Laktester för riskbedömning av förorenade områden

underlagsrapport 2a och 2b

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HÅLLBAR SANERING

Laktester för riskvärdering av förorenade områden – Underlagsrapport 2a: Laktester för oorganiska ämnen

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Förord

Ett av riksdagens miljömål är Giftfri miljö, och i detta mål ingår att efterbehandla och sanera förorenade områden. Brist på kunskap om risker med förorenade områden och hur de bör hanteras har identifierats som hinder för effektivt saneringsarbete. Naturvårdsverket har därför initierat kunskapsprogrammet Hållbar Sanering.

Den här rapporten redovisar projektet ”Laktester för riskbedömning av förorenade områden” som har genomförts inom Hållbar Sanering. I projektet har man tagit fram ett förslag till metodik för val, utförande och tolkning av laktester som verktyg i miljö- och hälsoriskbedömningar för förorenade områden.

Redovisningen är omfattande och presenteras i tre rapporter som innehåller:

1) huvudrapport och underlagsrapport 1a (Laktester för oorganiska ämnen).
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2) underlagsrapport 2a (Laktester för organiska ämnen) och 2b (Tester för bedömning av oral biotillgänglighet vid intag av jord).
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Naturvårdsverket har inte tagit ställning till innehållet i den här rapporten. Författarna svarar själva för innehåll, slutsatser och eventuella rekommendationer.

Naturvårdsverket juni 2006

Innehåll

Underlagsrapport 2b: Tester för bedömning av oral biotillgänglighet vid intag av jord

Summary and Conclusions

This report has been written as background information for “Laktester för riskbedömning av förorenade områden”. The report deals with leaching test for determination of leaching properties of organic compounds from contaminated soils. It provides a short introduction to the processes controlling the release of organic compounds in soils followed by an overview of leaching methods for organic compounds reported in the literature. The leaching principles are discussed and critical test conditions are highlighted.

Today, decisions concerning corrective actions at contaminated sites are traditionally based upon measurements of the total content of contaminants in soils. However, it is well known that for both inorganic and organic compounds only part of the total content of contaminants may be available for leaching to groundwater or surface water. Real measurements of the release of contaminants from soils will thus provide a much better input for the impact assessment. In response to that, leaching tests for organic compounds have been developing during the last 5 years. Several leaching methods been published for testing the leaching of organic compounds from soil. These methods are yet not official, standardised methods and in some cases critical test issues still need to be addressed. However, the leaching tests for organic compounds are as they appear today already better tools for impact assessment than measurements of total contents in solid phase.

In this report focus has been on leaching test for non-volatile organic compounds (e.g. PCBs, Dioxins and Furans, 2,4-dinitritoluene, PAH, Aliphatic hydrocarbons (especially the higher hydrocarbons), Aromatic hydrocarbons (other than BTEX and PAH)). These compounds are regarded as relevant for leaching tests as they have physico-chemical properties (and ageing effect) that makes it complicated if not impossible to predict the release by theoretical considerations.

Several leaching principles have been reported in the literature and the table below summarizes basic principles of some leaching methods regarded as most relevant for contaminated sites. Critical test conditions related to these principles are highlighted. Other leaching principles like availability test and pH-static leaching tests have been suggested also for non-volatile organic compounds. These tests are aiming at more scientific purposes and the interpretation of the results is not yet straight forward.

Leaching principles	Description	Output	Advantages	Cautions	Needs
Column with recirculation of eluate	The test is performed in glass columns at a fixed L/S ratio depending on the properties of the test material (between 1 and 2 l/kg). A continuous vertical up-flow is applied. The eluent consists of a diluted CaCl2 solution (with sodiumazide to prevent degradation). The eluent is recirculated until equilibrium is obtained. The eluate is collected as one single fraction.	An equilibrium concentration of contaminants from which an equilibrium pore water concentration can be estimated. This test provides an estimate of the present release of contaminants. The results obtained from this test is equivalent to results from a batch test	This method is developed with focus on leaching of non-volatile hydrophobic organic compounds and thus this test is designed to avoid critical conditions in the procedure		• This procedure needs to be validated further for example against other procedures (e.g. a percolation test).
Batch leaching test (standards in preparation ISO 21268-1 and 2	A batch test for non-volatile organic compounds is conducted in a glass container at a fixed liquid to solid ratio (2 or 10 l/kg). The eluent is a solution of either demineralised water or CaCl2. The container is agitated for a prefixed time to obtain equilibrium between contaminants in solution and contaminants in the soil. The eluate is separated from the solid by centrifugation	An equilibrium concentration of contaminants from which an equilibrium pore water concentration can be estimated. This test provides an estimate of the present release of contaminants.	The batch concept is well known from testing of inorganic compounds	• Separation of solid and eluate is a critical step in the procedure. If the separation is insufficient the leached amount of contaminants may for hydrophobic compounds significantly be overestimated. • Handling of eluate must be minimised to avoid losses of contaminant due to sorption onto test equipment	• The separation techniques used for separating soil and eluate needs to be developed further before this test is applicable for contaminated site impact assessment • A standardised method needs to be validated
Percolation test (standards in preparation ISO 21268-3)	Contaminated soil is packed in columns. The eluent consists of either demineralised water or a diluted CaCl2 solution. The flow direction is upward and the flow rate should be relatively low in order to ensure local equilibrium. The eluate is collected in several fractions (often like for inorganic column test).	This test procedure provides valuable information of the release of contaminants as a function of time. The leachate quality may be described in short and long term.	The column concept is well known from testing of inorganic compounds		• Local equilibrium is essential for interpretation of the results. Thus, a maximal flow rate may be defined at which local equilibrium is obtained for any soil. • A standardised method needs to be validated

As for inorganic constituent the objective of conducting leaching tests for organic compounds must be identified before choosing the leaching principles. The table below shows some common objectives and recommended leaching principles.

Objectives of testing	Recommended leaching principles
Present release of contaminants (snapshot of the release of contaminants)	Batch leaching or column with recirculation of eluate
Quality control / compliance testing	Batch leaching or column with recirculation of eluate or percolation leaching
Time dependence release / Leachate quality as a function of time	Percolation leaching
Accumulated leached amount of contaminant	Percolation leaching

Basically, three major elements are defined for impact assessment; source term characterisation, transport of contaminants, and impact at end target. Suitable tools for source term characterisation (e.g. leaching tests) are now available also for non-volatile organic compounds leaching from contaminated soils. There are still some few specific issues that need to be addressed:

- Degradation of contaminants must be avoided in the leaching tests. It may be done by adding chemicals like sodiumazide. The effect of these chemicals on the leaching results may be further investigated. Alternatively an on-line extraction applied.
- It needs to be settled if separation of soil and eluate is possible by means of centrifugation in the batch leaching procedure. The results of the batch leaching tests should provide reliable and meaningful results comparable to results from other leaching procedures. The separation procedure of solid and solute also affect results from availability test and pH-static leaching test.
- It need to be justified that local equilibrium in percolation test are/ or can be obtained.
- In general standardisation and validation (repeatability, reproducibility and ruggedness) of these leaching tests for non-volatile organic compounds are needed.

The next step in the impact assessment is transport of contaminant from the source to the end target. For this purpose transport models traditionally used for impact assessment may be used. This may be detailed transport model like MIKE-SHE or MOD-FLOW or a simplified approach. However, if a generally accepted concept for impact assessment is not easily available it may be valuable to develop an easy to operate tool for this purpose. Otherwise the transport of contaminants from source to end target may be an obstacle for implementing leaching test for contaminated site investigations.

1 Background and objectives

In impact assessments of contaminated sites human health effects, groundwater and surface water impacts are of primary concern. Conventionally, decisions are based upon measurements of the total content of contaminants in soils which may be combined with analysis of groundwater or surface water. However, it is well known that for both inorganic and organic compounds in soils only part of the total content of contaminants may be available for leaching to groundwater or surface water.

During the last 15 years leaching tests for inorganic compounds have been developed and standardized. These leaching methods have primarily been developed for waste materials but they have to some extent been adjusted for use on contaminated soils. However, until now the use of leaching tests for impact assessment at contaminated sites has been limited. This may partly be due to the fact that for most contaminated soils organic contaminants are also of concern and no standardized leaching methods are available at present for these compounds.

In response to the need for leaching tests, development of test methods for organic compounds has been carried out during the last 5 years (e.g. Bjuggren et al. 1999, Hjelmar et al., 2000,Danish EPA (2000), Comans et al., 2001, Hansen et al., 2004, Danish EPA 2004, Enell et al. 2004).

In this report different leaching methods for organic compounds will be described and the applicability of the methods for contaminated soils and impact assessment will be discussed.

2 Leaching of organic compounds

2.1 Advantages of incorporation of leaching tests in risk assessment

When assessing a contaminated site the rules described in the SNV Report no. 4638, ”Generella riktvärden för förorenad mark”(199) apply: the contamination can be assessed based on the quality criteria listed or a more or less elaborate site-specific risk assessment can be carried out. The basis for the site-specific assessment is for one part the assessment of the possible migration of the contaminants specific for the site. The different migration paths are shown in Figure 2.1 below.

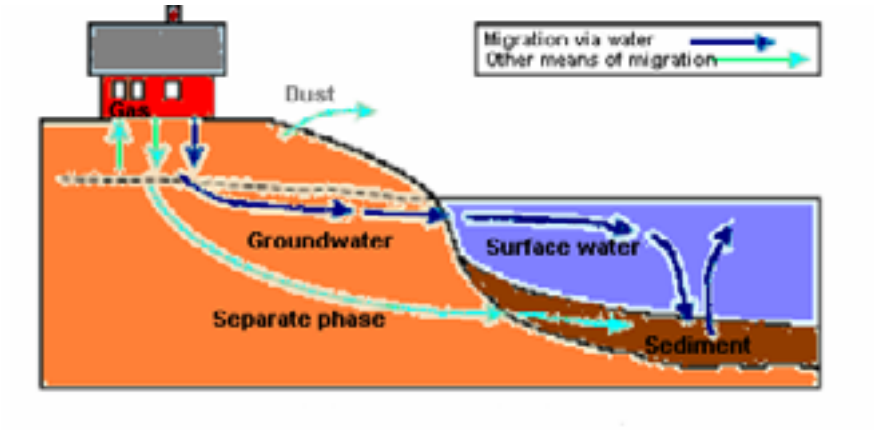


Figure 2.1. Migration paths (SNV, 199)

In relation to risk assessment of groundwater and surface water contamination the use of leaching tests can give a better estimate of the migration potential and of the actual level of impact. The leachability of an organic contaminant in soil will depend on the type of soil, the mix of contaminants and the age of the contamination (which affects the extent to which chemical changes, e.g. biodegradation have taken place). How these factors will affect the specific situation cannot be estimated on the basis of theoretical knowledge alone due to the complexity of the interaction of the factors. Thus knowledge of the total concentration may not give a proper indication of the actual leachability in a specific soil. This is shown in Figure 2.2 below, where fluoranthene concentrations in soils from a number of contaminated sites are compared with the leachable amount measured in a leaching test.

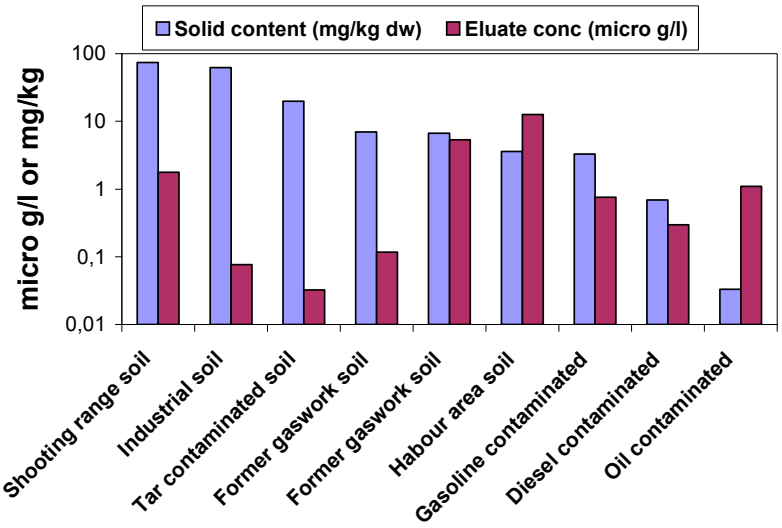


Figure 2.2. Comparison of solid content and leached concentration of fluoranthene for different soils contaminated from different activities. Data is from Gamst et al. 2005, Hansen et al. 2004 and unpublished data from Technical University of Denmark.

2.2 Organic compounds of relevance

To determine which organic compounds it would be relevant to conduct leaching tests for, a number of aspects will be of interest:

- organic compounds that have often been detected in surface and groundwater
- organic compounds that are regulated in relation to limit values (disposal criteria, soil quality criteria, groundwater criteria or drinking water criteria)

Referring to Swedish guidelines for soils, groundwater or surface water this would lead to a list encompassing the following compounds (it should be noted that the use of leaching tests could be relevant for other organic compounds, e.g. pesticides):

Phenol + cresol
Chlorophenols
Chlorobenzenes
PCBs
Dioxins and Furans
Bromomethanes
Bromochloromethanes
Carbon Tetrachloride
Chloromethanes
Chloroethylenes
Chloroethanes
2,4-dinitritoluene
BTEX
PAH

Aliphatic hydrocarbons
Aromatic hydrocarbons (other than the above mentioned)
MTBE

These compounds have very different physico-chemical properties. Some are quite volatile, others are very soluble or even miscible with water, and again others are fairly hydrophobic. For the soluble and water miscible compounds leaching tests often do not give a result that differs markedly from what can be calculated using theory. This is due to the fact that the amount of compound bound to the soil matrix is relatively small, which means that the influence of the mechanisms governing the sorption and desorption of compound only affect a small percentage of the total amount of compound present (at least at water saturated and near water saturated soil conditions).

The volatiles compounds are difficult to sample and test without loss of compound. Typically these compounds are also fairly soluble. The combination of these issues leads to the fact that theoretical calculations may lead to more reliable estimates of the actual leachability unless very rigid measures are taken to ensure that no loss of compound takes place, neither during sampling nor testing. This is of course only true if the parameters needed for the theoretical calculations are well known, e.g. from actual field measurements.

This leaves a number of organic contaminants where the use of leaching tests can give a better estimate of the actual site-specific impact, since the leaching of these compounds to groundwater or surface water will be very much governed by sorption to soil particles which is in general very difficult to assess theoretically for a specific soil and contamination situation. The different compounds can of course be found in mixtures with each other, but the following applies to the listed compounds or mixtures containing them.

The compounds from the above list for which leaching tests are most relevant are thus:

PCBs
Dioxins and Furans
2,4-dinitritoluene
PAH
Aliphatic hydrocarbons (especially the higher carbons)
Aromatic hydrocarbons (other than BTEX and PAH)

Based on this the report will in the following chapters deal with leaching tests for non-volatile organic compounds only.

3 Physical and chemical processes controlling leaching

For non volatile organic compounds three major processes control the concentration in the aqueous phase in batch or column test systems (or natural soil systems): dissolution, sorption, and the presence of dissolved organic matter and colloids (organic and inorganic). If there is a free organic phase in the contaminated soil dissolution of the organic compounds from the free phase into the aqueous phase controls the concentration in the aqueous phase. If there is no free phase, the concentration in the aqueous phase is controlled by sorption and by the dissolved organic carbon and colloids. Figure 3.1 shows a simplified picture of the partitioning of PAH between the particulate and water phase (from Comans et al., 2001). As this picture illustrates the leachable fraction, which consists of “free” organic compounds, compounds complexed with dissolved organic matter and compounds bound to natural colloids

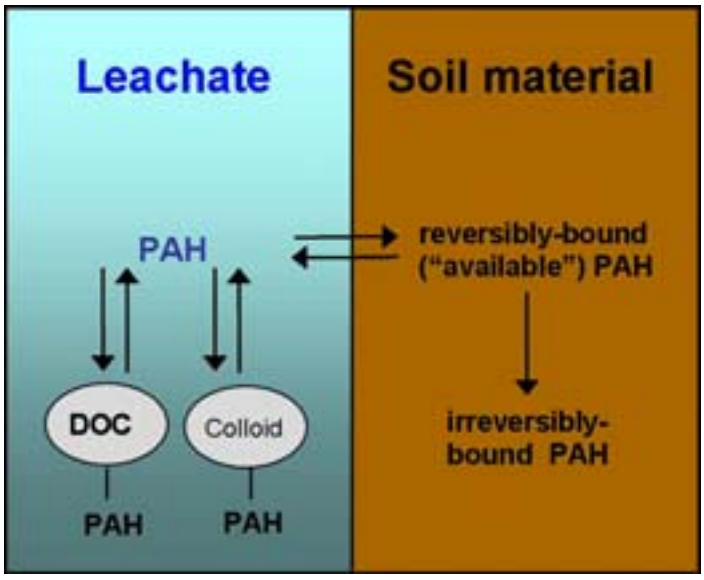


Figure 3.1. Partitioning processes controlling the leaching of organic contaminants from a soil (modified from Comans et al. 2001)

3.1 Dissolution

Dissolution of organic compounds from an organic phase into an aqueous phase is only important in contaminated soils with a free phase. If the free phase only contains one organic compound, the concentration in the aqueous phase or in the pore water corresponds to the aqueous solubility. If the free phase contains more than one organic compound, the equilibrium concentration in the aqueous phase or in the pore water can be estimated using Raoult's law:

$$C_i = x_i \cdot S_i$$

where

Ci is the equilibrium concentration of compound in the aqueous phase
xi is the mole fraction of compound i in the free phase
Si is the aqueous solubility of compound i.

In several studies concentrations of organic contaminants exceeding the solubility of the organic compounds have been observed (Weiß, 1998, Comans, 2001; Gamst et al., 2004). This might however be related to complex formation with dissolved organic carbon or association of contaminants with colloids (e.g. Knabner et al., 1996), confer section 3.3 and 3.4.

3.2 Sorption

Sorption and desorption by soils and sediments are fundamental processes controlling fate and transport of less polar and hydrophobic organic compounds in surface aquatic and groundwater systems.

Sorption of organic compounds to soils is often described in very simplified manner using the K_d concept. However, the simplification makes it easier to quantify sorption on the basis of only a few parameters. The traditional description is based on some simplifying assumptions:

- The adsorption isotherm is a standard tool for characterization of the partitioning of the organic compounds between the soil and sediments and the aqueous solution, basically describing how solute concentration is related to the adsorbed concentration. The simplest isotherm model is the linear adsorption isotherm that has been used frequently (e.g. Bouchard et al. 1990; Larsen et al. 1992; Kan et al., 1994), and is based on the principle that the sorption capacity of the sorbent is infinite. This means that there always is a fixed ratio between the concentration in the aqueous phase (Cw) and the concentration on the soil (Cs). That ratio is called the distribution coefficient (K_d and its unit is l/kg) and is independent of the concentration of the organic compound.
- Generally, it has been recognized that sorption of organic compounds from aqueous solution to soil is dominated by the fraction of natural organic carbon in the soil unless this fraction is extremely small (e.g. Karickhoff et al., 1979; Pignatello, 2000). Consequently, the partitioning of organic compounds between solute and natural organic carbon has been intensively investigated during the past decades (A review is given by Huang et al., 2003). It is, however, still not fully understood but limited studies showed that black carbon and kerogen exhibited nonlinear sorption for hydrophobic organic compounds and they may dominate the overall nonlinear sorption by soils (Huang et al., 2003). However, most transport models in soils use simple linear equilibrium expressions. Sorption onto minerals (clay, metal oxides, and metal hydroxides) is normally considered negligible unless the fraction of natural organic carbon is ex-

tremely small (Grathwohl, 1998; Pignatello, 2000). The type of natural organic carbon is not taken into account.

- The distribution coefficient is proportional to the content of natural organic carbon in the soil (foc) and the distribution coefficient between the organic carbon and water (Koc):

$$K_d = f_{oc} \cdot K_{oc}$$

where Koc is the partitioning coefficient between water and organic carbon (l/kg) estimated from the widely used Koc-Kow (octanol water partitioning coefficient) relations as proposed by Karickhoff et al. (1981), Abdul et al. (1987) or others.

- Sorption is reversible, e.g. the sorption process where an organic compound is adsorbed to the solid phase from aqueous phase and the process where an organic compound is desorbed from the solid phase to the aqueous phase can be described by the same K_d-value.
- Sorption is instantaneous, e.g. the mass transfer of an organic compound from the aqueous phase to the solid phase (or the reverse) to obtain equilibrium is so fast that it can be considered instantaneous.
- The effects of other organic compounds or colloids on sorption are negligible. Thus there is no competition for sorption sites between different organic compounds when more than one organic compound is present simultaneously.

This simple approach to describe sorption is often not valid and it has been shown frequently that sorption is a slow continuing process (e.g., Wu and Gschwend, 1986; Ball and Roberts, 1991a; Pignatello and Xing, 1996; Grathwohl, 1998; Valsaraj and Thibodeaux, 1999, Gamst et al., 2004). The sorption process is believed to be slow because the particles in the soil may contain an internal structure, in which the organic compounds diffuse and adsorb (referred to as intraparticle diffusion). This diffusion process is slow because the pores are narrow and the diffusion process thus becomes hindered by the size of the pores. Diffusion in and out of an internal structure of soil particles thus slows down the apparent sorption. Many hypotheses regarding slow sorption kinetics have been proposed, although hindered intraparticle diffusion through the narrow pore network of the soil particles and/or through the soil organic matter seems to be the dominating theories (Wu and Gshwend, 1986; Ball and Roberts, 1991a and b; Miller and Pedit, 1992; Grathwohl and Reinhard, 1993; Grathwohl, 1998; Brusseau et al., 1991; Pignatello and Xing, 1996; Weber and Huang, 1996). These explanations have been shown to fit experimental results.

The reversibility of sorption is another assumption that is not always fulfilled. Lower mass transfer rates are often observed for desorption than for adsorption. This is usually even more apparent in older than in more recently contaminated soils. This observation is often explained as being caused by non-attainment of sorption equilibrium (Pignatello, 2000; Gratwohl, 1998; Allen-King et al., 2002).

Some researchers have claimed that irreversible bindings to the soil organic matrix may also be the explanation (Alexander, 1995).

Nonlinear sorption has been observed frequently (e.g., Kishi et al., 1990; Weber et al., 1992), and some studies have shown that nonlinearity increases with increasing sorption time (Pignatello and Xing, 1996; Weber and Huang, 1996; Huang and Weber, 1998; Gamst et al., 2004).

3.3 Dissolved organic carbon

Dissolved organic carbon (DOC) is known to play a major role for dissolution of hydrophobic organic compounds. To understand the controlling processes of leaching of hydrophobic compounds focus has been on understanding the role of DOC (Comans et al., 2001 and Chin et al., 1990). Chin et al (1990) studied the distribution between organics in solid phase and a solute phase containing DOC. They concluded that organic contaminants can bind very strongly to DOC, resulting in a strong increase their water-solubility. Comans et al. (2001) showed that the leaching of PAH from a contaminated gas works soil increased strongly towards alkaline pH and coincided with the increase in the solubility of DOC in that pH-range. In addition PAH concentrations in the eluates were analyzed before and after removal of DOC by flocculation and the results clearly showed that leached PAH are predominantly presented in a form associated with DOC. Size exclusion chromatography of alkaline eluates showed that particularly the high-molecular fraction of DOC is responsible for the solubility enhancement and leaching of PAH. Thus, the behaviour of DOC is a major factor to be considered in both the development and interpretation of leaching tests.

The binding properties of DOC with respect to hydrophobic contaminant is still subject to ongoing research (*e.g.* at ECN in the Netherlands). Recently Laor and Rebhun (2002) suggested that linear partitioning or site complexation in the presence of excess available sites can not fully describe the interactions of hydrophobic compounds with dissolved humic material. Site-specific hydrophobic interactions at limited interior or external molecular surfaces may be considered. Similar binding properties of DOC have been suggested for heavy metals (Benedetti et al., 1995).

3.4 Colloids

Colloids are microscopic or submicroscopic organic or inorganic particles that are suspended in an aqueous phase. Colloids in porous media of interest in relation to leaching tests may be particles of biological origin (bacteria, viruses, and organic material), minerals (clay minerals, mineral precipitation, metal oxides, metal hydroxides), or combinations of these, e.g. clay minerals with humic substances adsorbed to the surface. Usually colloids are defined on the basis of their size. Particles with a diameter larger than 1 µm are considered as the upper size limit for colloids. The lower size limit for colloids is on the borderline of soluble molecules at approximately 1 nm (Stumm and Morgan, 1995). Thus, colloids constitute an additional separate phase in a water-saturated porous media, which usually is re-

garded as consisting of only two phases, water and solids (in the absence of air and a free phase). Due to the relatively large surface area pr. mass unit the capacity for sorption of organic compounds is often much larger than for the porous media. A contributing cause may also be that the colloids generally have a larger content of natural organic carbon. The naturally occurring colloids may have the following effects on transport and leaching of organic compounds:

- A larger fraction of the organic compounds than theoretically assumed is present in the aqueous phase due to the binding to the colloids.
- The organic compounds are potentially more mobile, because they are transported with the pore water adsorbed to the colloids.

As far as the strongly sorbing organic compounds (e.g. PAH and PCB) are concerned, the presence of colloids have to be taken into consideration when performing leaching tests (batch or column tests). It is important to define the objective of the test. If the colloids are considered as contributing to the leachable portion from a contaminated soil, the colloids should be measured as part of the aqueous phase. If the colloids are not considered as contributing to the leachable portion from a contaminated soil, the colloids should be separated from the aqueous phase to obtain the concentration in the real aqueous phase.

Problems with contaminants associated with colloids and dissolved organic carbon in the solute (e.g. Knabner et al., 1996) sometimes results in concentration measurements that exceed the solubility (Weiß, 1998, Comans, 2001; Gamst et al., 2004). In case of contaminants associated with dissolved organic carbon or mobile colloids the measured concentration in the eluate represents the mobile fraction of contaminant. However, if the separation of the aqueous phase from the soil phase has been insufficient (e.g. solid particles remains in the eluate) the contaminants bound in the soil particles will contribute to the measured concentration and using this for representing the mobile fraction of contaminants the results will be biased. Insufficient separation of the aqueous phase from the soil phase appear to be the dominating reason for measurement of concentration that exceed the solubility of a component in batch techniques (Gamst et al., 2004).

4 Assessing leaching of organic compounds from soil

4.1 Purpose of testing

The overall aim when conducting leaching tests is to determine the expected concentration of contaminants in solution when contaminated soil comes into contact with water. However, the results of the leaching tests may be used for different purposes. Two major purposes are testing for impact assessment and testing for compliance purposes.

Impact assessment: The major elements of impact assessment are shown in Figure 4.1.

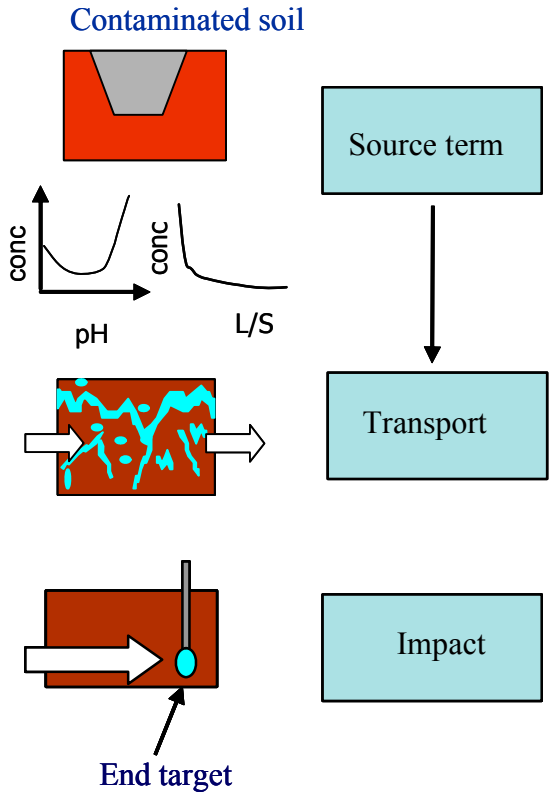


Figure 4.1. Major elements of impact assessment (from Comans 2004).

For impact assessment leaching tests are suitable tools for characterization of the source term, e.g. the present releases and long term releases of contaminants from soils. Some leaching tests also provide detailed information on processes controlling the release of contaminants. In order to obtain information on any aspect of the source term more than one test is needed. However, at this point the leaching behaviour of soil differs from the leaching behaviour of waste. Once the leaching properties of a waste stream have been characterized simpler and less extensive leaching test program can be used for the following lots of waste. For contaminated soils the leaching properties will most probably change from site to site and even

within one site. Thus, the leaching properties of soils can not be characterized once for all as can be done for a waste stream. Therefore, in praxis the soil source term characterization may in many cases be based on a less ambiguous leaching test program than for waste. In any case a direct measurement of the release of contaminants from a specific polluted soil provides a much better background for impact assessment than a measurement of the total content of contaminants in the soil, which is today the common praxis when it comes to management of contaminated soil (both for organic and inorganic pollutants).

Compliance testing: Leaching tests may be use for routine control purposes, for example in relation to some regulatory requirements (e.g. acceptance criteria for landfilling). Leaching tests for compliance testing should be fairly simple to perform, relatively cheap and the testing time low.

For both purposes the performance requirements should be strict with respect to repeatability, reproducibility and robustness. For compliance testing these aspects are key issues and for organic contaminants leaching tests aiming at equilibrium are suitable.

The leaching methods used for compliance testing may also be used for impact assessment as compliance tests should provide meaningful results that may be compared to e.g. regulatory requirements defined on basis of an impact assessment. In other words leaching tests used for compliance testing provide valuable information for an impact assessment of contaminated soil.

Leaching tests which can be used for impact assessment and for compliance testing can all be standardized. However, in the literature leaching methods have been applied on soils for many different purposes and in general tests have been developed in an attempt to simulate leaching behaviour in practice. This has led to a wide range of test recipes and leaching agents. These test methods are called simulation tests and they can not be standardized because they are developed for a specific purpose or scenario. Simulation tests will not be discussed further in this report.

4.2 Choice of leaching tests

The choice of leaching test method depends in the first place on whether the purpose of testing is assessing the impact of a contaminated soil on groundwater, surface water and soil or if the objective is compliance testing where the leaching methods often is prescribed. For impact assessment the choice of leaching test methods may be more complicated and, in the following section, some guidelines for this purpose are given.

The first step is always to formulate the questions that should be answered from the information provided by leaching tests. Some help can be found in the guidance document EN 12920. This European standard describes a methodology for assessing the leaching behaviour of waste. With some modifications the first steps of this procedure is relevant as a help for formulating the questions based on which the leaching methods are chosen. EN 12920 contains several steps, some descriptive steps and some of which make use of chemical, biological, physical and

leaching tests. Together these steps provide the information necessary to decide for corrective actions in a specific scenario. Another useful document for assessing the leaching behaviour of contaminated soils is in preparation in ISO/TC 190/SC 7/WG 6. Below the first steps of the methodology given in EN 12920 are described modified in order to be suitable for contaminated soil and with the aim of giving guidance for selecting suitable leaching test methods for organic contaminants in soil.

Step 1: Definition of the problem and the solution sought

In relation to contaminated soils leaching problems may be divided into two categories:

A: Investigation of a contaminated site. In this case the contaminated soil is often still at the site where the contaminating activities have been ongoing. At these types of sites the leaching tests may be a supplementary tool to existing methods for assessment of the impact of contaminants on groundwater or surface water. The leaching tests are used to characterize the source term in accordance with the scenario. This issue is the main area of interest within this work.

B: Excavated soils (*e.g.* soils for construction works). These soils may either be reused for specific purposes, taken to remediation or disposed at landfills. For reuse of contaminated soils leaching tests may be used for impact assessment of groundwater or surface water in relation to a specific scenario. The leaching tests are used to characterize the source term in accordance with the scenario. If soils are taken to remediation or disposal leaching tests may be used for control purposes, for example in relation to regulatory requirements (e.g. acceptance criteria for landfilling).

Step 2: Description of the scenario

This step consists of describing the normal and exceptional conditions which may influence leaching properties of the soil. This includes:

- the time frame,
- physical and chemical conditions,
- biological conditions,
- hydrogeological and climatic conditions
- mechanical and geotechnical conditions

The exposure pathways and end targets must also be defined.

Step 3: Description of the contaminated soil

In this step present properties of the soil are described. Relevant information includes:

- historical data related to the site from which the contaminated soil originate (earlier polluting activities, what kind of activities have been ongoing during the past, which contaminants are expected to be presented in the soil etc).
- total chemical composition
- physical properties like density, porosity, water content etc.

Step 4: Determination of the influence of parameters on leaching behaviour within the specified time frame

In this step the information from step 1–3 is gathered and the influence of key issues (chemical, physical, and geotechnical, mechanical and biological parameters) on relevant properties of the soil in the considered scenario is determined. Based on this knowledge the appropriate tests to assess release under the specified conditions are selected and performed.

In the table 4.1 some generally used objectives of testing are listed and leaching principles for each objective are suggested.

Table 4.1. Aspects of leaching behaviour of organic contaminants in soils and suggestion for suitable leaching principles that provides information on these aspects.

Objectives of testing	Recommended leaching principles
Source term	
Time dependence release / Leachate quality as a function of time	Percolation leaching
Accumulated leached amount of contaminant	Percolation leaching
Maximum leachability / Content of leachable contaminants	Availability leaching
Present release of contaminants (snapshot of the release of contaminants)	Batch leaching (e.g. recirculation of eluate in a column) or Percolation leaching
pH sensitivity of release	pH dependence release
Speciation of contaminants / binding properties	pH dependence release
Quality control / compliance testing	Batch leaching (e.g. recirculation of eluate in a column) or Percolation leaching

However, often a combination of two or more leaching methods will provide a more complete picture of the leaching properties of the contaminated soil. The decision on the extent of the leaching program will strongly depend on each scenario and in addition to the scenario parameters like amount of contaminated soil, budget and possibility of alternative solutions will often affect the decision.

5 Leaching methods – overview

5.1 Leaching tests

Leaching tests for organic compounds are not yet standardized, which implies that many different ways have been used to assess the release of organic compounds from contaminated soil. In this chapter leaching methods applicable for non-volatile hydrophobic organic compounds will be described and relevant references will be given.

5.1.1 Up-flow percolation column test (Dynamic column test)

The standardized up-flow percolation method for inorganic components (CEN/TS 14405) has been template for procedures used for organic compounds (mainly PAH and PCB). A set-up of dynamic column tests for organic compounds is shown in figure 5.1.

Figure 5.1. Example of up-flow percolation column test for organic compounds (photo from DHI).

In table 5.1 the key information related to a dynamic column test for organic compounds in contaminated soil is shown.

Table 5.1. Key information on dynamic column tests for organic compounds in soils

Objectives	Applicable for assessing time dependent release of organic contaminants
Basic principles	Contaminated soil is placed in columns made of glass or stainless steel. Tubes and connections shall also be made of inert materials. The eluent consists of either demineralised water or a weak CaCl2 solution to simulate a soil solution with respect to dominating cations. The flow direction is upward. The flow rate should be relatively low in order to ensure local equilibrium in the column. The eluate is collected in several fractions (often like for inorganic column test).
Critical conditions	• Biological degradation may take place if no precautions are taken to prevent this. One way to prevent this is to add NaN3 to the eluent. However the effect of adding NaN3 on releases of organic compounds from soil is not well documented yet. • All equipment must be made of glass or stainless steel in order to minimize loss of hydrophobic compounds by sorption to equipment. However sorption of hydrophobic compound can not completely be avoided. • If local equilibrium in the column is not obtained it is difficult to interpret the results unless the flow rate can be related to the specific scenario.
Advantages	• Leaching test methods provide useful information on composition of eluate at low L/S ratios which is the closest it is possible to get to a pore water composition by testing (except from simulation tests). In addition this leaching test provides information on long term leaching behaviour of the soil. • The main principles are well known from testing of inorganic compounds.
Disadvantages	The interpretation of the results from the column test requires that local equilibrium was obtained at any time in the column. Depending on the properties of the soil material the contact time required to obtain local equilibrium may vary. This means that predictions of short term leaching properties based on the first eluate fractions could underestimate the leaching. This issue remains to be investigated

In the literature several reports on dynamic leaching test methods for hydro-phobic organic compounds may be found. In table 5.2 a summary of selected test methods are given. Most of the test methods described in table 5.2 except from one aim at local equilibrium in the column. As can be seen from table 5.2 the test conditions used differ, which makes it very difficult to compare the column methods and it emphasizes the need for a standardized and validated column leaching method.

The method DIN V 19736 aims at maximum fluxes (non equilibrium). When interpreting the results of the dynamic column test the distinction between equilibrium and non-equilibrium conditions is an important issue.

	Scope	Compounds	Column material	Test conditions	Comments
ISO/DIS 21268-3 **In preparation**	To measure the release of inorganic and organic constituents from soil and soil materials. This test method produces eluates, which can subsequently be characterized bv physical, chemical and ecotoxological methods	Inorganic and organic constituents. Not suitable for volatile constituents	Glass column with an internal diameter of 5 cm or 10 cm and filling height of about 30 cm	Flow rate: linear velocity 15 cm/d through an empty column Eluent: 0,001 M $CaCl_2$ + (NaN_3)	This standard is in preparation and significant changes in the standard may occur during finishing and validating the standard
NVN 7376.	Valid for solid earthy and stony materials	PAH, PCB, OCP, EOX, phenol and cresole	Glass column with an internal diameter of 5 cm	Flow rate not specified Eluent: Ultra-pure water	This standard is only available in Dutch. Therefore details are not included in this table.
Enell et al. (2004)	To develop a column leaching test method for hydrophobic organic contaminants from soil	PAH	All materials consisted of glass or stainless steel. Filters were made of borosilicate (particle cut off at 0,7 µm)	Sterile water was pumped up-wards through the column. The estimated contact time was 30 min. Fine particles were settled in a sedimentation chamber which was monitored on the top of the column. The eluate was filtered and passed through an on-line solid phase extraction cartridge. Samples were collected after L/S steps of approximately 50 l/kg. To prevent biological degradation HgCl2 was used	Leaching experiments showed that after L/S 50 l/kg a steady state was reached. The occurrence of a steady state concentration can result from either mass transfer limitations or distribution equilibrium between the leachate and the contaminated soil. To interpret the leaching results it is essential to know if steady state concentration reflects equilibrium or mass transfer limitations.
DIN V 19736 (German prestandard)	Determination of desorption or dissolution rates of contaminants from various materials		Glass column	Flow velocity is about 1 m/day Eluent: degassed drinking water, On-line extraction in cyclohexane was used.	Interpretation of results as maximum fluxes

Comans et al. 2001	To develop leaching test to characterise leaching of organic compounds from soil and waste materials	PAH, PCB, Chlorophenols	Column of stainless steel	Pore water velocity 26 and 130 cm/day Eluent: 0,001 M $CaCl_2$	
Reemtsma and Mehrtens 1997	To examine the leaching of organic compounds from a soil.	PAH	All material was made of glass or PTFE	Flow rate 50–60 ml/h Eluent: 50 mM CaCl2 Glass fiber filters were used. On-line solid phase extraction was used.	The columns were operated under saturated flow with a 2 cm layer of eluent kept above the soil surface (Down-flow leaching)

5.1.2 pH-static test

pH is an important parameter, when it comes to leaching of organic compounds from soil. This is mainly due to the fact that DOC is generally strongly dependent on pH. Batch pH-static leaching experiments have been performed to investigate the effect of DOC on the leaching of PAH from soil and waste material (Comans et al. 2001). The pre-standards for pH-static leaching test with continuous pH-control and initial acid/base addition (prEN 14997 and prEN 14429) have been template for procedures used for organic compounds. Table 5.3 contains key information on pH-static leaching tests.

Table 5.3. Key information on pH-static test for organic compounds in soils

Objectives	Leaching of organic compounds as a function of changes in pH
Basic principles	This test method is based on the pre-standard for inorganic constituent prEN 14997. The soil is suspended in a solution made of either demineralised water or 0,001 M CaCl2 at a liquid to solid ratio of 10 l/kg. pH is monitored and adjusted to pre-selected set points in the range of 4–13 with acid or base. After a contact time at 48 hours the eluate is separated from the solid by centrifugation.
Critical test conditions	• The separation of the solid and the eluate is a critical test condition (see section 5.2.2) • Degradation of organic compounds should be prevented
Advantages	• The procedure with continuous acid/base addition is found to be easier to control and perform than the procedure with initial acid/base addition (Nordtest 2005) • This method may be suitable for investigating basic processes controlling leaching as for example the role of DOC. For this purpose leaching in the pH range 4–13 is relevant. • For soils with low buffering capacity the changes in leaching properties as function of changes in pH may be relevant. Generally in that case a pH-range between 5 and 9 is relevant.
Disadvantages	• No standardized and validated procedure is available. • If the separation step of solid and eluent is insufficient to separate all colloids from the eluate the batch test may overestimate the leaching of hydrophobic organic compounds • The results of the test may depend on the test conditions used (for example the method for separation of solid and liquid).
Relevant references	• The International standardization organization (ISO) is preparing a standard for pH-static leaching (ISO/CD 21286-4) with initial addition of acid or base. This standard is today (May 2005) a committed draft. Significant changes may occur before this standard is finished and validated. • Comans et al. (2001)

5.1.3 Availability test

An availability test for assessing the total available amount of organic pollutant for leaching has been developed (Comans et al., 2001). This leaching procedure is based on the concept used in the assessment of inorganic compounds leaching where leaching over time may ultimately approach the ”availability” as the maximum amount that may be released from the soil. The basic information on the availability test for organic compounds is given in table 5.4.

Table 5.4. Key information on availability test for organic compounds.

Objectives	The purpose of the test is to indicate the quantity of an organic compound that might leach out from a soil if exposed to extreme conditions (e.g. in the long term)
Basic principles	The availability for leaching is determined by extracting a soil sample with a solution of a commercial (Aldrich) humic acid at a high L/S ratio of 100 L/kg and a pH of 12. This high pH-value is necessary to keep the DOC in solution by preventing its adsorption to the soil. The quantities of the various organic compounds present in the soil that are available for leaching may be calculated on the basis of the results of this availability test (from Comans et al. 2001).
Critical test conditions	• The separation of the soil and eluate may be critical to the test results (se section 5.2.2)
Comments	• The concept ”availability” is for organic compounds not yet well described in the literature and the interpretation of the test results is not clear
Relevant references	• Comans et al. (2001) • Roskam and Comans (2003)

5.1.4 Equilibrium column test (recirculation of eluate)

The leaching methods described above are time consuming and relative expensive. Thus, the use of these methods may be limited for contaminated sites in case of either limited budget or in case of contaminated sites where amounts of contaminated soil are limited. There is, therefore, a need for a relative cheap, quick and easy to operate leaching test for non-volatile organic compounds, which provides reliable results. These results should be meaningful and applicable for both simple impact assessment of contaminated soil and for compliance testing of soil. For this purpose an equilibrium column test with recirculation of eluate has been developed for non-volatile organic compounds. In Figure 5.2 a picture and a sketch of the test system is shown and table 5.5 contain some key information on the test principles.

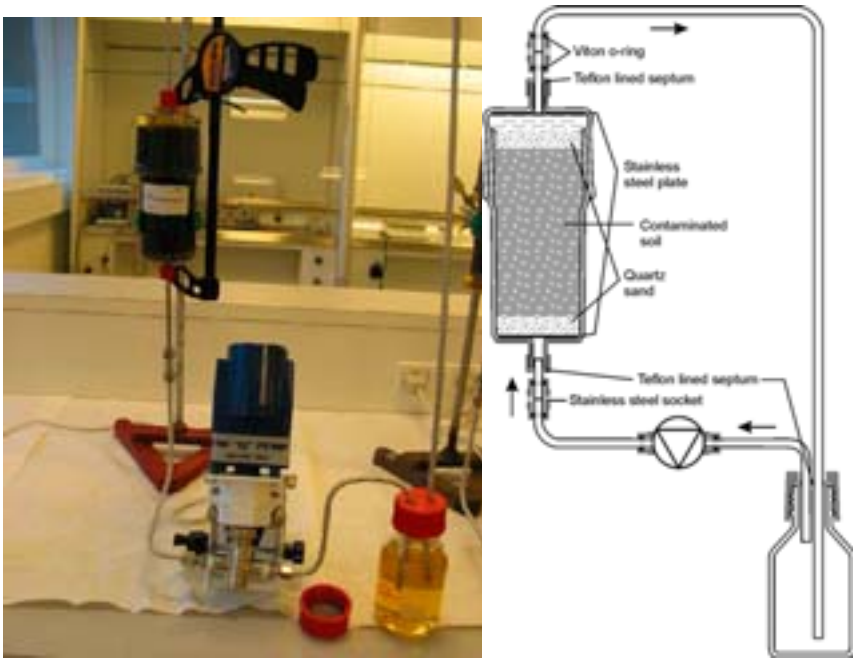


Figure 5.2. Photo and sketch of the equilibrium column test for organic compounds. The eluate is recirculated through the column for 7 days (Hansen et al. 2004 and Gamst et al. 2005).

The equilibrium column test with recycling of the eluent can be regarded as an alternative to the traditional batch leaching test. With this test some problems of the batch leaching test concerning hydrophobic compounds (e.g. grinding of soil material during agitation, separation of solid and liquid) have been solved. There is a need for a standardized leaching procedure for organic compounds which produces useful and reliable results for impact assessment and for compliance testing. A standardized test should be validated.

Table 5.5. Key information on the ”recycling equilibrium column leaching test” described by Hansen et al. (2004) and Gamst et al. (2005).

Objectives	This test method provides a determination of the "equilibrium" concentration of non-volatile organic compounds in the eluate
Basic principles	This column test is performed in glass columns at a fixed L/S ratio depending on the properties of the test material (between 1 and 2 l/kg). A continuous vertical up-flow is applied, so that the column is water saturated. The eluent consists of 0,005 M CaCl2 containing 0,5 g/l NaN3 (to prevent degradation) and is recirculated in the test system for 7 days to obtain equilibrium. The flow velocity is approximately 0,7cm/h (darcy velocity). The eluate is collected as one single fraction after 7 days of recirculation.
Critical test conditions	• Biological degradation must be prevented by biocides. Sodium azide and mercury chloride are common biocides. However, the effect of adding biocides to the leachant is not well documented (introduction of high ionic strength in the system) • The material used for test equipment has to be either glass or stainless steel. Also the pump used must be made of inert materials.
Advantages	• Fairly simple and easy to perform. The repeatability and reproducibility is within the range known from testing of inorganic compounds (Hansen et al. 2004) • The test material is treated very gently during testing and no grinding of the material will occur during leaching. • The influence of sorption onto equipment on the test results is minimized. Equilibrium between surfaces of the leaching devise, eluent and soil is obtained during the contact time. • No additional treatment of the eluate is needed after leaching before it can be characterised. Colloids present in the eluate after leaching through the column many times are expected also to be mobile in a natural situation. Thus, the concentration of organic compounds in the eluate represent an equilibrium concentration taking into account the potential influence of dissolved organic carbon and colloids on the leachability. • Test results are meaningful and can be used for impact assessment as well as for compliance testing
Disadvantages	• Limited amount of eluate is available for chemical analysis of organic compounds and often it will be necessary to set up several test to obtain enough eluate.

Table 5.5 contains relevant references on the recycling equilibrium column concept.

Table 5.5. Relevant references for the ”recycling equilibrium column test”

	Scope	Compounds	Column material	Test conditions	Comments
Hansen et al. 2004 and Gamst et al. 2005	To develop leaching test methods for non volatile organic compound applicable for impact assessment and for compliance testing	PAH	The column test is performed in a glass column (size: (~15 cm length and ~6 cm diameter, ~425 cm3). Tubes are made of stainless steel	The eluent that consists of 0,005 M CaCl2 containing 0,5 g/l NaN3 (to prevent degradation) The flow velocity is 0,7cm/h (darcy velocity). A continuous vertical up-flow is used and eluate is recirculated in the test system for 7 days to obtain equilibrium	The eluate is not further treated by centrifugation nor filtration after the leaching has ended. For analysis of organic compounds the extraction is performed directly in the receiving vessel from the test system. This test method was found to be suitable for non-volatile organic compounds especially hydrophobic compounds.
Maraqa (2001)	Determination of sorption equilibrium parameters using natural soil samples Different techniques were compared	Dimethylphthalate, diethylphthalate and dipropylphthalate	Columns of stainless steel (1.1 cm ID and 15.4 cm long) with stainless steel porous end plates.	The eluent was a 5mM CaCl2 with 0,05% sodium azide solution Flow rate 0,6 cm/min Contact time unknown	This leaching method was used for determination of sorption distribution coefficients and the results were compared to a batch leaching technique. Good agreement between these techniques was observed.

5.1.5 Batch test

Batch leaching test is a well known concept from leaching of inorganic compounds (EN 12457) but also known for organic compounds as sorption/desorption tests (Maraqa 2001, Bowman et al 2002). Figure 5.3 shows an example of a batch leaching container for organic compounds.

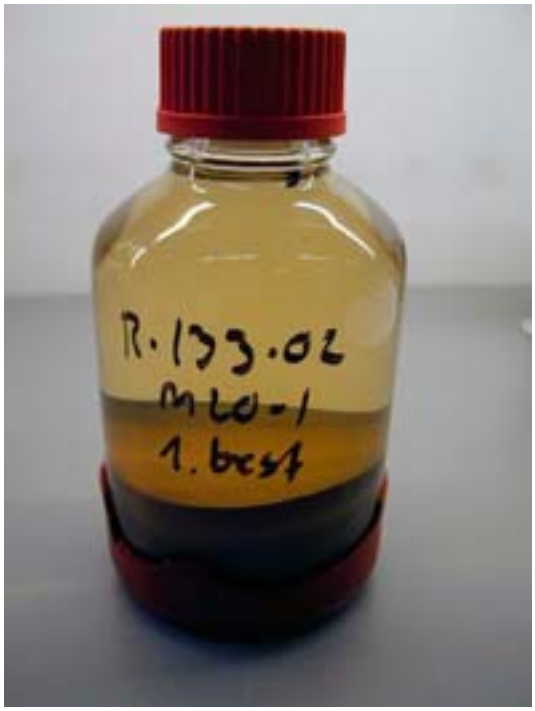


Figure 5.3. A batch leaching test for organic compounds.

In table 5.7 some of the key aspects related to a batch leaching test for non-volatile organic compounds are summarized.

Table 5.7. Key information on batch leaching concept.

Objectives	This test method provides an estimate of the "equilibrium" concentration of non-volatile organic compounds in the eluate
Basic principles	A batch test for non-volatile organic compounds is a technically fairly simple test, which is conducted in a glass container (or a container of another inert material) at a fixed liquid to solid ratio (often 2 l/kg or 10 l/kg). The eluent is a solution of either demineralised water or CaCl2. The container is agitated for a prefixed time to obtain equilibrium between contaminants in solution and contaminants in the soil. The eluate is separated from the solid by either centrifugation or filtration.
Critical test conditions	• Separation of the eluate from the soil is for hydrophobic compounds recognized to be a critical step in the procedure due to sorption onto colloids (organic and inorganic). The choice of separation technique may be essential to the test results. • Degradation should be prevented even if the contact time is low. Degradation of PAH in soil/water system has been observed within short time (Smith et al. 1997)
Advantages	• Main principles are well known from testing of inorganic compounds and the method is simple and easy to perform. • The repeatability of the batch test is found to be at the same order of magnitude as for inorganic compounds described by van der Sloot et al. 2001 (Hansen et al. 2004).
Disadvantages	• During agitation the soil grains will undergo grinding and “artificial colloids may be created and dispersed. This grinding process may create new surfaces for sorption and thus the distribution of hydrophobic compounds between solid and liquid will change and the test results may be biased (confer section 5.2.2.) • If the separation step of solid and eluent is insufficient to separate all colloids from the eluate the batch test may overestimate the leaching of hydrophobic organic compounds • The results of the batch leaching test may depend on the test conditions used (for example the method for separation of solid and liquid). • Sorption of highly hydrophobic compounds onto equipment may be significant.

In the literature several batch leaching experiments has been conducted on contaminated soil. Table 5.8 contains a summary of selected references. In ISO standardization of batch leaching tests for organic compounds are in preparation (ISO/DIS 21268-1 and 21268-2). Table 5.8 also contains principles of these tests. However, it must be recognized that the purpose of these test methods developed in ISO are to produce eluates for subsequent chemical and ecotoxicological testing. Using these procedures for impact assessment precautions must be taken regarding biodegradation and the separation method for soil and eluate must be chosen carefully in order to obtain useful and meaningful results. From table 5.8 it can be seen that different test conditions have been used in different studies and the results of the batch leaching tests would be more or less influenced by these test conditions. Thus it may be difficult to interpret the results in relation to impact assessment. This illustrates the need for a standardized method developed for hydrophobic compounds with the objective to produce meaningful results for both impact assessment and compliance testing. A standardized test should be validated.

Table 5.8 References on batch leaching test for organic compounds

	Scope	Compounds	Test conditions	Comments
ISO/DIS 21268-1 In preparation	To measure the release of inorganic and organic constituents from soil and soil materials. This test method produces eluates, which can subsequently be characterised by physical, chemical and ecotoxological methods	Inorganic and organic constituents. Not suitable for volatile constituents	Inert material for leaching vessels Eluent 0,001M CaCl2-solution L/S-ratio 2 l/kg Contact time 24 hours Centrifugation (high speed: suggested 27.000 g for 30 min or similar force).	This standard is in preparation and significant changes in the standard may occur during finishing and validating the standard
ISO/DIS 21268-2 In preparation	To measure the release of inorganic and organic constituents from soil and soil materials. This test method produces eluates, which can subsequently be characterised by physical, chemical and ecotoxological methods	Inorganic and organic constituents. Not suitable for volatile constituents	Inert material for leaching vessels Eluent 0,001 M CaCl2-solution L/S-ratio 10 l/kg Contact time 24 hours Centrifugation (high speed: suggested 27.000 g for 30 min or similar force).	This standard is in preparation and significant changes in the standard may occur during finishing and validating the standard
Hansen et al. 2004	To develop leaching test method for non-volatile organic compounds. Effect of centrifugation force and time was investigated and results of batch test compared to recycling equilibrium column test. A minor round robin test was performed	PAH	Glass vessels Eluent 0,005 M CaCl2-solution L/S-ratio 2 l/kg Contact time 24 hours Centrifugation (high speed centrifugation 27.000 g for 30 min or 6200 g for 60 min).	Batch leaching test results obtained for two waste material and two soils were compared to results from equilibrium column leaching test. For soil some disagreement between results from batch and equilibrium column test were observed.
Fortkamp et al. 2002	Development of leaching tests as a part of a methodology for impact assessment related to contaminated sites	Hydrocarbons (oil)	Stainless steel tubes with teflon top Eluent: deionised water L/S-ratio 4 l/kg Contact time 24 hours Centrifugation (3000 g for 20 min).	This investigation concludes that leaching tests for organic compounds still have to be developed and documented.
Maraqa (2001	Evaluate different technique for determination of sorption distribution coefficients	Dimethylphthalate, diethylphthalate and dipropylphthalate	Conducted in 20 ml glass vials Eluent 0,005 M CaCl2-solution + 0,05% NaN3 L/S-ratio 1.1 and 4 l/kg	

			Contact time: a 13 day sorption rate study was conducted Centrifugation (3000g for 30 min).	
Comans et al. 2001	To develop leaching test to characterise leaching of organic compounds from soil and waste materials. A two step batch leaching procedure was described A limited round robin test was performed	PAH, PCB, Chloro-phenols	Inert material for leaching container Eluent 0,001 CaCl2-solution L/S-ratio 2 l/kg and L/S 10 l/kg Contact time 24 hours Centrifugation (high speed: suggested 27.000 g for 30 min or similar force).	Compared to results obtained from pH-static leaching test consistent leaching results were obtained for the two-steps batch leaching procedure. Repeatability and reproducibility were acceptable.
Bjuggren et al. 1999	Development of leaching test for leaching of organic compounds from contaminated soil		Leaching tubes consist of stainless steel Eluent Deionised water with NaN3 (2 g/l) L/S-ratio 5 and 10 l/kg Contact time: from 6 hours to 24 hours was found to be suitable for obtaining equilibrium. Centrifugation (4000g for 20 min) Filtration of centrifuged samples through 0.45 um filters.	Filtration of sample before analysis can influence the measured concentrations significantly
Wahlström et al. 1994	To examine the leaching of organic compounds from a soil	BTEX, PAH, Chlorophenols, Total petroleum hydrocarbons	Conducted in glass bottles Eluent deionised water L/S-ratio 10–100 l/kg Contact time: ? Filtration was used. Different filters were tested and it was found that test results were strongly influenced by the type and size of filters used.	The test methods applied was based on methods for leaching of inorganic compounds. It was concluded that the development of leaching tests for organic compounds were still in an early stage and there was still a lot of work to be done to understand the processes controlling leaching. There is a need for standardisation

5.2 Critical test conditions

5.2.1 General critical conditions

POTENTIAL LOSSES OF NON-VOLATILE ORGANIC COMPOUNDS

Hydrophobic components may be lost from the eluent during leaching due to sorption onto test equipment when it comes into contact with the eluent (Bauw et al., 1991; Reemtsma and Mehrtens, 1997). Thus, to minimise losses by sorption, all equipment must be made of inert materials such as glass and stainless steel. However, investigations have showed that losses still may be an issue (Larsson, 2002). Larsson (2002) found that the total loss of PAH in the eluates was estimated to be between 0.2 % (w/w) and 20 % (w/w) and Gamst et al. (2005) found that the amount of PAH adsorbed corresponded to 9 % of totally leached PAH. The results obtained by Gamst et al., (2005) also showed that larger PAH sorbs more strongly to most materials, which is in accordance with other results (Bauw et al., 1991; Reemtsma and Mehrtens, 1997; Comans, 2001).

When focusing on organic leachable compounds, it is of major importance to minimise any degradation of such compounds both during the leaching test and before analysis. Degradation by photolysis may easily be minimised by covering the test equipment and sample bottles with aluminium foil. However, microbial degradation may not be avoided as easily. Chemical agents such as sodium azide (NaN3) or mercury chloride (HgCl2) (normally 0.2 g/l NaN3 or 2 %(w/w) HgCl2) have been used to reduce/minimise biodegradation (e.g. Enell et al. 2004, Wolf et al. 1989). However, the influence or bias such additives may have on the leaching procedure is not well understood. For example, it is known that NaN3 may increase the pH in soil (Skipper and Westermann, 1973; Wolf et al., 1989). Changes of pH to very low or very high pH may increase the content of leached PAH as a result of an increase in leaching of dissolved humic substances (Comans et al., 2001, Wahlström et al., 1994). The introduction of non-toxic conditions by physical pretreatment of the eluent may possibly be an alternative to the application of chemical additives (experiences from DHI, DTU and SGI). However, such measures will only prevent aerobic degradation. Potential anaerobic degradation processes will not be affected, but anaerobic degradation is often slower and may need significantly longer lag-phase time, compared to aerobic degradation. Studies of PAH degradation have shown that the smaller PAH (2–4 ring) are readily degradable (Hestbjerg et al., 2003; Muncnerova and Augustin, 1994) while the larger PAH remain recalcitrant (Hestbjerg et al., 2003; Schneider et al., 1996).

More research is needed on this area to quantify the potential biodegradation rates of the organics as a function of different conditions of the leaching test.

5.2.2 Test specific test conditions

FLOW RATE OF THE LEACHANT (DYNAMIC COLUMN LEACHING TEST)

The flow rate chosen for the dynamic column test may be a critical factor in relation to the results of the test. This column leaching test aims at local equilibrium between compounds distributed between soil and eluate at any time of the test duration. If local equilibrium is not obtained the leached concentration may depend

on the flow rate used (confer section 3.2). It should be noted that the dynamic column leaching test is not aiming at simulating flow conditions in the field. This leaching test provides a conservative estimate of the leached amount of organic compounds from the soil at various L/S ratios.

A low linear velocity of the eluent in the column is more likely to ensure that the local equilibrium conditions are fulfilled. However, in order to be able to perform the leaching test within a reasonable time a lower limit of flow rate is relevant, especially if the test is running to high L/S-ratios. The upper limit of flow rate would be relevant in order to make sure that local equilibrium is obtained for any organic component and any type of soil. These issues need to be investigated in details.

GENERATION OF COLLOIDS (BATCH TEST, AVAILABILITY TEST, PH-STATIC TEST)

During agitation or stirring of soil and eluate in batch test concepts the structure of the soil material may be altered due to grinding of soil particles. The effect of grinding on test results has been investigated by different researcher . Gamst et al. (2004) observed in a study for naphthalene that results obtained from batch sorption experiments conflicted with observations from gas diffusion experiments on the same soil (e.g. higher concentrations of naphthalene in the batch tests) (Gamst et al., 2003). It was suggested that the different observations might be due to the intra aggregate diffusion of naphthalene in soil aggregates in the column leaching experiment, whereas such aggregates are predominantly destroyed by physical disruption in the batch experiment. Further, Bergendahl and Grasso (1998) quantified the production of, and the mechanisms behind the release of, colloids in a batch leaching test of a coal tar contaminated soil. The study quantified the generation of colloid fractions during testing and particle count data indicated that the concentration of 0.72 – 0.83 µm diameter colloids in the filtrate increased with agitation time. It was concluded that colloid generation during batch testing resulted in an increase in total colloidal surface area in the filtrate. An increase in colloidal surface area may result in an over prediction of the aqueous phase concentration of hydrophobic contaminants. Work conducted for the Danish EPA (2003) indirectly indicated generation of colloids during agitation of batch tests. For two different soils increasing eluate concentrations of selected PAH-compounds were found as a function of contact time. At the same time increases in the turbidities were detected indicating the production of colloids during agitation by grinding of the soil materials.

It is not yet clearly demonstrated if these problems with “artificial” colloids in the batch concept can be solved. One way to solve the problem may be to use high speed centrifugation. Comans et al. (2001) obtained consistent results by using high speed centrifugation.

SEPARATION OF SOLID AND LIQUID (BATCH TEST, AVAILABILITY TEST, PH-STATIC TEST)

In the batch leaching procedures it is important to remove colloids potentially generated during the test for the eluates. Otherwise the batch tests will over predict the leaching of hydrophobic compounds from the soil. It is, however, not possible to define a particle cut off where particles below that limit will be mobile and above immobile. Mobilisation of particles will among other parameters depend on soil properties and site specific conditions. Therefore, the procedures used for separation of solid and eluate in these leaching test will operational define the leachable fraction of contaminant. The procedures used should thus be chosen carefully and if possible validated. Separation of soil and eluate has traditionally been done by either filtration or centrifugation. Both filtration and centrifugation techniques yield operationally defined particle cut-offs.

Separation by filtration: Previous investigations have focused on separation of solid and liquid by filtration in relation to organic compounds (Comans et al., 2001, Enell et al. 2004, working document ISO/TC 190/SC 7/WG6). Many different filters are commercially available but not all filters if any are suitable for hydrophobic compounds as these compounds may adsorb strongly to the filter materials. In the literature studies focusing on testing different types of filters can be found (Hjelmar et al., 2000, Rødsand and Rike, 1999, Rood et al., 1994, Bauw et al., 1991) all showing that losses of hydrophobic compounds during filtration are critical.

Separation by centrifugation: Separation of solid and liquid by centrifugation has often been in the batch procedures. However, numerous different centrifugation settings have been reported (e.g. Bouchard et al., 1990; Kan et al., 1994; Gamst et al. 2003, Hwang and Cutright, 2004). It is well known that different centrifugation force and time yields different particle cut-off in the solution and thus the content of colloids in the solutions are different when eluates are analysed. This may affect the content of hydrophobic compounds in the eluates and the results of the tests may thus be biased by such test specific differences.

In order to obtain useful leaching results for hydrophobic compounds from batch leaching tests it is essential that a suitable procedure for separation of soil and eluate is found. Investigations show that this procedure probably will be a centrifugation procedure at high speed (e.g. 27.000 g in 30 min.). This needs to be verified for different compounds and a number of different soils.

6 Leaching tests as a tool for impact assessment

6.1 Release of organic contaminants from soil

Non-volatile organic compounds may be released from a contaminated soil either by dissolution from a free phase or by desorption . The release pattern that may occur is illustrated in figure 6.1.

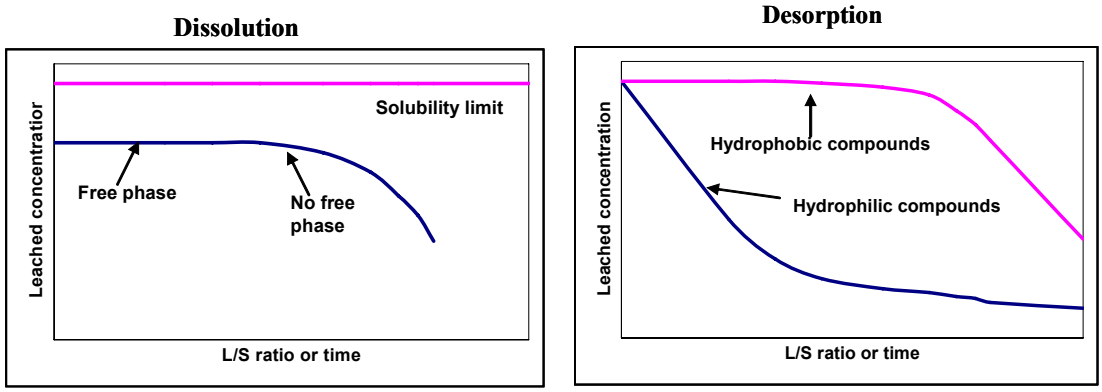


Figure 6.1. Ideal release of organic compounds from soils as a function of liquid to solid ratio or in other words time. The release pattern controlled by dissolution or desorption is illustrated.

In presence of a free organic phase in the contaminated soil the leached concentrations of contaminants in soils will be close to the solubility limit (confer section 3.1). It is, however, difficult to identify the presence of a free phase as the composition of the free phase is often unknown and thus the solubility limit of a certain compound is difficult to calculate.

As an example of difficulties linked to determine the leaching controlling mechanisms figure 6.2 shows the leached concentrations of PAH from 2 different Danish soils. Soil A is a sandy soil originating from a harbour area and Soil B is from a former gas production facility (Danish EPA 2004). From the figure different release pattern from different compounds can be observed even for different compounds within the same soil. For Soil A the leached concentration of fluoranthene is almost constant within the L/S range investigated (1–18 l/kg) whereas for benz(a)pyrene and benz(b,j,k)fluoranthene the leached concentration decrease significant after L/S 10 l/kg, which may be opposite than expected from the hydrophobicity of the compounds. For all three compounds Soil B shows almost constant leached concentrations until L/S 18 l/kg followed by a small decrease in leached concentration, which is more like expected. This different release pattern for different compounds and different soils justify the need for leaching test for organic compounds.

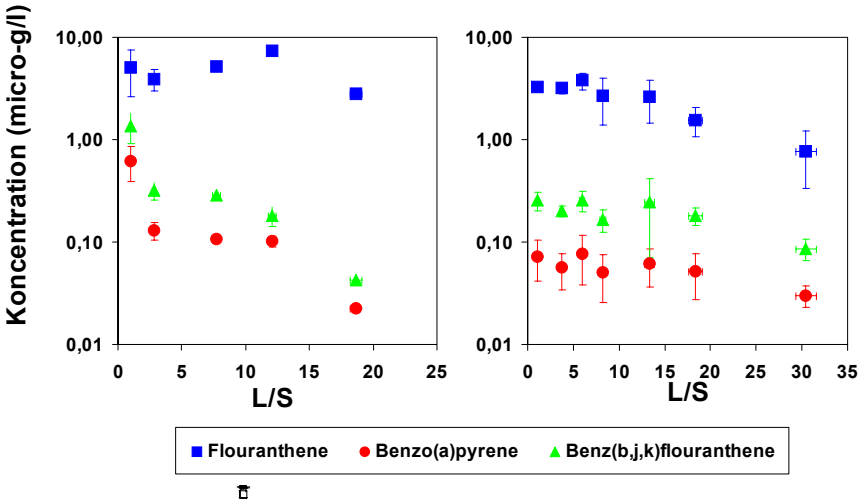


Figure 6.2. Leached concentrations of PAH from a sandy soil (A) and a more humic rich soil (B) (Danish EPA 2004).

6.1.1 Equilibrium controlled release contra diffusion limited release

In contaminated soils without free organic phases the controlling release mechanisms most often can be described in terms of either equilibrium controlled or diffusion limited release. Equilibrium controlled release may occur for slow percolation through porous or granular materials. Different leaching tests for estimating the equilibrium controlled release of non-volatile organic compounds has been suggested and selected methods are summarized in chapter 5. For a given scenario, leachate concentrations based on equilibrium will always be greater than or equal to those based on diffusion rates. Thus, equilibrium release estimates may be a conservative approximation to the release of contaminants from soils. In addition leaching tests based on equilibrium conditions are less sensitive to small changes in test conditions.

However, in many cases, organic contaminants are with time embedded in the soil matrix (ageing effect) and the release of the contaminants to the pore water may be very slow. In this case the contaminant release will be diffusion limited and the pore water concentration will be lower than an equilibrium concentration. In case of diffusion limited release the pore water concentration will depend on the actual pore water flow rate and with it the contact time between the contaminated soil and the pore water. To estimate the diffusion limited release of contaminants from soil the test methods will have to be based upon site specific conditions which, are valid for the specific scenario only. The use of such methods for contaminated site investigations would in most cases be too time consuming and expensive.

Equilibrium based leaching tests are, thus, regarded as the most suitable tools for contaminated site impact assessment, but it should be kept in mind that the measured release may be a conservative estimate of the release in the real scenario.

6.1.2 Test conditions versus field conditions

As pointed out several times in this report the leaching principles presented do not simulate the actual field conditions that occur at a specific contaminated site. How-

ever, when interpreting the results, the effect of the test conditions applied should be evaluated in relation to the field conditions. Table 6.1 shows leaching test conditions that might be different from field conditions. In addition table 6.1 provides a justification for choice of leaching conditions and the effect

Table 6.1. Leaching test conditions that may differ from field conditions and the effect on release of contaminants expected from these differences.

Test condition	Justification	Field condition	Effect
Biological degradation prevented by e.g. NaN3 and darkness	It is not possible to simulate field conditions in a leaching test similar to how biological degradation could occur in the field	Most organic compounds will be more or less degradable	In the field, the leaching from the contaminated soil may be lower than estimated by the leaching test due to biological degradation.
Release of organic compounds at equilibrium or local equilibrium	Equilibrium release would be equal to or a worst case estimate of the release of organic compounds	Release of organic compounds are controlled by either equilibrium release or diffusion limited release	The release of organic compounds may be lower in the field than estimated by a leaching test if the release is diffusion limited
Saturated leaching conditions	Leaching test must be conducted under controlled conditions, and water saturated leaching can be controlled much easier than unsaturated leaching	In the field the leaching may be unsaturated with wet and dry periods	The effect is unknown. However, wet and dry periods could probably cause significant changes in the leached concentrations. Lysimeter leaching experiments may be useful for the investigation of this topic
Reduced conditions may be applied	For organic compounds changes in redox conditions mainly affect the biological processes. If biological activity is prevented, changes in redox condition do not affect leaching significantly	Different stages of redox conditions may occur in the field	Desorption of most compounds are not significantly sensitive to redox changes. However, reduced conditions may be applied in the leaching test if necessary

6.2 Quality control

The use of leaching tests for contaminated soil in impact assessments at contaminated sites requires that a set of data quality principles is established. As a minimum requirement it should be possible to obtain relevant information about test variability (repeatability) from the leaching tests conducted on a specific contaminated site. At a contaminated site samples for leaching testing may be collected in different ways depending on the overall purpose of testing (this issue will also be addressed in the Guideline). However, it is important already in the planning of the sampling to include the quality control procedure, since it influences how and where samples should be collected. Figure 6.3 shows an example of how the sampling plan includes the quality control procedure. At one site two positions are sampled and at each position two samples are collected. In the laboratory one of the samples are divided into two samples. All together 5 samples are collected for

leaching testing. Following the scheme given in figure 6.3 it will be possible to derive information about

- Site variability (samples from two positions)
- Small variabilities at one position / sampling variability
- Test variability

The positions at which samples are collected for leaching tests may be chosen based on knowledge concerning for example total contents of contaminants, geology, or contamination history.

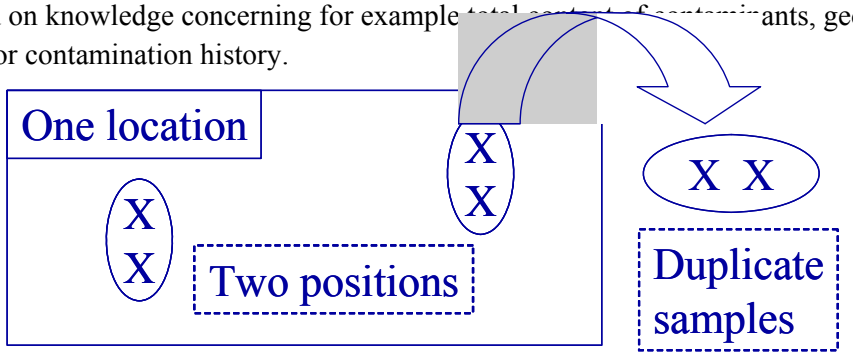


Figure 6.3. Example of combining the sampling plan and the quality control procedure for evaluation of a contaminated site based upon leaching testing (Hansen et al., 2005)

6.3 Leaching test results as input for impact assessment

6.3.1 Estimation of a pore water concentration

A concentration of organic contaminants in the pore water may be estimated based on the results of leaching tests. The calculations shown below are only valid for non volatile organic compounds (2-phase system) and when the release of contaminants is controlled by desorption.

The leaching test will in most cases be conducted at a higher L/S ratio than relevant for field condition. Based on mass balances following expression may be obtained

$$S \cdot C_{soil} + L_{field} C_{field} = S \cdot C_{soil,test} + L_{test} \cdot C_{test} \qquad 6.2$$

S soil is the amount of soil (kg dw)
C_{soil} is the solid concentration of contaminant under field condition (mg/kg dw)
L_{field} is the amount of pore water under field condition (l)
C_{field} is the pore water concentration under field condition (mg/l)
C_{soil}, test is the soil concentration of contaminant after leaching (mg/kg dw)
L_{test} is the amount of eluent used in the leaching test (l)
C_{test} is the leached concentration (eluate concentration) (mg/l)

The distribution of contaminant between the solid phase and the liquid phase may be expressed in terms of a distribution coefficient K_d.

$$K_d = \frac{S}{C} \qquad 6.3$$

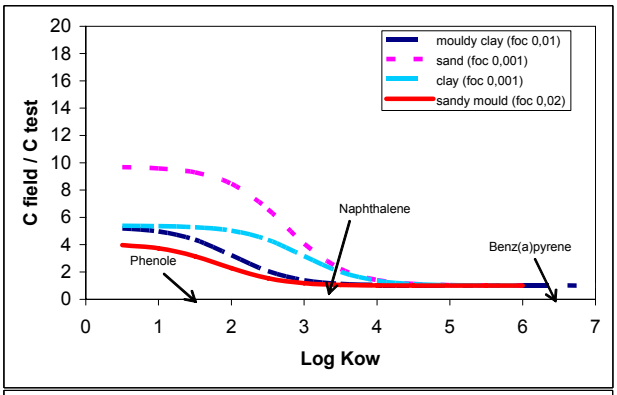
Assuming that the distribution coefficient determined in the leaching test is identical to the distribution of contaminants under field conditions following expression can be obtained by combining equation 6.2 and 6.3.

$$\frac{C_{field}}{C_{test}} = \frac{\left(S \cdot K_d + L_{test}\right)}{\left(S \cdot K_d + L_{field}\right)} \qquad 6.4$$

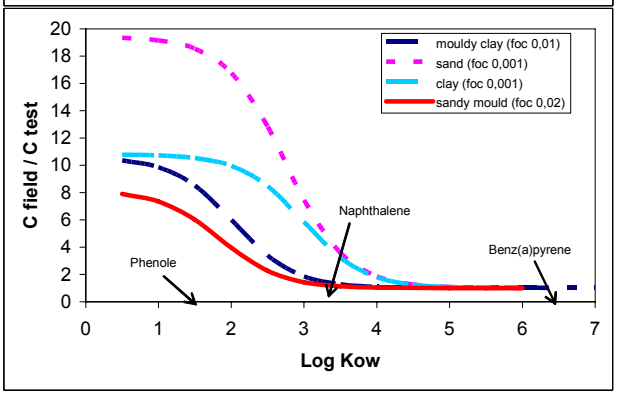
It should be emphasized that the K_d values determined in leaching test is based upon desorption processes. Leachate concentration consists partly of truly dissolved contaminants and partly of contaminants bound to DOC and colloids. Thus, the assumption of identical K_d values in leaching test and in field implies that the presence of colloids and DOC in leaching test and in field is identical, which is in most cases probably a rough assumption, as the soil has been disturbed by sampling, treatment and testing. However, research is needed in order to address this issue.

Figure 6.5 shows the relation between the pore water concentration and the leached concentration as a function of Log K_{ow} (octanol/water ratio). The K_d-value used in formula 6.4 is calculated based on Abdul's formula (Abdul et al., 1987) and assuming f_{oc} (fraction of organic matter) varying between 0,001 and 0,02 and water content between 0,15 and 0,35 lowest for sand and highest for sandy mould (The scenarios are defined in Jagg-model (Danish EPA, 1999). The calculations have been conducted for different L/S-ratio under which the leaching test was conducted. This figure illustrates that the hydrofobicity of the organic compounds and the content of soil organic matter have a significant influence on the relationship between the leaching test concentration and the pore water concentration. For compounds that are strongly bound in soils the leaching test concentration is almost identical to the pore water concentration.

L/S 1,0 l/kg



L/S 2,0 l/kg



L/S 10 l/kg

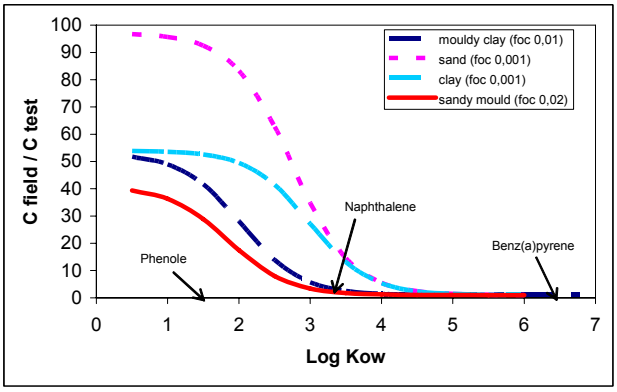


Figure 6.5. Relation between the pore water concentration and the leached concentration as a function of Log Kow calculated for different L/S ratio at which the leaching tests are conducted (L/S 1, 2 and 10 l/kg). The soil properties are defined in the Danish Jagg model (Danish EPA 1999).

However, in each case where a specific contaminated site is investigated the pore water concentration has to be estimated based on leaching test results and using formula 6.4 with site specific data in the calculations.

The estimated pore water concentration may be used to calculate the flux of contaminants from the contaminated soil.

It should be emphasised that the distribution coefficient determined in the leaching test can not be used for describing retardation of contaminant in unpolluted soil when the contamination is transported through the vadose zone and the aquifer.

6.3.2 Flux based evaluation

A flux based evaluation of the leaching test results require that a specific scenario is given. In case of using leaching test for evaluating contaminated sites the specific sites from where samples are collected constitute the scenario.

The flux of contaminant expresses the amount of contaminant released per surface area per time and can be calculated from 6.1

$$J = C_{field} \cdot N \cdot 10^{3} \qquad (6.1)$$

Where
J is the flux (mg/m^2 *year)
C_{field} is the pore water concentration estimated from the results of the leaching test (mg/l)
N is the net precipitation (m/year)

This flux of contaminants may be used as input for impact assessment models as for example done in the TAC model (see underlagsrapport 3).

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Underlagsrapport 2b: Tester för bedömning av oral biotillgänglighet vid intag av jord

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NATURVÅRDSVERKET

Summary and recommendations

Most quality criteria and cleanup levels (maximum contaminant levels, MCL's) for soil contaminants are based upon oral exposure and toxic effect studies with contaminants as pure chemical substances ingested with water or with food. When ingested with soil, the oral bioavailability of substances such as metals and polycyclic aromatic hydrocarbons (PAH) is likely to be different from that in the studies that the MCL's were based upon.

Dissolution of food, soil and contaminants take place throughout the human gastro-intestinal system. Uptake of the contaminants predominantly takes place in the small intestine, where conditions range from the slightly acidic, high chloride gastric conditions just after transit from the stomach to subsequent neutral to slightly alkaline, high phosphate intestinal conditions. The chemical conditions in the gastrointestinal tract are complicated and vary between individuals of different physiology, age, health etc. and for each individual with parameters such as feeding conditions, activity etc.

All metals can occur in different mineral forms and associations, and as constituents (species) depending upon the source of contamination and the weathering of the contaminated soil, and these differences will impact the human, oral bioavailability of the metals from soil. Similarly, PAH are expected to exhibit reduced availability after aging of a PAH contaminated soil.

Oral bioavailability of soil contaminants can only be measured using experimental animals with oral uptake physiology resembling that of humans (or in humans): in vivo studies. Documented and accepted *in vivo* methods are available for measuring the bioavailability of lead from soils using e.g.: juvenile swine and the methods have been applied also for cadmium and arsenic. Bioavailability will vary with the experimental animals, the experimental set up and the calculation methods used, and the measurements are associated with the variability inherent in all work with biological systems. No accepted method is available for *in vivo* measurement of the bioavailability of organic contaminants such as PAH from soils, primarily because of the problems associated with the metabolization of such compounds during digestion and uptake. 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 report, but reduced oral bioavailability has been reported for at the least arsenic, cadmium, lead and PAH.

In vivo bioavailability studies with experimental animals are costly and associated with ethical concerns. Therefore, simulation of the dissolution of soil contaminants in the human gastrointestinal tract in laboratory tests has been suggested (*in vitro*) to provide an upper limit of human, oral bioavailability: the oral bioaccessibility.

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

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. Still, the data material is not sufficient to establish whether, to what degree and for which contaminant bioaccessibility is rate or dissolution limiting.

Data are available from the open literature on bioaccessibility of soil contaminants, in particular for lead and arsenic, to some degree for cadmium, but very limited for nickel and for organic contaminants such as PAH, PCB and dioxins. The overall picture is that reduced soil bioaccessibility is very likely for cadmium and lead, likely for arsenic, and possible for nickel and PAH, PCB and dioxins. The oral bioaccessibility of the contaminants is highly variable even within the same soil type, source type and test, as far as can be concluded from the limited data available.

A number of different *in vitro* test methods are available to measure oral bioaccessibility of soil contaminants, but the results are not generally comparable between methods. Bioaccessibility methods are associated with the variability inherent in all test procedures. The data on quality of the bioaccessibility test methods are limited but methods of required quality are available or can be made available.

It is mandatory and urgent for the future use of bioaccessibility testing of soil contaminants that one single method is agreed upon internationally. 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.

The selection of oral bioaccessibility test methods should emphasize that the methods are:

- justifiable (simulate relevant processes)
- robust (can be repeated with “the same” result)
- relevant (can be correlated to uptake measured in animals or humans)

As an example, the RIVM fasted state method simulates the digestions processes in the mouth, oesophagus, stomach and upper small intestine of fasted children and is thus aiming at a precautionary approach (“realistic worst case”) for metal bioaccessibility. The RIVM fed state method simulates the digestions processes in the mouth, oesophagus, stomach and upper small intestine of fed children and is thus aiming at a precautionary approach (“realistic worst case”) for bioaccessibility of apolar, organic contaminants such as PAH.

Implementation and validation of the RIVM tests for the metals cadmium, lead, nickel and the PAH benzo(a)pyrene (BaP) and dibenz(a,h)anthracene (DBahA) at the DHI laboratories for the Danish Environmental Protection Agency (DEPA) has demonstrated that established quality objectives could be met, yielding satisfactory test analytical detection limits and linearity, as well as reasonable precision, whereas “trueness” could not be evaluated due to lack of reference materials or interlaboratory studies addressing the methods selected. For lead, an unsatisfactory between series and between laboratories precision was obtained for the RIVM fasted state

test method. A quality control scheme and a sampling and sampling plan enabling assessment of site variability and test precision has been suggested. During implementation and validation of the test methods, the need for careful evaluation of the quality of analytical methods, sample preservation and test methods has been demonstrated.

In application of the RIVM test methods for 7 Danish contaminated sites, relative bioaccessibilities well below 100 % were found for most of the contaminants tested for. Table 0.1 shows the range of reduction factors for bioaccessible concentrations from total concentrations that can be expected based upon the data from the 7 Danish sites. It should be noted that the reduction factor for lead is probably overestimated due to the excessively low bioaccessibilities obtained with the RIVM fasted state method for lead, see below.

Table 0.1. Realistic reduction factors for estimation of bioaccessible concentrations from total soil concentrations based upon data from 7 Danish sites

	Cd	Pb	Ni	BaP	DBahA
Range of reduction factors	1–3	(1–4)[1]	1–10	1–20	1–20

Furthermore, the application of bioaccessibility tests to the 7 Danish sites demonstrated that the variability of bioaccessible soil contaminant concentrations was of the same order of magnitude as the variability of the total soil concentrations.

In the literature, correlation between in vivo bioavailability data and *in vitro* bioaccessibility data have been demonstrated with some tests for lead, and to some degree for cadmium and arsenic. The correlation for lead reported in the literature is best, if test methods with a stomach segment only are considered.

The correlation obtained in the study for the DEPA for cadmium RIVM fasted state in vitro bioaccessibility data and *in vivo* bioavailability data (published data, soils made available by the scientists responsible) demonstrated a satisfactory correlation. For lead, the correlation was not satisfactory (non-linear and low in vitro test data). For nickel and PAH, soil samples with *in vivo* bioavailability data of accepted quality were not retrieved and the correlation thus not evaluated.

The poor between laboratory and between series variability, as well as the poor *in vitro* to *in vivo* correlation for the RIVM fasted state method lead data were attributed to the high and insufficiently stable pH in the stomach and intestinal segment of the test, as well as to a lead precipitating effect of other soil constituents.

As alternatives, the *in vitro* to *in vivo* correlation of a version of the RIVM fasted state test with mouth/oesophagus and stomach segments only (RIVM fasted state stomach only), i.e.: without the intestinal segment, and the SBRC method widely used in the US was studied for cadmium and lead. For cadmium, the alternative test methods did not provide improved correlation. For lead, the SBRC provided linear in vitro to in vivo correlation with high *in vitro* data, and the RIVM fasted state stomach only also provided linear correlation with slightly more realis-

[1] Reduction factor probably overestimated, see text.

tic bioaccessibilities, if soil samples giving too high pH in the test solution were excluded.

For all contaminants except for lead, a linear correlation was found between the RIVM test data and the data obtained with other *in vitro* bioaccessibility test methods, and the RIVM data were in general similar to or higher than data obtained with other methods. For lead, a linear correlation of RIVM data was found for most methods, but the RIVM data were low compared to the data obtained with other methods. For the alternative RIVM fasted state stomach only, the data were linearly correlated to *in vitro* data obtained with other test methods for cadmium, but not for lead (all samples including pH outliers). For the alternative SBRC method, the data were just linearly correlated to *in vitro* data obtained with other test methods for lead, but not for cadmium.

The overall evaluation of the applicability of the bioaccessibility test methods for use in risk assessment of contaminated sites for oral exposure is summarized in Table 0.2.

Table 0.2. Summary of the applicability of the bioaccessibility test methods for risk assessment of selected soil contaminants for oral exposure

	Cd	**Pb**	**Ni**	**Arsenic**	**Organic contaminants**
RIVM fasted state	Quantitative applicability	Not applicable	Qualitative applicability	Not evaluated	Not evaluated
RIVM fasted state stomach only	Not applicable	Quantitative applicability with reservations	Not evaluated	Not evaluated	Not evaluated
RIVM fed state	Not evaluated	Not evaluated	Not evaluated	Not evaluated	Qualitative applicability
SBRC	Not applicable	Quantitative applicability with reservations	Not evaluated	Not evaluated	Not evaluated
IVG, literature based	Quantitative applicability	Quantitative applicability	Not evaluated	Quantitative applicability	Not evaluated

Test methods are evaluated as suitable for quantitative application, if satisfactory test robustness and in vivo correlation has been demonstrated. Test methods are evaluated as suitable for qualitative application, if satisfactory test robustness has been obtained and in vivo data have not been available for correlation. The "reservations" for lead are that a pH stable RIVM fasted state stomach only test would be preferable but in the absence of such a method, the SBRC is a, conservative, alternative.

In risk assessment, it is suggested to evaluate the MCL's against the bioaccessible concentrations, C_{ba}, of the contaminants in the soils calculated as:

$$C_{ba} = \frac{C * RAC(\%)}{100(\%)}$$

In the equation, C is the total soil contaminant concentration and RAC is the bioaccessibility in % of the contaminant in the site soil samples relative to the bioaccessibility of the contaminant in soluble form comparable to that used in toxicity studies behind the MCL.

Reduced bioaccessibility and/or bioavailability have 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. Endorsement of bioaccessibility tests as part of risk assessment of contaminated soils (oral, human exposure) is under consideration in the Environmental Protection Agencies of the US and Denmark, and studies have been initiated by the environmental authorities in the UK, Germany and the NL.

The general conclusion is that correction of soil contaminant concentrations for bioaccessibility in evaluation of compliance with soil quality criteria and cleanup levels based upon reduced oral bioavailability/bioaccessibility of the contaminants may be recommended in a site specific risk approach. Conversely, the data available at present do not allow for general regulation of soil quality criteria and cleanup levels for specific contaminants, soil types or sources.

As short term recommendations, it is suggested to:

- endorse the use of bioaccessibility testing for those contaminants and those test methods that are robust and exhibit proven correlation between *in vivo* and *in vitro* data
    - purpose: to ensure utilization of accessible information on contaminant availability in risk assessment of contaminated soils in order to achieve cost efficient and safe remediation
- prepare guidelines describing test methods to be used, quality control, data quality objectives and practical use in risk assessment
    - purpose: to ensure that the tests are applied in a uniform and transparent form with sufficient but not excessive test quality
- establish a national set of reference values of bioaccessibilities for the typical, important sites where availability is expected to be included in risk assessment (oral exposure based)
    - purpose: to provide the site investigator with the background for deciding for or against including bioaccessibility test in the study, and the administrator with the background of evaluating the obtained bioaccessibility data
- establish stable and homogenous reference materials certified to the selected test(s) for mandatory use in all *in vitro* bioaccessibility test series intended for use in risk assessment
    - purpose: to enable the laboratories and the data users to evaluate and compare test quality

Selection of contaminants for test endorsement and accompanying measures should take into account the national significance of each compound as soil contaminant (toxicity and occurrence).

As a long term recommendation, it is suggested to perform:

- 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, BARGE, US EPA, UK EA, DEPA, RIVM)
    - 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 important contaminants

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

- identification of *in vivo* segment of contaminant uptake
    - purpose: to enable precise selection of test segment conditions (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 of redox sensitive species
- 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 limiting for bioavailability
- development of robust, validated and accepted in vivo methods for measurement of bioavailability in relevant experimental animals for contaminants without such methods available, in particular for organic contaminants such as PAH
    - purpose: to enable validation of in vitro bioaccessibility test methods against in vivo data for a broader selection of contaminants

1 Introduction

Soil quality criteria and cleanup levels for soil contaminants are frequently based upon toxicity studies of oral exposure with soluble, highly bioavailable contaminant forms ingested with water or with food. When ingested with soil, metals and PAH are likely to be less bioavailable than in the toxicity studies. Reduced bioavailability of soil contaminants may reduce the risk at a contaminated site and therefore, the Environmental Agencies and research institutions of several countries have worked to provide methods to assess the soil contaminant bioavailability for human, oral exposure.

Assessment of oral contaminant bioavailability requires uptake studies performed in experimental animals or humans (*in vivo* data) but as these data not readily available, laboratory tests for dissolution of soil contaminants in the human gastro-intestinal system are currently under development, *i.e.: in vitro* bioaccessibility tests.

This summary report is intended to give the rationale for applying bioaccessibility tests in risk assessment for human, oral exposure of selected soil contaminants, to present and discus test methods, and to provide examples of test data and their validity. The report is primarily based upon three reports prepared for the Danish Environmental Protection Agency (DEPA):

- Human bioaccessibility of heavy metals and PAH from soil /1/
- Human bioaccessibility of soil contaminants /2/
- In vivo bioavailability and in vitro bioaccessibility of soil contaminants /3/

and one report prepared for the Environment Agency, United Kingdom (UK EA):

- Test for bioaccessibility of metals and PAH from soil, test selection, validation and application /4/

The reports summarize data from the open literature and projects results. Besides upon these reports, the text in the current report is based upon a number of reviews and textbooks that are not explicitly quoted /5–20/, in addition to published methods and studies quoted with precise references.

The first literature review soil /1/ and its recommendations were discussed with an international reference group:

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

Selected results of the bioaccessibility testing /2;3/ were presented and discussed at workshops arranged by UK EA (Oxford, UK, March 15, 2005) and the Bioaccessibility Research Group of Europe (BARGE, Hørsholm, Denmark, April 19–20, 2005).

The report is subdivided into 8 main sections:

- Bioavailability and bioaccessibility of soil contaminants in risk assessment
- Physiology of the human contaminant uptake
- Chemistry of selected soil contaminants
- Bioavailability (study methods)
- Bioaccessibility (test methods)
- Bioaccessibility (data)
- Bioaccessibility (correlation to bioavailability)
- Developments and perspectives

The emphasis in the Danish projects has been upon the soil contaminants cadmium, lead, nickel (metals), benzo(a)pyrene (BaP) and dibenz(a,h)anthracene (DBahA, polycyclic aromatic hydrocarbons, PAH). Information on other soil contaminants has to a limited extent been included in the literature review, but more extensive studies on other contaminants such as arsenic are available in the literature and in reports, see e.g.: /16;21–26/.

The report has been prepared by Christian Grøn, DHI Water & Environment for Statens Naturvårdsverk under the funding program Hållbar Sanering.

2 Bioavailability and bioaccessibility of soil contaminants in risk assessment

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

2.1 Toxicity and exposure

The limit values for soil (the maximum contaminant limits for soil, MCL's) are generally calculated on the basis of a (provisional) tolerable daily intake value (TDI) or a (provisional) tolerable weekly intake (TWI), 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. Examples of applied risk levels are doses comparable to excessive risks of 10^{-5} or 10^{-6} *i.e.:* a calculated hypothetical risk of one extra cancer outcome among 100.00 or 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_{al}) 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 /27/, and MCL's may be developed based upon oral uptake by children /28/. The MCL for soil ingestion is obtained by dividing the TDI (corrected for allocation) with the estimated daily soil exposure (EDE):

MCL (mg contaminant/kg soil) =
TDI (mg contaminant/person/day) x f_{al}/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 was administrated to the animals mixed in the feed or in drinking water (the vehicle or transporter of the contaminant). The amount of contaminant needed to produce adverse health effects in the animal is then recorded. As an alternative, epidemiological studies relating observed human health effects to recorded exposures have been used[2]. Most toxicological studies report the total ingested amount only and do seldom indicate exact values for the bioavailability of the substances administered.

[2] 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 %) /56/.

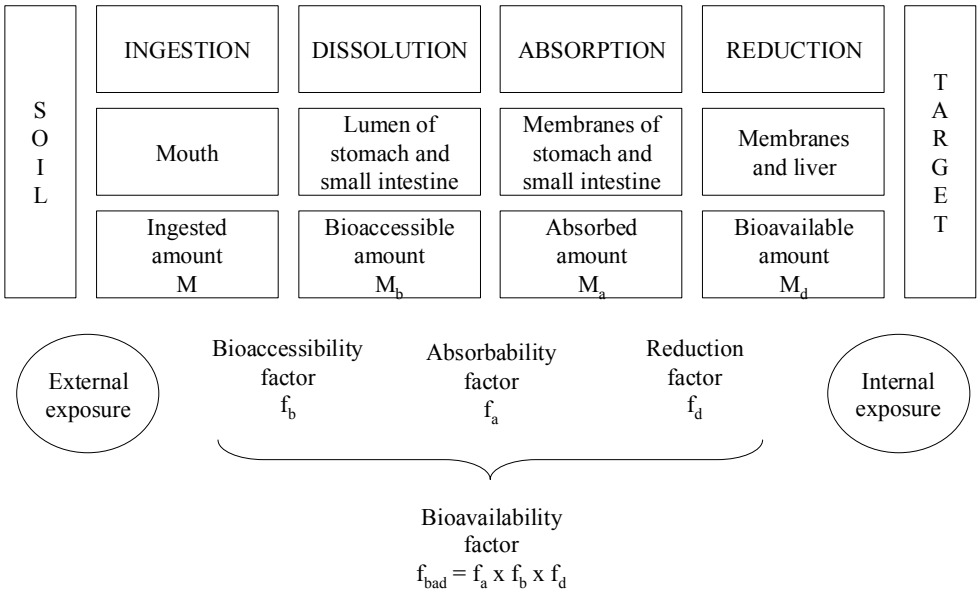
2.2 Bioavailability

When extrapolating from such experimental conditions to other conditions e.g.: to intake of contaminated soil, this approach requires, that the uptake 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 = 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, or reaches relevant target organs. 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 or an internal target organ, 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, see also chapter 3.

Figure 2.1 Schematic presentation of oral uptake processes.



2.3 Relative bioavailability

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

RAF = amount taken up from soil matrix/amount of soluble contaminant 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 or relevant target organ when ingested with *e.g.:* soil and the amount in circulation or target organ when ingested in the toxicity experiment.

2.4 Application of bioavailability in risk assessment

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 generic RAF value could be found and agreed upon, this would then result in a proportional change in the MCL:

$MCL_{true} = MCL/RAF$

Alternatively, the concentration held against the MCL is not the total soil contaminant concentration, C, but the concentration of bioavailable soil contaminant, Cb:

$C_b = C \times RAF$

In this approach, the original, exposure allocation and toxicity based MCL is maintained and furthermore, the approach can be applied in site specific risk assessment.

For substances where the critical toxic effect is not systemic toxicity but local toxicity (*i.e.:* local irritation, intestinal cancers), the toxic effect is considered to be dependent of the concentration in the gastrointestinal tract, and the MCL will depend directly upon bioaccessibility, see section 2.5, rather than the bioavailability.

It should be noted that although most relative bioavailabilities are less than 1 and would result in an increased MCL ($MCL_{true} > MCL$) or a lower bioavailable soil concentration than total soil concentration ($C_b < C$), RAF values above 1 could be found that would result in a demand for a decreased MCL and thus for increased requirement for intervention.

The US EPA allows for using the concept of relative bioavailability in risk assessment /29/, but does not give guidance to the practical implementation yet. Still, according to recent reviews /10/,/11;30/ several state regulatory agencies have issued guidance documents. In the US and in Canada, RAF values have been used

to increase cleanup levels after risk assessment on a case by case basis. Adjustment of cleanup levels based upon bioavailability studies has been reported from the US for arsenic, lead, mercury, PAH, PCB and dioxins /10;11;30/ and from Canada for lead and nickel /31/.

Adjustment of the bioavailability is an option in the US EPA model for risk assessment of lead uptake in children /32/. Site specific *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 site specific in vivo studies with experimental animals resembling humans, e.g.: with immature or juvenile swine /10;31/.

A general guidance /6/ on whether to include bioavailability studies would be:

- if a bioavailability significantly lower than 1 is likely to result from a bioavailability study,
- if the total soil concentrations do not exceed the MCL by too much,
- and if the costs of cleanup are sufficiently large,

a bioavailability study is worth considering.

2.5 Bioaccessibility

The bioaccessible fraction of a soil contaminant is the fraction that can be dissolved in the gastrointestinal tract of the organism in question, compare Figure 2.1. The bioaccessible concentration is considered the upper limit of the contaminant concentration that can reach systemic circulation and thus cause a systemic toxic impact, again compare Figure 2.1, factors f_a and f_d are in the range 0–1.

Bioaccessibility is generally measured using an in vitro laboratory test simulating the conditions in the gastrointestinal tract of humans, but in vivo measurements of soil contaminants in the stomachs and intestines of animals have been reported, see e.g.: /33/.

The use of *in vitro* bioaccessibility tests is a possible substitute for *in vivo* bioavailability data in risk assessment for human, oral exposure. Thus in Europe, the emphasis in risk assessment is currently on developing *in vitro* tests for bioaccessibility as an estimate of the bioavailability of soil contaminants /18/. Also, the US EPA is moving towards accepting “validated” *in vitro* tests for lead. 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

2.6 Application of bioaccessibility in risk assessment

As requirements for bioaccessibility test methods to be applied in risk assessment of contaminated soils it should be considered that the methods are:

- justifiable
- robust
- relevant

With "justifiable" is meant that the test simulates the appropriate processes in the human gastrointestinal tract, *i.e.:* is based upon human physiology. The physiology of the human gastrointestinal tract has been reviewed elsewhere (see *e.g.:* /18;34/) and a short introduction is given in section 3. For physiologically based test methods, the selection of the segments (mouth, oesophagus, stomach, small intestine, colon etcetera) to include and the conditions in each segment should reflect the selected target (fasted child, fed adult etcetera) and the physiology of contaminant uptake.

"Robust" methods can be reapplied at the same laboratory or at another laboratory giving approximately the same result for the same soil, *i.e.:* the within laboratory and between laboratory variations are sufficiently small. Robustness evaluation should also include that the test can be applied with method detection limit resembling what is required considering the MCL's in question.

The term "relevant" means that the test yields results that reflect the in vivo bioavailability of the soil contaminants, *i.e.:* that there is a linear correlation between the in vivo bioavailability as obtained in experiments with accepted animals (or humans) and the *in vitro* test results and preferentially, that the *in vitro* test results are in general equal to or slightly higher than the *in vivo* bioavailabilities in order to respect the precautionary principle.

In risk assessment, the total soil concentrations, C, and the relative bioaccessibilities, RAC, are used to calculate the bioaccessible concentrations, Cba, of the contaminants in the soils according to:

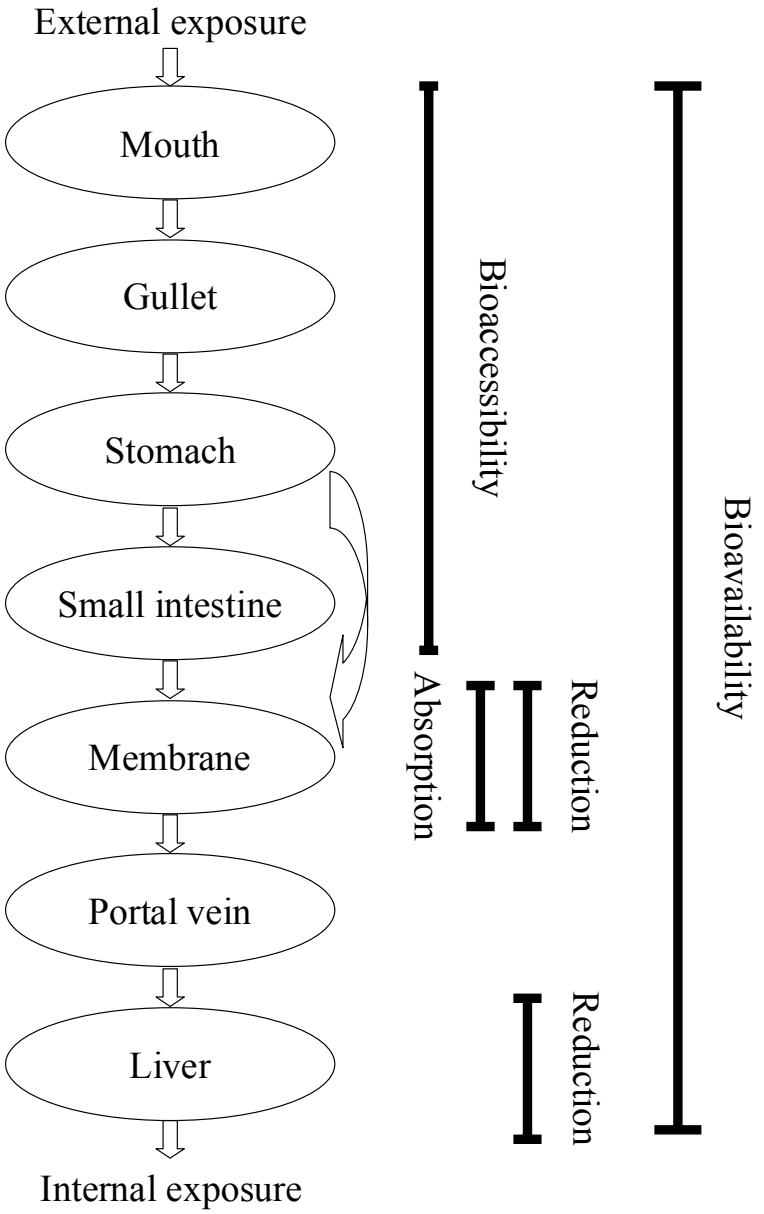
$$C_{ba} = \frac{C * RAC(\%)}{100(\%)}$$

The relative bioaccessible concentrations are subsequently held against the MCL instead of the total soil concentration, compare section 2.4.

3 Physiology of the human contaminant uptake

A series of segments are involved in human uptake of ingested soil contaminants, Figure 3.1.

Figure 3.1 Segments 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).

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 /35/. The environment in the segments differs and accordingly impacts the bioaccessibility process differently, Table 3.1.

Table 3.1 Functions and conditions in the segments involved in bioaccessibility processes, combined from /10;13;18;35/

Segment	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[3]	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 Solubilization 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 solubilized by bile Cationic metals are solubilized 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 (½-time for emptying) in the stomach varies similarly from 8–15 minutes to ½–3 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.

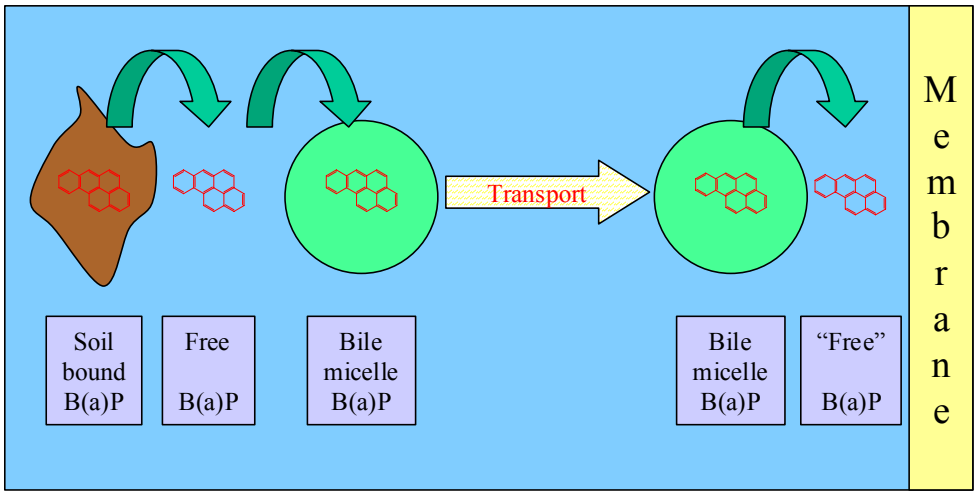
It should be noted, that the gastrointestinal tract constitute a complex ecosystem with aerobic and anaerobic microorganisms /34/. The density of microrganisms is less in the human stomach and in the upper part of the small intestine but

[3] Gullet also: oesophagus.

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 /36/. Sulfate reducing bacteria have been detected in the human large intestine /37/ but on the other hand, high concentrations of oxygen have been detected throughout the gastrointestinal tract of pigs /38/. 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.

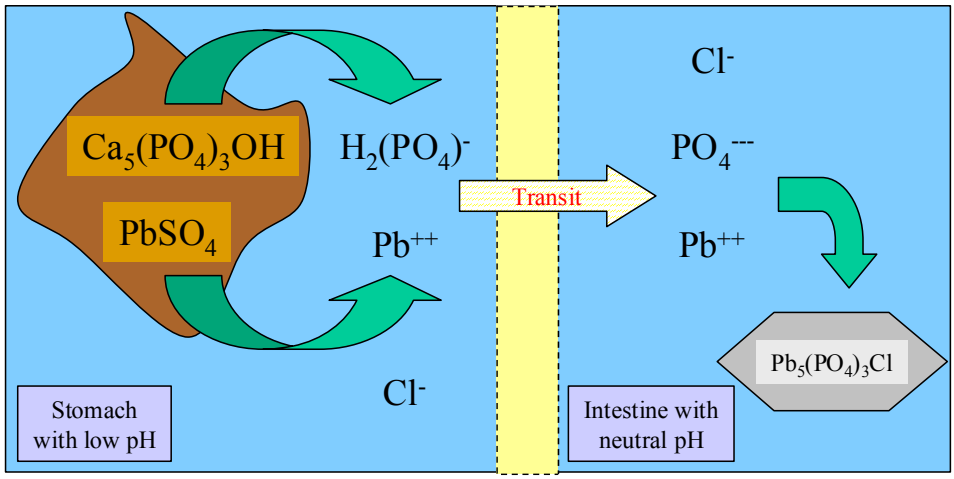
Absorption requires that the contaminants are dissolved (free or bound to a dissolved carrier such as bile), transported to the gastrointestinal wall and, if bound to a carrier, released at the surface of the gastrointestinal membrane for absorption, Figure 3.2. The carrier mechanisms can be dissolution 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. 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, $Pb_5(PO_4)_3Cl$) /39/. The phosphate can originate from digested food or from the soil.

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

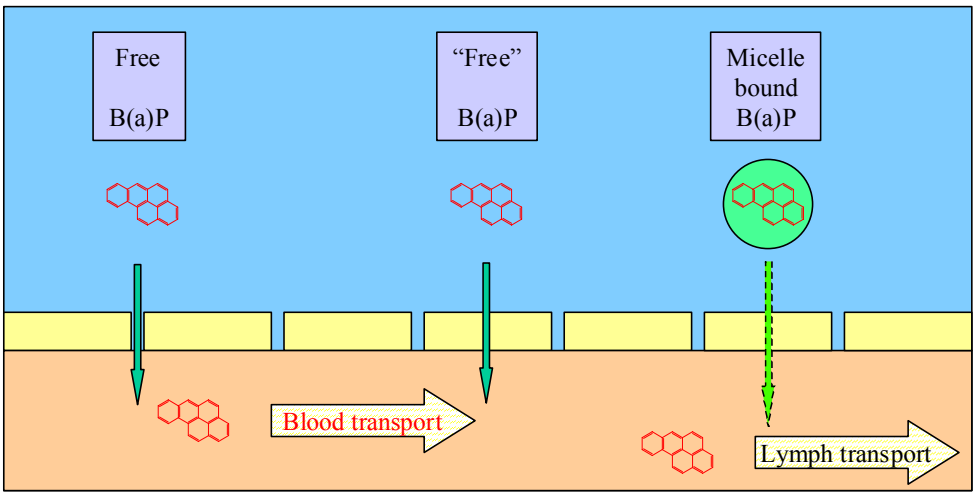


Phosphate minerals, such as hydroxyapatite, $Ca_5(PO_4)_3OH$, will dissolve in the low pH of the stomach, but dissolution will be slower and less complete with higher pH in the stomach (as occurring after food ingestion). If stomach transit is fast (as occurring under fasting conditions), the hydroxyapatite 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 chloro-leadphosphate. 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, chemistry of simultaneously ingested food and feeding conditions 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 /13/ 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, reincorporation of the contaminants in mixed micelles with lipids followed by secretion of these into the lymphatic circulation /40/. 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 /41/. 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 /42/.

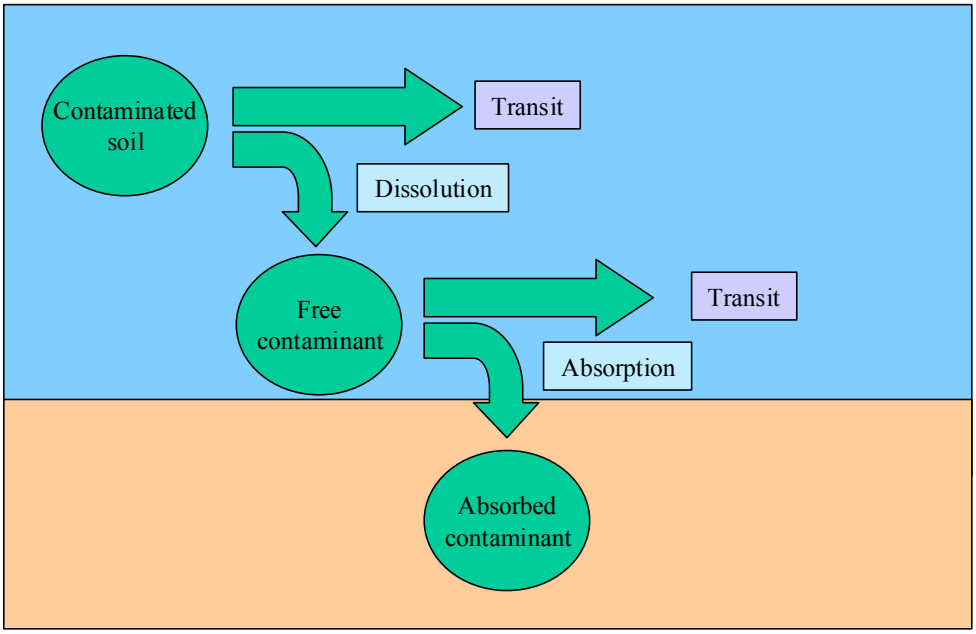
Reduction and transformation of the absorbed contaminant concentrations takes place in the epithelium membranes (binding and exclusion) and cells (degradation and transformation of organic contaminants), as well as in the liver (degradation and transformation of organic contaminants, transformation of 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, see section 2.3, 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 to slow to be completed before transit), f_a will be "in charge" of relative bioavailability.

Figure 3.5 Dissolution and absorption as rate limiting processes of human uptake of contaminants, modified from /43/.



A test for bioaccessibility of contaminants in soil should thus 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.

4 Chemistry of selected soil contaminants

The human uptake is highly dependent upon the chemical conditions encountered during digestion, see chapter 3, but also upon the matrix and chemical form (speciation) of the contaminants. The specific physiochemical 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). This can be illustrated considering common soil contaminants such as polycyclic aromatic hydrocarbons (PAH) and metals (As, Pb, Cd, Cr, Cu, Ni and Zn, where As strictly speaking is a metalloid and not a metal but for simplicity, the term metals is used for all the inorganic elements in this review).

Figure 4.1Structures of selected PAH.

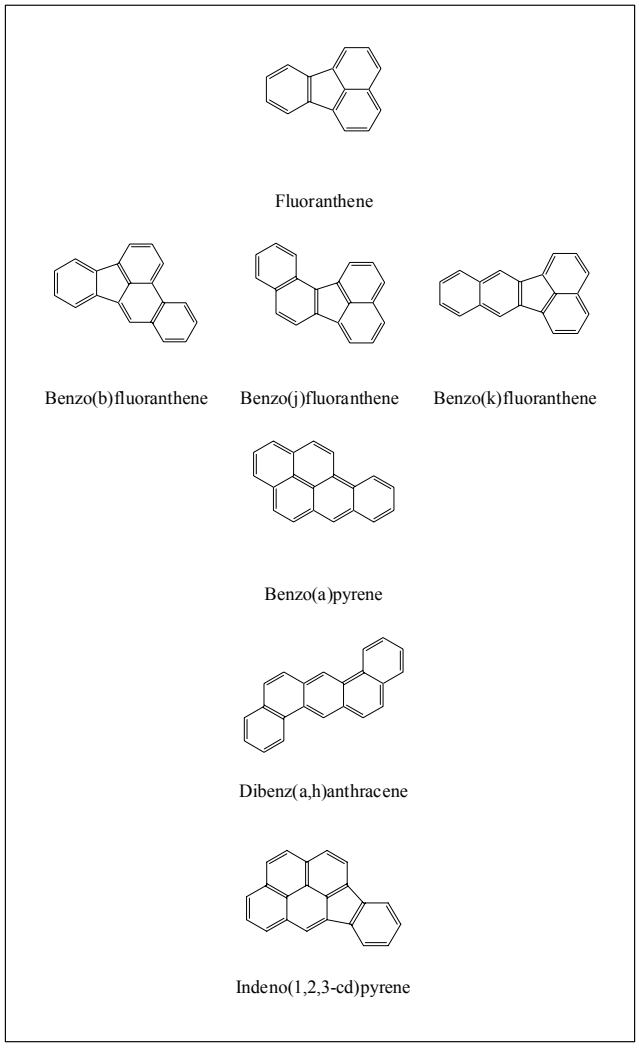


Table 4.1 Physiochemical data of selected PAH /44/

Property	Fluoran-thene	Benzo (b+j+k) fluoran-thene	Benzo(a) pyrene	Dibenz (a,h)anthracene	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 pres-sure 10^{-6}Pa	1300	0.013–0.5	0.73	0.013[4]	0.017[2]
Water solu-bility µg/L	210	0.8–3	3.8	0.5	0.19[2]
Partitioning 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 Physiochemical data of selected metals /5;7–9;11;17;44/

	Arsenic	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
	As	Cd	Cr	Cu	Pb	Ni	Zn
Aqueousspecies I	H_3AsO_3 $H_2AsO_3^-$	Cd^{++}	Cr^{+++}	Cu^{++}	Pb^{++}	Ni^{++}	Zn^{++}
Oxidation state	III	II	III	II	II	II	II
Aqueous species II	$H_2AsO_4^-$ $HAsO_4^{--}$	None	$HCrO_4^-$ CrO_4^{--}	None	None	None	None
Oxidation state	V		VI	-	-	-	-

Most metals are thus cationic species in aqueous solutions such as the human digestive juices, but arsenic and chromium may also be anionic.

Both organic contaminants such as PAH and metals are found in different phases and forms in soils and the differences will impact the dissolution of the contaminants in the human gastrointestinal tract and thus the bioaccessibility.

As 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. This means that the test should be able to dissolve all those species completely that would also be dissolved in the human gastrointestinal tract, but should also leave those species undissolved that would persist against the digestive juices.

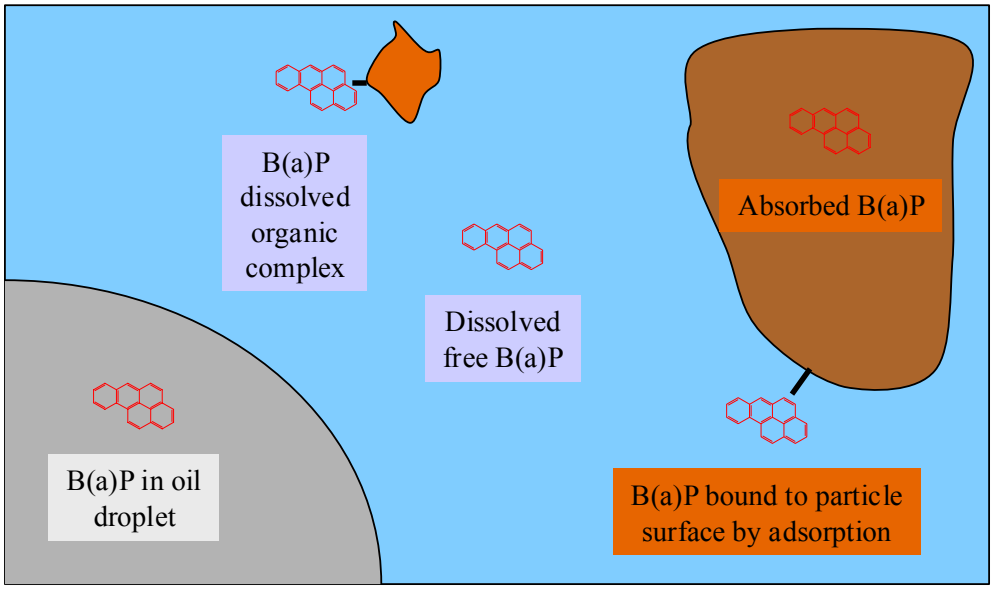
[4] Reference: /93/.

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 physiochemical 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.

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



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.:* /45/) and a more detailed discussion is beyond the scope of this report. Still, it should be noted that as low as 10 % bioavailability has been measured (as mutagenic activity) for benzo(a)pyrene in soil /46/, suggesting a significant effect of aging. Bioavailability reductions varying from 5 % to 50 % have been measured (as biodegradation) for 16 different soils /47/, suggesting large differences in the magnitude of the aging effect among different soils. Certainly, it has been suggested /48/ /49/ 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, will the bioaccessibility of separate phase benzo(a)pyrene differ from the accessi-

bility 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 further differ, depending upon the stability of the organic "complex" in the gastro-intestinal lumen, see section 3.

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 ("surfactant 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

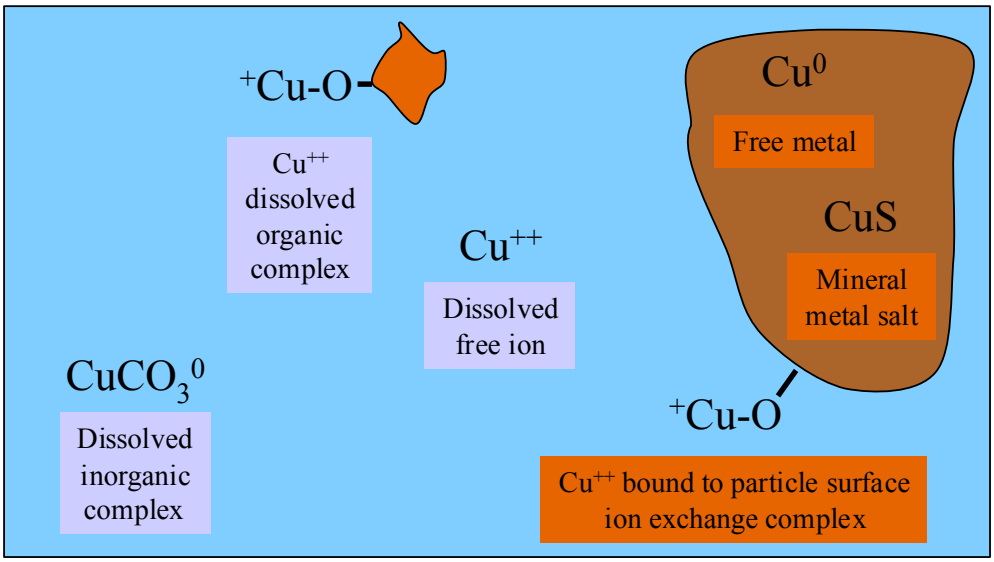
In assessing bioaccessibility of metals in soil, three major obstacles are encountered:

- most metals occur naturally at varying concentrations and in varying chemical forms
- chemical form (species) of the original metal (source) may vary from free metal to aqueous solution of a salt
- chemical forms are interchangeable depending upon the soil conditions and history

Assessment of the bioaccessibility 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) of metals in soils has been observed for As(V) /50/ and Cr /51/. For As(V), the aging 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 /50/. For Cr(VI), the aging effect 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 /51/. For Cr(III), the aging effect can be explained by slow (50 days) transformation of comparatively bioaccessible Cr^{+++} bound in particle surface ion exchange complexes to less bioaccessible $Cr(OH)_3$.

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. Also, due to their different physiochemical properties, the mechanisms for reduced bioaccessibility of metals differ among the metals.

The primary mechanism for reduced bioaccessibility of metals from soil will thus be presence of low solubility species (or for some metals even free form), adsorption to ironoxyhydroxides, soil organic matter and clay minerals, and binding in minerals. The most important factors for release from the soil will be acidic dissolution of species and minerals, alkaline dissolution of soil organic matter and “complexation” by bile acids and other organic and inorganic complex binders. Reduction of metals to more soluble species may be important as well.

5 Bioavailability study methods

In terms of relevance, the ultimate bioavailability measurements are studies in humans, followed by animal experiments (both *in vivo*) and then by *in vitro* tests.

In vivo studies 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, see Figure 2.1 and Figure 3.1.

Different approaches have been taken for in vivo bioavailability studies and three of these are frequently applied with soil contaminants /6;18;52/:

- excretion measurements
- blood kinetics
- target tissue measurements

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 considered the bioavailable fraction. Pros et contras are:
+ dissolution and transport under real conditions
+ removal by absorption under real conditions
+ the transport over the epithelium membrane during absorption is 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 metabolized /7/.

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 contras are:
+ dissolution and transport under real conditions
+ removal by absorption under 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 analyzed 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

One bioavailability study has been performed applying blood measurements in humans on the bioavailability of lead from a contaminated soil /53/.

In target tissue measurements, the contaminated matrix is ingested and after due delay, the resulting concentration is measured in a target tissue such as the liver, if liver cancer is the effect driving the MCL. Pros et contras are:
+ dissolution and transport under real conditions
+ removal by absorption under 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
+ distribution and potential tissue accumulation included

- metabolites not considered, unless specifically analyzed 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 /7/. Use of this method is valid only for contaminants where the liver is the major organ for distribution and metabolization and this should be verified in advance.

All *in vivo* methods for bioavailability measurements thus address the overall bioavailability including both bioaccessibility and absorption, 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 or correction, compare the US TDI for arsenic, see section 2.1.

A selection of *in vivo* methods that have been used for determining bioavailability of soil contaminants is given in Table 5.1, see also section 2.3 for explanation of the concept of relative bioavailability.

Table 5.1 Examples of experimental animals and study principles applied to estimate bioavailability of selected soil contaminants

Experimental animal	Targets	Principles	Contaminants	References
Juvenile swine	Weighted concentrations in blood and organs, for some contaminants selected organs or blood only	Bioavailability relative to soluble metal salt in feed	Lead[5]	/54/
Mini pigs	Concentrations in selected organs, secreted amounts with urine or with faeces	Absolute bioavailability or bioavailability relative to soluble form added with feed	Lead, nickel, cadmium, arsenic and PAH	/55/
Sprague-Dawley rats	Blood concentrations	Bioavailability relative to soluble metal salt in feed	Lead and arsenic	/56/
Sprague-Dawley rats	Weighted concentrations in blood and organs	Bioavailability relative to soluble metal salt in feed	Lead	/57/
New Zealand white rabbits	Blood	Bioavailability relative to soluble metal salt in feed	Lead	/33/
Humans	Blood	Absolute bioavailability applying isotope dilution	Lead	/53/
Mice	Excreted amounts in urine	Bioavailability relative to extract of soil PAH from same soils in feed	PAH	/58;59/

It should be noted that no generally accepted method exists for estimation of the bioavailability of organic contaminants such as PAH from soils due to the complexity imposed by the metabolization of most organic compounds.

In vivo bioavailabilities obtained with different experimental animals have been reported for one soil sample and for different soils samples from the same 4 sites, Table 5.2.

Table 5.2 Relative in vivo bioavailability of lead obtained with different experimental animals /33;56;60/

	Rats	Juvenile swine	Rabbits
One sample			
Butte MW-1	0.093	-	0.48
Same site, different samples			
Butte	0.093–0.23	0.19	0.48
Bingham Creek	0.36	0.28–0.31	-
Murray	0.41	0.53–0.71	-
Joplin	0.34	0.59–0.67	-

Evidently, the bioavailability found for soil lead depends upon the experimental animal/study method used. The US Environmental Protection Agency (US EPA) recently concluded in a study on stabilization of lead in contaminated soils that the

[5] Also used for cadmium and arsenic.

data obtained for relative bioavailabilities in different experimental animals and in humans did not show a clear correlation between the different bioavailabilities obtained /61/.

The effect of selected target organ upon in vivo bioavailability can be seen from the different relative bioavailabilities obtained for lead from the same soil samples, Table 5.3.

It can be argued that the target should be the organ relevant from a toxicological point of view as *e.g.:* blood for lead and kidneys for cadmium. Alternatively, lead bioavailability can be calculated as a weighted average of blood (weight 3), liver, bone and kidney (each weight 1) in order to obtain a robust, concentration independent (see below) relative bioavailability estimate /54/.

Table 5.3 Relative bioavailability measured in different target organs in vivo in rats and swine and in two different soil samples /57;62/

	Blood	Kidneys	Liver	Bone
Rats				
Joplin	0.34	0.48	0.27	0.34
Juvenile swine				
Butte	0.22	0.13	0.090	0.13

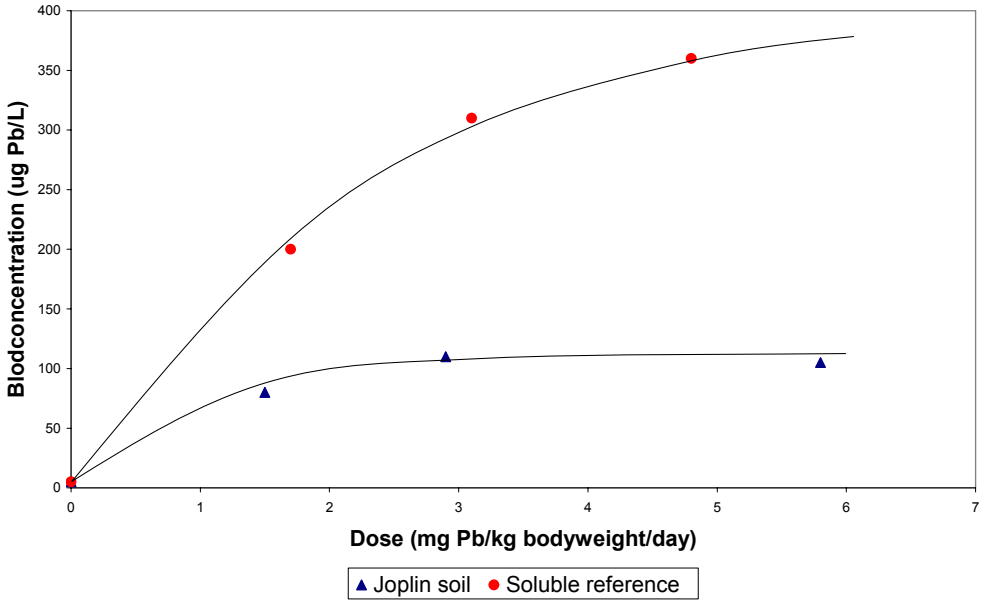
A linear dose response is generally seen with organs such as liver, kidney or bone as target organs but with blood as the target "organ", a non linear response has been observed with decreasing blood lead response at increasing exposure dose, Figure 5.1 /54;57/.

The uptake in blood is best fitted to an exponential function:

$$C_b = k_1 + k_2 * (1 - e^{(-k_3 * C_d)})$$

with k1, k^2 og k^3 as constants, C_b the blood concentration (µg Pb/L blood), and C_d the administered dose (mg Pb/kg bodyweight/day). It has been suggested that the non-linearity of the blood lead dose response curve is to some extent due to processes in the blood system rather than to non-linear uptake from the gastrointestinal system, considering also the linear dose response relationship seen for the other organs /63/.

Figure 5.1 Blood concentration of lead in response to dose administered as contaminated soil or as soluble lead salt, redrawn from/57/



As can be seen from Figure 5.1, both the absolute and the relative bioavailability of lead from both soil and soluble reference decrease with increasing dosage, as seen also in other studies, see *e.g.:* /64/. In order to account for this effect, it has been suggested to calculate the relative bioavailability of lead as the ratio between the maximum (plateau) blood concentration (k_2 in the exponential function) obtained from soil and from the soluble reference /57/.

To illustrate the different in vivo bioavailabilities obtained with different calculation points and methods can be mentioned, that the relative bioavailability of lead from Joplin soil was 0.44 calculated at 60 µg Pb/L blood, 0.32 at 100 µg Pb/L blood and 0.34 calculated from the maximum concentrations /57/.

All in vivo methods for bioavailability measurements are subject to large variability, as are all biological systems. As an example can be mentioned a range of relative standard deviations of 8–53 % for lead bioavailability measured for 6 soils in the same experimental animal species, with the same study design and using the same calculation methods /55/.

In summary, the relative bioavailability of *e.g.:* lead depends upon the experimental animal, the target organ and the calculation method employed.

6 Bioaccessibility test methods

In a recent review /1/, a range of factors for consideration in design of a suitable of in vitro bioaccessibility test method simulating human physiology were identified:

- buffered low pH (pH < 2) high chloride gastric segment
- buffered slightly alkaline (pH > 7) phosphate containing intestinal segment
- aerobic followed by anaerobic conditions (stomach and intestine, respectively, optional)
- separate assessment of bioaccessibility in the two segments (gastric and gastric followed by intestinal, optional, depends upon contaminant)
- addition of enzymes, bile and milk powder (or similar food constituent)
- sufficient reaction time in each segment (3 hours in gastric compartment, 10 hours in intestinal compartment)
- liquid to solid ratio (L/S) stability (L/S > 100)

No currently available method satisfies all these requirements, and the design of an *in vitro* bioaccessibility test method must thus be a compromise between a series of factors derived from contaminant chemistry, human digestion physiology and practical test considerations, in addition to factors derived from the anticipated use of the results in risk assessment of contaminated soils.

It should be noted, that most *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 /20/.

At the least 10 different methods for bioaccessibility testing of soil contaminants were identified in a recent review /1/. For 8 of these methods, the relevance of the test results, see section 2.6, can be evaluated by comparison with in vivo bioavailability data for one or more contaminated soils, see Table 6.1.

The details of the different test methods have been summarized previously, see *e.g.:* /1;20;65/.

Three different methods are currently widely used for routine testing:

- SBRC (Solubility/Bioavailability Research Consortium method developed from the Physiologically Based Extraction Test, PBET, method) /66/
- DIN (Deutsches Institut für Normung) /67/
- RIVM (National Institute of Public Health and the Environment) /68;69/

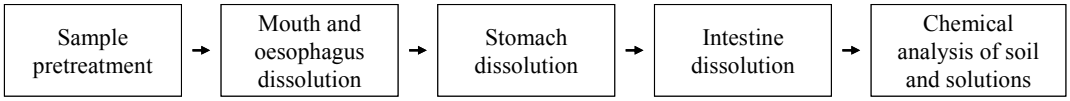
Table 6.1 Bioaccessibility test methods with in vivo bioavailability data for one or more contaminated soils

Method	Segments included	Addition of food	Principle	Contaminants tested	Reference
PBET	Stomach and intestine	None	Simplified based upon human physiology	Lead, arsenic	/56/
SBRC	Stomach, intestine optional	None	Simple buffered acid, aiming at robust worst case	Lead, arsenic	/70;71/
IVG	Stomach or intestine after stomach	Optional	Simplified based upon human physiology	Lead, arsenic and cadmium	/21;62;72/
MB	Saliva, stomach and intestine	None	Simplified based upon human physiology	Lead	/73/
RIVM	Saliva, stomach and intestine	Optional, part of fed state version of the test for organic contaminants	Corresponding to human physiology	Lead	/69;74/
DIN	Stomach and intestine, saliva optional	Optional	Corresponding to human physiology	Lead, cadmium, nickel, arsenic and PAH	/75/
SHIME	Stomach and small intestine, colon optional	Optional	Corresponding to human physiology	Lead	/76/
TIM	Stomach and intestine	Included	Dynamic simulation of human physiology	Lead	/76/

The SBRC method is also known as the Drexler method and is available though the Internet /71/. The original PBET method is still used at some European laboratories. The RIVM method comprises two versions, a fasted state version for metals such as lead and a fed state version for organic contaminants such as PAH, both of which are discussed in this report.

The general steps of the three test methods are presented in Figure 6.1 and Table 6.2, and details of the methods are summarized in the appendix to this report.

Figure 6.1 Outline of steps or segments in in vitro bioaccessibility test methods.



The three methods are all initially based upon human gastrointestinal physiology, considering factors such as composition of digestion juices, relative amounts of juices during digestion and ratios soil to digestion juices. The link to human physiology is maintained for the RIVM and DIN methods /68;77/, whereas the SBRC

method is simplified with the emphasis on obtaining a good correlation in vitro bioaccessibility to in vivo bioavailability with the most simple test /78/.

Table 6.2 Steps in the three selected in vitro bioaccessibility test methods

Test method	SBRC	RIVM	DIN	
		Fasted	Fed	
Mouth and oesophagus	No	Yes	Yes	Optional
Stomach	Yes	Yes	Yes	Yes
Intestine	Optional	Yes	Yes	Yes
Food addition	No	No	Yes	Optional
Contaminants	Metals	Metals	Organic	Metals and organic

The SBRC method with a stomach segment only is used widely in the US for lead, cadmium and arsenic bioaccessibility testing, and the test data are well correlated to juvenile swine in vivo bioavailability data, chapter 8, /79/ and /71/, for lead and correlated for arsenic. The stomach segment based SBRC test is also recommended for nickel, whereas addition of the optional intestinal segment is recommended for chromium and mercury /66/.

The RIVM fasted state method is described as a ”realistic worst case” test method /68/. In the presentation of the RIVM method /68/, it is stated that the small intestinal segment is included only and not the more distal parts of the intestine, because dissolution of e.g.: lead is primarily occurring in this part of the gastrointestinal system.

At this point, it should be emphasized that different test methods with different compositions of test solutions will inevitably yield different test results as also demonstrated employing five different test methods with each of three different soils /65/, see Table 6.3. Still, different test methods with different test solution compositions may all provide justifiable, robust and relevant test results as defined above, see section 2.6, just with different correlations between in vitro bioaccessibility test results and in vivo bioavailability data from animal studies.

Table 6.3 Bioaccessibilities of lead, cadmium and arsenic from 3 soils as obtained with 3 selected in vitro test methods /65/

	SBRC	DIN	RIVM
Lead			
Oker 11	0.56	0.16	0.29
Montana 2711	0.90	0.46	0.11
Flanders	0.91	0.31	0.66
Cadmium			
Oker 11	0.92	0.62	0.51
Montana 2711	0.99	0.45	0.40
Flanders	0.92	0.38	0.78
Arsenic			
Oker 11	0.11	0.11	0.19
Montana 2711	0.59	0.41	0.59
Flanders	0.50	0.30	0.95

The differences in bioaccessibilities obtained with different methods are obvious from the data in the table. Overall, the simple methods with a stomach segment seem to give higher bioaccessibilities than more physiologically correct methods with intestinal segments included. Still, which method is most "correct" depends upon the criteria set up for an acceptable method, see section 2.6, and in most cases upon the correlation between the in vitro bioaccessibilities and in vivo bioavailabilities, see chapter 8.

A more detailed description of each step in bioaccessibility tests including discussion and recommendations can be found in /4/, and a more detailed discussion of the processes behind in /1/.

For the implementation of in vitro bioaccessibility test methods in Denmark, a set of quality objectives were identified, Table 6.4, for the bioaccessibility tests /2/.

Table 6.4 Data quality objectives for bioaccessibility tests of soils.

Matrix		**Metals**		**PAH**	
Parameter	Cadmium	Lead	Nickel	Benzo(a) pyrene	Dibenz(a.h) anthracene
Linear range	From detection limit to 5 times the MCL for the contaminant tested for				
Relative standard deviation	<15%				
Analytical limit of detection (mg/kg dw)[6]	0.5	4	3	0.01	0.01

During the Danish first implementation of the RIVM fasted and fed state *in vitro* bioaccessibility test methods at DHI, the test quality was validated, Table 6.5 and Table 6.6.

Table 6.5 Test validation for RIVM fasted state method applied for the metals lead, cadmium and nickel

	Cadmium	**Lead**	**Nickel**
Analytical detection limit (mg/kg dw)	0.2	2	0.8
Precision total (%RSD)	7.2	20	8.8
Precision between series (%RSD[7])	6.9	19	8.4
Precision within series (%RSD)			
Soil	-	5.7	7.4
Test spiked to 1 x MCL	1.9	5.0	2.2
Test spiked to 5 x MCL	2.9	3.2	4.3
Recovery (% of added)			
Test spiked to 1 x MCL	99	61	99
Test spiked to 5 x MCL	98	80	98
Linearity (mg/kg dw)	25	(160)	130

[6] Set to 1/10 of the MCL to be tested against.
[7] RSD: relative standard deviation.

It should be mentioned that the RIVM fasted state test validation for lead with soil in the test did exhibit higher RSD (poorer precision) and lover recoveries than obtained for the other metals.

Table 6.6 Test validation for RIVM fed state applied for the PAH benzo(a)pyrene (BaP) and dibenz(a,h)anthracene (DBahA)

	BaP	**DBahA**
Analytical detection limit (mg/kg dw)	0.01	0.005
Precision within series (%RSD)		
Soil	13	-[8]
Soil spiked to 1 x MCL	14	15
Soil spiked to 5 x MCL	11	14
Recovery (% of added)		
Soil spiked to 1 x MCL	84	86
Soil spiked to 5 x MCL	73	78
Linearity (mg/kg dw)	0.01–50	0.005–50

Overall, the RIVM tests did exhibit satisfactory quality data compared with the quality objectives established for the purpose, except for the test quality for lead.

[8] Results below analytical detection limit.

7 Bioaccessibility data

7.1 Literature bioaccessibility data

In a recent review, the bioaccessibilities of 7 metals and 7 PAH reported in the literature were summarized and the bioaccessibility ranges to expect were estimated Table 7.1.

Table 7.1 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

It should be noted that the typical intervals in Table 7.1 are overall range estimates that should not be used for setting general bioaccessibility values. Data are compiled 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. No generic correlation between contaminant bioaccessibility and compound, soil or source properties can currently be deduced from the data.

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.

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 7.1. 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 source. Furthermore, bioaccessibility from gastric conditions is higher or much higher than from intestinal conditions for cadmium and lead, respectively.

A few bioaccessibility studies of other organic contaminants than PAH from soil have been published and a selection of data are quoted below for perspective, Table 7.2.

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 /80/, but the range of bioaccessibilities was 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 /77/.

Table 7.2 Selected data sets on bioaccessibilities of organic contaminants other than PAH from soil with partitioning coefficients from /81/

Compound group	Partitioning coefficients log (K_{ow})	Soils and sources	Test	Bioaccessibility	References
Dioxins[9]	6–12	Soil with air emissions as source	PBET[10]	Stomach and intestine 20–34%	/80/
Dioxins[11]	6–12	Slag from copper ore processing	Digestive tract model	Stomach and intestine 44–52%	/77/
PCB[12]	4–8	Spiked artificial soil	RIVM	Stomach and intestine 34–40%	/82/
PCB[13]	4–8	Soils	Digestive tract model	Stomach and intestine 32–83%	/83/
Lindane	4	Spiked artificial soil	RIVM	Stomach and intestine 57%	/82/
Pesticides[14]	3–6	Spiked soil	PBET	Stomach or stomach and intestine 2–44%	/84/

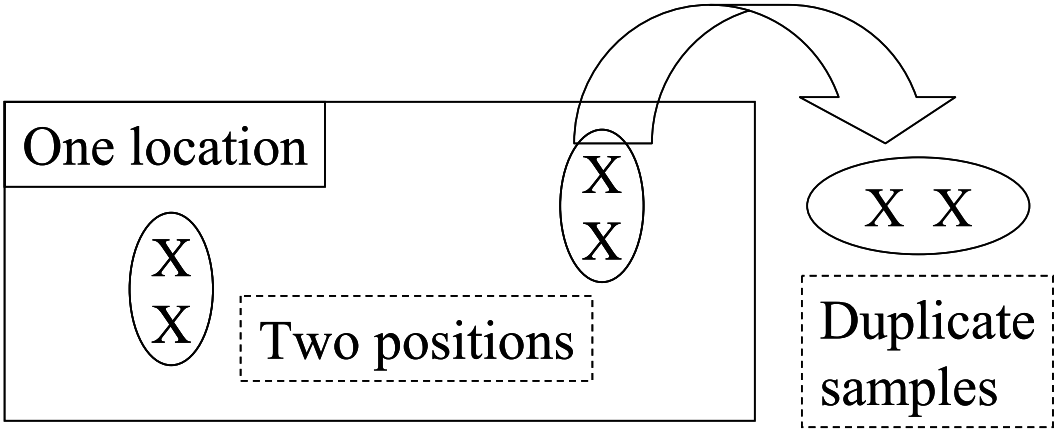
[9] 7 polychlorinated dibenzodioxins and 10 polychlorinated dibenzofurans included.
[10] Modified for use with organic contaminants.
[11] 5 polychlorinated dibenzodioxins and 5 polychlorinated dibenzofurans included.
[12] 4 polychlorinated biphenyl congeners included.
[13] 6 polychlorinated biphenyl congeners included.
[14] 6 pesticides included: diazinon, malation, chlorpyrifos, *trans*-chlordane, cis-chlordane and p,p'-DDT.

7.2 Experimental bioaccessibility data

In a study done for the DEPA /2/, 7 Danish sites contaminated with one or more of the target contaminants of this report were sampled following a sampling plan as shown in Figure 7.1. The samples were taken in the most contaminated depths, in most cases 0.1–0.3 m below the surface, using a hand auger. Soil samples were dried, sieved and homogenized and then subjected to analysis for total soil concentrations and to test for bioaccessibility of the metals cadmium, lead and nickel, and the PAH benzo(a)pyrene (BaP) and dibenz(a,h)anthracene (DBahA). The test methods were RIVM fasted state for metals and RIVM fed state for PAH, see /2/ for detailed method descriptions.

The calculations of relative bioaccessibilities in this application study were done relative to experimental references with soluble contaminants added.

Figure 7.1 Sampling plan for the Danish field sites.



The sampling and test plan, Figure 7.1, was designed for use in site risk assessment in order to allow for separate estimation of site variability and test reproducibility from analysis of the relative ranges, see *e.g.:* /85/ for the statistical method.

Based upon the total soil concentrations, Table 7.3, five of the sites would require intervention based upon concentrations of one or more contaminants exceeding the Danish intervention values, whereas the remaining two sites would be considered contaminated for exceeding the Danish soil quality criteria.

During the bioaccessibility testing, samples from two positions at the metal casting site and from one position from the metal slag site exhibited pH values in the test solutions above 2 in the stomach segment and/or above 7 in the intestinal segment for the fasted state test, and these samples accordingly exhibited lower lead bioaccessibilities than the other samples tested. Still, there was no general correlation between test pH values and lead bioaccessibilities.

The relative bioaccessibility data demonstrated that almost all contaminants had bioaccessibilities well below 100 %, lowest for nickel and PAH and with the highest site to site variability for lead.

Table 7.3 Total soil concentrations at the Danish field sites

(mean±standard deviation)	**Cd** (mg/kg dw)	**Pb** (mg/kg dw)	**Ni** (mg/kg dw)	**BaP** (mg/kg dw)	**DBahA** (mg/kg dw)
Danish soil quality criteria	0.5	**40**	**30**	**0,1**	**0,1**
Danish intervention values	**5**[15]	400	30	1	1
Area with >100 years of urban history	1.3 ±0.64	680 ±110	10 ±1.3	3.9 ±1.7	0.80 ±0.47
Urban soil close to highway	0.47 ±0.28	73 ±72	10 ±6.1	0.22 ±0.31	0.08 ±0.06
Urban soil close to metal industry	2.2 ±0.60	330 ±110	15 ±2.5	-	-
Rural area with fishing net tarring	-	-	-	5.4 ±9.1	0.99 ±1.7
Urban soil with metal slags	28 ±16	3.900 ±1.800	48 ±21	-	-
Urban soil with metal casting sand	2.2 ±1.2	710 ±410	69 ±36	-	-
Urban soil with ashes from porcelain factory	0.71 ±0.54	160 ±170	11 ±3.7	1.8 ±1.1	0.33 ±0.22

Table 7.4 Relative bioavailabilities at the Danish field sites

(mean±standard deviation)	**Cd** (%)	**Pb** (%)	**Ni** (%)	**BaP** (%)	**DBahA** (%)
Area with >100 years of urban history	54±9.9	78±20	29±4.8	15±3.1	14±3.3
Urban soil close to highway	68±4.5	29±17	19±2.4	38±27	40±24
Urban soil close to metal industry	57±3.6	107±18	16±2.2	-	-
Rural area with fishing net tarring	-	-	-	5.7±0.03	12±9.1
Urban soil with metal slags	52±7.1	43±48	22±19	-	-
Urban soil with metal casting sand	35±13	53±28	32±14	-	-
Urban soil with ashes from porcelain factory	43±5.7	27±10	22±2.5	16±2.1	20±6.0

The soil texture was determined for the 7 sites and the soils classified according to Danish standard methods /86/,/87/ but no correlation could be found between texture and relative bioaccessibilities.

Considering the bioaccessible concentrations of the soil contaminants only, Table 7.5 and see section 2.6 for calculations of bioaccessible concentrations from total soil concentrations and relative bioaccessibilities, three sites would require intervention, three sites would be considered contaminated and one site would be

[15] The MCL relevant to bioaccessbility (i.e.: based upon human, oral exposure) is given in bold.

considered uncontaminated. In this context, the term contaminated is defined as imposing a risk for human, oral exposure.

Table 7.5 Bioaccessible soil concentrations at the Danish field sites

(mean±standard deviation)	**Cd** (mg/kg dw)	**Pb** (mg/kg dw)	**Ni** (mg/kg dw)	**BaP** (mg/kg dw)	**DBahA** (mg/kg dw)
Danish soil quality criteria	0.5	**40**	**30**	**0.1**	**0.1**
Danish intervention values	**5**	400	30	1	1
Area with >100 years of urban history	0.67 ±0.42	500 ±170	3.0 ±0.66	0.53 ±0.26	0.10 ±0.04
Urban soil close to highway	0.22 ±0.22	24 ±19	1.6 ±1.6	0.08 ±0.07	0.02 ±0.02
Urban soil close to metal industry	1.2 ±0.23	360 ±130	2.4 ±0.65	-	-
Rural area with fishing net tarring	-	-	-	0.40 ±0.81	0.07 ±0.12
Urban soil with metal slags	17 ±9.2	1.900 ±2.200	12 ±12	-	-
Urban soil with metal casting sand	0.84 ±0.60	460 ±260	21 ±7.5	-	-
Urban soil with ashes from porcelain factory	0.25 ±0.20	59 ±52	2.4 ±0.98	0.33 ±0.20	0.08 ±0.06

The contribution to the overall uncertainty from introducing the bioaccessibility correction was estimated based upon the duplicate sampling and testing plan, Table 7.6. The variability analysis demonstrated, as expected, that the primary contribution to data variation was the field variation at either location or position.

Table 7.6 Variability analysis for bioaccessibility at the 7 Danish sites

Mean relative range	**Cd** (%)	**Pb** (%)	**Ni** (%)	**BaP** (%)	**DBahA** (%)
Test variation	13	14	12	11	24
Position variation	13	50	39	46	52
Location variation	37	66	36	24	48

A comparison of the overall relative standard deviations for total soil concentrations and bioaccessible soil concentrations for the 7 Danish sites (data not shown) demonstrated accordingly that even with the inevitable variation originating from the tests, the total variation in the data intended for risk assessment did not increase considerably.

8 *In vitro* bioaccessibility to *in vivo* bioavailability correlations

In order to be relevant, an in vitro bioaccessibility test method can be expected to provide data correlated to data obtained with in vivo bioavailability data, see section 2.6. This approach does have limitations:

- *in vivo* data for bioavailability may not have been obtained in the same experimental animals as the toxicity data behind the MCL
- *in vitro* tests for bioaccessibility aim at simulating the dissolution in the human gastrointestinal tract, whereas the *in vivo* bioavailability methods are based upon the conditions in the experimental animals used
- *in vivo* methods include both dissolution of contaminants from soils, uptake through the gastrointestinal walls and any subsequent excretion and degradation/transformation, whereas the *in vitro* test include dissolution only
- *in vitro* test bioaccessibility data depends upon the test method used
- *in vivo* bioavailability data depends upon the experimental animals, dosages, target organs and calculation methods
- both *in vivo* and *in vitro* results are subject to variation due to soil inhomogeneity, method performance variations and for *in vivo* results in addition to biological variability

In other words: *in vitro* bioaccessibility test methods and in vivo bioavailability studies will give different results as they are designed to investigate different parts of the oral uptake of contaminants from soils, and the results from both types of studies are associated with variation as well as with systematic and random errors. Still, a valid *in vitro* bioaccessibility test method should reflect differences in solubility of soil contaminants in the gastrointestinal system and thus relate to an upper limit of the potential for uptake of the contaminants from the soil tested, as compared to other soils and to soluble forms of the contaminant. If the rate limiting step of contaminant uptake is the dissolution from soil, there will be a linear relation between bioaccessibility and bioavailability, see chapter 3.

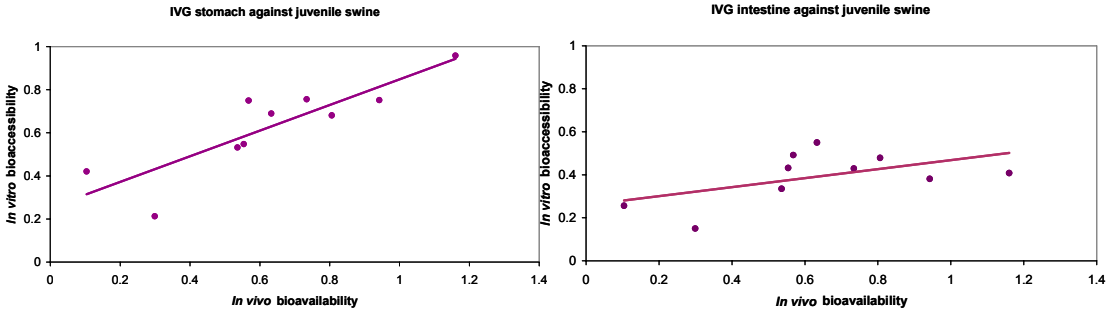
A correlation is expected for in vitro bioaccessibility to in vivo relative bioavailability rather than to absolute bioavailability. Accordingly, the in vivo data presented in the subsequent sections are relative bioavailabilities, if nothing else is noted.

8.1 Literature *in vitro* bioaccessibility to *in vivo* bioavailability correlation

8.1.1 Cadmium

The correlations in vitro to in vivo for cadmium reported in one published study with a test method with a stomach segment and for tests with a subsequent intestinal step are shown in Figure 8.1, see Table 5.1 and Table 6.1 for explanations and references for applied methods.

Figure 8.1 In vitro bioaccessibility of cadmium from soils against in vivo bioavailability as reported in the literature for an in vitro test method with a stomach segment (left) followed by an intestinal segment (right) /72/



A linear relationship *in vitro* to *in vivo* and generally higher bioaccessibility than bioavailability was found for the bioaccessibility test method with a stomach segment, whereas the linear relationship was less evident and the bioaccessibility data lower for test in a subsequent intestinal segment. It should be noted that the IVG bioaccessibility test method applies an intestinal step after removing the contaminants dissolved in the stomach step, and this method is thus expected to provide lower test results than methods that does not use this approach.

In vitro bioaccessibility and *in vivo* bioavailability data were also reported for the DIN test against mini pigs /55/, but the set up of the bioavailability study did not allow for calculation of the relative bioavailability of cadmium (no soluble references included) and the data can thus not be evaluated here.

8.1.2 Lead

The correlations in vitro to in vivo for lead reported in the literature are shown for test methods with a stomach segment only in Figure 8.2 and for tests with an intestinal step in Figure 8.3, see Table 5.1 and Table 6.1 for explanations and references for applied methods.

Figure 8.2 In vitro bioaccessibility of lead from soils against in vivo bioavailability as reported in the literature for in vitro test methods with a stomach segment only /9;56;88;89/

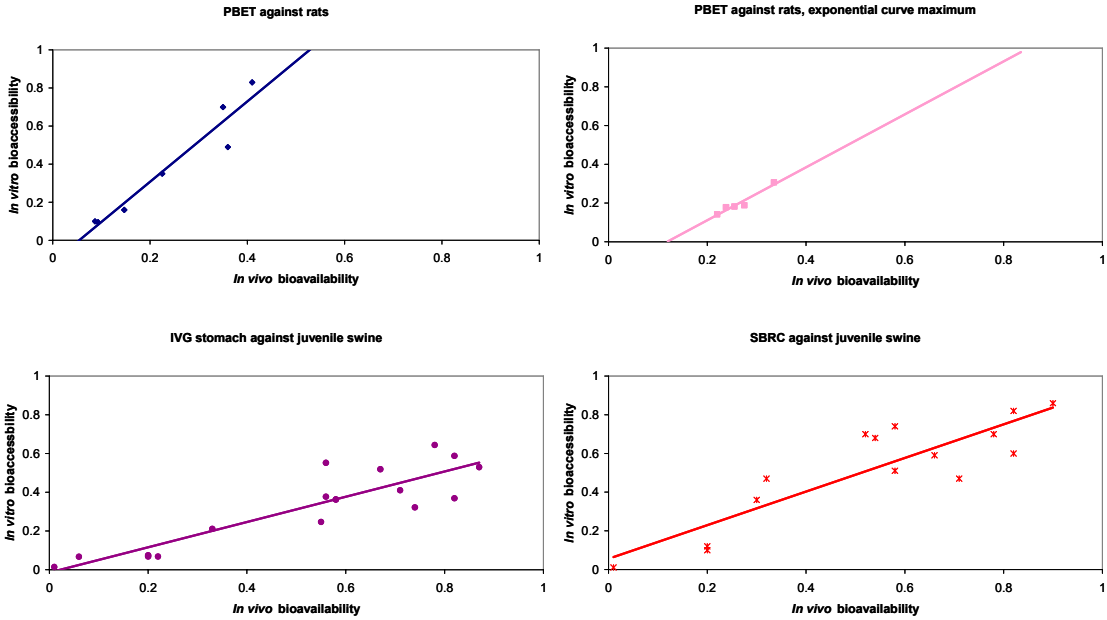
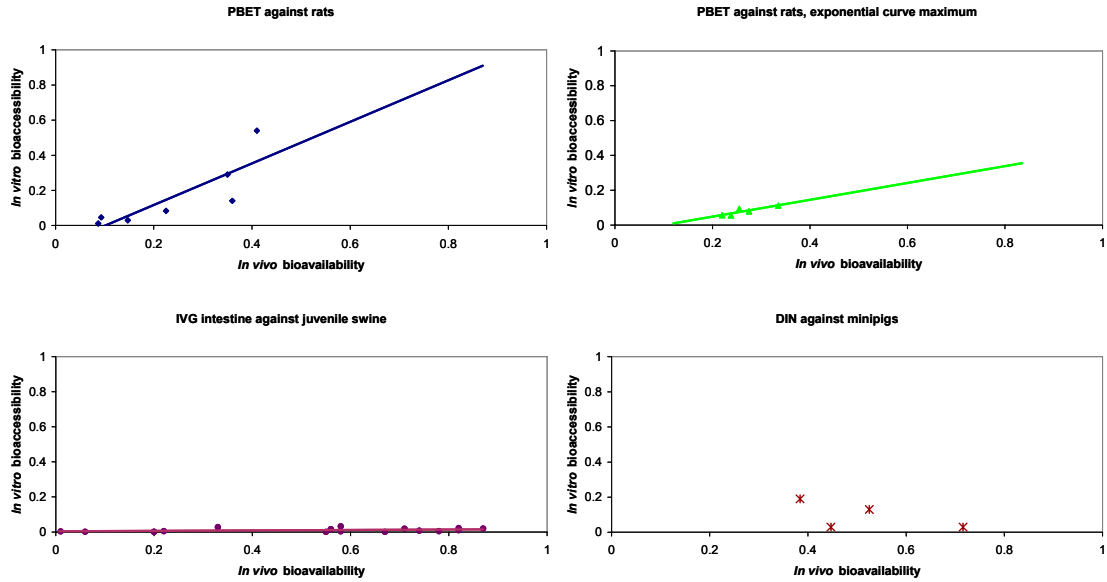


Figure 8.3. In vitro bioaccessibility of lead from soils against in vivo bioavailability as reported in the literature for in vitro test methods with an intestinal segment /88–91/



The published correlations clearly demonstrate, that:

- different bioavailability calculation methods yield different *in vitro* to *in vivo* relationships for the same bioaccessibility test method and the same experimental animals (top figures, Figure 8.2 and Figure 8.3)
- different bioaccessibility test methods yield different *in vitro* to *in vivo* relationships for the same bioavailability method and experimental animal (bottom figures, Figure 8.2)
- bioaccessibility test methods with an intestinal segment yield lower *in vitro* to *in vivo* relationships than methods with a stomach segment only for the same bioavailability methods and experimental animas (Figure 8.2 and Figure 8.3)
- some combinations of *in vitro* bioaccessibility and *in vivo* bioavailability test methods provide poor correlation (bottom right, Figure 8.3)

Again, it should be noted that the IVG bioaccessibility test method applies an intestinal step after removing the contaminants dissolved in the stomach step, and this method is thus expected to provide lower test results than methods that do not use this approach.

Poor *in vitro* to *in vivo* correspondence was reported for the NIST 2710 certified reference material soil using the mass balance bioaccessibility test method and rats for bioavailability studies /73/.

Table 8.1 demonstrates that *in vitro* test methods such as RIVM and TIM in fasted state versions could yield bioaccessibility data that for one soil sample corresponded to the *in vivo* uptake in humans, whereas other methods did show very significant discrepancies see Table 6.1 for explanations and references for applied methods.

Table 8.1 In vitro bioaccessibilities of lead from the Bunker Hill soil against the in vivo bioavailabilities measured in humans /76/, refer to Table 6.1 for explanations and references for applied methods.

Method	Bioaccessibility		Bioavailability humans	
	Fasted	Fed	Fasted	Fed
PBET	13	22	26	2.5
RIVM	32	24		
DIN	14	29		
SHIME	2.0	24		
TIM	28	3.8		

8.1.3 Nickel

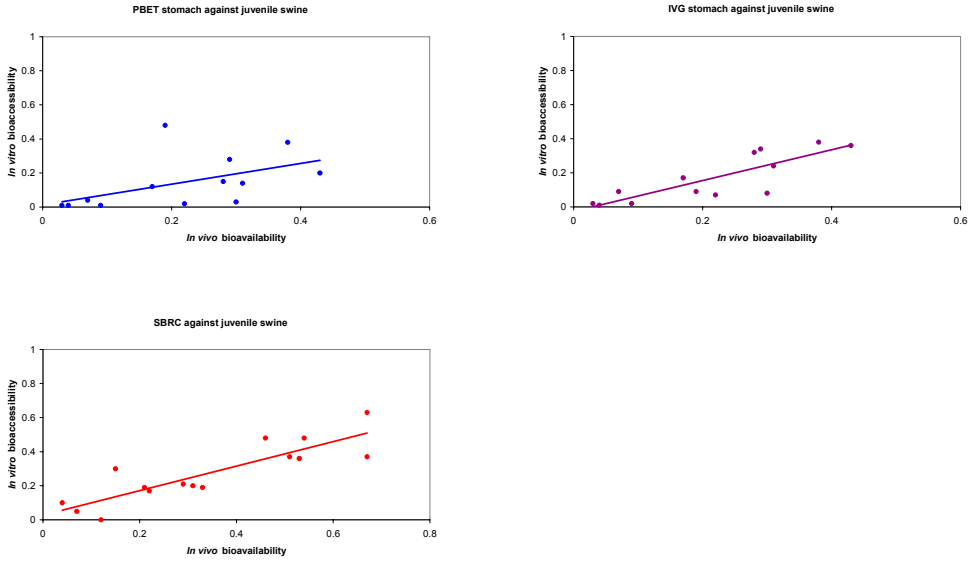
Two sets of in vitro bioaccessibility and in vivo bioavailability data were reported for nickel from soil. Data from the DIN test against mini pigs /55/ did not allow for calculation of the relative bioavailability of nickel (no soluble references included) and the data can thus not be evaluated here. For a Canadian study, the data could not be released for publication.

8.1.4 Arsenic

The correlations in vitro to in vivo for arsenic reported in the literature are shown for test methods with a stomach segment only in Figure 8.4 and for tests with an intestinal segment in Figure 8.5, see Table 5.1 and Table 6.1 for explanations and references for applied methods. It should be noted, that the in vivo data used for correlation with the IVG, PBET and DIN in vitro data were based upon urinary excretion as customary for arsenic studies, whereas the target organs behind the in vivo data used for correlation with the SBRC in vitro data were not stated in the reference.

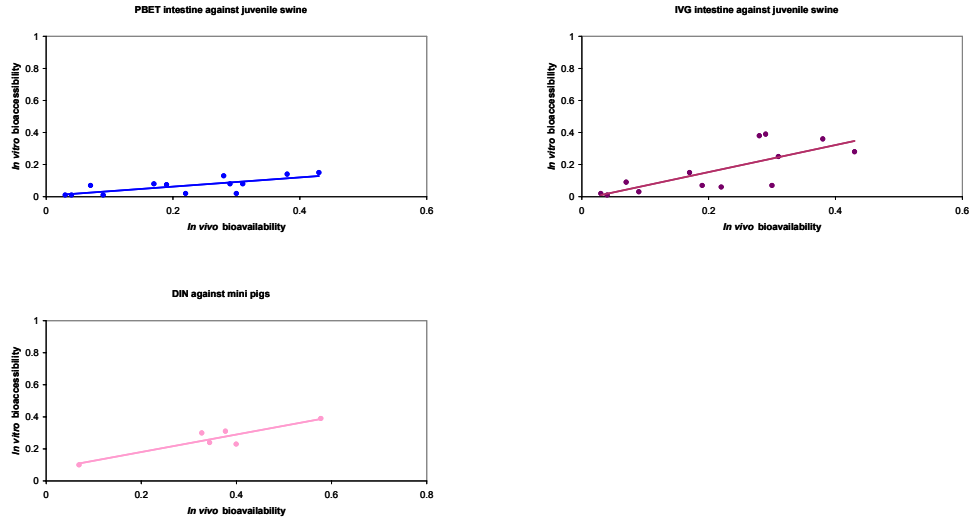
The tests with a stomach segment only all showed linear correlation in vitro to in vivo, SBRC better than IVG stomach better than PBET, but with slightly higher in vivo than in vitro data. The tests with an additional intestinal segment also show linear correlation in vitro to in vivo, PBET better than IVG intestine with very few data for the DIN test method. The in vivo are higher or slightly higher than in vitro data for PBET and IVG, respectively.

Figure 8.4 In vitro bioaccessibility of arsenic from soils against in vivo bioavailability as reported in the literature for in vitro test methods with a stomach segment only /21;71/.



Again, it should be noted that the IVG bioaccessibility test method applies an intestinal step after removing the contaminants dissolved in the stomach step, and this method is thus expected to provide lower test results than methods that do not use this approach.

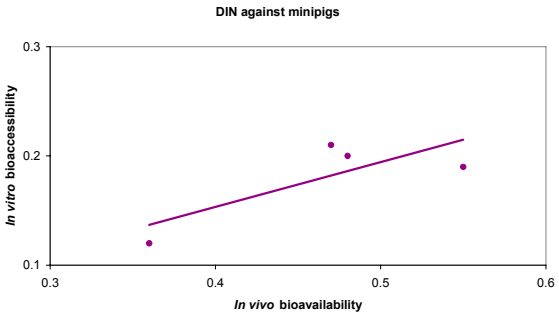
Figure 8.5 In vitro bioaccessibility of arsenic from soils against in vivo bioavailability as reported in the literature for in vitro test methods with an intestinal segment /21;55/



8.1.5 PAH

In vitro bioaccessibility and relative in vivo bioavailability data were reported for the PAH compound benzo(a)pyrene (BaP) as obtained with the DIN test against mini pigs Figure 8.6. Only a limited number of soil samples were tested (4), and the relative in vivo bioavailability was found as the amount of the compound not excreted with faeces. The bioavailabilities must thus be considered an upper limit of the fraction of the compound reaching systemic circulation in the mini pigs.

Figure 8.6 In vitro bioaccessibility of benzo(a)pyrene from soils against in vivo bioavailability as reported in the literature for in an vitro test method with a stomach segment and an intestinal segment /55/.



A more detailed analysis /1/ of the in vitro bioaccessibilities and absolute in vivo bioavailabilities (again obtained as upper limits of bioavailability from PAH not excreted with faeces) from this study /55/ demonstrated that for 4 of the PAH in all 4 soils and for all of the 12 PAH in one soil, a reasonable in vitro to in vivo correlation was obtained, but this was not the case considering all PAH in all 4 soils.

For pyrene, the bioavailability can be estimated from the amount not excreted with faeces as mentioned above, but the data in the report fra /55/ also allows for

estimation of the bioavailability from the amounts of the metabolite 1-hydroxypyrene excreted with urine, Table 8.2.

Table 8.2 Bioavailability and bioaccessibility of PAH from 4 different soils given ordered relative to the most bioavailable/bioaccessible soil for each method, calculated from /55/

Soil identification	Bioavailability order calculated from non excreted PAH with faeces		Bioavailability order calculated from excreted 1-hydroxypyrene with urine	Bioaccessibility order
	Pyrene	BaP	Pyrene	Pyrene
Bruchsal	0.39	0.65	0.73	0.24
Carl 1	0.79	1.00	0.60	0.28
Lothringen 1	0.75	0.86	0.53	0.36
Lothringen 2	1.00	0.85	1.00	1.00

Evidently, the order of the bioavailabilities and bioaccessibilities differ among the methods applied and the compounds considered.

It should be noted, that the variability in the in vivo bioavailability data was considerable (op to 100 % relative standard deviation) in this study and that for one experimental group, the data from one of four animals were omitted from the data treatment due to excessive variation.

8.2 Experimental *in vitro* bioaccessibility to *in vivo* bioavailability correlation

In a study done for the DEPA, soil samples with internationally documented in vivo bioavailability data were tested using the unmodified RIVM fasted state and fed state bioaccessibility test methods for metals (Cd, Pb and Ni) and PAH (BaP and DBahA), respectively /3/, and furthermore using a modification of the RIVM fasted state method without the intestinal step (RIVM fasted state stomach only) and the SBRC method.

Soils for the study were identified partly via the international Bioavailability Research Group Europe (BARGE) that among its members today counts most international research groups active within bioaccessibility testing, partly via a search of the scientific literature and published reports. The samples from the BARGE were identified through personal communication, and the literature search was done at the Technical Knowledge Centre of Denmark with the following search profile:

(bioavailability or uptake or oral or absorption)

and

soil

and

(polycyclic aromatic hydrocarbons or PAH or 50-32-8 or 53-70-3 or 7440-43-9 or 7439-92-1 or 7440-02-0)

and

(vivo or human or infant# or animal# or pig# or swine# or rat# or mice or mouse or sheep# or rabbit# or primate# or monkey# or hamster#)

in the on line literature bases:

MEDLINE, BIOSIS and CAPPLUS

with:
>1995

and

not (vegetable# or crop# or plant# or translocation# or phyto# or dermal)

The literature search thus covered the recent chemical, biological and medical publications on the oral uptake of the specified contaminants (by name or CAS number) from soil, but excluding dermal uptake and plant uptake.

In all 356 titles and abstracts were identified, 82 papers and reports were retrieved leading to the identification of 15 research groups and 3 authorities with relevant, published activities. All were contacted at the least 3 times in order to identify soil samples with in vivo bioavailability data leading to totally:

- 44 samples for lead
- 16 samples for PAH
- 13 samples for cadmium
- 2 samples for nickel

retrieved from totally 8 sources:

- Stan Casteel, University of Missouri
- Mike Ruby, Exponent
- Nick Basta, Ohio State University
- William Brattin, Syracuse Research Corporation
- Agnes Oomen, National Institute for Public Health and the Environment
- Jürgen Wittsiepe, Ruhr-Universität Bochum
- Eric Weyand, Maple City Research Inc.
- National Institute of Standards & Technology (commercially available soil reference material with published bioaccessibility test data)

Furthermore, access was obtained to a soil sample tested for bioavailability of lead to humans by Dr. Mark Maddaloni, now United States Environmental Protection Agency (US EPA).

One Canadian set of samples with data for nickel were unfortunately not available to the study due to confidentiality restrictions.

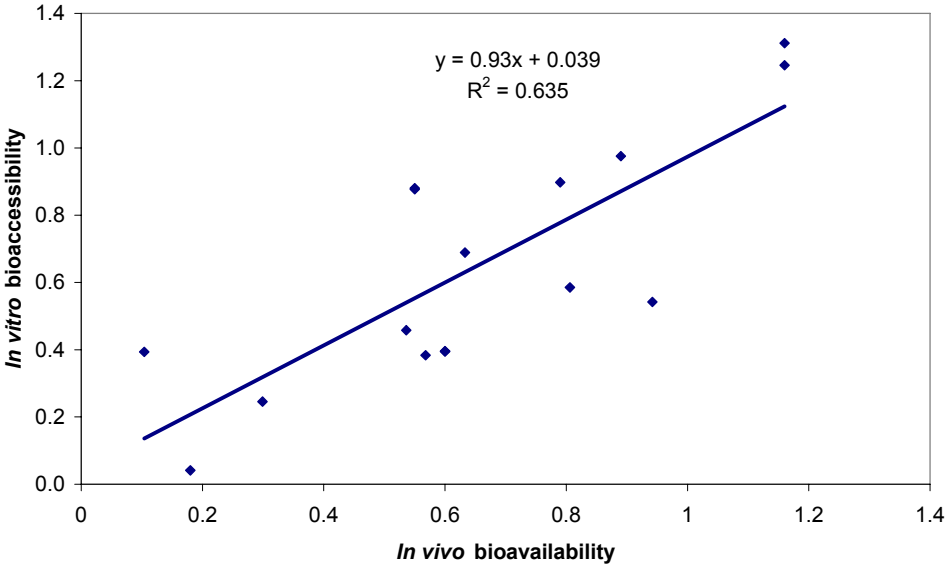
8.3 Application of RIVM unmodified bioaccessibility test methods

The RIVM fasted state (metals) and fed state (PAH) were applied to retrieved soil samples. The calculation of relative bioaccessibilities in this application study was done using the correction functions for final pH in the tests.

8.3.1 Cadmium

In all, 13 samples with cadmium in vivo bioavailability, all obtained for juvenile swine, were retrieved and tested with the RIVM fasted state in vitro bioaccessibility test method, Figure 8.7.

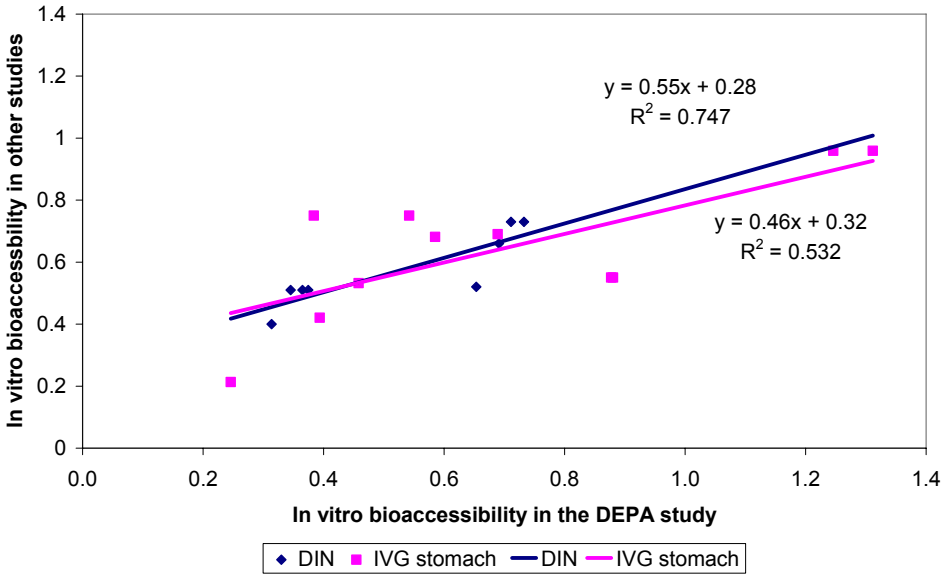
Figure 8.7 In vitro bioaccessibility of cadmium obtained with the RIVM fasted state test method against in vivo bioavailability obtained with juvenile swine.



There was a reasonable linear relationship between the in vitro and the in vivo data, and the overall trend was slightly higher bioaccessibilities than bioavailabilities.

For some of the retrieved soils, in vitro bioaccessibility data obtained with other methods (IVG /72/ and DIN /55/) have been available for comparison with the data obtained in the DEPA study with the RIVM fasted state test, Figure 8.8. The data obtained with the three bioaccessibility test methods exhibit reasonably linear correlation with the RIVM fasted state data as the highest for high bioaccessibilities.

Figure 8.8 In vitro bioaccessibility of cadmium obtained with other test methods /55;72/ against in vitro bioaccessibility obtained with the RIVM fasted state test method.



A small number of samples (5) have been tested in different series in the DEPA study, Table 8.3, where series 1 and 2 are separated by approximately 6 months, and series 2a and 2b are separated by a few weeks.

Table 8.3 In vitro bioaccessibility of cadmium obtained with the RIVM fasted state method in different test series of the DEPA study.

Sample	Series 1	Series 2a	Series 2b
Oker 11	0.54±0.006	0.75±0.007	0.72±0.01
Bunker Hill	nt[16]	0.71±0.01	0.72
Urban soil with metal slags	0.69	nt	0.56
Urban soil with metal casting sand	0.26	nt	0.38
Urban soil with ashes from porcelain factory	0.45	nt	0.52

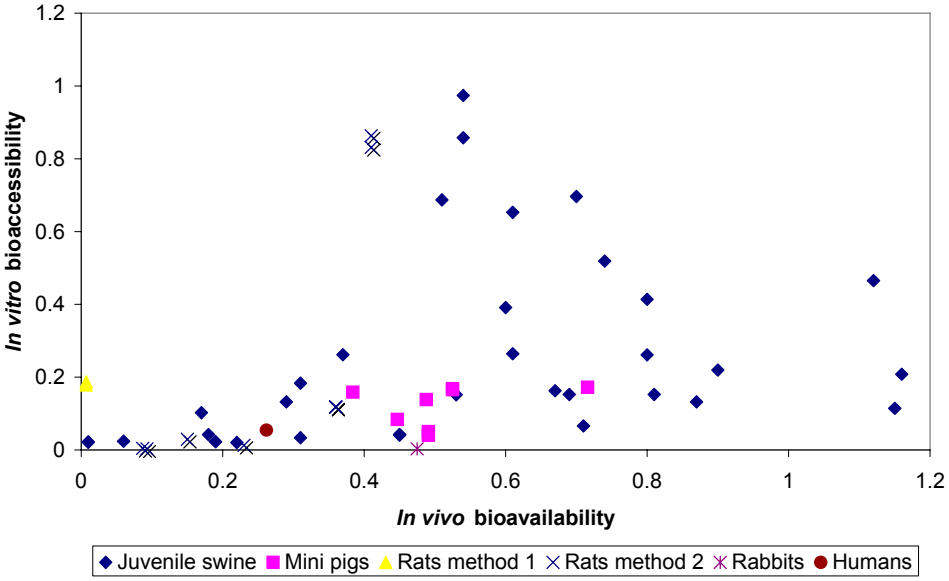
The variations in bioaccessibilities as obtained in different series separated by longer time were considerable, whereas the variations in data obtained within a small time interval were also small. It should be noted that the soil sample Oker 11 was tested from two different batches in series 1 and series 2.

8.3.2 Lead

In all, 44 samples with lead in vivo bioavailability obtained for juvenile swine, mini pigs, rats (two methods) and rabbits were retrieved and tested with the RIVM fasted state in vitro bioaccessibility test method, Figure 8.9.

[16] nt: not testet

Figure 8.9 In vitro bioaccessibility of lead obtained with the RIVM fasted state test method against in vivo bioavailability obtained with juvenile swine, mini pigs, rats and rabbits.



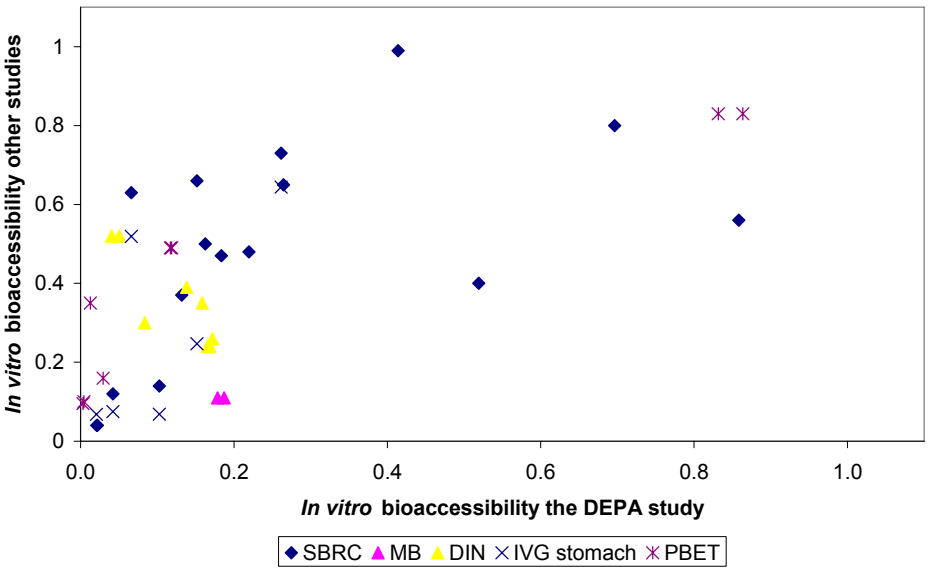
Evidently, the bioaccessibility data obtained with the RIVM fasted state method were not correlated to the bioavailability data. Furthermore, the bioaccessibilities were generally much lower than the bioavailabilities. Separation of bioavailability data obtained with one experimental animal and one method did not improve the correlation, as would also not be expected from Figure 8.9.

Exclusion of soil samples with high pH (> 1.8) in the stomach segment did not improve the correlation. A number of samples were mine waste and thus not strictly soil samples for which the RIVM method was developed. Still, exclusion of all samples that were not identified as soil samples did not improve the correlation. Here, it should be remembered that some soil samples are contaminated with mine waste and the distinction between soils and mine wastes may thus be somewhat artificial.

For some of the retrieved soils, in vitro bioaccessibility data obtained with other methods (IVG stomach and intestine /72/, DIN /55/), MB /73/, SBRC /63/, PBET /56/) have been available for comparison with the data obtained in the DEPA study with the RIVM fasted state test, Figure 8.10.

The bioaccessibilities obtained with the RIVM fasted state method for lead are correlated to the data obtained with the other methods, except for the highest values and the DIN method, but the RIVM data are generally lower than those obtained with the other methods.

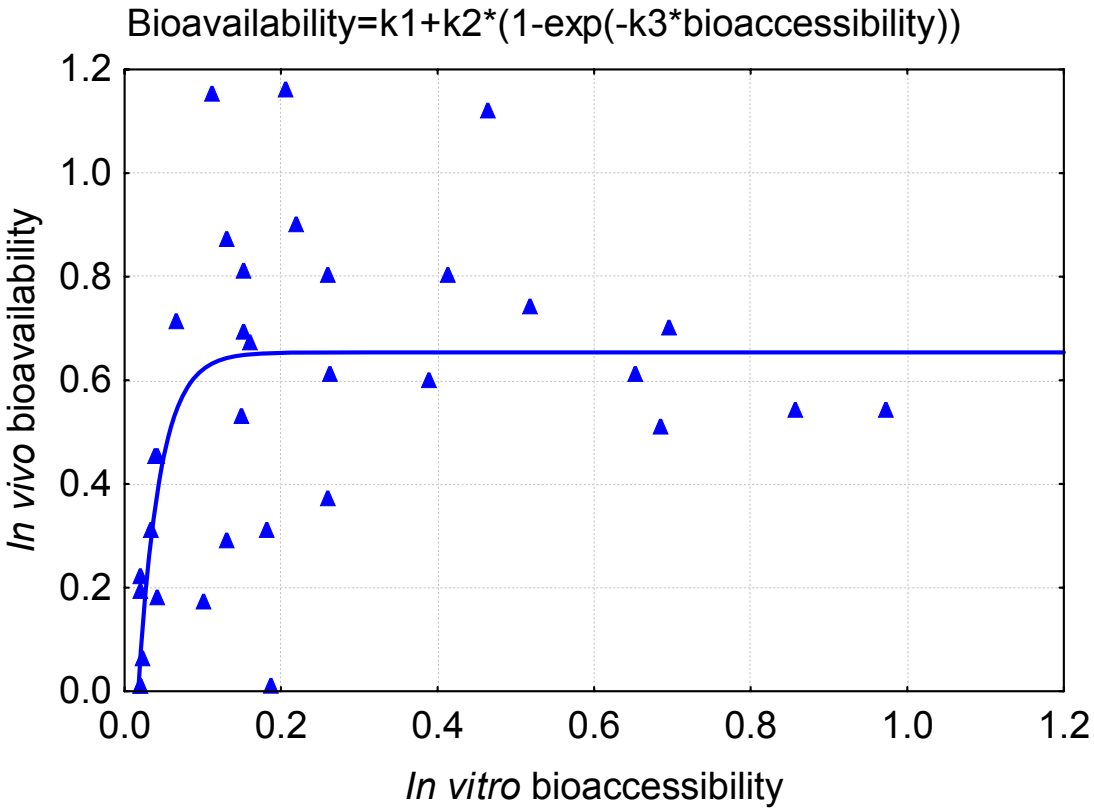
Figure 8.10 In vitro bioaccessibility of lead obtained with other test methods /72/, /55/), /73/,/63/ /56/ against in vitro bioaccessibility obtained with the RIVM fasted state test method.



The data included in Figure 8.10 were all, except for the DIN method, obtained with the method versions without an intestinal segment, as those are the versions generally applied. Comparison of the RIVM fasted state method data that does include also an intestinal segment with data obtained with versions of the other methods applying their intestinal segments (PBET, IVG, MB and DIN) did not provide better correlations (data not shown). Still, the RIVM fasted state method did in reality provide the best correlation to bioavailability data compared to data obtained with other methods in versions with intestinal segment included (data not shown).

Part of the explanation of the poor correlation obtained between the lead bioaccessibilities obtained with the RIVM fasted state method and the juvenile swine bioavailabilities may be that the *in vivo* data could be biased. Figure 8.11 shows the *in vivo* data plotted against *in vitro* data, and the plot resembles the blood lead to dose curve shown in Figure 5.1. The blood uptake, chapter 5, exponential function do to a reasonable degree explain the data variability ($R^2 = 0.402$). Even though this may question the validity of the juvenile swine bioavailability data, it does not justify the very low bioaccessibilities obtained for the majority of samples with the RIVM fasted state method.

Figure 8.11 In vitro bioavailability of lead obtained with the juvenile swine method against bioaccessibility obtained with the RIVM fasted state method.



For one soil sample tested in the DEPA study, the Bunker Hill soil, a set of data is available also with RIVM fasted state data after stomach and after intestinal segments /76/ and also in vivo bioavailability obtained in humans /53/, Table 8.4.

Table 8.4 Absolute bioaccessibility obtained with the RIVM fasted state test method at RIVM /76/ and in the DEPA study, and absolute bioavailability as obtained in humans /53/

	In vitro RIVM stomach segment only	In vitro RIVM stomach segment followed by intestinal segment	In vitro RIVM stomach segment followed by intestinal segment, the DEPA study	In vivo in fasted humans
Bunker Hill	0.71±0.01	0.32±0.03	0.031	0.26±0.081

Evidently, bioaccessibility of lead is decreased by the addition of the intestinal segment, but in the study done at RIVM not to a value below the *in vivo* value. In the DEPA study, the *in vitro* value was clearly below the *in vivo* value. Very careful comparison of the test practical test performance at RIVM and in the DEPA study did not reveal any discrepancies that might explain the very large variations in data obtained for the same samples at the two laboratories (RIVM and DHI).

A small number of samples (6) have been tested in different series in the DEPA study, Table 8.5, where series 1 and 2 are separated by approximately 6 months, and series 2a and 2b are separated by a few weeks.

Table 8.5 In vitro bioaccessibility of lead obtained with the RIVM fasted state method in different test series of the DEPA study

Sample	Series 1	Series 2a	Series 2b
Oker 11	0.40±0.02	0.19±0.01	0.17±0.003
Bunker Hill	nt[17]	0.063±0.004	0.055
Urban soil with metal slags	0.054	nt	0.13
Urban soil close to highway	0.11	nt	0.18
Urban soil with metal casting sand	0.15	nt	0.11
Urban soil with ashes from porcelain factory	0.13	nt	0.36

The variations in bioaccessibilities as obtained in different series separated by longer time are considerable, whereas the variations in data obtained within a small time interval are also small. The variation appears random rather than systematic. It should be noted that the soil sample Oker 11 was tested from two different batches in series 1 and series 2.

8.3.3 Nickel

No soil samples could be retrieved with relative in vivo bioavailability data from soil available. A series of soil samples from a Canadian site were not available, and for two soil samples from a bioavailability study in mini pigs /55/, no soluble nickel reference was included in the experiments and only absolute bioavailabilites could thus be obtained.

A linear relationship was obtained for the bioaccessibility data from the DEPA study against data obtained with the DIN method for the same 6 samples, with highest results seen for the RIVM fasted state test in the DEPA study, Figure 8.12.

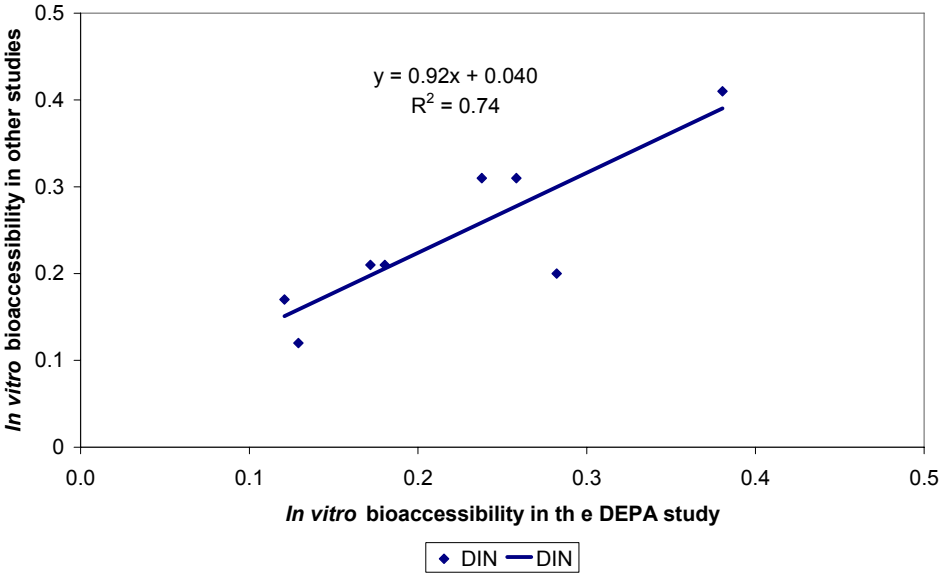
A small number of samples (4) have been tested in different series in the DEPA study, Table 8.6, where series 1 and 2 are separated by approximately 6 months, whereas series 2a and 2b are separated by a few weeks.

Table 8.6 In vitro bioaccessibility of nickel obtained with the RIVM fasted state method in different test series of this study

Sample	Series 1	Series 2a	Series 2b
Oker 11	0.23±0.01	0.26±0.007	0.25±0.01
Urban soil with metal slags	0.024	nt[18]	0.22
Urban soil with metal casting sand	0.47	nt	0.37
Urban soil with ashes from porcelain factory	0.26	nt	0.23

[17] nt: not tested.
[18] nt: not tested.

Figure 8.12 In vitro bioaccessibility of nickel obtained with other test methods /55/) against in vitro bioaccessibility obtained with the RIVM fasted state test method.



The variations in bioaccessibilities as obtained in different series separated by longer time are considerable, whereas the variations in data obtained within a small time interval are also small. The variation appears random rather than systematic. It should be noted that the soil sample Oker 11 was tested from two different batches in series 1 and series 2.

8.3.4 PAH

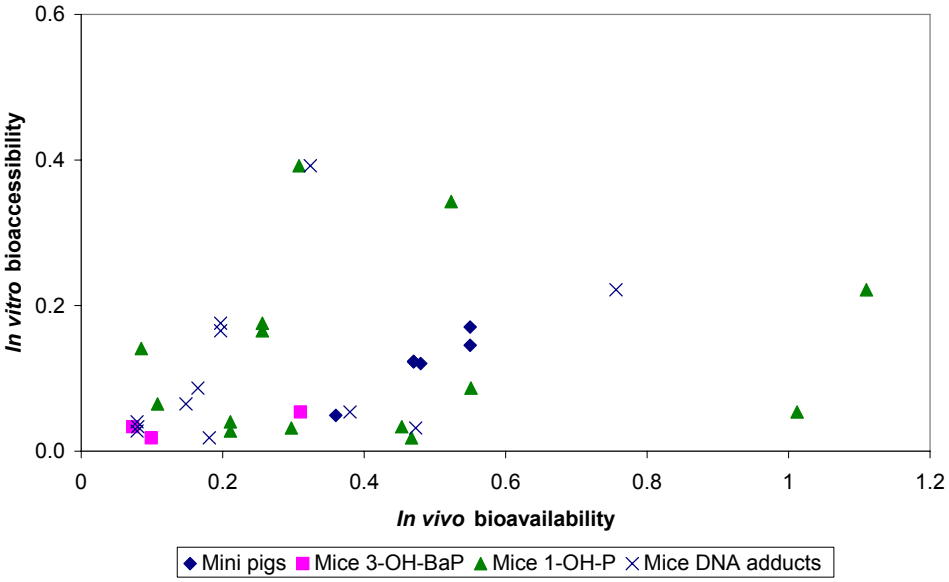
A series of 4 soil samples with in vivo bioavailability data from a study in mini pigs /55/ were retrieved. Only for benzo(a)pyrene (BaP), a soluble reference was included in the experiments. Dibenzo(a,h)anthracene could thus be evaluated from absolute bioavailability data only, and the other PAH included in this *in vivo* study /55/ were not part of the DEPA bioaccessibility study.

Additionally, 13 soil samples were retrieved with in vivo bioavailability data from mice, for 3 samples from excretion of the BaP metabolite 3-hydroxybenzo(a)pyrene (3-OH-BaP), all with urine excretion data for the pyrene metabolite 1-hydroxypyrene (1-OH-P) and 10 with data for formation of PAH DNA adducts.

At this point, it should be recalled that an accepted and validated method for in vivo determination of PAH bioavailability is currently not available.

The *in vitro* bioaccessibilities obtained for BaP in the DEPA study are plotted against all *in vivo* data in Figure 8.13.

Figure 8.13 In vitro bioaccessibility of BaP obtained with the RIVM fed state test method against in vivo bioavailability obtained with mini pigs and with different methods in mice.



Evidently, there is no general correlation *in vitro* to *in vivo*, but at linear correlation is indicated for the four samples tested in mini pigs, and the bioavailabilities were higher than the bioaccessibilities. Here, it should be recalled that the data from the mini pigs are upper limits to PAH bioavailability as they are based upon the amount of PAH not excreted with faeces and thus do not include processes such as metabolization of the PAH in the gut.

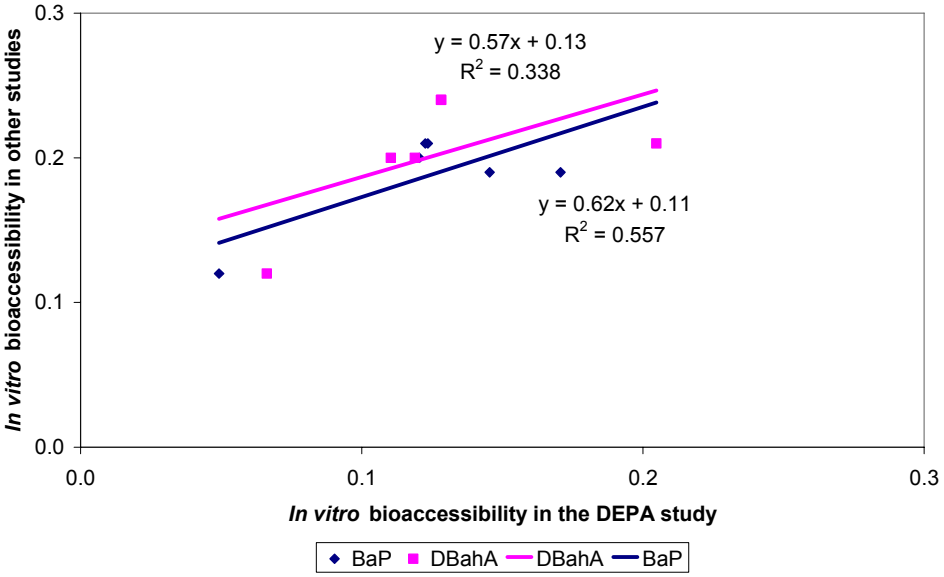
The number of data points with bioavailabilities calculated from urinary excretion of 3-OH-BaP is too limited to allow for analysis of the correlation and for the bioavailabilities based upon urinary excretion of the pyrene metabolite 1-OH-P, no correlation was seen.

The correlations of the bioaccessibilities of DBahA to *in vivo* data were not better than for BaP. For this correlation analysis, the *in vivo* data from mini pigs as absolute bioavailabilities were used based upon an assumption of close to 100 % bioavailability of the soluble reference.

The information available on the different soils is too limited to allow for an analysis of the reasons for the poor *in vitro* to *in vivo* correlations.

Linear relationships were indicated for the bioaccessibility data from the DEPA study against data obtained with the DIN method for the same 4 samples, with highest results seen for the RIVM fed state test in the DEPA study for high bioaccessibilities and lowest for low bioaccessibilities, Figure 8.14.

Figure 8.14 In vitro bioaccessibility of PAH obtained with another test method /55/) against in vitro bioaccessibility obtained with the RIVM fed state test method.



No soil samples were tested for PAH in different series in the DEPA study, and reproducibility over time of the test can thus not be evaluated.

8.4 Application of alternative bioaccessibility test methods

Two alternative methods were applied to samples tested *in vivo* for bioavailability in order to identify a test method with a more satisfactory correlation *in vitro* to *in vivo* for lead. Among the samples with *in vivo* data, only samples with data obtained in juvenile swine or minipigs were selected for this supplementary correlation study. In addition, 6 soil samples from the Danish sites were selected in order to illustrate the different bioaccessibilities obtained with different methods for the Danish soils.

The alternative methods selected were a version of the RIVM fasted state bioaccessibility test without the intestinal segment (stomach only) and the SBRC method as published by John Drexler /71/.

The RIVM fasted state stomach segment only was chosen because the high pH of the intestinal segment of the RIVM fasted state method was identified as one of the reasons for the poor *in vitro* to *in vivo* correlation for this method.

The SBRC method was chosen because this method is aiming at being a robust, low pH stomach only test with published acceptable *in vitro* to *in vivo* correlation for lead (see e.g.: /9;71/). A further advantage is that this method is expected to comply with requirements set for a bioaccessibility test method to be endorsed by the US EPA for use in site risk assessment in the US.

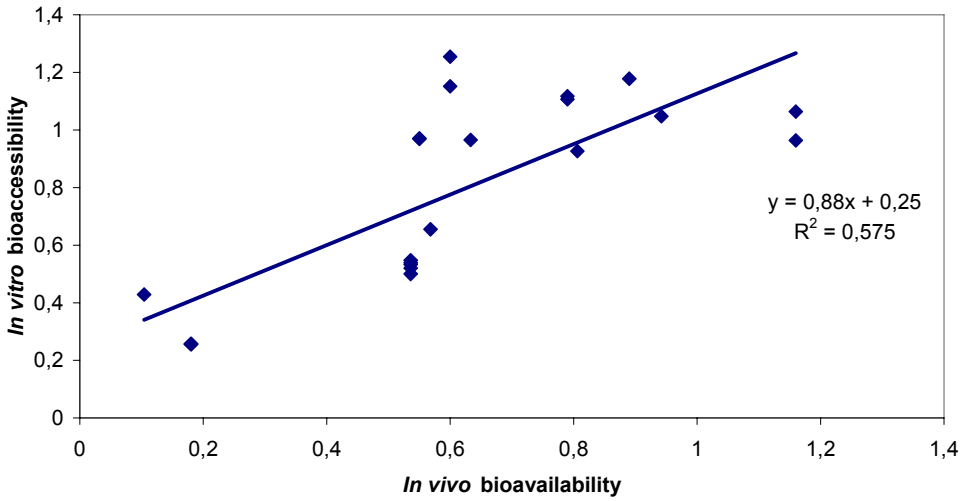
The calculations of relative bioaccessibilities in this correlation study were done relative to experimental references with soluble contaminants added.

8.4.1 Application of the RIVM fasted state stomach segment only method

The RIVM fasted state test method /74/ excluding the intestinal segment and with the amount of soil sample reduced from standard 0.6 g to 0.3 g was used. The liquid to solid ratio of 22.5 for the total mouth/oesophagus and stomach segments of the RIVM test method was maintained.

In all, 12 samples with cadmium in vivo bioavailability for juvenile swine were tested, Figure 8 15.

Figure 8 15 In vitro bioaccessibility of cadmium obtained with the RIVM fasted state stomach segment only test method against in vivo bioavailability obtained with juvenile swine



There is a linear correlation between the *in vitro* and the *in vivo* data, and the overall trend is higher bioaccessibilities than bioavailabilities.

For some of the tested soils, *in vitro* bioaccessibility test data obtained with another method (IVG /72/) have been available for comparison with the RIVM fasted state stomach only data, see Figure 8 16. The data show linear correlation between the results from the two methods with higher IVG results in the low bioaccessibility range and higher RIVM results in the high range.

No samples have been tested for cadmium bioaccessibility with this method in different series in this study.

In all, 18 samples with lead in vivo bioavailability for juvenile swine were tested, Figure 8 17.

Figure 8 16 In vitro bioaccessibility of cadmium obtained with another test method /72/ against in vitro bioaccessibility obtained with the RIVM fasted state stomach only test method.

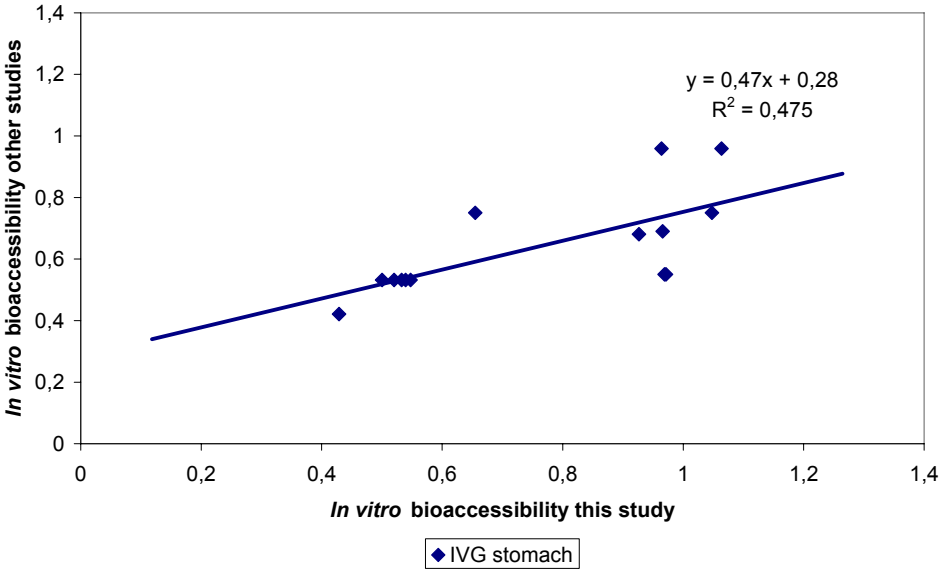
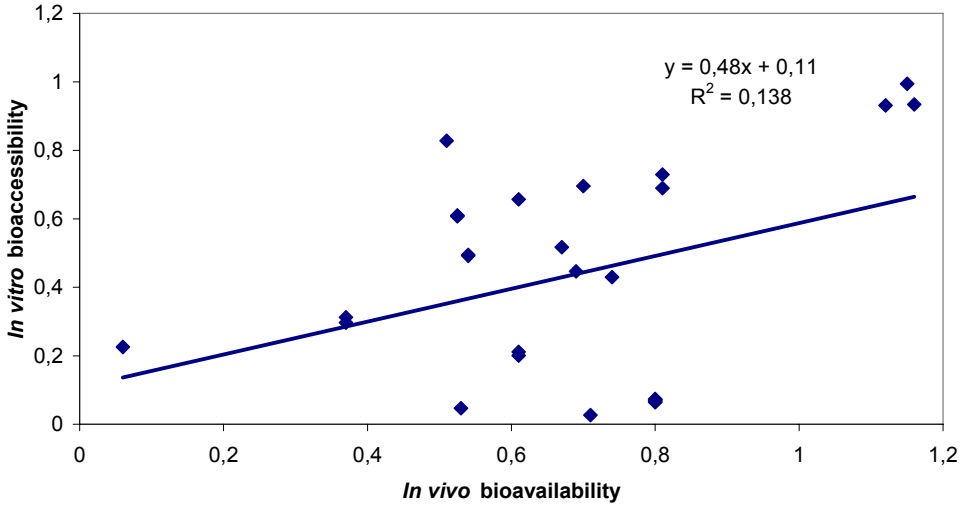
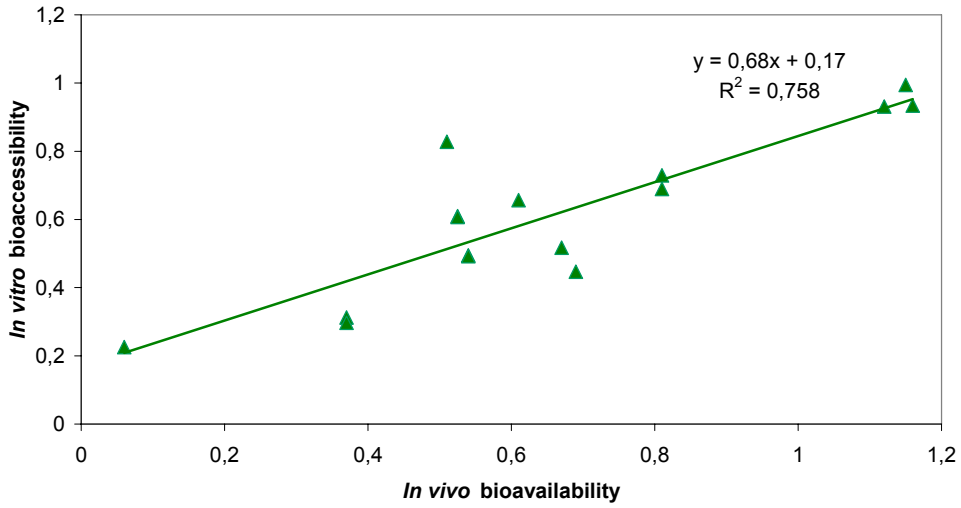


Figure 8 17 In vitro bioaccessibility of lead obtained with the RIVM fasted state stomach segment only test method against in vivo bioavailability obtained with juvenile swine.



There is not a linear correlation between the *in vitro* and the *in vivo* data. For 6 of the samples tested, the final pH after stomach test was above the target value of 1.8. The correlation of the bioaccessibility obtained for the remaining 12 samples with final pH < 1.8 with *in vivo* bioavailability is good, Figure 8 18, with overall higher bioaccessibility than bioavailability in the low bioavailability range and lower bioaccessibility in the high range.

Figure 8 18 In vitro bioaccessibility of lead obtained with the RIVM fasted state stomach segment only test method against in vivo bioavailability obtained with juvenile swine, final pH < 1.8.



The data suggest that the poor correlation obtained for all soil samples, Figure 8 17, was due to too high pH after the stomach segment and consequently, that a good correlation might be obtained for all samples if the pH was kept below 1.8 by adjustment of the test procedure.

For some of the tested soils, *in vitro* bioaccessibility test data obtained with other methods (IVG /72/, DIN /55/, SBRC /92/) have been available for comparison with the RIVM fasted state stomach only data. The data showed no linear correlation between the results obtained with the RIVM fasted state stomach only method and the data obtained with the tree other *in vitro* methods, data not shown. The number of data for the RIVM test satisfying the pH < 1.8 requirement was too limited (3) to allow for an evaluation of the correlation with the three other methods.

No samples have been tested for lead bioaccessibility with this method in different series in this study.

8.4.2 Application of the SBRC method

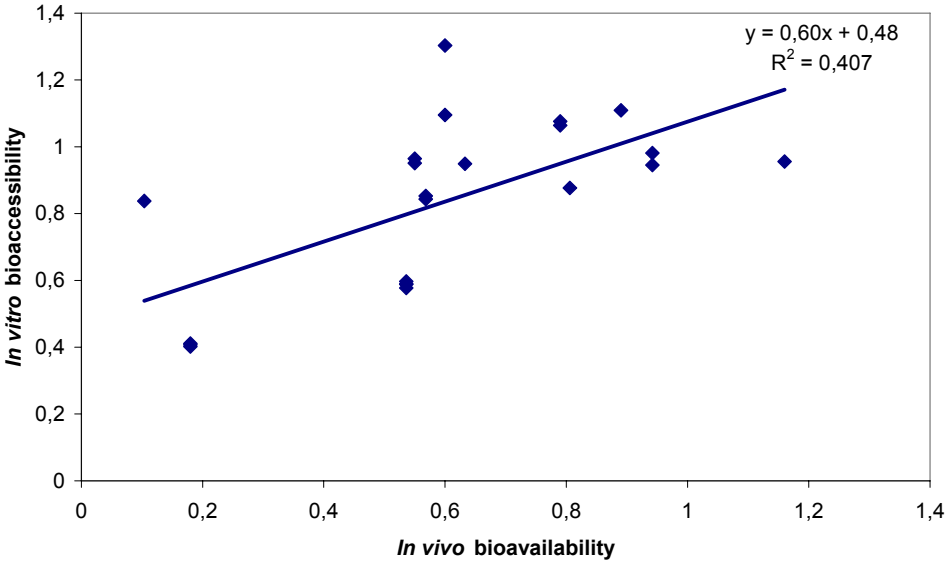
The SBRC test method /71/ with the amount of soil sample reduced from standard 1.0 g to 0.5 g was used. The liquid to solid ratio of 100 for the SBRC test method was maintained.

The calculations of relative bioaccessibilities in this correlation study were done relative to experimental references with soluble contaminants added.

In all, 12 samples with cadmium *in vivo* bioavailability for juvenile swine were tested, Figure 8 19.

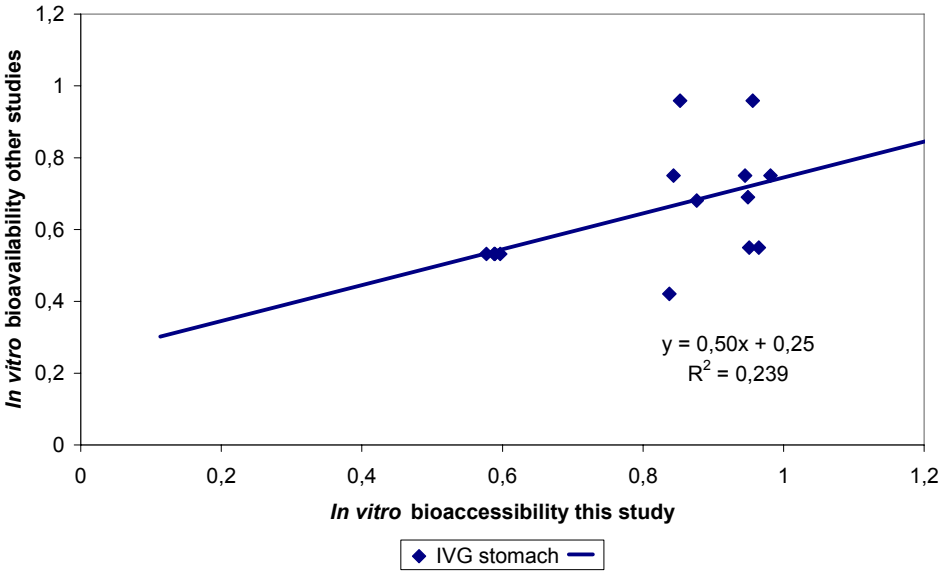
There is not a linear correlation between the *in vitro* and the *in vivo* data, and the overall trend is higher bioaccessibilities than bioavailabilities with most bioaccessibilities in the range 0.8–1.2, even for some soil samples with in vivo bioavailabilities at or below 0.6.

Figure 8–19. In vitro bioaccessibility of cadmium obtained with the SBRC test method against in vivo bioavailability obtained with juvenile swine.



No samples have been tested for cadmium bioaccessibility with this method in different series in this study.

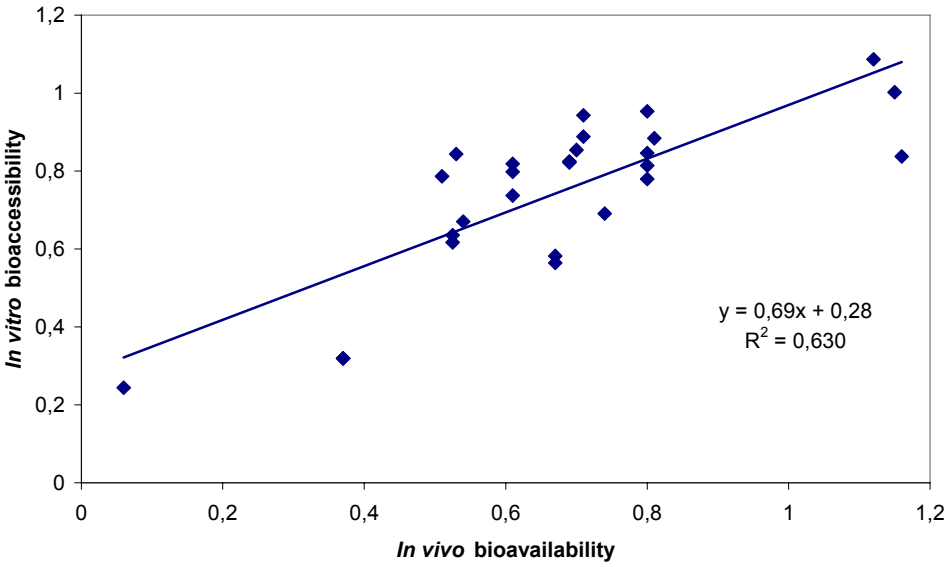
Figure 8 20 In vitro bioaccessibility of cadmium obtained with another test method /72/ against in vitro bioaccessibility obtained with the SBRC test method



For some of the tested soils, in vitro bioaccessibility test data obtained with another method (IVG /72/) have been available for comparison with the RIVM fasted state stomach only data, see Figure 8 20. The data do not show linear correlation between the results obtained with the two methods, and the SBRC results are in the range 0.8–1 except for one sample.

In all, 18 samples with lead in vivo bioavailability for juvenile swine were tested, Figure 8–21.

Figure 8–21. In vitro bioaccessibility of lead obtained with the SBRC test method against in vivo bioavailability obtained with juvenile swine

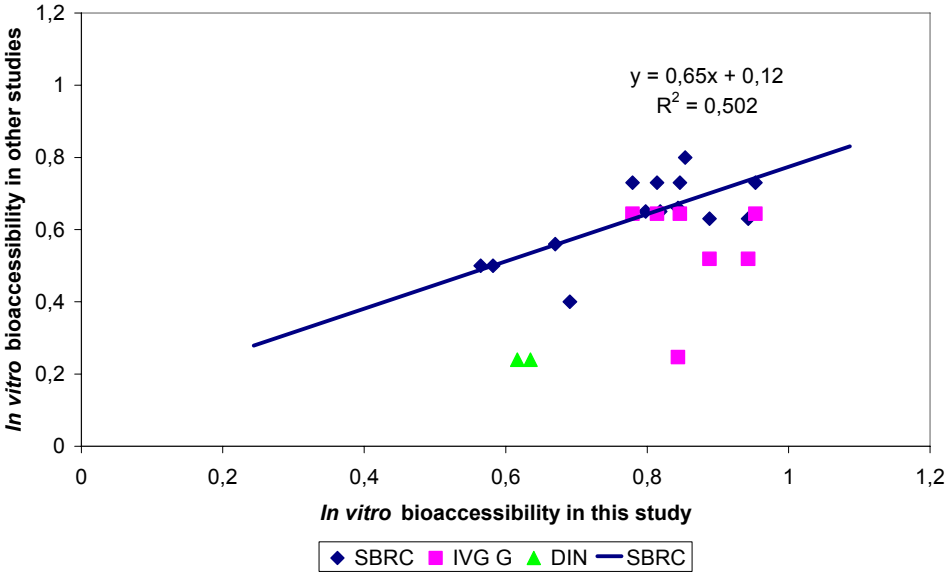


There is a linear correlation between the in vitro and the in vivo data and the overall trend is higher bioaccessibilities than bioavailabilities, in particular for low bioavailabilities.

For some of the tested soils, in vitro bioaccessibility test data obtained with other methods (IVG /72/, DIN /55/, SBRC /92/) have been available for comparison with the SBRC data from the DEPA study. The data showed linear correlation between the results obtained with the SBRC method in the DEPA study and those obtained previously with the same method /92/ with higher bioaccessibilities generally obtained in this study, Figure 8–22. There was no correlation between the SBRC and the data obtained with the IVG stomach in vitro method and the correlation could not be evaluated for DIN (one sample only).

In a section specifying the quality control procedures for the SBRC method /9/, it is required that the test solution lead concentration from test of the NIST 2711 certified reference material should be 9.22±1.50 mg Pb/L. In this DEPA study, the test solution concentration from NIST 2711 was 9.7 mg Pb/L (duplicate tests) and thus well within the required range.

Figure 8–22. In vitro bioaccessibility of lead other test /55;72;92/ against in vitro bioaccessibility obtained with the SBRC test method



No samples have been tested for lead bioaccessibility with this method in different series in this study.

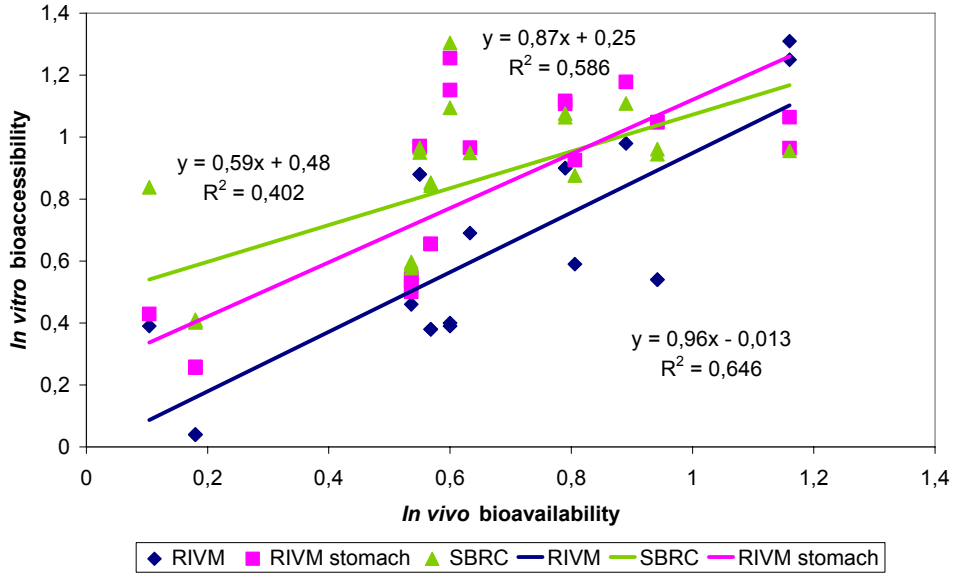
8.4.3 Comparison of different bioaccessibility test methods applied to soils with internationally documented in vivo bioavailability data

A comparison of the three bioaccessibility test methods applied to soil samples with internationally documented in vivo bioavailability data from juvenile swine, Figure 8 23, shows that there is a linear correlation for cadmium between *in vitro* and *in vivo* data for the RIVM fasted state (best correlation) and RIVM fasted state stomach only tests but not for SBRC.

For RIVM fasted state stomach only and SBRC both simulating the stomach dissolution only, all samples with *in vivo* relative bioavailabilities above approximately 0.6 gave *in vitro* relative bioaccessibilities close to 1, *i.e.:* complete dissolution of soil cadmium. Also, the *in vitro* data obtained with these methods were above or much above the *in vivo* data, whereas the data obtained with the full RIVM fasted state test method were closer to the *in vivo* data.

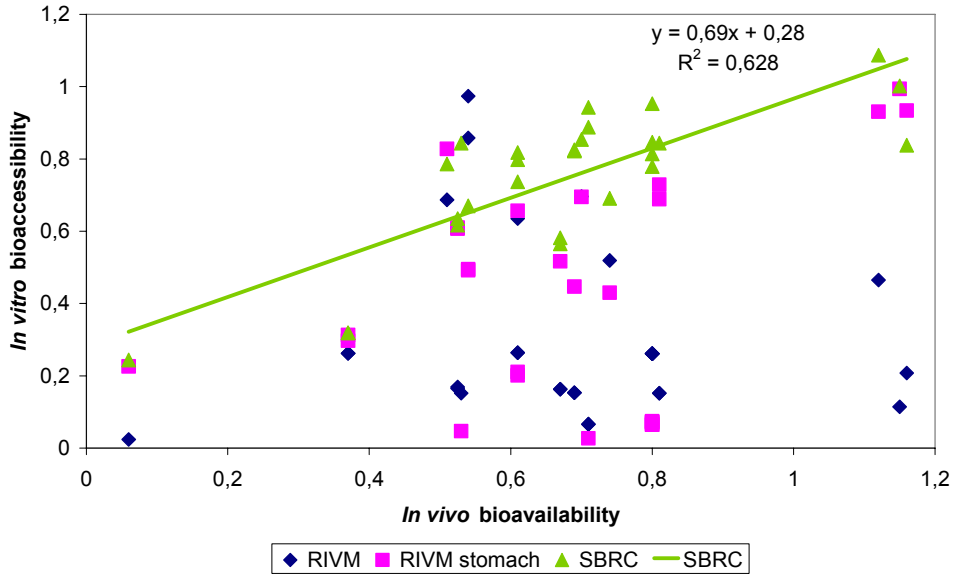
Considering also the *in vitro* to *in vivo* correlation demonstrated previously for the full set of soil samples with Cd *in vivo* data, Figure 8.7, with the RIVM fasted state method, this method is considered to provide the best and satisfactory *in vitro* to *in vivo* correlation for cadmium.

Figure 8–23. In vitro bioaccessibility of cadmium as obtained with the three test methods against in vivo bioavailability as obtained in juvenile swine.



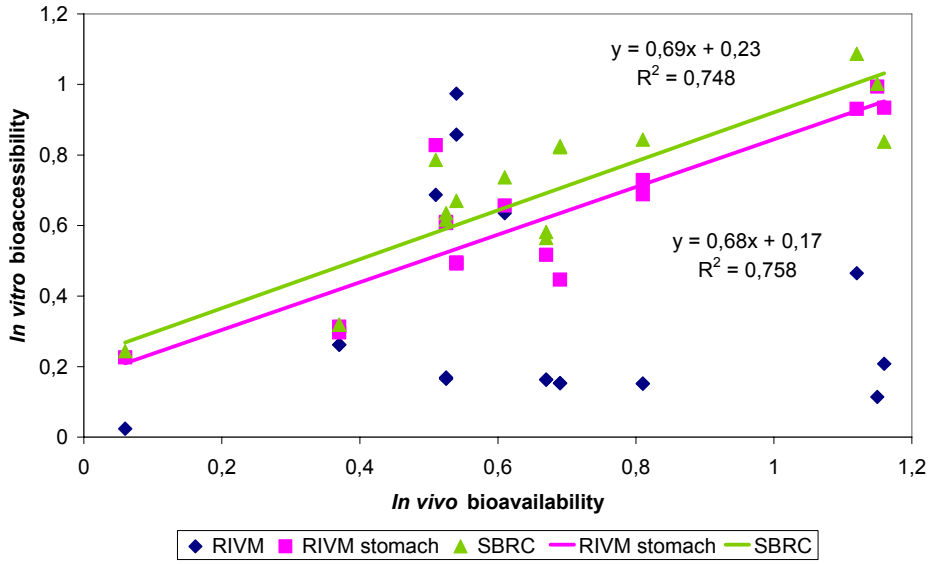
A comparison of the three bioaccessibility test methods applied to soil samples with internationally documented in vivo bioavailability data for lead from juvenile swine or mini pigs, Figure 8 24, shows that only the SBRC method provides linear correlation between in vitro and in vivo data for lead.

Figure 8–24. In vitro bioaccessibility of lead as obtained with the three test methods against in vivo bioavailability as obtained in juvenile swine or mini pig.



If only samples with pH after the RIVM stomach segment below the required value of 1.8 are included, both RIVM stomach only and SBRC provides linear correlation with similar correlation coefficients (R2), Figure 8 25, with the RIVM stomach only test giving in vitro data that are on the average slightly closer to the in vivo data.

Figure 8–25. In vitro bioaccessibility of lead as obtained with the three test methods against in vivo bioavailability as obtained in juvenile swine or mini pigs, data with pH after RIVM stomach only below 1.8.



The full RIVM fasted state test method including the intestinal segment still exhibited a large fraction of samples with apparent discrepancy between *in vitro* and *in vivo* data. As the samples with deviating pH after the stomach segment are now excluded and the effect of deviating pH after the intestinal segment is compensated in the calculation of the relative bioaccessibility, other factors must cause this apparent bias of the bioaccessibility data. A reduction in recovery of lead added to soil recorded in the full RIVM test suggest that constituents added with the soil (such as e.g.: phosphate) may have caused the excessively low lead bioaccessibilities of lead from some soil samples.

Considering also the poor *in vitro* to *in vivo* correlation demonstrated previously for the full set of soil samples with Pb in vivo data with the RIVM fasted state method, this method is not considered to provide satisfactory *in vitro* to *in vivo* correlation for lead. The SBRC and the RIVM fasted state stomach only methods are both considered to provide satisfactory *in vitro* to *in vivo* correlations, with the RIVM data being closest to in vivo data. Still, this conclusions is based upon the assumption that the RIVM fasted state stomach only can be modified to ensure pH after the stomach segment below 1.8 without changing the overall *in vitro* data.

8.4.4 Application of alternative bioaccessibility test methods to Danish soils

In all 6 soil samples from each of 6 Danish sites have been subjected to the RIVM fasted state, the RIVM fasted state stomach segment only and the SBRC test methods for cadmium and lead, Table 8–7 and Table 8–8.

Table 8 7 Relative bioaccessibilities and relative standard deviations for test of 6 Danish soil with three different test methods, cadmium

	RIVM fasted state	RIVM fasted state stomach only	SBRC
Area with >100 years of urban history	0.50	1.22	1.16
Urban soil close to highway	-[19]	1.02	1.17
Urban soil close to metal industry	0.64	0.92	0.89
Urban soil with metal slags	0.56	0.83	0.95
Urban soil with metal casting sand	0.38	0.85	0.91
Urban soil with ashes from porcelain factory	0.52	1.11	1.09
Overall relative standard deviation (%)	13	7.2	12

Considering the evaluation of the three *in vitro* methods, see section 8.4.3, the *in vitro* relative bioaccessibilities obtained with the RIVM fasted state method is evaluated to provide the best estimate, Table 8–7.

In accordance with the findings in section 8.4.3, the cadmium bioaccessibilities obtained with the two other methods are higher and probably not a good prediction of cadmium bioavailability of from these soils. The test precisions (including sample inhomogeneity), Table 8–7, were similar for RIVM fasted state and SBRC, but better for RIVM fasted state stomach only.

Considering the evaluation of the three *in vitro* methods, see section 8.4.3, the *in vitro* lead relative bioaccessibilities obtained with the RIVM fasted state method is considered to have provided too low values, Table 8–8, in accordance with the findings in section 8.4.3, and the data are probably not good predictions of lead bioavailabilities from these soils. For the 4 soil samples with test pH in the required range (< 1.8), the RIVM fasted state stomach only is considered to provide the lead relative bioaccessibility values best estimating *in vivo* relative bioavailabilities, whereas the in vitro results for the remaining two samples are probably too low. Finally, the data obtained with the SBRC test method are probably reasonable but high estimates of relative bioavailability of lead from the 6 soils.

[19] -: bioaccessibility test gave result below test detection limit.

Table 8–8. Relative bioaccessibilities and relative standard deviations for test of 6 Danish soil with three different test methods, lead

	RIVM fasted state	RIVM fasted state stomach only	SBRC
Area with >100 years of urban history	0.88	0.65	0.90
Urban soil close to highway	0.18	0.64	0.62
Urban soil close to metal industry	1.08	0.87	0.90
Urban soil with metal slags	0.13	0.24[20]	0.76
Urban soil with metal casting sand	0.11	0.25[21]	0.75
Urban soil with ashes from porcelain factory	0.36	0.51	0.72
Overall relative standard deviation (%)	14	10	2.2

The test precisions (including sample inhomogeneity), Table 8–7, were similar for the two RIVM methods, but considerably better for the SBRC method.

[20] pH after stomach segment above required limit of 1.8.
[21] pH after stomach segment above required limit of 1.8.

9 Developments and perspectives

It is generally acknowledged, that many soil contaminants are less available for humans via oral exposure than presumed when setting maximum contaminant levels for soil quality or intervention values for remediation. This is primarily the case for a range of metals (*e.g.:* lead, cadmium, arsenic) and for apolar organic contaminants such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and polychlorinated dibenzodioxins and –furans (dioxins).

There is an increasing awareness of the reduced availability of these soil contaminants from contaminated soil and the importance of considering this in setting cost effective and safe remediation goals is becoming accepted internationally. Environmental agencies (DK, UK, US, NL, D) are currently working in this direction, and an international research group, the Bioaccessibility Research Group of Europe (BARGE), is working with this goal as well.

The main outstanding issue is to develop, implement, validate and apply suitable methods for quantifying the differences in availability for practical risk assessment, preferentially in an international consensus on methods.

An accepted *in vivo* method for studying the bioavailability of metals (applied for lead, cadmium, arsenic) from contaminated soils in juvenile pigs is available, but the costs of using the methods for all but the largest sites are excessive. There is a need for research to establish a suitable *in vivo* method for studying the bioavailability of organic contaminants such as PAH, PCB and dioxins.

Several test methods exist for *in vitro* measurement of metals (primarily applied for lead, cadmium, arsenic) bioaccessibility from contaminated soils and a few methods have been applied for organic contaminants (primarily for PAH, PCB, dioxins and selected pesticides).

As the bioaccessibility test results will inevitably depend upon the method applied, international consensus on one common set of methods is urgently needed in order to enable establishment of a shared data on test application and quality. It should be emphasized here, that different methods can be equally ”good” as long as they are robust and relevant, even though they provide different results. The important point is to know the test quality (robustness) and the correlation to *in vitro* bioavailability (relevance) for the method applied, and to ensure use of the same method within each jurisdiction in order to attain conformity and transparency in legal decisions.

In the BARGE, the current development towards a common in vitro bioaccessibility test method for Europe has been accelerated and is believed to provide such a method by the end of 2005.

In order to control and document the bioaccessibility test quality, there is an urgent need for production of reference materials for quality control of the tests and for laboratory proficiency tests for control of interlaboratory comparability. Furthermore, there is a need for access to a wider selection of soils and contaminants with *in vivo* bioavailability data from experimental animals than currently available in order to allow for ensuring the *in vitro* to *in vivo* correlation (the relevance).

These activities are best organized in an international cooperation in order to keep costs at the lowest level possible and to provide the best data for quality evaluation.

Finally, the availability of soil contaminants can currently only be incorporated in soil risk assessment on a site specific basis due to the very large variability of bioavailability/bioaccessibility for different site sources, soils and contaminants. Therefore there is a need for establishing also a national set of reference values of bioaccessibilities for the typical, important sites where availability is expected to be included in risk assessment. Finally, the procedures for risk assessment including bioaccessibility of soil contaminants should be described emphasizing important issues such as method to apply, contaminants accepted for testing, required test quality, number of samples to be tested, calculation procedures, MCL compliance rules etc.

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Appendix:
Test summaries for three commonly applied in vitro bioaccessibility test methods

Test conditions in mouth and oesophagus segments

Procedure	SBRC	RIVM Fasted/fed state versions[22]	DIN	Comments
Test solution[23]				
Inorganic constituents				
KCl		896	1500	
KSCN		200	500	
NaH_2PO_4		888	2000	
Na_2PO_4				
NaCl		298	1667	
NaOH		72/-		
Na_2SO_4		570	1833	
NaHCO3		-/1694	500	
CaCl2			500	
Organic constituents				
Urea		200	333	
α-Amylase		145/290	833	
Uric acid		15	33	
Galactaric acid		50/25	2500	
pH		6.5±0.2/6.8±0.2	6.4	

[22] Only shown where the conditions differ among the two version.
[23] If not stated otherwise: mg/L used for the test solution.

Procedure	SBRC	RIVM	DIN	Comments
Test conditions				
Sample mass		0.6 g/0.4 g	2 g	
Test solution volume		9 mL/6 mL	30 mL	
L/S ratio		15	15	
Test time		5 minutes	30 minutes	
Temperature		37±2°C	37°C	

Test conditions in stomach segments

Procedure	SBRC	RIVM Fasted/fed state versions[24]	DIN	Comments
Test solution[25]				
Inorganic constituents				
HCl	≈0.36 N, until pH = 1,50	0.1 N/0,078	Until pH = 2.0	
KCl		824	700	
NaH_2PO_4		266	270	
NaCl		2752	2900	
$CaCl_2 . 2H_2O$		400		
NH_4Cl		306		
Organic constituents				
Urea		85		
Galactaric acid		3000	3000	
Glycine	30.03			
Glucose		650		
Glucuronic acid		20		
Glucosamine hydrochloride		330		
BSA[26]		1000		
Pepsine		1.000/2500	1000	

[24] Only shown where the conditions differ among the two version.
[25] If not stated otherwise: mg/L used for the test solution.
[26] Bovine serum albumin, a blood protein.

Procedure	SBRC	RIVM	DIN	Comments
Food addition		-/4500 mg baby food	10000 mg milk powder	Addition of milk powder is optional in the DIN method
pH	1,50±0,05	1.07±0.07/1.30±0.02	2.0	
Test conditions				
Sample mass	1,00±0,05 g	0.6 g/0.4 g	2 g	
Test solution volume	100±0,5 mL	13.5 mL (Σ[27] 22.5)/12.0 mL (□ 18)	100 mL	
L/S ratio	100	22.5(Σ 37.5)/ 30(Σ 45)	50	
Test time	1 hour	2 hours	2 hours	
Temperature	37±2°C	37±2°C	37°C	
Test mixture pH		1.2/2-2.5	2.0	The DIN method requires readjustment of to pH = 2.0

[27] Including previous segments.

Test conditions in intestine segments

Procedure	SBRC	RIVM Fasted/fed state versions[28]	DIN	Comments
Test solution[29]				Duodenal juice and bile are separately prepared in the RIVM methods, whereas one solution for both digestion solutions is used in the DIN method
Inorganic constituents, duodenal juice				
HCl		0.0022 N		
KCl		559	300	
NaH_2PO_4		79		
NaCl		6943		
$NaHCO_3$		3355	1000	Solid $NaHCO_3$ followed by titration to required pH is used in the DIN method
NaOH	Until pH = 7.0 ± 0.2			
$MgCl_2$		49.5	200	
$CaCl_2$		197.8	500	
Inorganic constituents, bile				
HCl		0.0022 N/0.0018 N		

[28] Only shown where the conditions differ among the two versions.
[29] If not stated otherwise: mg/L used for the test solution.

Procedure	SBRC	RIVM	DIN	Comments
KCl		364		
NaCl		5207		
$NaHCO_3$		5728		
$CaCl_2$		220		
Organic constituents, duodenal juice				
Urea		99	300	
BSA[30]		990		
Pancreatine	500	2970/8911	9000	
Lipase		495/1485		
Trypsin		-	300	
Organic constituents, bile				
Urea		248		
BSA		1782		
Bile	1.750	5940/29703	9000	
pH, duodenal juice		7.8±0.2/8.1±0.2		The pH of the test solutions is not controlled in the DIN method, but the soil solution text mixture pH is controlled
pH, bile		8.0±0.2		

[30] Bovine serum albumine, a blood protein.

Procedure	SBRC	RIVM	DIN	Comments
pH adjustment				
$NaHCO_3$		-/9411		
Test conditions				
Sample mass	1.00 ± 0.05 g	0.6 g/0.4 g	2 g	
Test solution volume	95	27 mL duodenal juice and 9 mL bile (Σ[31] 58.2)/ 12 mL duodenal juice, 6 mL bile and 2 mL sodium bicarbonate solution (Σ 38)	100 mL (Σ 200)	
L/S ratio	95	60 (Σ 97)/ 50 (Σ 95)	50 (Σ 100)	
Test time	4 hours	2 hours	6 hours	
Temperature	37±2°C	37±2°C	37°C	
Test mixture pH	7.0 ± 0.2	>5.5/6.5-7.0	7.5	In the SBRC method, the intestinal pH is initially established by titration to pH = 7.0

[31] Including previous segments.

Practical test performance

Procedure	SBRC	RIVM Fasted/fed[32]	DIN	Comments
Mixing	30±2 rpm[33] all over	55 rpm all over	200 spm[34] shaker or 300 rpm magnetic stirrer (2 cm magnet)	
Adjustment of pH	In the stomach segment, pH adjustment is with test solutions applied, but if final pH in the test solution has increased to above 2, the test must be repeated with stepwise additions of hydrochloric acid to maintain low pH. In the intestinal segment, the test mixture is titrated manually with sodium hydroxide solution to pH = 7.5 ± 0.2	pH adjustment is with test solutions applied, but final pH in the test solution is controlled	Adjustment of pH is continuous with an autotitrator, alternatively with continuous, manual titration	
Phase separation	Filtration through 0.45 µm pore diameter disposable cellulose acetate filters	Centrifugation at 3000 g[35] for 5 minutes	Centrifugation at 7000 g for 10 minutes, optional filtration through 20 µm stainless steel sieve (organic contaminants) or 30 µm nylon sieve (metals) for removal of floating particles	

[32] Only shown where the conditions differ among the two versions.
[33] rpm: rounds per minute.
[34] spm: strokes per minute.
[35] g: gravity acceleration.

Quality control requirements

Procedure	SBRC	RIVM Fasted/fed[36]	DIN	Comments
Reagents blanks	1 per reagents preparation and at the least 1 per 20 samples max. 25 µg Pb/L	Not required	Not required	
Test blanks	1 per series and at the least 1 per 20 samples, max. 50 µg Pb/L	1 per series	Not required	
Replicates	1 per series and at the least 1 per 10 samples, max. 20 % CV[37]	All samples in triplicate	Not required	
Control samples, synthetic	1 per series and at the least 1 per 20 samples, recovery 85–115 % for lead	Not required	Not required	
Control samples, matrix	1 test of Montana 2711 standard reference soil from NIST[38] per 50 samples, recovery experience value 84–116 % for lead	1 test of Montana 2711 standard reference soil from NIST per series for metals/		
none currently	Not required			
Mass balance	Not required	For 1 sample per series	For 1 per 20 samples, max. 10 % deviation from 100 % mass recovery	

[36] Only shown where the conditions differ among the two versions.
[37] Coefficient of variation or relative standard deviation.
[38] NIST: National Institute of Standards and Technology.

Analytical methods for soil and test solutions

Procedure	SBRC	RIVM	DIN	Comments
Metals, soil	Digestion of samples with 2:1 HNO_3/H_2O, followed by 30 % H_2O_2, and ICP analysis	Digestion with aqua regia (HNO_3/HCl) followed by ICP MS[39] analysis	Digestion with aqua regia followed by AAS analysis	
Metals, test solutions	ICP of test solutions	Dilution x 10 of test solutions in 0,1 M HNO_3 and ICP MS analysis	Dilution of test solution x 5–10 and analysis by ICP-MS or ICP OES[40]	For analysis of test solutions with AAS, digestion is required
PAH, soil	Not specified	HPLC[41] for benzo(a)pyrene	Soxhlet extraction with hexane/acetone, 4:1, followed by GC[42]-MS analysis or corresponding methods	
PAH, test solutions	Not specified	HPLC for benzo(a)pyrene. For other organic contaminants extraction with hexane, addition of methanol, centrifugation, concentration by evaporation and analysis by GC-ECD[43]	Extraction of test solution with hexane followed by addition of NaCl (aq., sat.) and acetone, analysis by GC-MS or corresponding, SPE[44] not suitable due to interference from added milk powder	Saponification of milk powder constituents can be required to obtain full recovery of PAH. Addition of saturated sodium chloride solution and acetone, as well as centrifugation can be required to break solvent to test solution emulsions

[39] MS: mass spectrometry, a detection method used for both metals and organic compounds.
[40] OES: optical emission spectroscopy, a multielement detection method used primarily for metals.
[41] HPLC: high performance liquid chromatography, a method used for analysis (separation) primarily of organic compounds.
[42] GC: gas chromatography, a method used for analysis (separation) primarily of organic compounds.
[43] ECD: electron capture detector, a method used for analysis (detection) primarily of organic compounds.
[44] SPE: solid phase extraction, a method used to concentrate and purify extracts primarily of organic compounds as part of their analysis.

Soil pretreatment methods

Procedure	SBRC	RIVM	DIN	Comments
Drying	<40°C	Air drying for one week	According to ISO[45] 11464 og ISO 14507	
Removal of large particles	-	Removal of pebbles and twigs	<1 mm ensured by removal of particles or by sieving	
Grinding/milling	Not specified	I Retsch mill	Not specified	
Sieving	<250 µm	<1 mm	<1 mm ensured by removal of particles or by sieving	
Homogenization	Sample divider	Mixing with a shovel and sub-sampling with teflon coated stainless steel spoon	Not specified	

[45] International Standardization Organisation.

Laktester för riskbedömning av förorenade områden

underlagsrapport 2a och 2b

RAPPORT 5557

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Laktester används ofta i riskbedömningar för att uppskatta ett förorenat materials lakningsegenskaper eller för att bedöma fastläggningsegenskaper för olika föroreningar. I dag saknas en gemensam metodik för hur olika laktester bör utföras och tolkas vid riskbedömning av förorenad mark. Detta kan ge stora variationer när olika platser bedöms och kan i värsta fall leda till felaktiga prioriteringar. Här redovisas ett förslag till metodik för val, utförande och tolkning av laktester som verktyg i miljö- och hälsoriskbedömningar för förorenade områden. Rapporten beskriver olika typer av laktester och författarna ger rekommendationer på hur de bör tolkas i riskbedömningssammanhang. Vidare diskuteras hur resultaten kan utnyttjas som indata till riktvärdes- och spridningsmodeller samt osäkerheter och känsliga antaganden. En utvärdering som bygger på sammanställningar från svenska och danska saneringsprojekt visar att det finns osäkerheter i hur resultat från dagens standardiserade laktester ska användas och tolkas vid riskbedömning. Rapporten ges ut i tre delrapporter.

Naturvårdsverket har inte tagit ställning till innehållet i den här rapporten. Författarna svarar själva för innehåll, slutsatser och eventuella rekommendationer.

Kunskapsprogrammet Hållbar Sanering samlar in, bygger upp och sprider kunskap om förorenade mark- och vattenområden. Genom Hållbar Sanering kan myndigheter, forskare och företag söka bidrag för utredningar, seminarier och utvecklingsprojekt som täcker kunskapsluckor på kort och lång sikt. Hållbar Sanering styrs av en programkommitté som består av representanter från Banverket, Göteborgs stad, KTH, Linköpings Universitet, Länsstyrelsen i Kalmar, Naturvårdsverket, Norges Teknisk- Naturvetenskaplige Universitet; SGI, SLU, Sydkraft SAKAB och Umeå Universitet.

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