100 \text{ mg/kg dw}$$

All tested site soil samples were well below the revised soil quality criterion MCL_{rev} .

The costs of testing can be estimated for 10 samples (1.500 DKR per sample) to totally 15.000 DKR with an additional 2.000 DKR for test evaluation and

commenting. Total costs are estimated to approximately 17.000 DKR for the bioaccessibility study.

10 Discussion and recommendations

Most quality criteria and cleanup levels (maximum contaminant levels, MCLs) for soil contaminants are based upon oral exposure and effect studies with contaminants as pure chemical substances ingested with water or with food. When ingested with soil, the bioaccessibility of substances such as the metals and PAH reviewed here is likely to be different from that in the studies that the MCLs are based on.

Bioaccessibility of the soil contaminants depends upon the contaminant chemistry, the soil properties and the chemical conditions in the gastrointestinal system.

In inorganic form, the metals cadmium, copper, lead, nickel and zinc occur as divalent cations only, whereas chromium can occur both as a trivalent cation and as an anion, and arsenic occurs as one of two anionic species. All metals can occur in different mineral forms and associations and with soil constituents depending upon the source of contamination and the weathering of the contaminated soil. Similarly, PAH is expected to exhibit reduced availability after aging of a PAH contaminated soil. However, at the same time some studies indicate increased mutagenic activity from metabolites of PAH in contaminated soils.

Uptake of the contaminants predominantly takes place in the small intestine, where conditions range from the acidic, high chloride gastric conditions just after transit from the stomach to subsequent neutral to slightly alkaline, high phosphate intestinal conditions. The importance of uptake from the acidic high chloride conditions of the stomach and of aerobic/anaerobic processes is not clear. The chemical conditions in the gastrointestinal tract are complex and vary between individuals of different physiology, age, health etc and for each individual with parameters such as feeding conditions, activity etc.

A number of different *in vitro* test methods are available to measure bioaccessibility of soil contaminants, but the results are not generally comparable between methods. The data on quality of the bioaccessibility test methods are limited but it would be expected that methods of required quality are available or can be made available. A suitable bioaccessibility method simulating human physiology must include all important dissolution processes and emphasize all important test details, most important:

- buffered low pH (pH < 2) high chloride gastric compartment
- buffered slightly alkaline (pH > 7) phosphate containing intestinal compartment
- aerobic followed by anaerobic conditions (stomach and intestine, respectively, optional)
- separate assessment of bioaccessibility in the two compartments (gastric and gastric followed by intestinal)
- addition of enzymes, bile and milk powder (or similar food constituent)

- sufficient time in each compartment (3 hour in gastric compartment, 10 hours in intestinal compartment)
- L/S stability ($L/S > 100$)

No currently available method satisfies all these requirements, but several methods will require only limited adjustment in order to do so: PBET (different versions), Digestive tract model, DIN and RIVM.

It is mandatory and urgent for the future use of bioaccessibility testing of soil contaminants that one single method is agreed upon. Alternatively, a set of methods applicable each to different purposes (*e.g.*: heavy metals and organic contaminants) should be the aim. To reduce costs and complexity of testing, the lowest number of tests possible should be aimed at.

Data are available on bioaccessibility of soil contaminants, in particular for lead and arsenic, to some degree for cadmium and chromium, but very limited for copper, nickel, zinc and PAH. The overall picture is, that reduced soil bioaccessibility is very likely for cadmium, lead and chromium (III) (uptake in small intestine), likely for arsenic and chromium (VI), and possible for copper, nickel, zinc and PAH. The degree of uptake in humans for cadmium and lead will though depend upon the degree to which uptake takes place in the stomach and in the first part of the small intestine prior to neutralisation of the gastric low pH and precipitation of metals. The bioaccessibility of all the reviewed contaminants is highly variable even within the same soil type, source type and test, as far as can be concluded from the limited the data available.

Bioaccessibility will impact human exposure if dissolution of the soil contaminants is rate limiting compared to absorption or if only one fraction (*e.g.*: mineral species) of the soil contaminant is readily bioaccessible and another fraction, that might be 100%, is not. Correlation between bioavailability and bioaccessibility has been demonstrated for lead, arsenic and PAH in some test system but not in others. Still, the data material is not sufficient to establish whether, to what degree and for which contaminant bioaccessibility is rate or dissolution limiting.

A large number of bioavailability *in vivo* studies with experimental animals have been published, a review of these is outside the scope of the present review, but reduced bioavailability has been reported for at the least arsenic, cadmium, lead and PAH.

Reduced bioaccessibility and/or bioavailability has been taken into consideration in site specific regulation of cleanup levels for contaminated sites in the US and Canada, in particular for mine waste and ore processing sites.

The general conclusion is that regulation of soil quality criteria and cleanup levels based upon reduced bioavailability/bioaccessibility of the contaminants is recommended after site specific risk assessment. Conversely, the data available at present does not allow for general regulation of soil quality criteria and cleanup levels for specific contaminants, soil types or sources.

A short term and a long term model for implementation of bioaccessibility in risk assessment of contaminated sites can be suggested.

The short term approach (figure 10.1) can be employed for arsenic, lead and PAH where bioaccessibility has been shown to be rate limiting for bioavailability. For other contaminants, the rate or dissolution limiting role must be established prior to application of the bioaccessibility approach, either on a site specific basis or as a general study.

The long term approach (figure 10.2) requires establishment of “calibration” curves of *in vivo* bioavailability versus *in vitro* bioaccessibility but has the advantage of being based upon a direct, proven link between the measured quantity (bioaccessibility) and the toxicological interpretation (bioavailability). A 1:1 correlation can not be expected due to potential interspecies differences between bioavailability studies using experimental animals and bioaccessibility tests simulating the human physiology.

Figure 10.1 Short term approach to implementation of bioaccessibility of soil contaminants in risk assessment

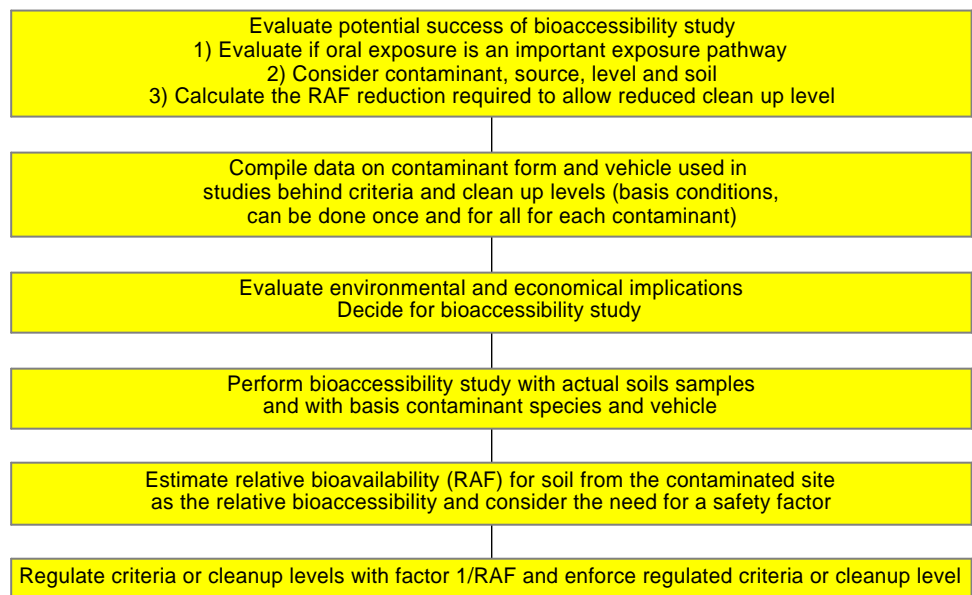
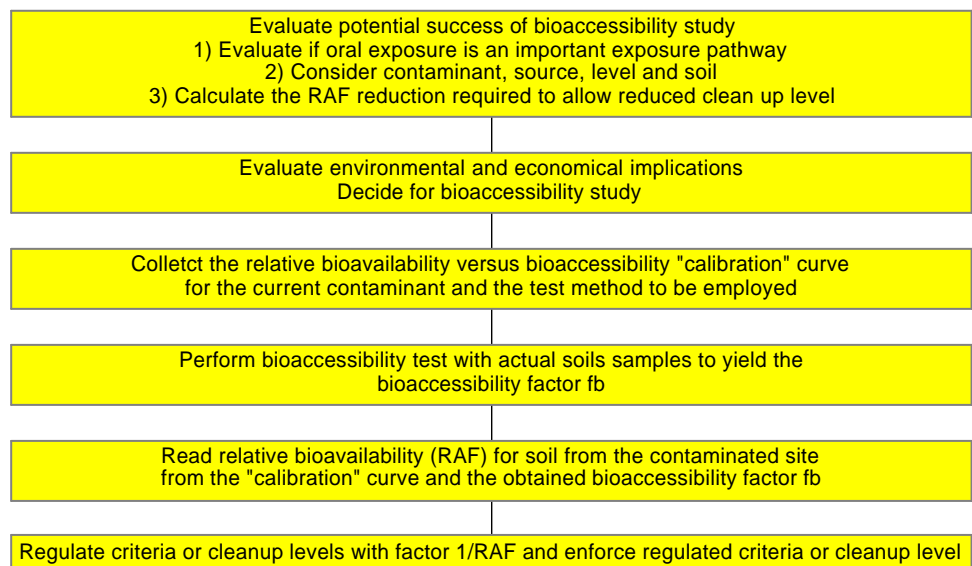


Figure 10.2 Long term approach to implementation of bioaccessibility of soil contaminants in risk assessment



In order to implement the short term approach in Denmark, see also section 9.5, the following is required:

- compilation of *in vivo* bioavailability data for the contaminants from soil available in the literature
 - ? purpose: to secure that reduced bioavailability is occurring for all contaminants that are candidates for bioaccessibility testing
- compilation of the contaminant forms, vehicles and bioavailability used in toxicity studies behind current criteria and cleanup levels
 - ? purpose: to produce the conditions required for bioaccessibility testing yielding relative bioaccessibility factors
- selection, implementation and validation of one test method for relative bioaccessibility of soil contaminants
 - ? purpose: to give access to a reliable method for testing
- testing of a selection of contaminated soils for relative bioaccessibility
 - ? purpose: to produce a Danish reference set (contaminants, soil types, sources) for deciding if bioaccessibility testing is likely to yield regulation of criteria and cleanup levels

With use of different bioaccessibility test methods in different countries, access to stable and homogeneous subsamples of soils used around the World in bioavailability studies is essential in order to allow for extrapolation of data obtained in these studies to the sites where bioaccessibility tests are used.

In order to implement the long term approach, international collaboration is required in order to accomplish what is needed:

- selection, implementation, validation and interlaboratory comparison of one test method or one set of test methods for bioaccessibility of soil contaminants (European or preferentially transatlantic scale, ISO, CEN and US EPA)
 - ? purpose: to give access to a reliable method or set of methods for testing as common reference and to ensure compliance of all future data
- production of corresponding high quality *in vivo* bioavailability and *in vitro* bioaccessibility data for the important contaminants, soil types, sources and speciations (European or preferentially transatlantic scale)
 - ? purpose: to produce relative bioavailability versus bioaccessibility “calibration” curves and demonstrate bioaccessibility as rate limiting factor for bioavailability for more contaminants

As research tasks, further refinement of the theory behind implementation of bioaccessibility and bioavailability in risk assessment of soil contaminants should include:

- identification of *in vivo* compartment of contaminant uptake
 - ? purpose: to enable precise selection of test compartment (stomach or stomach and intestine) to be used for bioaccessibility testing of different contaminants
- evaluation of gut redox conditions and impact upon bioaccessibility
 - ? purpose: to enable selection of aerobic/anaerobic conditions for bioaccessibility testing

- description of the mechanisms of uptake, in particular the kinetics of dissolution and absorption in different compartments, with different vehicles etc
 - ? purpose: to ensure that the conceptual model of human uptake used is correct and that the bioaccessibility is *de facto* rate or dissolution limiting for bioavailability
- investigation in the use of the pharmaceutical approach with time dissolution profiles in soil contaminant bioaccessibility testing
 - ? purpose: to develop a more precise tool for bioavailability predictions and to identify the rate limiting process (dissolution or absorption)

Selection of target contaminants should take into account the significance of each compound as soil contaminant (toxicity and occurrence).

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Appendix

Data for As

Soil	Source	Speciation	Concentration (mg/kg dw)	Test ¹	Bioaccessibility	Reference
Soil 4 mountain types	mine waste	-	140-350	DIN	stomach 1,2-34% stomach and intestine 2,2-40%	(1)
Soil 12 types	wood preservatives	-	57-63	DIN	stomach 0,48-100% stomach and intestine 6,5-29%	(1)
Soil 21 types	-	-	2-690	DIN	stomach and intestine 5-60%	(2)
Soil 3 types	waste and urban impact	-	4,8-680	DIN ²	stomach 1,6-82 stomach and intestine 2,9-85	(1)
Soil	mine waste	-	230-18.000	IVG/PBET	stomach 17%/12% stomach and intestine 15%/8,3%	(3)
Soil mountain ³	mine waste	-	630	Mass-balance	stomach 69% stomach and intestine 66%	(4)
Soil urban	wood treated with copper-chromium arsenate	-	160	Mass-balance	stomach and intestine ⁴ 41%	(5)
Soil 10 types ⁵	mine waste	-	19-47	PBET	stomach 23-48% stomach and intestine 8,5-56%	(6)
Soil urban	mine waste	oxides iron oxides	410-3.900	PBET	stomach 49-55% stomach and intestine 44-50%	(7)
Dust	mine waste	oxides	170	PBET	stomach 34%	(7)

¹ See table 6.3 of main report for method descriptions

² Modified according to Rotard

³ NIST SRM 2710 standard reference material

⁴ The method allowed for separate determination of stomach and small intestine dissolution

⁵ Data presented for ungrounded bulk samples presented only

Soil	Source	Speciation	Concentration (mg/kg dw)	Test ¹	Bioaccessibility	Reference
house		silicates sulphides			stomach and intestine 32%	
Soil urban	steel industry	-	15-160	PBET	stomach and intestine 6-15%	(8)
Soil urban	mine waste	-	19-94	PBET	stomach and intestine 2-9%	(8)
Soil	mine waste and industry	-	250-70.000	PBET	stomach and intestine 0,5-42%	(8)
Soil agricultural	-	-	59-170	PBET	stomach and intestine 6-15%	(8)
Soil	-	-	101-205	PBET	stomach and intestine 7-17%	(8)
Soil mountain	mine waste	sulphides (enargite, tennanite and arsenopyrite)	1.400	PBET ⁶	stomach and intestine 12%	(9)
Soil	-	-	17-100	PREP	stomach and intestine 10-56%	(10)
Soil mountain	mine waste	sulphide (arsenopyrite)	1.400-2.100	PREP ⁷	stomach 13-16% stomach and intestine 16-25%	(11)
Soil agricultural and paddy	mine waste	sulphide (arsenopyrite)	6,7-76	SBET	stomach 18-20% ⁸	(12)
Soil	-	-	55-77	SBET DIN/RIVM/TIM	stomach 50% stomach and intestine 44-96%	(13)
Soil mountain	-	-	210-240	SBET DIN/RIVM/TIM	stomach 11% stomach and intestine 15-19%	(13)
Soil mountain	-	-	88-100	SBET DIN/RIVM/TIM	stomach 59% stomach and intestine 50-59%	(13)
Soil 36 types	added AsO ₄ ³⁻	As(V)	81-100	SBET	stomach 2,6-100% ⁹	(14)

⁶ Early version

⁷ < 125 µm particles only

⁸ Mean for paddy soil and agricultural soils, respectively, ranges 4,7-32%

⁹ 6 months after spiking

Data for Cd

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil 4 mountain types	mine waste	-	0,43-2,1	DIN	stomach 4,8-100% stomach and intestine 10-22%	(1)
Soil 12 types	wood preservatives	-	0,26-0,80	DIN	stomach 37-100% stomach and intestine 4,3-10%	(1)
Soil 22 types	-	-	0,3-65	DIN	stomach and intestine 35-83%	(2)
Soil 3 types	waste and urban impact	-	0,33-7,1	DIN ¹⁰	stomach 8,9-100% stomach and intestine 46%	(1)
Soil mountain	mine waste	-	22	Mass-balance ¹¹	stomach and intestine 48%	(4)
Soil 10 types	mine waste	-	1,2-5,3	PBET	stomach 65-86% stomach and intestine 3,8-79%	(6)
Soil native	spiked	soluble salt	not specified	PBET ¹²	stomach and intestine 64%	(15)
Soil	-	-	2,3-32	PREP	stomach and intestine 0,3-16%	(10)
Soil agricultural and paddy	mine waste	-	0,9-6,1	SBET	stomach 62-73% ¹³	(12)
Soil	-	-	14-17	SBET DIN/RIVM/TIM	stomach 92% stomach and intestine 57-78%	(13)
Soil mountain	-	-	23-27	SBET DIN/RIVM/TIM	stomach 92% stomach and intestine 50-90%	(13)
Soil mountain	-	-	35-39	SBET DIN/RIVM/TIM	stomach 99% stomach and intestine 40-79%	(13)

¹⁰ Modified according to Rotard

¹¹ Modified method, Cd not dissolved or precipitated during ingestion measured

¹² Modified version developed for food uptake studies

¹³ Mean for paddy soil and agricultural soils, respectively, ranges 50-80%

Data for Cr

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil 3 mountain types	mine waste	-	7,8-81	DIN	stomach 1,5% stomach and intestine _14	(1)
Soil 12 types	wood preservatives	-	29-39	DIN	stomach 2,7-7,6% stomach and intestine -	(1)
Soil 22 types	-	-	37-180	DIN	stomach and intestine 4-54%	(2)
Soil 3 types	waste and urban impact	-	0,5-21	DIN ¹⁵	stomach 17% stomach and intestine 0,6%	(1)
Soil urban	construction waste burning	-	4,9-100	Mass-balance	stomach 2,2-15% stomach and intestine 2,9-15%	(16)
Soil urban	slag	-	2.400	Mass-balance	stomach and intestine 34%	(5)
Soil mountain ¹⁶	-	-	39	Mass-balance	stomach 3,7% stomach and intestine 3,0%	(17)
Soil	added CrCl ₃	Cr(III)	0,33	PBET	stomach 37-72%	(18)
Soil	added K ₂ CrO ₄	Cr(VI)	0,33	PBET ¹⁷	stomach 18-46%	(18)
Soil	-	-	37-210	PREP	stomach and intestine <1%	(10)
Soil mountain	added K ₂ CrO ₄	Cr(VI)	200-400	SBET	Stomach 48-108%	(19)
Soil mountain	added K ₂ CrO ₄	Cr(III) ¹⁸	200-400	SBET	Stomach 3,2-14%	(19)
Soil 35 types	added CrCl ₃	Cr(III)	740-17.000	SBET	Stomach 1,5-35% ¹⁹	(14)

¹⁴ No detections above unspecified limit of detection

¹⁵ Modified according to Rotard

¹⁶ NIST SRM 2710 standard reference material

¹⁷ Modified version, stomach step only

¹⁸ Cr(III) from added Cr(VI)

¹⁹ 100 days after spiking

Data for Cu

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil 4 mountain types	mine waste	-	73-1.700	DIN	stomach 3,7-78% stomach and intestine 2,9-100%	(1)
Soil 12 types	wood preservatives	-	13-29	DIN	stomach 7,1-52% stomach and intestine 13-43%	(1)
Soil 3 types	waste and urban impact	-	14-38	DIN ²⁰	stomach 8,6-95% stomach and intestine 13-100%	(1)
Soil urban	municipal and coal incinerator ashes, de- icing salt, snow disposal, metal industry waste	elemental	135-4.400	GJST	stomach 11-15%	(20)
Soil 10 types	mine waste	-	250-820	PBET	stomach 0,7-47% stomach and intestine 8,8-60%	(6)
Soil	-	-	37-3.700	PREP	stomach and intestine 0,8-110%	(10)
Soil agricultural and paddy	mine waste	-	6,0-99	SBET	stomach 41-54% ²¹	(12)

²⁰ Modified according to Rotard

²¹ Mean for paddy soil and agricultural soils, respectively, ranges 28-102%

Data for Ni

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil 4 mountain types	mine waste	-	5,4-17	DIN	stomach 2,8-54% stomach and intestine 8,8-41%	(1)
Soil 12 types	wood preservatives	-	19-34	DIN	stomach 2,4-22% stomach and intestine 1,9-22%	(1)
Soil 22 types	-	-	11-110	DIN	stomach and intestine 8-54%	(2)
Soil 3 types	waste and urban impact	-	5,7-23	DIN ²²	stomach 9,5-85% stomach and intestine 7,8-90%	(1)
Soil 10 types	mine waste	oxide	1.800-7.300	PBET	stomach 11-28% stomach and intestine 11-24%	(6)
Soil	-	-	190-900	PREP	stomach and intestine <0,2-2,0%	(10)

²² Modified according to Rotard

Data for Pb

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil 4 mountain types	mine waste	-	31-3.000	DIN	stomach 0,1-78% stomach and intestine 1,5-5,7%	(1)
Soil 12 types	wood preservatives	-	36-180	DIN	stomach 2,5-84% stomach and intestine 0,20-2,7%	(1)
Soil 22 types	-	-	19-6.400	DIN	stomach and intestine 11-70%	(2)
Soil 3 types	waste and urban impact	-	8,0-880	DIN ²³	stomach 18-84% stomach and intestine 1,3-3,4%	(1)
Soil urban	municipal and coal incinerator ashes, de- icing salt, snow disposal, metal industry waste	carbonate oxide phosphate tin oxide carbonate iron oxyhydroxide silicate	920 – 7.500	GJST	stomach 18 – 58%	(21)
Soil urban	construction waste burning	-	16-1.100	Mass-balance	stomach 70-110% stomach and intestine 3,0-54%	(16)
Soil mountain ²⁴	-	-	5.500	Mass-balance	stomach 76% stomach and intestine 11%	(4)
Soil urban	wood treated with copper-chromium arsenate	-	68	Mass-balance	stomach and intestine 69%	(5)
Soil urban	mine waste	-	2.900	Mass-balance	stomach and intestine 70%	(5)
Soil urban	slag	-	1.200	Mass-balance	stomach and intestine 39%	(5)
Soil urban	-	-	5.000	Mass-balance	stomach 34-96% intestine 19-68%	(17)
Road dust	traffic	-	2.600	PBET	stomach 77%	(22)
Soil	diffuse	-	2.800	PBET	stomach 51%	(22)

²³ Modified according to Rotard

²⁴ NIST SRM 2710 standard reference material

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
urban						
Soils 10 types	mine waste	-	190-1.200	PBET	stomach 50-86% stomach and intestine 1,3-9,5%	(6)
Soil mountain	mine waste	sulphate (anglesite) sulphide (galena)	3.900	PBET	stomach 4,2% intestine 0,7%	(23)
Soil mountain	mine waste	liron oxides iron sulphates manganese oxides	1.000	PBET	stomach 0,5% intestine <0,2%	(23)
Soil mountain	mine waste	phosphate iron sulphates	5.800	PBET	stomach 5,6% intestine 0,1%	(23)
Soil mountain	mine waste	phosphate manganese oxides oxide and hydroxide silicates	1.800	PBET	stomach 2,1% intestine 0,6%	(23)
Soil mountain	mine waste	sulphate (anglesite) sulphide (galena) phosphate manganese oxides	3.900	PBET	stomach 9,5-35% stomach and intestine 1,0-4,0%	(7)
Soil urban	mine waste	sulphate (anglesite) phosphate manganese oxides iron oxides	1.400	PBET	stomach 69% stomach and intestine 12%	(7)
Soil urban	mine waste	sulphate (anglesite) lead phosphate iron oxides oxides	2.100	PBET	stomach 83% stomach and intestine 25%	(7)
Soil and sediment	mine waste	sulphate (anglesite) iron sulphates carbonate (cerussite)	6.900-10.000	PBET	stomach 16-49% stomach and intestine 0,4-8,0%	(7)
Soil mountain	mine waste	sulphides (galena) sulphate (anglesite)	3.900	PBET ²⁵	stomach 0,87% stomach and intestine 0,18%	(9)
Soil native	spiked	soluble salt	not specified	PBET ²⁶	stomach and intestine 47%	(15)
Soil	mine waste	carbonate (cerussite)	4.400	PBET ²⁷	stomach 36%	(24)

²⁵ early version

²⁶ Modified version developed for food uptake studies

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
		oxide sulphides (galena) sulphate (anglesite)				
Soil	mine waste	carbonate (cerussite) oxide sulphides (galena) sulphate (anglesite)	2.700	PBET ²⁶	stomach 48%	(25)
Soil	-	-	22-9.200	PREP	stomach and intestine 0-43%	(10)
Soil 15 urban types	urban impact	-	180-2.500	RIVM	stomach and intestine 2-83% ²⁸	(26)
Soil	-	-	50-2.400	RIVM	stomach and intestine 28-74%	(27)
Soil agricultural and paddy	mine waste	sulphide (galena)	19 - 950	SBET	stomach 51-62% ²⁹	(12)
Soil	-	-	610-730	SBET DIN/RIVM/TIM	stomach 91% stomach and intestine 13-66%	(13)
Soil mountain	-	-	5.500-6.400	SBET DIN/RIVM/TIM	stomach 56% stomach and intestine 4-29%	(13)
Soil mountain	-	-	1.000-1.100	SBET DIN/RIVM/TIM	stomach 90% stomach and intestine 11-68%	(13)

²⁷ Modified, stomach step only

²⁸ Data biased by uncertainty concerning the total soil lead concentrations

²⁹ Mean for paddy soil and agricultural soils, respectively, ranges 28-76%

Data for Zn

Soil	Source	Speciation	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil 4 mountain types	mine waste	-	41-260	DIN	stomach 30-97% stomach and intestine 6,5%	(1)
Soil 12 types	wood preservatives	-	70-104	DIN	stomach 15-46% stomach and intestine -	(1)
Soil 3 types	waste and urban impact	-	75-720	DIN ³⁰	stomach 35-83% stomach and intestine 14%	(1)
Soil urban	municipal and coal incinerator ashes, de- icing salt, snow disposal, metal industry waste	carbonate oxide	450-4.600	GJST	stomach 27-45%	(20)
Soil	-	-	230-4.200	PREP	stomach and intestine 0-4,7%	(10)
Soil agricultural and paddy	mine waste	-	56-570	SBET	stomach 35-50% ³¹	(12)

³⁰ Modified according to Rotard

³¹ Mean for paddy soil and agricultural soils, respectively, ranges 13-61%

Data for PAH

Soil	Source	PAH	Concentration (mg/kg dw)	Test	Bioaccessibility	Reference
Soil	-	16 PAH	190	Digestive tract model	stomach 9% stomach and intestine 23%	(28)
Soil 22 types	-	16 PAH	20-5.000	Digestive tract model	stomach and intestine 7-95%	(28)
Soil	waste	11 PAH	37-200	Digestive tract model	stomach and intestine 11-15%	(29)
Soil 4 types	coal mine waste and waste	fluoranthene	50-850	DIN	stomach and intestine 11-50%	(2)
Soil 4 types	coal mine waste and waste	benzo(b)fluoranthene	5,6-210	DIN	stomach and intestine 16-27%	(2)
Soil 4 types	coal mine waste and waste	benzo(k)fluoranthene	3,3-120	DIN	stomach and intestine 12-26%	(2)
Soil 4 types	coal mine waste and waste	benzo(a)pyrene	5,8-200	DIN	stomach and intestine 12-21%	(2)
Soil 4 types	coal mine waste and waste	dibenzo(a,h)anthracene	0,9-20	DIN	stomach and intestine 12-24%	(2)
Soil 4 types	coal mine waste and waste	indeno(1,2,3-cd)pyrene	3,0-130	DIN	stomach and intestine 15-26%	(2)

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