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Bioaccessibility of contaminants from ingested soil in humans

Method development and research on the bioaccessibility of lead and benzo[a]pyrene

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Abstract

In most human risk assessments of soil contamination, oral bioavailability of a contaminant from soil is considered equal to the bioavailability of the contaminant from the matrix as used in toxicity studies upon which risk assessment is based. In toxicity studies typically food and liquid matrices are used. In literature it is suggested that oral bioavailability of contaminants from soil is significantly lower. As a consequence, risks may be overestimated substantially.
A simple *in vitro* digestion model, representative for human physiology, was developed in order to investigate the effects of a soil matrix on oral bioavailability. This model allows measuring bioaccessibility of a contaminant, i.e. the fraction of the dose ingested that becomes available for absorption into the human body. The *in vitro* digestion model may, after validation to *in vivo* data, be used as a tool to assess site specific bioaccessibility and thus site-specific *relative* bioavailability factors.
With the *in vitro* digestion model the effects of several parameters on bioaccessibility were investigated. Lead and benzo[a]pyrene were chosen as test contaminants. Effects of 1) type of contaminant, 2) contamination level, 3) type of soil, 4) pH of soil, 5) ageing of the soil, and 6) metal speciation on bioaccessibility were investigated. Also a start was made with investigating the differences between artificially and historically contaminated soil. It was tried to describe the data by means of a mechanism-based mathematical model. The results suggest in many cases a non-linear relationship between the level of lead contamination and the amount of contaminant mobilised from soil into digestion juice, i.e. chyme, or a relationship with a maximum level of benzo[a]pyrene in chyme, i.e. a precipitation level. In addition, bioaccessibility seems to depend on the type of contaminant and the type of soil. The other tested parameters seemed to have little or no influence on the bioaccessibility. Further experiments are needed in order to validate the mathematical model and to make the model applicable for forecasting bioaccessibility of a contaminant from a certain soil sample. Ultimately, the mathematical model may be employed to estimate bioaccessibility values based on contaminant and soil characteristics.

Contents

Samenvatting

In risicoschatting van gecontamineerde bodem wordt de orale biobeschikbaarheid van een contaminant uit bodem vaak gelijkgesteld aan de orale biobeschikbaarheid van die contaminant uit de matrices zoals gebruikt in toxiciteitstudies, welke de basis vormen voor humane risicobeoordeling. In toxiciteitstudies worden vrijwel altijd voedings- of vloeistofmatrices gebruikt. In de afgelopen jaren zijn er echter aanwijzingen gekomen dat de orale biobeschikbaarheid uit bodem significant lager kan zijn, hetgeen tot overschatting van de risico's leidt. Het onderzoek beschreven in dit rapport heeft zich daarom gericht op de ontwikkeling van een systeem waarmee een deelproces van de orale biobeschikbaarheid van een contaminant uit bodem kan worden geschat en voorspeld.
Orale biobeschikbaarheid kan worden onderverdeeld in 4 processen, i.e. 1) ingestie van een matrix + contaminant, 2) bioaccessibility, de extractie van een contaminant uit zijn matrix ten gevolge van het digestieproces in het maagdarmkanaal, 3) intestinale absorptie, het transport van vrijgemaakte contaminant uit het darmlumen in het bloed, en 4) metabolisme in de lever. Bioaccessibility wordt bepaald door de matrix, in dit geval bodem, waarin de contaminant aanwezig is. Daarom is een simpel *in vitro* digestiemodel ontwikkeld, representatief voor de humane fysiologie, waarmee bioaccessibility kan worden bepaald. Ontwikkeling en optimalisatie van dit *in vitro* digestiemodel zijn beschreven in dit rapport. Er is voorkeur aan een *in vitro* model boven een *in vivo* model gegeven, omdat met een *in vitro* model op eenvoudigere wijze een groot aantal monsters en variabelen zijn te testen.
Het rapport beschrijft tevens de resultaten van studies naar de bioaccessibility van het anorganische lood en het hydrofobe organische benzo[a]pyreen. Deze contaminanten zijn enerzijds, in overleg met de opdrachtgever, gekozen vanwege hun beleidsrelevantie en anderzijds vanwege de verwachting dat de bioaccessibility van anorganische stoffen door andere factoren wordt beïnvloed dan de hydrofobe organische stoffen. Als overige variabelen zijn gekozen: 1) contaminatie-niveau, 2) bodemtype, 3) pH van bodem, 4) het verouderen van de bodem (alleen voor lood), en 5) de speciatie van lood in het uitgangsmateriaal. Daarnaast is voor lood een begin gemaakt met de vergelijking tussen bodems die kunstmatig of historisch gecontamineerd zijn.
De bioaccessibility uit geteste bodems is minimaal 6% en maximaal 72% voor lood, en minimaal 2% en maximaal 63% voor benzo[a]pyreen. De resultaten laten vaak een niet-lineair verband zien tussen bioaccessibility en contaminatieniveau in de bodem (Pb), of een verzadigingsniveau voor de bioaccessibility van benzo[a]pyreen, wat verklaard zou kunnen worden door precipitatie in het digestiesap. Bij de data-analyse is gebleken dat de conventionele statistische testen, zoals multipele variantie-analyse en multipele regressie-analyse, eigenlijk niet geschikt waren voor analyse van de verworven datasets. Daarom is in een vroegtijdig stadium een voorlopige versie van een mathematisch model ontwikkeld en toegepast voor data-analyse. Dit mathematisch model, dat gebaseerd is op theorieën over binding van contaminanten aan bodemdeeltjes, staat een niet-lineair verband tussen de bioaccessibility van

lood/benzo[a]pyreen en het contaminatieniveau toe. In de helft van de gevallen worden de data voor lood beter beschreven met een niet-lineair verband volgens het mathematisch model, dan met een lineair verband. Vanwege het verzadigingsniveau zijn voor benzo[a]pyreen te weinig data over vóór het verzadigingspunt om een uitspraak te doen over het verband tussen bioaccessibility en contaminatieniveau. Daarnaast blijkt uit de data dat bioaccessibility afhankelijk is van de contaminant en van het bodemtype. De pH van de bodem heeft geen direct effect op de bioaccessibility. De slecht oplosbare loodspeciatie $PbSO_4$ vertoont in de huidige experimenten slechts een lichte neiging tot lagere bioaccessibility dan het goed oplosbare $Pb(NO_3)_2$. Na veroudering van de bodem gedurende 1½ jaar nam, afhankelijk van de bodem, de bioaccessibility enigszins toe of bleef gelijk. Mogelijk hangt dit samen met de bewaarcondities van de bodem (gedroogde bodem, 4 ºC). De bioaccessibility van lood van historisch gecontamineerde bodem blijkt vergelijkbaar met waarden voor kunstmatig gecontamineerde bodems. Een uitgebreidere vergelijking tussen historisch verontreinigde bodems en in het laboratorium gecontamineerde bodem moet gemaakt worden, zodat hiertussen een link kan worden gelegd. Onderzoek hiernaar zal in toekomstige studies aandacht moeten krijgen, evenals validatie van het *in vitro* model met *in vivo* data, en vergelijking van het *in vitro* model met andere *in vitro* digestiemodellen.

Conclusies t.b.v. risicoschatting:
1) Er kan geen nauwkeurige uitspraak worden gedaan over *de* bioaccessibility van een contaminant in bodem omdat de bioaccessibility afhankelijk is van het bodemtype en mogelijk van het contaminatieniveau. Het zou wel mogelijk zijn om voor een bepaalde stof een generieke waarde te bepalen waaronder de bioaccessibility in ieder geval ligt. Een verdere mogelijkheid tot verfijning is m.b.v. het mathematisch model. Met het mathematisch model zou voor een contaminant de relatie tussen bioaccessibility en contaminatieniveau kunnen worden beschreven. Met het mathematisch model kan dan een schatting gemaakt worden van de relative biobeschikbaarheidsfactor op basis van de contaminant en het bodemtype. Eerst zijn nog aanvullende experimenten noodzakelijk om het mathematisch model te valideren.
2) Wel lijkt het mogelijk om locatiespecifiek de bioaccessibility van een contaminant in bodem, en daarmee locatiespecifiek de biobeschikbaarheid, te bepalen. De resultaten die worden verkregen met het *in vitro* digestiemodel moeten met de nodige voorzichtigheid worden behandeld zolang validatie aan de *in vivo* situatie niet heeft plaatsgevonden. Indien rekening wordt gehouden met een relatieve biobeschikbaarheidsfactor, kan het risico van bodemcontaminanten aanmerkelijk lager zijn dan wat gewoonlijk op basis van contaminatieniveau in de bodem en gegevens uit toxiciteitstudies afgeleid wordt. Dit wordt geïllustreerd door de bioaccessibility waarden van de geteste bodems, welke tussen 6 en 72% zijn voor lood, en tussen 2 and 63% voor benzo[a]pyrene.

Summary

In risk assessment on contamination of soil, oral bioavailability from soil is assumed to be equal to bioavailability of the contaminant from the matrix used in toxicity studies upon which human risk assessment is based. In toxicity studies typically food and liquid matrices are used. In literature it is suggested that oral bioavailability of contaminants from soil can be significantly lower. As a consequence, risks from soil may be overestimated substantially. On the basis of this knowledge it was aimed to focus our research on development of a tool for estimation and prediction of one process from bioavailability of contaminants from soil.
Oral bioavailability can be divided into four major processes, i.e. 1) oral intake of matrix + contaminant, 2) bioaccessibility, which represents the extraction of a contaminant from its matrix by digestion, 3) intestinal absorption, which represents transport of the extracted contaminant from intestinal lumen into blood, and 4) metabolism in liver. Bioaccessibility is affected by the matrix in which the contaminants are ingested, in this case soil. Therefore a simple *in vitro* digestion model, representative for human physiology, was developed allowing measurement of bioaccessibility. Development and optimisation of this *in vitro* digestion model is described in the present report. An *in vitro* model was preferred over *in vivo* studies since it facilitates the possibilities to investigate a large number of samples and variables.
The present report also describes the results of studies on the inorganic contaminant lead and the hydrophobic organic contaminant benzo[a]pyrene. The choice of these contaminants was based on the relevancy for policy and on the assumption that bioaccessibility of inorganic and hydrophobic organic contaminants will be affected differently by the variables. In addition, effects of 1) contamination level, 2) type of soil, 3) pH of soil, 4) ageing of soil (for lead), and 5) lead speciation on bioaccessibility are investigated. Furthermore, for lead preliminary experiments are performed to compare soils that were artificially or historically contaminated.
Bioaccessibility from the tested soils was at minimum 6% and at maximum 72% for lead, and at minimum 2% and at maximum 63% for benzo[a]pyrene. The experimental results show non-linear relationships between bioaccessibility and contamination level in the soil (Pb), or a level of saturation for the bioaccessibility of benzo[a]pyrene, which can be explained by precipitation in digestive juice. It appeared that conventional statistical tests, like multiple analysis of variance and multiple regression were inadequate to describe the data. Hence, a preliminary version of a mechanism-based mathematical model was developed and applied for data analysis. The mathematical model allows non-linear relationship between bioaccessibility of lead or benzo[a]pyrene and the level of contamination. The non-linear relationship according to the mathematical model describes the data in half of the cases for lead better than a linear relationship. Due to the saturation level for benzo[a]pyrene, not enough data are left before the point of saturation to discriminate between a linear or non-linear relationship between bioaccessibility and soil contamination level. In addition, bioaccessibility seems to depend on the type of contaminant and the type of soil. Soil pH did not have a direct effect on bioaccessibility. The hardly soluble lead speciation $PbSO_4$ displays in the present experiments only

slightly lower bioaccessibilities in comparison to the well soluble $Pb(NO_3)_2$. Bioaccessibility slightly increased or remained unchanged (depending on the soil) after ageing of the soil for 1½ year. It is possible that encapsulation of the contaminants did not occur due to the storage conditions of the soil (dried soil, 4 °C). Bioaccessibility of lead for historically contaminated soils is comparable to bioaccessibility values for artificially contaminated soils. This comparison will also be taken into account in future studies in order to link the experimental results. Further research should be focussed on the validation of the *in vitro* digestion model with *in vivo* data, and comparison of the *in vitro* digestion model to other *in vitro* digestion models.

Conclusion for risk assessment:
1) It is not possible to assess *the* bioaccessibility of a contaminant in soil since bioaccessibility depends on the soil type and possibly on the contamination level. It would be possible to determine a generic bioaccessibility value for a contaminant, which represents the upper level possible. Further refinement is possible with the mathematical model. With the mathematical model the relationship between bioaccessibility and contamination level can be described. The mathematical model can than be used to estimate a relative bioavailability factor depending on the contaminant and soil type. Further experiments are needed to validate the mathematical model.
2) It seems to be possible to estimate location-specific bioaccessibility values for a contaminant in soil, and thus location specific bioavailability. The results obtained with the *in vitro* digestion model should be interpreted with caution, as long as *in vivo* validation has not taken place.
If considering a relative bioavailability factor, the risk of soil contaminants can be considerably smaller than what usually is derived on the basis of contamination level and toxicity studies. This is exemplified by the bioaccessibility values from the tested soils, which were between 6 and 72% for lead, and between 2 and 63% for benzo[a]pyrene.

Glossary

B(a)P	=	benzo[a]pyrene
CV	=	Coefficient of Variation
EU	=	European Union
HPLC	=	High Pressure Liquid Chromatography
ICP-MS	=	Inductively Coupled Plasma-Mass Spectrometry
I.V.	=	Intervention Value
LLQ	=	Lower Limit of Quantification
LOI	=	Loss On Ignition
MPR	=	Maximum Permissible Risk
n.d.	=	not determined
OECD	=	Organisation for Economic Co-operation and Development
Pb	=	lead
$PbAc_2$	=	lead acetate, i.e. $Pb(CH_3COO)_2$
$PbCl_2$	=	lead chloride
$Pb(NO_3)_2$	=	lead nitrate
$PbSO_4$	=	lead sulphate
PBET	=	Physiologically Based Extraction Test
TOC	=	Total Organic Carbon
UF	=	Uncertainty Factor

1. Introduction

1.1 Rationale for investigating oral bioavailability from soil

In human health risk assessment, ingestion of soil is considered a major route of exposure to many soil contaminants [Sheppard et al., 1995; Paustenbach, 2000]. For that reason oral bioavailability of ingested soil contaminants is relevant to investigate. Oral bioavailability of soil contaminants is defined as the contaminant fraction that reaches the systemic circulation. Figure 1.1 describes the four major processes of oral bioavailability for soil contaminants. After soil ingestion, contaminants can be partially or totally released from the soil during digestion. The fraction of contaminant that is mobilised from soil into chyme is defined as the bioaccessible fraction [Ruby et al., 1999]. This fraction is considered to represent the maximum fraction of contaminant available for intestinal absorption. Bioaccessible contaminants can subsequently be absorbed, i.e. transported across the intestinal wall, and transferred into the blood (or lymph) stream. The compounds may be biotransformed and excreted in the intestinal epithelium or liver. This is referred to as first-pass effect. After these steps, the contaminants reach the systemic circulation and thereby the rest of the body, and may exert systemic toxicity. Consequently, oral bioavailability of soil-borne contaminants is the result of the four steps presented in figure 1.1: soil ingestion, bioaccessibility, absorption, and first-pass effect.

In vitro studies [Ruby et al., 1992; Ruby et al., 1993; Hack and Selenka, 1996; Ruby et al., 1996; Ellickson et al., 2001] and studies in experimental animals [Freeman et al., 1992; Dieter et al., 1993; Freeman et al., 1993; Freeman et al., 1994; Freeman et al., 1996; Ruby et al., 1996; Casteel et al., 1997; Ellickson et al., 2001] and humans [Maddaloni et al., 1998] suggest that oral bioavailability of contaminants from soil can be significantly lower than from matrices like water or food. Up till now in risk assessment, the oral bioavailability of a contaminant from soil is assumed to equal the oral bioavailability from the matrix applied in toxicity studies. In toxicity studies typically liquid and food matrices are employed. As a consequence, risks from soil may be overestimated substantially. Hence, introduction of a ***relative bioavailability factor*** for a contaminant, i.e. the bioavailability from a soil matrix with respect to the bioavailability from the matrix used in toxicity studies to assess the maximum tolerable risk (MTR), would lead to more accurate risk assessment. The background, the derivation, and how to use such relative bioavailability factors in human risk assessment is addressed in a report “Overzicht van werk tot nu toe en koppeling aan beleidsvragen” [Rompelberg and De Zwart, 2001]. A more general technical evaluation of the intervention values for soil/sediment and groundwater is presented in a report by Lijzen et al. [2001].

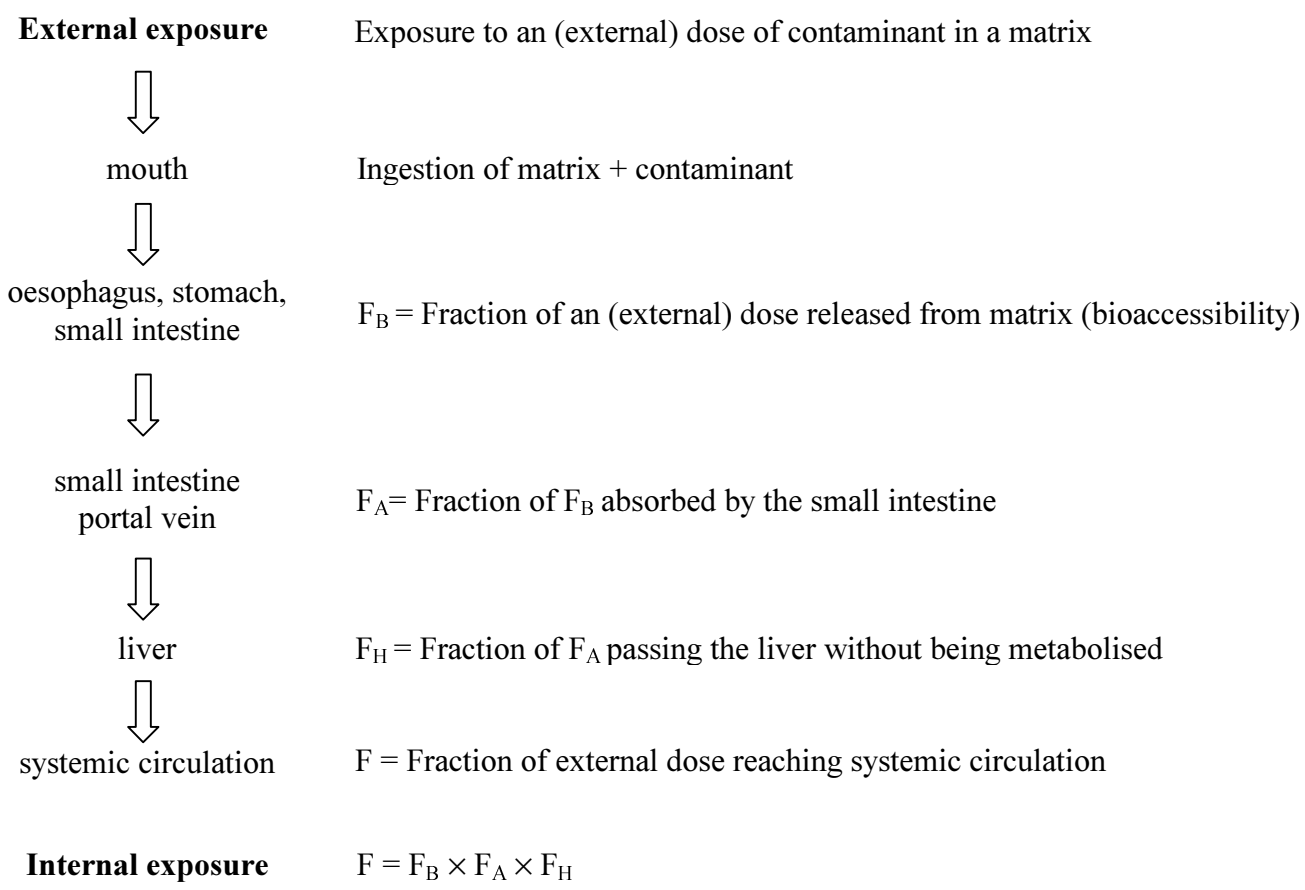

Figure 1.1. Processes in oral bioavailability.

Investigating (oral) bioavailability of contaminants from soil is especially relevant in case 1) (oral) bioavailability from the matrix applied in toxicity studies to assess NOAEL is expected to be significantly different from (oral) bioavailability from soil, 2) contamination levels are near or above the intervention value (I.V.), and 3) soil ingestion is a major exposure route.

1.2 Aim

The overall aim of the project is to develop a tool for estimating oral bioavailability of a contaminant from a certain soil sample in order to improve risk assessment of polluted soils.

It would be very expensive and time consuming to assess the oral bioavailability of each soil sample by *in vivo* studies. An *in vitro* model was also preferred over *in vivo* studies since it reduces the need for test animals. Hence, an *in vitro* digestion model based on human physiology has been developed as a simple, cheap and reproducible tool to investigate bioaccessibility, as an aspect of oral bioavailability of soil contaminants. This *in vitro* digestion model focuses on bioaccessibility, see figure 1.1, since this step is an indicator for human internal exposure. For example, when bioaccessibility is low, oral bioavailability will also be low. An estimate of absolute oral bioavailability of a contaminant from soil can be made on the basis of data on bioaccessibility in combination with absorption and metabolism data from literature. An estimate of the relative bioavailability factor that may be used for risk assessment can be obtained by dividing bioaccessibility for the soil matrix, $F_{B,soil}$, by the bioaccessibility for the matrix used in toxicity studies, $F_{B,tox\ study}$, on which the intervention value is based, i.e. $F_{B,soil}/F_{B,\ tox\ study}$. In this case it is assumed that F_A and F_H, the fraction absorbed and metabolised are not affected by the matrix of ingestion. Calculation of the absolute oral bioavailability and the relative bioavailability factor is further specified in the report “Overzicht van werk tot nu toe en koppeling aan beleidsvragen” [Rompelberg and De Zwart, 2001].

In literature it has been reported that bioaccessibility is significantly affected by various factors such as physical-chemical properties of the contaminant [Dieter et al., 1993; Freeman et al., 1996; Gasser et al., 1996; Ruby et al., 1996; Ruby et al., 1999], soil characteristics [Ruby et al., 1993; Ruby et al., 1996; Hamel et al., 1998; Hamel et al., 1999; Ruby et al., 1999], the composition of digestive juices [Guyton, 1991; Ruby et al., 1992; Oomen et al., 2000], and the presence of food constituents [Hack and Selenka, 1996; Oomen et al., submitted]. For that reason it is realistic to assume that there are too many variables to expect that one uniform value for oral bioavailability of a certain contaminant from any type of soil can be derived. Consequently, our research is focussed on development of a simple *in vitro* method to determine bioaccessibility of a certain soil sample to improve location specific remediation decisions. A second aim was developing a *mathematical model* for bioaccessibility which takes variables like the level of contamination, type of contaminant (metals versus organic compounds, metal speciation in soil) and physical characteristics of soil into account. Such a mathematical model can be used to derive I.V. more accurately, or as a first step in actual site-specific risk assessment. However, before such a mathematical model can be build structural information on bioaccessibility in relation to various variables is required. In table 1.1, a schematic overview of variables to be tested for obtaining this structural information, is depicted. All variables were tested in the *in vitro* digestion model.

Table 1.1. Scheme of variables to be tested in the in vitro digestion model.

Type of contaminant [a]	**Contamination level** [b]	**Type of soil** [d]	**Soil pH** [e]	**Ageing** [f]	**Lead speciation** [f]	**Contamination** [h]
Lead	blank	OECD-medium	pH 3	t=0	Lead sulphate	Artificial
Benzo[a]pyrene	0.5 × I.V. [c]	Appelscha sand	pH 4	t=1½ year	Lead nitrate	Historical
	1 × I.V.	Hulshorst sand A	pH 5		Lead chloride	
	3 × I.V.	Hulshorst sand B	pH 6		Lead acetate	
	5 × I.V. [c]	Bemelen silt	pH 7			
		Boskoop silt loam				
		Angeren silty clay loam				
		Akkrum loam				

a In the present report only data on lead and benzo[a]pyrene are described. Arsenic, cadmium and benzo[a]anthracene will be tested in future experiments.
b These contamination levels are determined for each variable (except for the experiments to compare artificially and historically contaminated soil).
c The within day variation and between day variation is determined for these contamination levels.
d The amount of lead and benzo[a]pyrene mobilised from soil is tested for all contamination levels for comparison to other soil types.
e Soil pH has been tested for lead and benzo[a]pyrene in OECD-medium only.
f Ageing of the soil has been tested for lead in OECD-medium and Dutch soil types at different contamination levels.
g Lead sulphate and lead nitrate were investigated in all types of soil, whereas lead nitrate and lead chloride were tested in OECD-medium only.
h A preliminary study is performed to compare artificially versus historically contaminated soil. To that end, bioaccessibility values for field soils that were historically contaminated with lead are compared to bioaccessibility values of soils that were artificially contaminated with lead.

In summary, the aim of the studies described in this report is:

1) The development of a simple physiologically based *in vitro* digestion model representative for human physiology. The model can be used as a tool to assess site-specific relative bioavailability factors, but results should be interpreted with case, as long as *in vivo* validation has not taken place.
2) Research on the effects of several variables (e.g. type of contaminant, contamination level, soil type, soil pH, ageing of the soil, speciation lead in soil, and historically versus artificially contaminated soil) on bioaccessibility of lead and benzo[a]pyrene (see table 1.1).
3) Development of a mathematical model. The mathematical model will be used to investigate the results of the different variables on bioaccessibility. Ultimately, the mathematical model may be employed to estimate bioaccessibility or relative bioavailability factors based on contaminant and soil characteristics.

2. Optimisation of *in vitro* digestion model

2.1 General overview digestion models

In recent years, various studies [Ruby et al., 1992; Ruby et al., 1993; Gasser et al., 1996; Hack and Selenka, 1996; Ruby et al., 1996; Hamel et al., 1998; Maddaloni et al., 1998; Hamel et al., 1999; Ellickson et al., 2001] have been reported on oral bioavailability of contaminants from soil. However, most of these studies were described for specific soil samples. As soil characteristics may affect oral bioavailability it is difficult to extrapolate the results of those studies to soil samples in general. Alternatively, bioaccessibility was investigated in order to obtain information on the influence of the soil matrix on oral bioavailability of lead and benzo[a]pyrene.

The number of studies on bioaccessibility is growing, but is still a developing area of investigation. In 1992, Ruby et al. were one of the first to report on bioaccessibility of lead from soil using a model under only simulated gastric conditions [Ruby et al., 1992]. The authors then already mentioned that dissolved lead concentrations in the digestate from only the stomach would provide an overestimation of soluble lead, since complexation and precipitation of this lead is to be expected in the intestine where pH values are higher. Other frequently applied methods on studying bioaccessibility are so-called leaching studies in which the total extractable contaminant concentration in soil is measured. These studies are based on leaching the soil with concentrated nitric acid (HNO_3) in a laboratory microwave extraction procedure (EPA method 3051) or with a mixture of nitric and hydrochloric acid (HCl) on a hot plate (EPA method 3050) [Hamel et al., 1998]. A toxicity leaching protocol (EPA method 3015) for assessing potential risk to soils at hazardous waste site employs a concentrated HCl solution and a low liquid to soil ratio [Hamel et al., 1998]. Unfortunately these methods will also overestimate the amount of contaminant that can be absorbed after ingestion of soil [Hamel et al., 1998], since conditions are much more aggressive than physiological conditions. Other studies mimicking leaching protocols were also reported [Gasser et al., 1996]. However, none of these studies represent human physiology.

In more recent years the persuasion that physiologically based tests are required has gained ground. In 1995, Rotard et al. [1995] proposed an *in vitro* digestion model using synthetically prepared digestion juices. Contrary to earlier reported models [Ruby et al., 1992; Gasser et al., 1996] the model covers the entire route from mouth to small intestine. Another, much more sophisticated model was introduced by Minekus et al. [1995]. This latter model is employs gradual transit from one compartment to another, and defines bioaccessibility as the fraction of test compound that diffuses through hollow fibre membranes. The model by Minekus was not especially validated for soil matrices, but was focussed on matrices like food and medicines. Another physiologically based extraction test (PBET) was introduced in 1996, again by Ruby et al. [1996]. This test was designed around paediatric gastrointestinal tract parameters

for a child 2-3 years old, believed to be at the greatest risk to metal exposure from accidental soil ingestion. The PBET has been used to mimic fasting conditions, which produce the most soluble heavy metal concentrations due to low gastric pH values, and, hence the most conservative conditions for accessing bioaccessibility. Test results were compared with results of oral bioavailability studies in rats, rabbits and monkeys [Ruby et al., 1996]. The relevance of this comparison is questionable since it is not clear whether these animal models represent the human gastrointestinal tract adequately. Interspecies comparison on characteristics of the gastrointestinal tract of humans, rats, rabbits and monkeys revealed various major differences [De Zwart et al., 1999]. For example, a study on pH values in the contents of the stomach of different species revealed major differences between monkeys, rabbits and rats.

In summary: Various *in vitro* models and test procedures have been described in literature to estimate bioaccessibility (and absorption). However, some test protocols take human physiology insufficiently into account, resulting in substantial overestimation of the risks (EPA method 3050 and 3051) [Ruby et al., 1992], where others are too complicated requiring advanced laboratory equipment [Minekus et al., 1995]. The model suggested by Rotard et al. [1995] seems to form a good starting point for a test procedure combining a simple model and a model representative for human physiology.

2.2 Criteria for testing

The *in vitro* digestion model, as suggested by Rotard et al. [1995] was taken as a starting point to study bioaccessibility. Subsequently, criteria for the required model were formulated:

1. The model has to represent human physiology.
2. The model should be focussed on physiology of children (until 7 years of age), for reasons of their high soil ingestion rate due to frequent hand-to-mouth contact [Duggan and Inskip, 1985; Davis and Waller, 1990; Van Wijnen et al., 1990; Calabrese et al., 1997].
3. The composition of the last compartment of the model has to represent fluid as found in the small intestine, i.e. chyme. The colon (large intestine) is not taken into account since only a minority of compounds will be absorbed substantially in this part of the intestine.
4. The test procedure should be easily applicable, since a number of variables and conditions should be tested.

2.3 Factors optimised

The model introduced by Rotard et al. [1995] was optimised for various factors. These optimisations were given from both a physiological point of view and a practical point of view.

1. **Factor for optimisation:** ***temperature***
   Physiologically, a temperature of 37 °C is preferred, since enzymes have their optimum activity at this temperature. Chemical characteristics like solubility are affected by temperature.
   Outcome: Experiments are performed at 37 °C.

2. **Factor for optimisation:** ***solid-to-fluid ratio***
   The Rotard model was scaled down from 10 g of soil to 1 g of soil. This scaling was performed mainly for practical reasons. In the original model the solid-to-fluid volume ratio amounted 1:60 (10 g in 600 ml) for soil and 1:240 (2.5 g in 600 ml) for soil dust. Soil to fluid ratios in the range of 1:5 to 1:25 have been observed to underestimate dissolution of metals in extraction procedures of this type [Ruby et al., 1992; Ruby et al., 1996], most likely due to diffusion-limited dissolution kinetics. Taking this in mind, the soil-to-fluid ratio was selected such that this parameter was not likely to control the test results. The ratio of the different digestive juices was based on physiology [Guyton, 1991]. The absolute volumes of the digestive juices were subsequently, for practical reasons, scaled to the content of the test flasks used.
   Outcome: In our model the solid-to-fluid ratio is 0.6 g dry matter: 57.5 digestion juices = 1: 96.

Table 2.1. Transit times optimised for physiological values.

transit times/compartment	optimised model (hr)	Rotard model (hr)
mouth (saliva)	0.08	0.5-2.5
stomach (gastric juice)	2	3-15
small intestine (bile + duodenal juice)	2	1.5-7.5 [a]
total experiment time	4.1	5-25

a) Bile and duodenal juice were added separately with a time interval of 2.5 hr.

3. **Factor for optimisation:** ***transit times***
   The Rotard model gives transit times through the various compartments which are long compared to physiological transit times as reported in literature. Moreover, this elongates the duration time of the experiments unnecessarily.
   Outcome: Transit times were chosen on basis of times reported in text books and review

publications [Degen and Philips, 1996; Daugherty and Mrsny, 1999]. Table 2.1 summarises the transit times in the Rotard model and in the optimised model.

4. **Factor for optimisation: *centrifugation***
   To separate chyme from digested soil, the method of Rotard et al. contained a centrifugation time of 2 hours at 6000 rpm. The duration and speed were based on the matrix, i.e. gravel dust. For our application it appeared to be unnecessary to use these conditions. In our model a centrifugation step of 5 minutes at approximately 3000*g* already was optimal, since a longer duration did not have any effect. We also looked at sedimentation of the soil matrix in the digestion juice, since we thought that that would represent physiology better than centrifugation. It appeared that sedimentation or centrifugation (up to 30 minutes) did not differ significantly.
   Outcome: At the end of the digestion process a 5 minutes centrifugation step (3000*g*) appeared to be sufficient.

5. **Factor for optimisation: *pH***
   The pH values of the various juices were corrected for physiology. As far as available, values representative for physiology in children were applied.
   Saliva: Data were obtained from literature on pH of saliva in adults and some scarce children data. Saliva pH can range between 6.2 and 7.4 with the higher pH exhibited upon increased secretion (due to food ingestion etc.) [Altman and Dittmer, 1968; Kedjarune et al., 1997]. In the present experimental set-up the pH of the saliva compartment was set to 6.5.
   Gastric juice: In children lowest gastric pH reported is 1 [Anderson et al., 1999]. Ruby et al. [1992] demonstrated dependence of bioaccessibility on gastric pH. pH values of 1.3, 1.6, 2.0, 2.5, and 3.0 were tested. It appeared that at lower pH lead mobilisation from soil in the stomach compartment increased [Ruby et al., 1992]. We tested the relation between gastric pH for lead and the bioaccessibility in the compartment resembling the small intestine. Gastric pH values of 0.9, 1.2, 1.3, 1.4, 1.8, 2.3, and 5.0 were employed. It appeared that bioaccessibility was significantly higher at lower pH, ranging from 43% bioaccessibility for pH 0.9, about 15% for gastric pH between 1.2 and pH 1.8, 9% for pH 2.3 and 6% for pH 5.0. These observations are in agreement with the observations of Ruby et al. [1992].
   Because paediatric gastric pH is quite variable among individuals and depends strongly on nutritional status, selecting an appropriate value is a difficult task. Research on paediatric gastric pH using both *in vivo* and *in vitro* stomach fluid followed by pH measurements resulted in mean fasting pH ranges from 1 to 4. This behaviour is consistent with adults, who have a mean fasting gastric pH of approximately 2. In the PBET of Ruby et al. [1996], the pH was set at 1.3 for the fasting state. In the present experimental set-up a gastric pH of 1 is employed, resulting in a pH of saliva + gastric juice of about 1.2. This represents a fasting state and will result in conservative bioaccessibility values.

Intestinal pH: pH in the intestine ranges from 4.5 in the first part (duodenum) to 7.5 in the last part (ileum) [Daugherty and Mrsny, 1999]. We decided to set the intestinal pH at 5.5, since our model mainly represents the duodenum and jejunum (based on composition of chyme and chosen transit times).
Outcome: The pH of the saliva, gastric juice, duodenum juice and bile was set to 6.5 ± 0.2, 1.07 ± 0.07, 7.8 ± 0.2 and 8.0 ± 0.2 respectively, resulting in a pH of the chyme at the end of the artificial digestion of about 5.5.

6. **Factor for optimisation: *mixing***
Ruby et al. [1992] already reported that the extent of mixing is critical in determining dissolution rates.
Outcome: Although we determine the dissoluted amount rather than the rate, it was preferred to develop a model in which the mixing conditions were constant. For that reason a rotator was placed in an incubator in which tubes were placed.

2.4 Optimised *in vitro* digestion model

A schematic representation of the optimised *in vitro* digestion model is shown in figure 2.1.

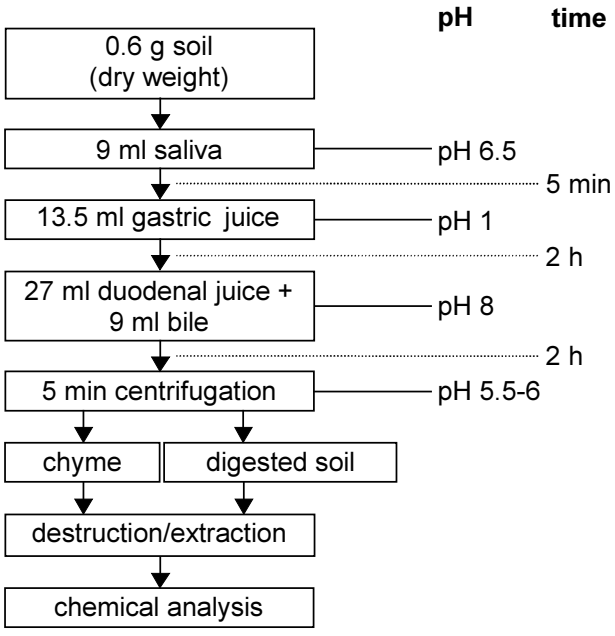

Figure 2.1. Schematic representation of optimised in vitro digestion model.

The exact procedure of an *in vitro* digestion, including the composition of the different digestive juices, is presented in appendix I.

3. Research on effects of several variables on bioaccessibility

3.1 Materials and methods

3.1.1 Study parameters

Various variables were studied, which may affect bioaccessibility of lead and benzo[a]pyrene from soil in humans.

a) Type of contaminant
b) Level of contamination
c) Soil type
d) Soil pH
e) Ageing of the soil (studied for lead)
f) Speciation in soil (studied for lead)
g) Artificially contaminated soil versus polluted soil (studied for lead)

The experimental set-up of these variables is addressed below. All soils were digested in triple with freshly prepared artificial saliva, gastric juice and duodenal juice and bile (see optimised model figure 2.1).

3.1.1.1 Type of contaminant

In the present report two types of contaminants are addressed. Lead was chosen as a representative of inorganic compounds (heavy metals) and benzo[a]pyrene (= B(a)P) as a representative of organic compounds. Both contaminants are relevant to current (Dutch) policy issues.

Results on arsenic, cadmium and benzo[a]anthracene will be reported in a subsequent report.

3.1.1.2 Level of contamination

OECD-medium as well as seven Dutch soil types were spiked at 0, 0.5, 1, 3 and 5 times I.V with lead or benzo[a]pyrene. ($I.V._{lead}$ = 530 mg Pb/kg dry matter soil, $I.V._{B(a)P}$ = 40 mg B(a)P/kg dry matter soil). To that end, the different lead salts were grounded and added to dried soil and subsequently mixed. For spiking with benzo[a]pyrene a stock solution in hexane was prepared and sprinkled on the dried soil, mixed, and left overnight in the fumehood to evaporate the hexane. After spiking, the seven Dutch soil types were stored at 4 ºC for at least 2 weeks. To the spiked OECD-medium water was added (50%, in accordance to its water holding capacity), and stored at 4 ºC for at least 2 weeks.

3.1.1.3 Soil type

In order to assess the effect of soil type on bioaccessibility, OECD-medium as well as seven soil types representing Dutch soil, spiked with lead or benzo[a]pyrene at 0, 0.5, 1, 3 and 5 times I.V., were tested. OECD-medium was chosen as a kind of reference soil. The Organisation for Economic Co-operation and Development (OECD) and the European Union (EU) require the use of this soil to assess the toxicity of chemicals on earthworms. OECD-medium consists of 10% dry peat (< 1mm), 20% clay, 70% sand and at most 1% $CaCO_3$. Moisture accounts for 50% of the dry weight matter. The pH_{KCl} normally is 5.
The Dutch soil represents a variety of soil types in the Netherlands. All soil types were air-dried, resulting in a moisture percentage between 0.03 and 3.5%. Table 3.2 summarises the characteristics of these samples, taken from the top layer of the soils. Background lead levels, e.g. in unspiked soil, have been determined and are presented in table 3.2. In two soil samples, Boskoop silt loam and Angeren silty clay loam, relatively high amounts of Pb were already present in soil before spiking (see table 3.2). The background concentration of benzo[a]pyrene of blank soil samples was never above the lower limit of quantification (LLQ).

Table 3.1. Characteristics of Dutch soil samples representing a variety of soil types in the Netherlands (analysed by TAUW, Deventer, The Netherlands).

Soil location	Texture	% clay < 2 µm	% silt 2-50 µm	% sand 50-2000 µm	TOC[a)]	pH_{KCl}	$CaCO_3$ (%)
Appelscha	sand	< 1	1-2	97	1.5	4.1	0.7
Hulshorst A	sand	< 1	< 1	100	0.3	4.7	0.4
Hulshorst B	sand	<1	2-3	92	5.3	3.8	0.7
Bemelen	silt	18	62	20	2.0	7.1	2.9
Boskoop	silt loam	24	16	59	29.8	5.1	3.6
Angeren	silty clay loam	29	32	39	13.0	7.0	10
Akkrum	loam	26	28	46	3.9	7.0	6.2

a) Total Organic Carbon, determined by Loss On Ignition (LOI) by the Soil and Ground Water Research Laboratory, National Institute of Public Health and the Environment.

Table 3.2. Background lead levels in unspiked soil determined at RIVM by means of destruction with HNO_3.

Soil location	Texture	µg Pb/g dry matter soil
Appelscha	sand	8
Hulshorst A	sand	< LLQ
Hulshorst B	sand	7
Bemelen	silt	14
Boskoop	silt loam	294
Angeren	silty clay loam	188
Akkrum	loam	21

3.1.1.4 Soil pH

The effect of soil pH on bioaccessibility was assessed in OECD-medium for lead and benzo[a]pyrene. These media were spiked with $Pb(NO_3)_2$, $PbSO_4$ or B(a)P at 0, 0.5, 1, 3 and 5 times the I.V. ($I.V._{lead}$ = 530 mg Pb/kg dry matter soil, $I.V._{B(a)P}$ = 40 mg B(a)P/kg dry matter soil), and subsequently brought to a pH_{KCl} of 3, 4, 5, 6, or 7. After spiking, the artificially contaminated soil samples were stored at 4 ºC for at least 2 weeks.

3.1.1.5 Ageing of the soil

It has been found in literature that ageing of soil may decrease the bioavailability of contaminants from soil, e.g. as a result of enclosure of the contaminant in the silica skeleton of soil [Alexander, 1995; Kukkonen and Landrum, 1998]. In order to study possible effects of ageing of soil on lead bioaccessibility, bioaccessibility was determined within weeks after contamination (but no earlier than within two weeks), and was determined again after 1½ years of storage. This was performed for artificially contaminated OECD-medium and Dutch soil samples at different contamination levels. The Dutch soil types were stored dry without addition of water, while after spiking water was added to OECD-medium to its water holding capacity (50%). Storage occurred at 4 °C.

3.1.1.6 Lead speciation in soil

In order to study the effects of metal speciation on bioaccessibility, two to four speciations of lead were used in the experiments. Lead sulphate ($PbSO_4$), lead nitrate ($Pb(NO_3)_2$), lead chloride ($PbCl_2$) and lead acetate ($PbAc_2$) were employed in OECD-medium at different I.V.s. $PbSO_4$ and $Pb(NO_3)_2$ were used in the Dutch soil types at different I.V.s. $PbSO_4$ was chosen as representative of lead angelsite, which is most frequently present in polluted soil [Ruby et al., 1999]. $Pb(NO_3)_2$, $PbCl_2$ and $PbAc_2$ have been chosen because mainly these lead speciations are administered (generally dissolved in water or added to chow) in animal studies.

3.1.1.7 Artificially versus historically contaminated soil

It was decided to test artificially contaminated soils first, because that was the only way to address the questions on the influence of certain variables, e.g. level of contamination, soil type, soil pH, lead speciation, ageing of soil, on bioaccessibility of contaminants. A pilot experiment was performed with historically polluted soils from the field in order to investigate the relationship between artificially contaminated soils and historically polluted soils. These lead-polluted soil samples were collected at different locations in three different towns in The Netherlands and offered for testing by mr. R. Theelen (TAUW Milieu B.V., Deventer, The Netherlands). Table 3.3 presents the characteristics of these soil samples. All soil types were air-dried, resulting in a moisture percentage ≤ 3.5%. Lead concentrations of the soil samples were determined by three different methods/laboratories and presented in table 3.4.

Table 3.3. Characteristics of lead-polluted Dutch soil samples

Soil sample	Clay (%) < 2 µm	Organic matter (%)	pH_{KCl}
sample 1, town A	2.1	4.6	6.6
sample 2, town A	2.6	4.2	4.8
sample 3, town A	8.2	11.5	6.9
sample 4, town A	4.8	6.6	7.4
sample 5, town A	2.6	6.6	5.4
sample 6, town A	2.4	7.3	5.7
sample 7, town A	4.7	6.4	7.2
sample 8, town A	16.4	14.9	6.1
sample 9, town B	1	3.3	7.0
sample 10, town B	6	10	7.3
sample 11, town B	2	2.6	7.3
sample 12, town B	3.1	2.9	5.0
sample 13, town B	3.5	15	7.6
sample 14, town C	10	3	7.6
sample 15, town C	11	18	7.3

Table 3.4. Lead concentrations in historically contaminated soil samples as determined by three different methods.

	µg Pb/g dry matter soil		
Soil sample	Method 1[a]	Method 2[b]	Method 3[c]
sample 1, town A	180	360	180
sample 2, town A	330	280	300
sample 3, town A	390	430	250
sample 4, town A	850	930	430
sample 5, town A	650	530	520
sample 6, town A	750	640	500
sample 7, town A	1300	1900	1320
sample 8, town A	2300	2300	670
sample 9, town B	400	700	460
sample 10, town B	450	690	390
sample 11, town B	650	1200	600
sample 12, town B	800	910	300
sample 13, town B	1400	1200	930
sample 14, town C	1000	1050	980
sample 15, town C	2500	1400	870

[a] Method 1: as determined by means of HNO_3/HCl destruction by TAUW Milieu B.V., Deventer, The Netherlands.

[b] Method 2: as reported by client to TAUW Milieu B.V., Deventer, The Netherlands.

[c] Method 3: total of recovered lead in the TNO gastrointestinal model (TIM) model, TNO Nutrition, Zeist, The Netherlands.

3.1.1.8 Methodological parameters

Parameters like within-day variability, between-day variability and stability of the samples are determined, at least for each soil type and for each contaminant. To that end, soils spiked with 0.5 × I.V. and 5 × I.V., were digested in six-fold. Between-day variation was determined over a period of 4 days.

3.1.2 Chemical analysis

All samples were analysed for Pb by means of ICP-MS at the Laboratory of Inorganic Analytical Chemistry of the National Institute of Public Health and the Environment. The lower limit of quantification (LLQ) of this analysis was 2 ng Pb/ml chyme (10 times diluted). This is approximately equivalent with 195 ng/g dry matter soil (2 ng Pb/ml chyme-diluted = 20 ng/ml chyme-undiluted ⇔ in the digestion model 58.5 ml chyme is present: 58.5 ml × 20 ng = 117 ng Pb/58.5 ml chyme ⇔ 0.6 g dry matter of soil was brought in 58.5 ml chyme:
117 ng Pb/0.6 g dry matter soil = 195 ng Pb/g dry matter soil).
Benzo[a]pyrene concentrations were determined in blank soil samples before digestion and in all chyme samples by means of HPLC with fluorescence detection at the Laboratory of Exposure Assessment & Environmental Epidemiology of the National Institute of Public Health and the Environment. The LLQ of this analysis was 0.63 ng B(a)P/ml chyme. This is approximately equivalent to 61 ng/g dry matter soil (0.63 ng B(a)P/ml chyme ⇔ 36.9 ng/58.5 ml chyme = 36.9 ng/0.6 g dry matter soil = 61 ng/g dry matter soil).

3.1.3 Data analysis

For each sample that was placed in the *in vitro* digestion model, bioaccessibility of the contaminant of concern was determined. Bioaccessibility is calculated as the percentage of contaminant that has been extracted from soil and was detectable in the chyme.

$$\text{Bioaccessibility}(\%) = \frac{\text{contaminant present in chyme } (\mu g)}{\text{contaminant present in soil before digestion } (\mu g)} \times 100\% \qquad \text{eq. 1}$$

Bioaccessibility represents the fraction of contaminant that is *available* for absorption in the small intestine (in children) under fasting conditions.
In addition, bioaccessibility can be calculated and expressed in µg/g dry matter soil, i.e. the amount of contaminant mobilised from soil during *in vitro* digestion per gram of soil introduced into the digestion tube.

Within-day and between-day variation (%) for the extraction of Pb and benzo[a]pyrene from artificially contaminated soil samples were calculated by means of ANOVA.

Initially, it was planned to analyse the data on the effect of the various parameters on the basis of a statistical analysis (e.g. multivariate analysis of variance or multiple regression analysis). The data however revealed already in an early stage of the studies a non-linear relationship between contamination level and bioaccessibility. Moreover, variance of the various data sets was not homogeneous, indicating that, from a statistical point of view, also non-linear multiple regression models should not be applied. These findings accelerated the need for developing a preliminary mathematical model.
With the mathematical model it was tried to identify whether the relationship between contamination level and the amount of contaminant mobilised from soil during digestion was linear or non-linear. Comparison was based on "log-likelihood" values. If the mathematical model can fit the data, the effect of the other parameters can be assessed by statistical evaluation of the fitted curves that were obtained under different circumstances.

3.1.3.1 Mathematical model

Modelling of bioaccessibility of environmental contaminants from soil started with a quantitative, realistic, description of the experimental system with which bioaccessibility was measured. In modelling the process, two approaches were discriminated. In the first approach the exchange of the contaminant from soil into juice is described dynamically, i.e. as a time-dependent process. Alternatively, the exchange process was described statically, i.e. in terms of the ultimate distribution of the contaminant between soil and juice. *A priori* neither of both ways was preferable to the other. In practice, however, the availability of suitable data sets that may be used for model calibration, i.e. the estimation of model parameters, steered the modelling approach. For example, calibration of a dynamic model requires time-dependent data of the exchange of the contaminant between soil and (successive amounts of) juice. These data were not available from the experiments described in the present report, since bioaccessibility was determined at the end of the digestion process. Therefore, the static modelling approach was chosen for the modelling of bioaccessibility from soil.

The mechanism of exchange of contaminants between soil and digestive juice depends, among others, on the physico-chemical properties of the contaminant. For example, when i types of metal ions of valence z^+ are dissolved in an aqueous solution, $C^{z+}_{i,\,juice}$, dispersed with soil particles containing j types of metal ions on their surface, $S^{z+}_{j,\,soil}$, these ions may pair-wise be exchanged against each other, or:

$$C^{z+}_{i,juice} \rightleftarrows S^{z+}_{j,soil} \qquad \text{eq. 2}$$

Hydrophobic organic compounds such as benzo[a]pyrene can either 'dissolve' in the organic matter of the soil, or bind to specific sites of the soil. The first mechanism can be described by

equilibrium partitioning, and would result in a strict linear relationship between the contaminant level in soil and the bioaccessibility. The present model focuses on the second mechanism that can be described by receptor binding, as the data suggest non-linear relationships. Similar to the model for metals, when i types of organic contaminants are dissolved in an aqueous solution, $C_{i,\,juice}$, dispersed with soil particles containing j types of receptor molecules on their surface, $X_{j,\,soil}$, these contaminants may bind to the receptor molecules present in the soil, or:

$$C_{i,juice} + X_{j,soil} \rightleftarrows (C_i X_j)_{soil} \qquad \text{eq. 3}$$

In the digestive system the interest is in the exchange of only one particular contaminant between soil and juice (i = 1). Unfortunately, the number of homovalent counter ions in soil or the number of receptors in soil with which this contaminant may be exchanged or to which it may bind is unknown. For reasons of simplicity it was therefore assumed that, in the case of the exchange of a particular metal ion, soil contains one ‘generic’ counter ion and, in the case of receptor binding of a particular organic contaminant, one ‘generic’ receptor, or:

$$C^{z+}_{1,juice} \rightleftarrows S^{z+}_{2,soil} \qquad \text{eq. 4}$$

and

$$C_{juice} + X_{soil} \rightleftarrows (CX)_{soil} \qquad \text{eq. 5}$$

It was further assumed that the exchange of a particular metal ion with its counter ion or the binding of a contaminant to its receptor has reached equilibrium at the end of the final extraction of soil with digestive juice. Given the assumptions mentioned, theory on liquid-soil ion exchange (Gapon equation) and chemical receptor binding (modified Scatchard equation) may be applied to the experimental data. The equations and their corresponding theoretical curves (figure 3.1 and 3.2) are described below.

Gapon equation (ion exchange)

$$\frac{C_{1,juice}^{z+}}{C_{2,juice}^{z+}} = K_{aff} \times \frac{S_{1,soil}^{z+}}{S_{2,soil}^{z+}} \qquad \text{eq. 6}$$

with: $C_{1,juice}^{z+}$ Equilibrium concentration of the counter ion in digestive juice

$C_{2,juice}^{z+}$ Equilibrium concentration of the contaminant in digestive juice

$S_{1,soil}^{z+}$ Equilibrium concentration of counter ion in soil

$S_{2,soil}^{z+}$ Equilibrium concentration of contaminant in soil

K_{aff} Affinity constant

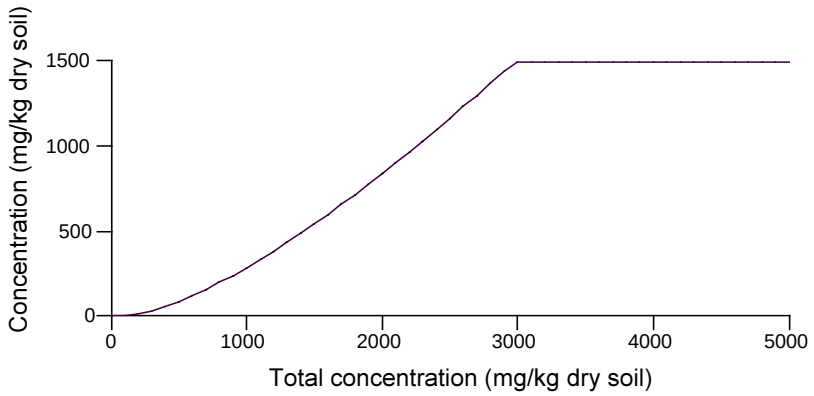

Figure 3.1. Simulation of the concentration of a metal ion in digestive juice (Concentration) after extraction of soil containing various concentrations of the ion (Total concentration) with digestive juice (Gapon like equation, i.e. eq. 6). The concentration at which the digestive juice becomes saturated with the ion was set at 1500 mg/kg dry soil.

Modified Scatchard equation (receptor binding)

$$C_{total} = C_{juice} + CX_{soil} = C_{juice} + \frac{S_{cap} \times C_{juice}}{K_{dis} + C_{juice}} \qquad \text{eq. 7}$$

with C_{total} Total concentration of the contaminant in the *in vitro* system

C_{juice} Concentration of the contaminant in digestive juice

CX_{soil} Concentration of the contaminant-receptor complex in soil

S_{cap} Maximal receptor binding capacity of soil

K_{dis} Dissociation constant for the binding of the contaminant to the soil receptor

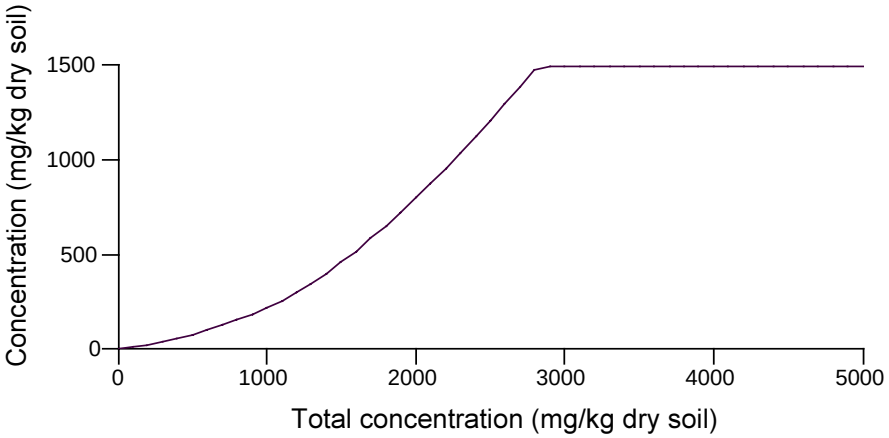

Figure 3.2. Simulation of the concentration of an organic contaminant in digestive juice (Concentration) after extraction of soil containing various concentrations of the contaminant (Total concentration) with digestive juice (Scatchard like equation, i.e. eq. 7). The concentration at which the digestive juice becomes saturated with the contaminant was set equivalent to 1500 mg/kg dry soil.

Both equations can be rewritten into an expression of the concentration of the metal ion/organic contaminant in the digestive juice as function of its initial amount in soil, i.e. the soil concentration at the start of the extraction process. It should be noted that the Gapon and Scatchard equations can become approximately linear.

The number of sites in the soil for metal complexation is obtained by the sum of $S_{1,soil}^{z+}$ and $S_{2,soil}^{z+}$. This is referred to as the cation exchange capacity, CEC. CEC and S_{cap} are typical soil characteristics. Similarly, K_{aff} and K_{dis} depend on the type of soil, as well as the contaminant and the digestive juice properties. Furthermore, the combinations K_{aff}/CEC and K_{dis}/S_{cap} are characteristic for a certain metal ion/soil or organic contaminant/soil combination. Our future aim is to obtain an expression of bioaccessibility as function of such model parameters. These parameters should, however, first be rewritten in terms of *a priori* known soil and/or compound characteristics (pH, organic soil content, clay content, etc.).

3.2 Results

3.2.1 Bioaccessibility of lead

3.2.1.1 Level of contamination

For OECD-medium a more or less constant bioaccessibility percentage at each contamination level was found. This may imply a linear (dose proportional) relationship between the amount of lead added to OECD-medium and the amount of lead mobilised from OECD-medium (see figure 3.3 and 3.4). However, *in vitro* digestion of soil samples that were artificially contaminated with lead resulted in bioaccessibility values that can range from 7 to 61% within one soil (see appendix II and III) instead of a constant percentage. Figure 3.3 and 3.4 show the amount of mobilised lead at the different contamination levels for the lead speciations $Pb(NO_3)_2$ and $PbSO_4$. Analysis of the contamination level versus the amount of lead mobilised from soil reveals that a non-linear relationship according to the mathematical model describes the data better than a linear relationship for half of the relationships of the Dutch soil types. Figure 3.5 represents an example of a data set for one soil, Akkrum loam spiked with $Pb(NO_3)_2$, that has been analysed with both a non-linear and a linear relationship. This figure illustrates that analysis of the relationship between bioaccessibility and contamination level can be ambiguous. The fact that not all data sets are clearly in favour of one type of relationship indicates that the available data do not allow a definitive conclusion whether or not the Gapon-based or a linear relationship is the model of choice for the quantification of bioaccessibility.
Remarkably, all Dutch soil samples show a lower bioaccessibility at 1 and 3 × I.V. than at 0.5 and 5 × I.V. Lead bioaccessibility from 'blank' soil samples and from the same soils spiked with 0.5 × I.V. demonstrated a similar order of magnitude for bioaccessibility, although lead in 'blank' soil samples origins from historical sources, whereas '0.5 × I.V.' was added in the laboratory.

When assuming that bioaccessibility of lead can be described by a Gapon equation, the effect of the other parameters can be assessed by statistical analysis of the model parameters (K_{aff} and CEC) for each variation. However, for two reasons such an analysis cannot be performed. 1) Estimation of **two** model parameters requires many and good data. These are presently not available, resulting in very large standard deviations so that the effect of the parameters cannot be assessed. 2) From the present data it remains inconclusive whether bioaccessibility can be described with a Gapon-based or a linear equation. Consequently, only a quantitative approach to assess the effect of soil type on bioaccessibility is possible.

3.2.1.2 Effect of soil type

The data suggest that bioaccessibilities vary per soil type. Appelscha sand, Hulshorst A sand and Hulshorst B sand display rather high bioaccessibilities, while soils like Angeren silty clay show lower bioaccessibility values (see appendix II and III, and figure 3.3 and 3.4).

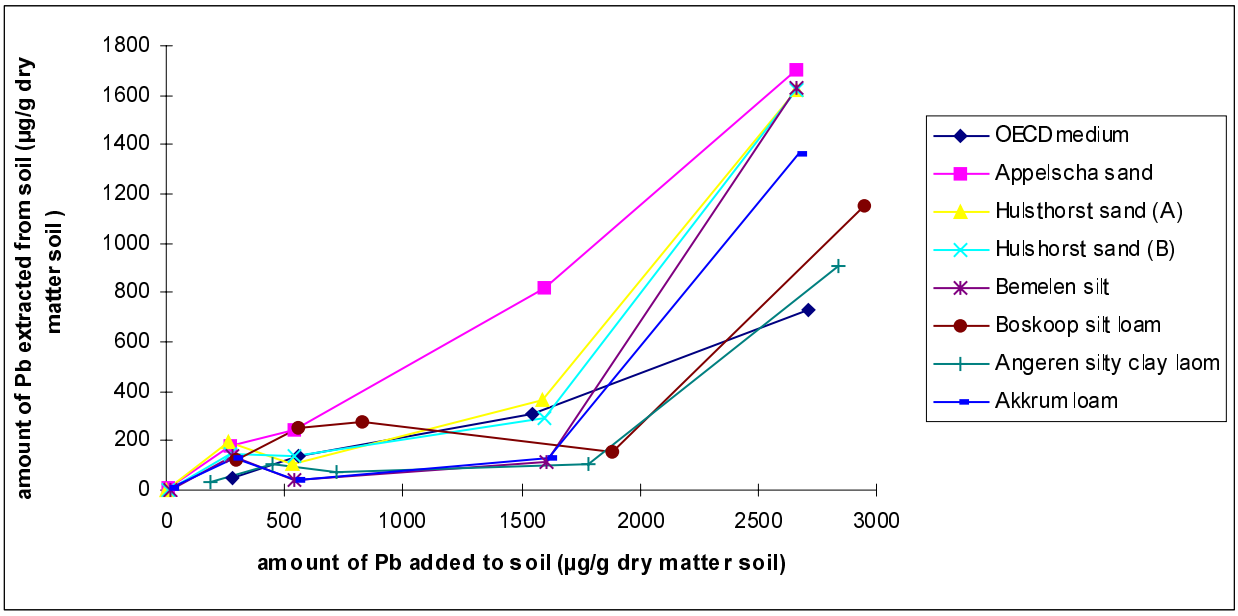

Figure 3.3. The amount of lead in chyme against the amount of lead present in OECD-medium and seven Dutch soil types before digestion for lead speciation ***$Pb(NO_3)_2$*** *(n=3).*

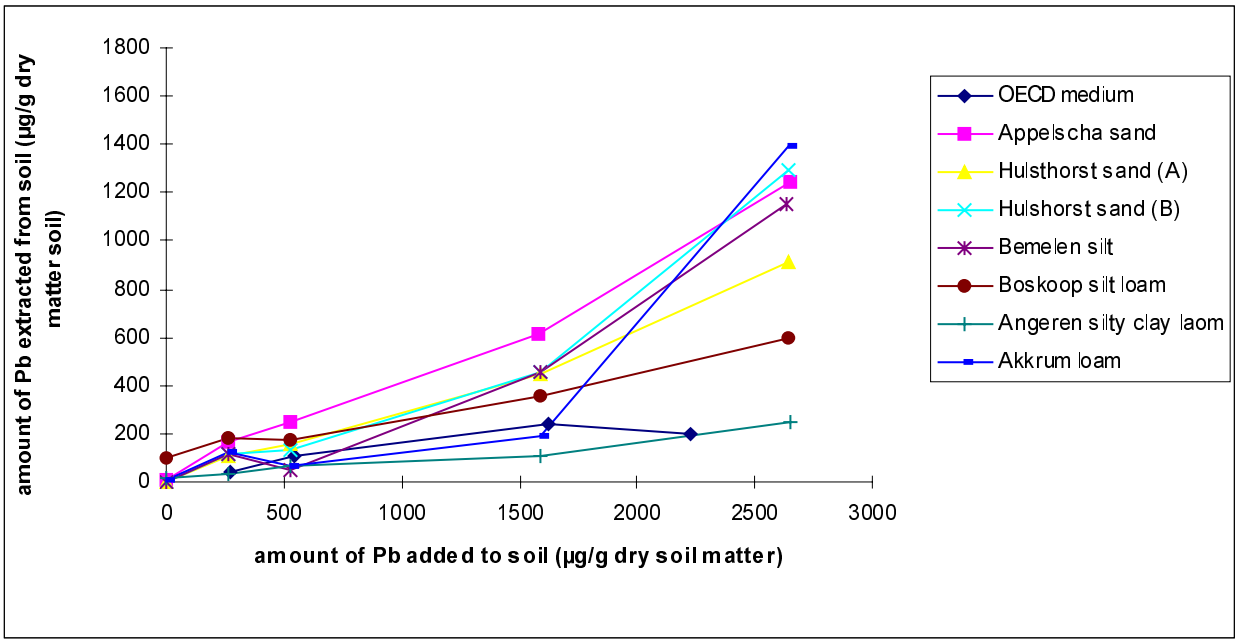

Figure 3.4. The amount of lead in chyme against the amount of lead present in OECD-medium and seven Dutch soil types before digestion for the lead speciation ***$PbSO_4$*** *(n=3).*

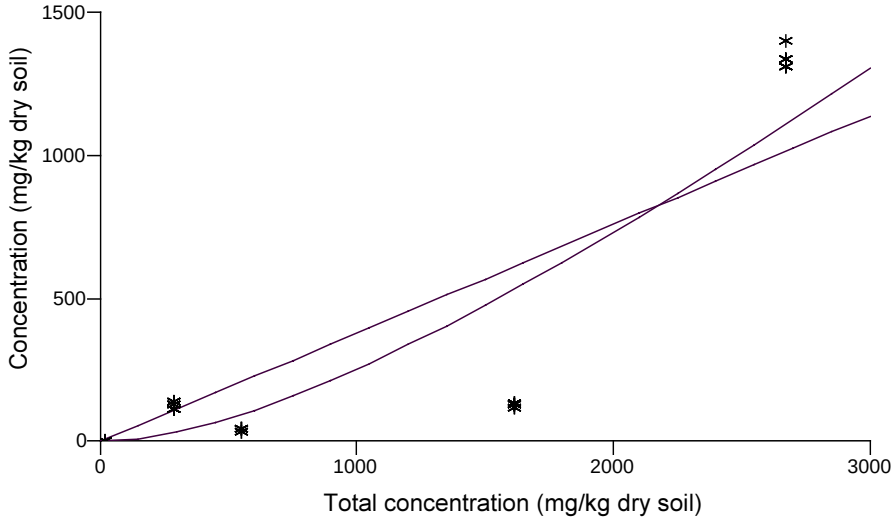

Figure 3.5. Example of the analysis of the relationship between the level of soil contamination (x-axis) and the concentration of Pb in chyme (= Pb mobilised from soil during in vitro digestion) (y-axis). Akkrum loam spiked with $Pb(NO_3)_2$ is used as example. The data are fitted with both a non-linear relationship according to the mathematical model and a linear relationship, showing (slightly) different values of the log-likelihood function. The linear relationship describes the data slightly better in the present example. This difference, however, does not warrant a definitive conclusion whether a linear or non-linear model describes the data best.

3.2.1.3 Effect of soil pH

The effect of soil pH on bioaccessibility of lead was studied in OECD-medium. A quantitative approach to assess the effect of soil pH on bioaccessibility is impossible because it remains inconclusive whether bioaccessibility can be described by a Gapon equation. Qualitatively, soil pH seems to have no effect on the bioaccessibility of lead, both for OECD-medium contaminated with $Pb(NO_3)_2$ and $PbSO_4$, see figure 3.6.

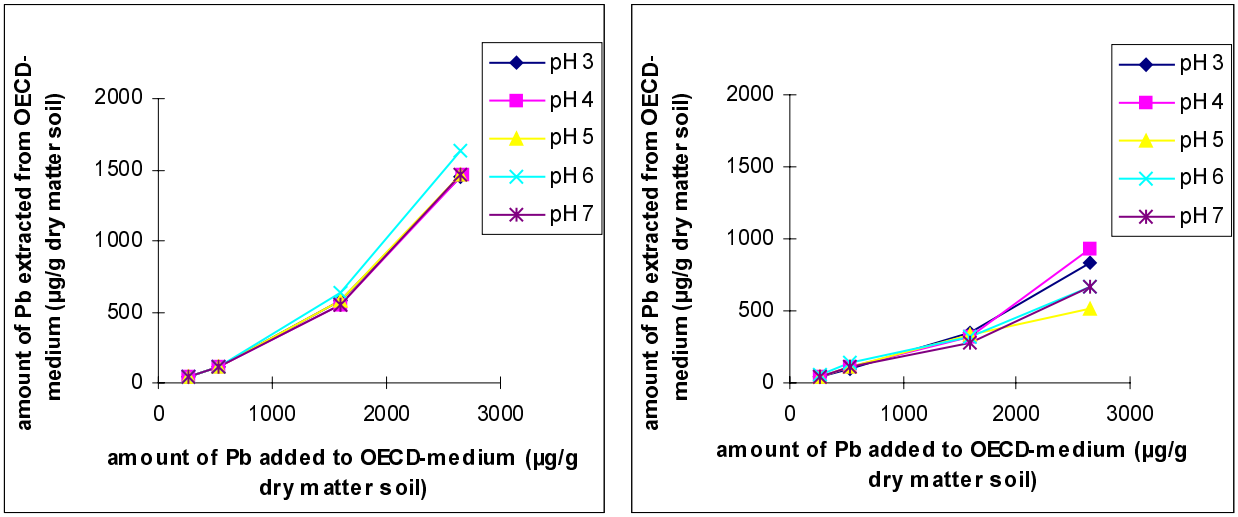

Figure 3.6. The amount of lead extracted from soil during in vitro digestion against the amount of lead ($Pb(NO_3)_2$ left panel; $PbSO_4$ right panel) added to OECD-medium with different soil pH values (n=3).

3.2.1.4 Effect of ageing of soil

The bioaccessibility of Pb ($PbSO_4$, $Pb(NO_3)_2$, $PbCl_2$ and $PbAc_2$) in artificially contaminated OECD-medium was found to increase after ageing during 1½ years, see appendix IV. Bioaccessibility of Pb in artificially contaminated Dutch soil samples did not change after ageing. More specifically, bioaccessibility of $PbSO_4$ was slightly increased in most soil types whereas bioaccessibility of $Pb(NO_3)_2$ was not changed after ageing. The circumstances of ageing differed between OECD-medium and artificially contaminated Dutch soil samples: the former soils were stored in wet conditions while the latter soils were dried before storage. This difference may have caused the different trends in bioaccessibility between OECD-medium and Dutch soil samples after ageing. Dried soil samples can be expected to change little in time as no transport medium for the contaminant is present.

3.2.1.5 Effect of lead speciation in soil

The effect of metal speciation on bioaccessibility of lead was studied in OECD-medium, which was artificially contaminated with four lead speciations, i.e. $PbSO_4$, $Pb(NO_3)_2$, $PbCl_2$ or $PbAc_2$. The results are shown in figure 3.7. Similar to the other variables, the effect of metal speciation can only be described qualitatively. Bioaccessibility after digestion with soil contaminated with $Pb(NO_3)_2$ tend to be slightly higher than in case of contamination with $PbSO_4$. This effect was particularly seen at the highest concentration tested.

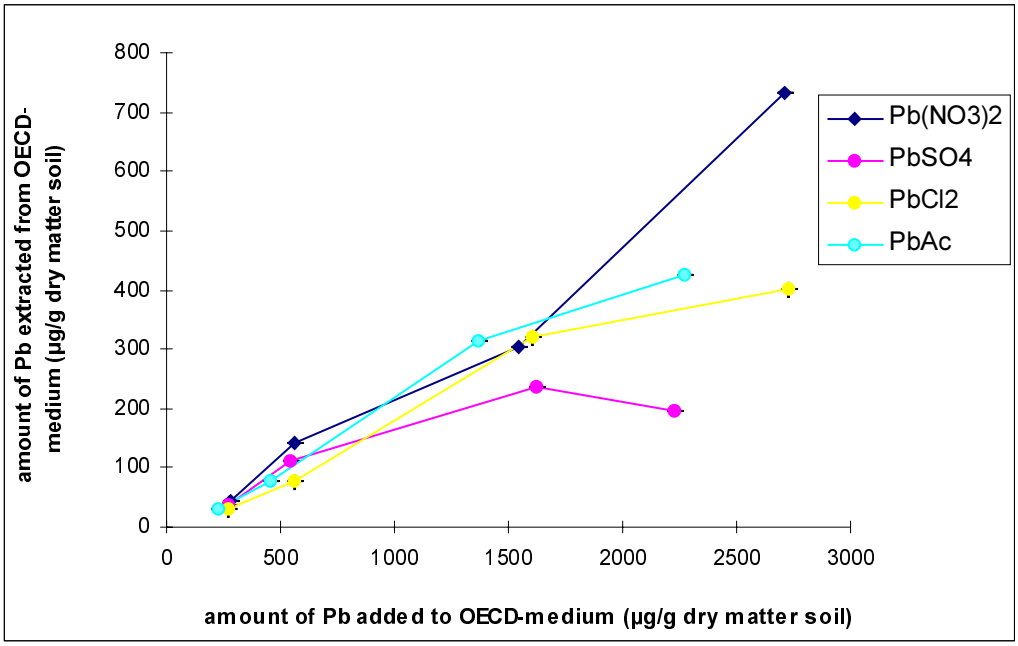

Figure 3.7. The amount of lead extracted from soil during in vitro digestion against the amount of lead present in OECD-medium for four different lead speciations.

In addition, the effect of metal speciation on bioaccessibility of a metal was studied in seven Dutch soil types, which were artificially contaminated with either $PbSO_4$ or $Pb(NO_3)_2$. Similar to the OECD-medium, bioaccessibilities in case of contamination with $Pb(NO_3)_2$ tend to be slightly higher than in case of contamination with $PbSO_4$ (see appendices I and II).

The bioaccessibility of lead added as $Pb(NO_3)_2$ and $PbSO_4$ to OECD-medium is about twice as low for the experiments on lead speciation (figure 3.7) compared to the experiments on soil pH (figure 3.6). A new bulk of OECD-medium was prepared for the latter experiments, which probably together with the between-day variation, explains the difference.

3.2.1.6 Artificially versus historically contaminated soil

The amount of lead present in soil that contained historically contaminated soil was determined at three external laboratories each using its own method of analysis. This resulted in substantial differences (see table 3.4: method 1, 2 and 3, and table 3.5: method 1 and 2), hindering a reliable interpretation of the bioaccessibility data. Method 3 seems to be unreliable because the matching bioaccessibility data are very high (in two cases >100%). This can be due to the indirect method that is used, since method 3 represents the amount of lead recovered from the TNO gastro-intestinal tract model TIM.
The bioaccessibility data obtained with method 1 and 2 ranged from 2-67% (83% is excluded because of high CV) whereas the contamination levels of the soil samples ranged from 0.3 to

4.7 × I.V.. Both ranges are comparable to the results obtained with artificially contaminated Dutch soils (appendix II and III).

Table 3.5. Bioaccessibility of Pb from historically polluted Dutch soil samples (n=3), calculated with total lead concentration levels in soil as determined by two different methods.

Soil sample	Bioaccessibility 1 [a]		Bioaccessibility 2 [b]	
	Mean (%)	CV (%)	Mean (%)	CV (%)
sample 1, town A	51	7	25	7
sample 2, town A	53	3	63	3
sample 3, town A	60	2	54	2
sample 4, town A	54	10	49	10
sample 5, town A	49	8	61	8
sample 6, town A	57	9	67	9
sample 7, town A	83	49	57	49
sample 8, town A	2	8	2	8
sample 9, town B	34	36	20	36
sample 10, town B	5	46	3	46
sample 11, town B	37	9	20	9
sample 12, town B	44	9	39	9
sample 13, town B	35	2	41	2
sample 14, town C	3	35	3	35
sample 15, town C	25	15	45	15

[a] Calculation based on lead concentration levels in soil as determined by means of HNO_3/HCl destruction by TAUW Milieu B.V., Deventer, The Netherlands.

[b] Calculation based on lead concentration levels is soil as reported by client to TAUW Milieu B.V., Deventer, The Netherlands.

3.2.2 Bioaccessibility of benzo[a]pyrene

3.2.2.1 Level of contamination

Bioaccessibility ranged from 14 to 50% within one soil type (see appendix V), instead of a constant percentage. Figure 3.8 demonstrates that especially OECD-medium, sandy soils (Appelscha and Hulshorst A and B) and silt soils (Bemelen) display a less than dose proportional relationship between the level of contamination and the amount of benzo[a]pyrene mobilised from soil. Data analysis of the contamination level versus the amount of benzo[a]pyrene mobilised from soil indicates that a linear and non-linear relationship cannot be discriminated from each other, based on the log-likelihood criterion. A relationship with precipitation describes the data better than a relationship without precipitation. An example of a data set of a soil, Hulshorst sand A, is presented in figure 3.9. The inability of discriminating a linear from a non-linear relationship results from the few data points left (2 to 3 data points) before the precipitation point is reached. As the available data do not allow a definitive conclusion on the relationship between soil concentration of benzo[a]pyrene and its respective bioaccessibility, the effect of the other variables on bioaccessibility cannot be assessed from a statistical quantitative point of view.

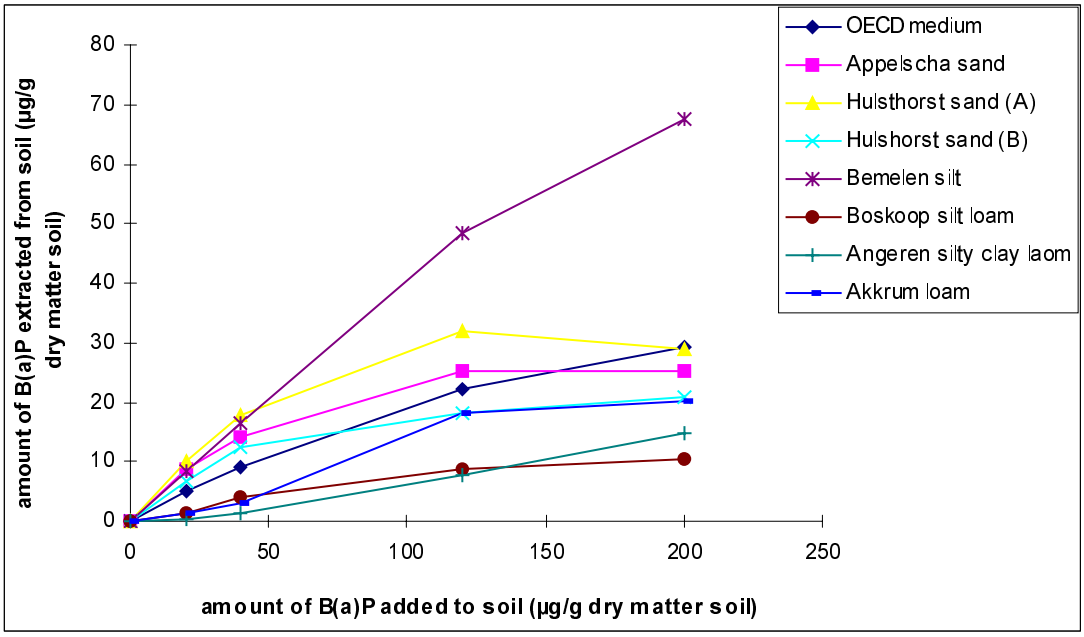

Figure 3.8. The amount of benzo[a]pyrene mobilised during in vitro digestion against the amount of benzo[a]pyrene added to OECD-medium and seven Dutch soil types (n=3)

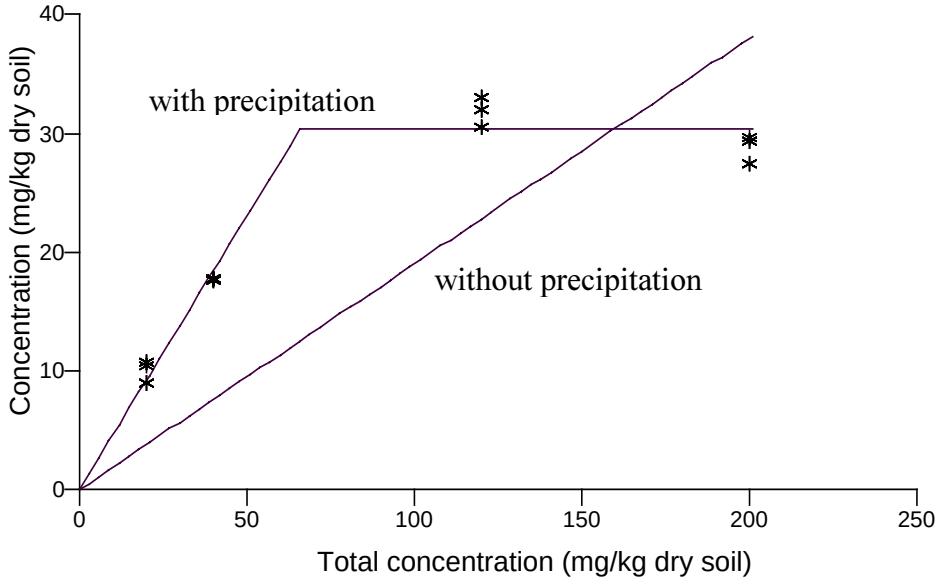

Figure 3.9. Example of the analysis of the relationship between the level of soil contamination (x-axis) and the concentration of benzo[a]pyrene in chyme (= mobilised from soil during in vitro digestion) (y-axis). Hulshorst sand A is used as example. The data are fitted with and without a level of precipitation. Based on the log-likelihood function a linear model and a non-linear (Scatchard like equation) model lead to exactly the same result, either with or without precipitation. The relationships with precipitation describe the data better than the relationships without precipitation.

3.2.2.2 Effect of soil type

The effect of soil type on bioaccessibility of benzo[a]pyrene has been studied in artificially contaminated OECD-medium and seven Dutch soil samples at different contamination levels (see figure 3.8). The data clearly indicate that the soil type influences the percentage of bioaccessibility (see appendix V). Bioaccessibility percentages were highest for sandy soils (Appelscha and Hulshorst A and B) and silt soils (Bemelen).

3.2.2.3 Effect of soil pH

The effect of soil pH on bioaccessibility of benzo[a]pyrene has been studied in artificially contaminated OECD-medium. The results presented in figure 3.10 indicate that soil pH has no effect on the bioaccessibility of benzo[a]pyrene. For each soil pH a less than dose proportional relationship between contamination level and bioaccessibility is observed.

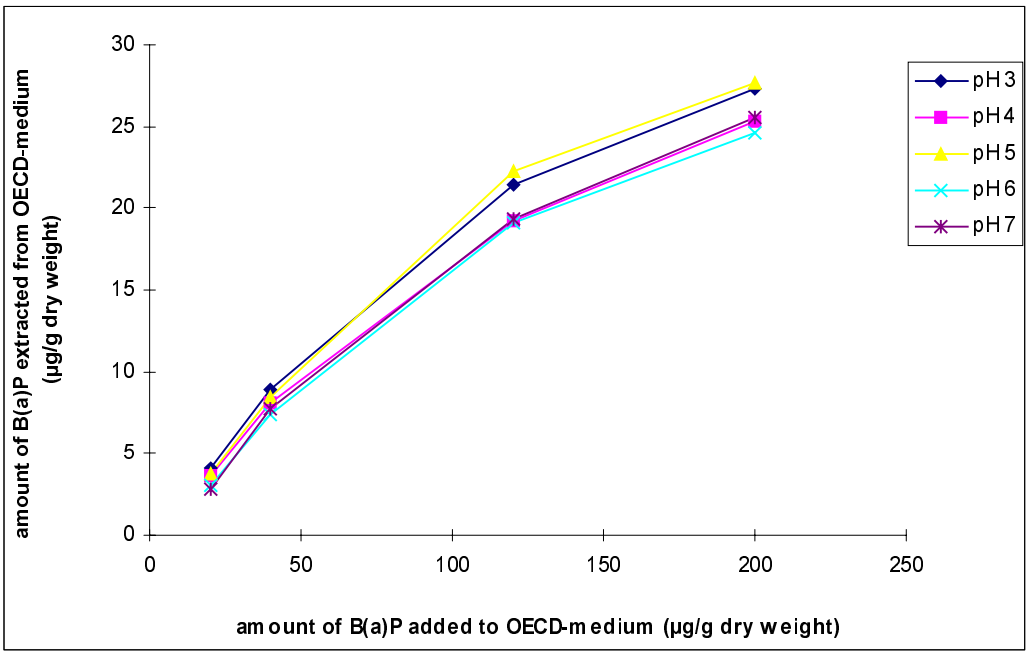

Figure 3.10. The amount of benzo[a]pyrene mobilised during in vitro digestion against the amount of benzo[a]pyrene added to OECD-medium with different soil pH (n=3).

3.2.3 Type of contaminant

The effect of the type of contaminant can only be assessed qualitatively. Bioaccessibility values appear to be different for lead and benzo[a]pyrene when comparing per soil (see appendix II, III and V). Furthermore, bioaccessibility appears to increase with increasing contamination level for lead (see figure 3.3, 3.4, 3.5 and 3.6), while a decrease is observed with increasing contamination level for benzo[a]pyrene (see figure 3.8, 3.9 and 3.10). Both relationships can be explained by the mathematical model for lead and benzo[a]pyrene.

3.2.4 Methodological parameters

3.2.4.1 Within-day variation

In general, the within-day variation for bioaccessibility of lead and benzo[a]pyrene for artificially contaminated OECD-medium and Dutch soil types is acceptable (see table 3.6), typically between 5 and 20%. In some cases high within-day variation values were determined, in particular for OECD-medium spiked with $PbCl_2$ at 0.5 × I.V., and Hulshorst B sand spiked with $PbSO_4$ at 0.5 × I.V..

Table 3.6. Within-day variation (%) for the extraction of Pb and benzo[a]pyrene from OECD-medium and 7 different Dutch soil types, artificially contaminated with different lead speciations or benzo[a]pyrene, calculated by means of ANOVA.

soil and soil type	contaminant (speciation)	0.5 × I.V. within-day variation (%)	5 × I.V. within-day variation (%)
OECD-medium	$PbSO_4$	29	20
	$Pb(NO_3)_2$	5	10
	$PbCl_2$	68	8
	$PbAc_2$	5	13
Akkrum, loam	$PbSO_4$	8	5
	$Pb(NO_3)_2$	14	8
Angeren, silty clay loam	$PbSO_4$	21	20
	$Pb(NO_3)_2$	15	10
Hulshorst B, sand	$PbSO_4$	97	17
	$Pb(NO_3)_2$	13	11
Appelscha, sand	$PbSO_4$	14	8
	$Pb(NO_3)_2$	12	11
Hulshorst A, sand	$PbSO_4$	11	10
	$Pb(NO_3)_2$	12	17
Boskoop, silt loam	$PbSO_4$	6	11
	$Pb(NO_3)_2$	9	15
Bemelen, silt	$PbSO_4$	19	4
	$Pb(NO_3)_2$	8	8
OECD-medium	B(a)P	5	5
Akkrum, loam	B(a)P	21	18
Angeren, silty clay loam	B(a)P	34	14
Hulshorst B, sand	B(a)P	6	6
Appelscha, sand	B(a)P	5	5
Hulshorst A, sand	B(a)P	7	4
Boskoop, silt loam	B(a)P	20	13
Bemelen, silt	B(a)P	8	6

3.2.4.2 Between-day variation

The between-day variation for the bioaccessibility of lead from artificially contaminated OECD-medium and various Dutch soil types ranged from 11% to 79%. Between-day variation for bioaccessibility of benzo[a]pyrene for the same soils was very variable: ranging from 6% to 154% (see table 3.7).

Table 3.7. Between-day variation (%) for the extraction of Pb and benzo[a]pyrene from OECD-medium and 7 different Dutch soil types, artificially contaminated with different lead speciations or benzo[a]pyrene, calculated by means of ANOVA.

soil and soil type	contaminant (speciation)	0.5 × I.V. between-day variation (%)	5 × I.V. between-day variation (%)
OECD-medium	$PbSO_4$	30	55
	$Pb(NO_3)_2$	27	51
	$PbCl_2$	79	48
	$PbAc_2$	20	11
Akkrum, loam	$PbSO_4$	16	11
	$Pb(NO_3)_2$	65	31
Angeren, silty clay loam	$PbSO_4$	65	41
	$Pb(NO_3)_2$	82	20
Hulshorst B, sand	$PbSO_4$	34	52
	$Pb(NO_3)_2$	33	21
Appelscha, sand	$PbSO_4$	21	39
	$Pb(NO_3)_2$	31	74
Hulshorst A, sand	$PbSO_4$	33	35
	$Pb(NO_3)_2$	45	26
Boskoop, silt loam	$PbSO_4$	29	50
	$Pb(NO_3)_2$	23	27
Bemelen, silt	$PbSO_4$	22	12
	$Pb(NO_3)_2$	28	16
OECD-medium	B(a)P	6	8
Akkrum, loam	B(a)P	154	77
Angeren, silty clay loam	B(a)P	107	19
Hulshorst B, sand	B(a)P	24	7
Appelscha, sand	B(a)P	13	10
Hulshorst A, sand	B(a)P	14	25
Boskoop, silt loam	B(a)P	72	40
Bemelen, silt	B(a)P	36	21

The within-day and between-day variations are in most cases acceptable and in some cases large. This may be due to inhomogeneity of the spiked soils, incomplete destruction of the soil samples for determination of the contaminant concentration, and variability within the digestion system. In future experiments, these aspects will be improved by 1) better homogenisation of soils and verifications of homogeneity, 2) destruction of soils by aqua regia instead of HNO_3 (for lead), and 3) inclusion of a reference soil for each digestion series.

4. Discussion

4.1 *In vitro* digestion model

The present report describes the development of an *in vitro* digestion model that can be used to estimate bioaccessibility, i.e. mobilisation of contaminants from soil during digestion, in humans. The *in vitro* digestion model is based on physiology of children. Increasing gastric pH values, i.e. less acid, resulted in decreasing bioaccessibility values for lead. The present *in vitro* digestion model employs a gastric pH of 1, which is the lowest physiological value. Hence, based on gastric pH, the model represents a worst case *in vitro* digestion model for lead.
The reproducibility of the bioaccessibility is acceptable and in some cases large, but is likely to improve when the soil samples are better homogenised. A homogenised soil does probably not represent the situation as encountered in the field. In the present study we are not interested in obtaining information on the bioaccessibility of inhomogeneous soils, but in the reproducibility of the *in vitro* digestion. Whether to use homogenised soils in future research should be discussed.
After validation of the model to *in vivo* data, the *in vitro* digestion model is an applicable way to assess location specific relative bioavailability factors soil-borne contaminants.

4.2 Study parameters

Oral bioavailability of contaminants from soil is difficult to determine since various factors seem to influence this process. In literature factors such as soil pH [Ruby et al., 1996], organic matter content [Ruby et al., 1996] and physico-chemical properties of the contaminant [Descotes and Evreux, 1984; Freeman et al., 1996; Hamel et al., 1998] are reported to be of interest. Unfortunately, many of these studies are performed as case studies in which only one or two variables are taken into account. We therefore performed a set of studies to assess the effects of 1) contaminant type, 2) contamination level, 3) soil type, 4) soil pH, 5) ageing of soil, 6) metal speciation, and 7) artificially versus historically contaminated soil, in a structural way. This approach is in first instance only to be resolved by using artificially contaminated soils (except for parameter 7). It needs to be emphasised that the authors of this report recognise the need for comparison on bioaccessibility between artificially contaminated soils and historically contaminated soils. Therefore, a preliminary study was performed. This comparison will be taken further into account in future studies.

Type of contaminant. Contaminants were classified either as organic compounds or as heavy metals, since it is to be expected that different factors affect the bioaccessibility of both groups

of contaminants. Bioaccessibility of hydrophobic organic compounds will probably be considerably affected by the amount of bile and other sorbing components in chyme [Oomen et al., 2000], whereas bioaccessibility of heavy metals is assumed to be considerably affected by the pH values in the gastro-intestinal tract [Ruby et al., 1996; Oomen et al., submitted]. Benzo[a]pyrene and lead were chosen as compounds to be investigated since risk assessment of both contaminants from soil needs to be investigated into more detail. Lead and B(a)P indeed appear to affect bioaccessibility in different ways. In order to include physico-chemical properties of contaminants in the mathematical model to be developed, bioaccessibility of other heavy metals and organic compounds will be investigated in future studies.

Level of contamination. One of the factors potentially affecting bioaccessibility is the level of contamination. In general, a linear (dose proportional) relationship between contamination level and bioaccessibility/bioavailability is taken as basic assumption. This assumption simplifies risk assessment, since it can be assumed that regardless of the level of contamination, a constant percentage of the contaminant will be bioaccessible/bioavailable. Especially in cases of a more than dose proportional relationship between the level of contamination and bioaccessibility/bioavailability, an incorrect judgement of potential risks is given. In these situations risks will be underestimated.
The data indicate that a non-linear relationship between the contamination level and the amount of contaminant mobilised from soil is likely. The results are obtained by analysing bioaccessibility by a mathematical model that can explain non-linear relationships on the basis of ion exchange or receptor binding. For lead half of the relationships were in support of non-linear relationships that were described by the underlying concept of the Gapon equation and half were not. For benzo[a]pyrene a relationship with precipitation describes the data better than a relationship without precipitation. Discrimination between a linear and a non-linear relationship according to the mathematical model cannot be performed because few data points can be used for analysis (2 to 3 data points) due to the precipitation level. In interpreting these results it should be kept in mind that the available data are rather scarce and do not yet allow a definitive conclusion to be made on the dose response relationship between the soil concentration of the contaminants and its accompanying bioaccessibility. Further investigations should be focussed on answering the question whether the present mathematical model (a non-linear model!) can be used to quantify bioaccessibility of lead and benzo[a]pyrene from Dutch soils. In order to shed light on this issue future experimental protocols should take more levels of contamination into account. The range of 0 to 5 × I.V. can be maintained.

Soil type. In literature, soil type is mentioned as a factor affecting bioaccessibility [Ruby et al., 1999]. In a study of Ruby et al. [1996] some soil characteristics like total organic carbon (TOC, determined by weight loss on ignition at 430 °C) were taken into account. It was concluded that organic carbon may provide a sorption surface for soil lead that will be readily desorbed in the gastric environment. In this way, elevated soil TOC may result in a greater

fraction of bioaccessible lead. This theory is in contradiction to the mechanism on which our mathematical model for lead is based. Our mathematical model would suggest lower bioaccessibility values with increasing TOC, as in that case more binding sites are present for the metal ions in the soil. The experimental results should be analysed by comparing the parameters of the mathematical model (K_{aff} and CEC) and their standard deviations. However, at the present the experimental results are not sufficient to conclude whether the mathematical model correctly describes the relationship between lead contamination level in soil and bioaccessibility. We therefore need to make a preliminary evaluation of the effect of soil type on bioaccessibility based on trends instead of statistical evaluation using the mathematical model. Our results of lead from various Dutch soils (for these soils TOC values were known, see table 3.1) and historically polluted Dutch soil samples (for these soils % organic matter were known, see table 3.2) show a trend in decreasing bioaccessibility with increasing TOC, although this relationship is not very clear. The trend is in accordance with our mathematical model. Nevertheless, the weak relationships indicate that the TOC content is not the only determinant for bioaccessibility. Reviewing the results of Ruby et al. it appears that they neither could demonstrate a strong relation between TOC and bioaccessibility. To our opinion bioaccessibility is determined by multiple factors of which TOC may be one.
Similar to the data of lead, the effect of soil type on bioaccessibility of benzo[a]pyrene can only be based on trends in relationships of benzo[a]pyrene contamination levels in soil and bioaccessibility. These relationships show a tendency for higher bioaccessibilities in sandy soils than in loamy soils was observed. One of the main differences between sandy (Appelscha, Hulshorst A and B) and loamy soils (Boskoop, Angeren and Akkrum) is a higher clay content in loamy soils than in sandy soils. However, in Bemelen silt and OECD-medium containing a high clay content (18% and 23%), also a relatively high bioaccessibility (34-42% and 15-25%) was measured. This indicates that the clay content will not be the ultimate predicting factor for bioaccessibility from soil.
In sandy soils a part of the process of bioaccessibility seems to be subject to saturation for benzo[a]pyrene. The mathematical model predicts that saturation may occur at different contamination levels for the soils, while the contaminant concentration in the chyme should be similar. The former can be observed from the experimental data (see figure 3.8). The latter is not observed from the present data, especially Bemelen silt shows amounts of extracted benzo[a]pyrene that are higher than saturation levels for other soil types (figure 3.8). This may be due to an experimental artefact or the mathematical model cannot describe the data and should be adjusted.
In conclusion, we think that it is necessary to gain insight into the binding process of lead and benzo[a]pyrene to components soil during *in vitro* digestion in order to explain the results observed. Information on these processes should subsequently be related to the mathematical model and the model parameters.

Soil pH. Similar to the soil type, the effect of soil pH on bioaccessibility can, at the present, not be assessed quantitatively. The soil pH appeared to have no effect on the bioaccessibility

of lead from OECD-medium. In a study by Ruby et al. [1996] it was concluded that acidic pH (2.4 - 4.9) of the test material resulted in decreased lead bioaccessibility, most likely due to formation of soil alteration lead phases such as anglesite and lead jarosite that are more stable in the acidic gastric environment. According to the authors neutral soil pH results in soil alteration phases such as cerussite and ferromanganese lead oxides that appear to have greater solubility in the gastric environment. The results of our study underscore these conclusions, since we demonstrated that the variable soil pH has no influence on bioaccessibility. Measuring the pH of soil will therefore only be useful if the pH is a good predictor for lead phases present in soil. The lead phases itself seem to be a better predictor for bioaccessibility. The acidity of soil does not seem to affect the bioaccessibility of benzo[a]pyrene from OECD-medium.

Ageing of soil. The effect of ageing of the soil is investigated for artificially contaminated soils. Bioaccessibility of lead after ageing increased for OECD-medium and was hardly affected for seven Dutch soil types. The increase in bioaccessibility after 1½ year of ageing is in contradiction to the expectation. The soil was dried before contamination and stored at 4 °C. Such circumstances may reduce the enclosure of contaminant in the silica skeleton or other changes in binding of the contaminant to the soil to a minimum. This, together with the between-day variation, may explain the results.

Lead speciation. The relationship between lead phases and bioaccessibility was studied by spiking soils with different lead speciations. Four different lead speciations were added to OECD-medium and two of these speciations, i.e. $Pb(NO_3)_2$ and $PbSO_4$, were also added to various Dutch soil types. In a study by Ruby et al. [1996] soil samples were among others characterised for the lead speciation present in the samples. Lead concentrations ranged from 1388 to 10230 mg/kg, i.e. approximately 3 to 20 × I.V. In all samples anglesite ($PbSO_4$) was the most frequently present speciation of lead. The investigators concluded that bioaccessibility was low in case the soil sample contained primarily less soluble lead phases (e.g., galena, anglesite, lead phosphate) and had a greater degree of lead phase encapsulation. Lead-bearing soil samples which contained more soluble lead phases (metal-lead oxide, lead oxide, cerussite) produced higher bioaccessibility. Our results do not entirely confirm the observations of Ruby et al. [1996]. $PbSO_4$ contaminated soils (including OECD-medium) demonstrated only tendencies to lower bioaccessibility compared to soils contaminated with the much more soluble $Pb(NO_3)_2$, may be due to the use of artificially contaminated soils, in which encapsulation may not occur.

Artificially contaminated soil versus historically polluted soil. Since we had hardly any data on the soil characteristics of the polluted soils, it is difficult to make a good comparison on bioaccessibility with artificially contaminated soils. The range of bioaccessibility measured in

the polluted soils was comparable to the range determined in the artificially contaminated soils. In future, this comparison should be investigated more into detail.
Note that large differences were observed in the determination of total lead in the soils as determined by different methods (table 3.4). This can have a large impact on the calculated bioaccessibility. Risk assessors should thus be aware of such problems.

5. Conclusions

In vitro digestion model. The present report describes the development of an *in vitro* digestion model that can be used to estimate bioaccessibility, i.e. mobilisation of contaminants from soil during digestion, in humans. The reproducibility of the *in vitro* digestion model is investigated by examining the bioaccessibility data. The reproducibility of the bioaccessibility is acceptable and in some cases large, but is likely to improve when the soil samples are better homogenised. The *in vitro* digestion model can be used as a tool to assess location specific relative bioavailability factors of soil-borne contaminants, but results should be interpreted with care, as long as *in vivo* validation has not taken place.
The bioaccessibility values of the soil samples as determined by the *in vitro* digestion model range between 6 and 72% for lead and between 2 and 63% for benzo[a]pyrene. Depending on the bioaccessibility of the contaminants from the matrix used in toxicity studies, such values may result in relative bioavailability factors <1, indicating that accounting for the matrix of ingestion via relative bioavailability factors may affect risk assessment.

Study parameters. The effect of several variables on bioaccessibility has been investigated with the *in vitro* digestion model. The results point in the direction of a non-linear relationship between amount of lead or benzo[a]pyrene mobilised from soil during digestion and the level of contamination. Moreover, bioaccessibility seems to depend on the type of contaminant and the type of soil. These conclusions might have great impact for risk assessment on contaminated soil. It means that it is impossible to pass judgement about *the* bioaccessibility, and thus about *the* bioavailability, of a contaminant from soil. This would mean that only site specific risk assessment, or risk assessment for specific conditions, can be performed. Of course, it remains possible to assess the upper bioaccessibility value covering all possible soils.
Soil pH did not affect lead bioaccessibility, while $PbSO_4$ shows only tendencies of lower bioaccessibility compared to soils contaminated with the much more soluble $Pb(NO_3)_2$. Ageing for 1½ years under the employed laboratory conditions (dried soil, 4 °C) did not have a large effect on the bioaccessibility. Comparison on bioaccessibility between artificially and historically polluted soils was difficult due to a lack of soil characteristics of the polluted soils. However, the range of bioaccessibility measured in the polluted soils was comparable to the range determined in the artificially contaminated soils.

Mathematical model. In order to analyse the experimental data, it is tried to develop a mathematical model. This mathematical model will take determining factors affecting bioaccessibility of a contaminant into account. Ultimately, the mathematical model can be used to estimate bioaccessibility and thereby determine more accurate intervention values based on contaminant and soil characteristics. The present report describes the initial steps of the development of such a mathematical model.

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Appendix I Instruction *in vitro* digestion

1 INTRODUCTION

The *in vitro* digestion model is an artificial system that is a simplification of the human gastrointestinal tract. The digestion juices are composed by adding a mixture of lipids, enzymes, electrolytes and other characteristics compounds to water. In this manner, artificial saliva, gastric juice, duodenal juice and bile are prepared.
The *in vitro* digestion model consists of three steps:
1. In a tube, soil is added to saliva and incubated at 37 °C in a rotating system.
2. After five minutes gastric juice is added and the mixture is incubated at 37 °C in a rotating system during two hours.
3. Finally, duodenal juice and bile are added and the mixture is incubated at 37 °C in a rotating system during two hours.

After the last incubation step, the mixture is centrifuged for five minutes, The supernatant represents the chyme. In the chyme, the contaminant concentration is determined. The pellet may be used to determine the mass balance.

2 EQUIPMENT AND AIDS

To give the trade name just means that the experiment was carried out with this equipment, but this is not necessary. However, the use of the called electrode is necessary.

2.1 Stove, type KTG 800, Heraeus.
2.2 Rotators, model L2, Labinco and type Reax 2, Heidolph.
2.3 Centrifugetubes, Nalgene,
2.3.1 Polycarbonate tubes with polyphenyleneoxide caps, polyphenylene plug and silicon O-ring, measures 38 x 102 mm, cat. nr. 3430-3870.
2.3.2 Polycarbonate tubes with polypropylene caps, measures 38 x 101 mm, cat. nr. 3118A-0085.
2.4 Spatula, coated with PTFE, art. nr. 762/T en 794/t, Omnilabo.
2.5 Volumetric flask and erlenmeyer flask
2.6 GFL waterbath, type 1086, Salm en Kipp.
2.7 Centrifuge Rotina 48 and Hettich 30 RF, Depex.
2.8 Dispensers.
2.9 Magnetic stirrer and a stirring rod.
2.10 pH meter, model 720A with Ross sure-flow combination pH electrode, art.nr. 8172BN, Orion.
2.11 Pipets, Gilson.
2.12 Analytische balance, Mettler, type AT 261.
2.13 Balance, Mettler, type PM 1200.

3 CHEMICALS AND SOLUTIONS

The solutions are of analytical purity unless otherwise stated. To mention the trademarks just means that the experiment was carried out using these trademarks, but these are not binding. All the necessary materials, such as glassware, plastic bottles must be free from trace elements for the determination of lead, arsenic and cadmium. To remove possible contamination, the material must be rinsed by a solution of hydrochloric acid (3.12.1). Afterwards the material must be cleaned carefully with demineralized water to remove all the acid. The concentrations of the solutions can be proportionately readjusted.

3.1 NaCl, Merck
3.1.1 Solution of NaCl, 350.6 g/2 l.
3.2 KSCN, Merck
3.2.1 Solution of KSCN, 10 g/500 ml
3.3 NaH_2PO_4, Merck.
3.3.1 Solution of NaH_2PO_4, 88.8 g/l.
3.4 Na_2SO_4, anhydrous, Merck.
3.4.1 Solution of Na_2SO_4, 28.5 g/500 ml.
3.5 KCl, Merck.
3.5.1 Solution of KCl, 89.6 g/l.
3.6 $CaCl_2 . 2H_2O$, Merck.
3.6.1 Solution of $CaCl_2 . 2H_2O$, 2.22 g/100 ml.
3.7 NH_4Cl, Merck.
3.7.1 Solution of NH_4Cl, 1.53 g/50 ml.
3.8 $NaHCO_3$, Merck.
3.8.1 Solution of $NaHCO_3$, 169.4 g/2 l.
3.9 KH_2PO_4, Merck.
3.9.1 Solution of KH_2PO_4, 8 g/l.
3.10 MgCl, Merck.
3.10.1 Solution of MgCl, 5 g/l.
3.11 NaOH, Merck.
3.11.1 Solution of NaOH, 2 g/50 ml.
3.12 HCl, 37 % g/g (sg 1,18 kg/l), Merck.
3.12.1 Solution of HCl, dilute 8 ml of HCl, 37 % g/g, with 992 ml demineralized water.
3.13 Urea, Merck.
3.13.1 Solution of urea, 25 g/l.
3.14 D+ Glucose, anhydrous, Merck.
3.14.1 Solution of glucose, 65 g/l.
3.15 D-Glucuronic acid, Fluka.
3.15.1 Solution of glucuronic acid, 2 g/l.
3.16 D-Glucosaminehydrochloride, Merck.
3.16.1 Solution of glucosaminehydrochloride, 33 g/l.
3.17 Uric acid, Merck.
3.18 α-Amylase, Merck.
3.19 Mucin, Roth.
3.20 Pepsin, Merck.
3.21 BSA(serum albumine bovine), Merck.
3.22 Pancreatin, Merck.

3.23 Lipase, Sigma.
3.24 Bile,Sigma.

The digestion juices: saliva; gastric juice; duodenal juice and bile, consist of organic and anorganic material. The organic and anorganic parts are made separately and mixed 1:1 for the 'final' digestion juice. Hereafter, enzymes, BSA, mucin and other characteristic compounds for the specific digestion juice concerned are added. The pH of the juices are adjusted at room temperature at the day of preparation.

The solutions may be made in advance, if made separetely. In this case, no HCl, NaOH, $CaCl_2$ and NH_4Cl solutions should be added. These solutions must be added on the day of preparation of the digestion juices.

The following variation is accepted in the weighing of the solid compounds:

Weight	Variation
< 500 mg	1 %
= 1000 mg	0,25 %
> 1000 mg	0,1 %

The following volumes can be adjusted to proportion:

3.25.1 **Saliva, anorganic**.
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.1.1	Potassiumchloride solution (3.5.1)	: 10	ml
3.25.1.2	Potassiumthiocyanate solution (3.2.1)	: 10	ml
3.25.1.3	Sodiumdihydrogenphosphate solution (3.3.1)	: 10	ml
3.25.1.4	Sodiumsulfate solution (3.4.1)	: 10	ml
3.25.1.5	Sodiumchloride solution (3.1.1)	: 1,7	ml
3.25.1.6	Sodiumhydroxide solution (3.11.1)	: 1,8	ml

3.25.2 **Saliva, organic.**
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.2.1	Urea solution (3.13.1)	: 8	ml

3.25.3 **Saliva "final",** pH 6,5 ± 0,2.
3.25.3.1 Mix 500 ml anorganic solution (3.25.1) en organic solution (3.25.2) in a erlenmeyer flask on a stirrer with a stirring rod.
3.25.3.2 Add 145 mg α-amylase (3.18) and dissolve.
3.25.3.3 Add 15 mg uric acid (3.17) and dissolve.
3.25.3.4 Add 50 mg mucine (3.19) and dissolve.
3.25.3.5 Check the pH. If there is a deviation from pH 6,5 ± 0,2 add sodiumhydroxide solution of 1 M (3.11.1) or concentrated HCl (3.12) until the right pH is reached.

3.25.4 **Pancreatic juice, anorganic**.
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.4.1	Sodiumchloride solution (3.1.1)	: 15,7 ml
3.25.4.2	Sodiumdihydrogenphosphate solution (3.3.1)	: 3,0 ml
3.25.4.3	Potassiumchloride solution (3.5.1)	: 9,2 ml
3.25.4.4	Calciumchloride solution (3.6.1)	: 18 ml
3.25.4.5	Ammoniumchloride solution (3.7.1)	: 10 ml
3.25.4.6	Hydrochloric acid 37 % g/g (3.12)	: 8,3 ml

3.25.5 **Pancreatic juice, organic.**
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.5.1	Glucose solution (3.14.1)	: 10 ml
3.25.5.2	Glucuronic acid solution (3.15.1)	: 10 ml
3.25.5.3	Urea solution (3.13.1)	: 3,4 ml
3.25.5.4	Glucoseaminehydrochloride solution (3.16.1)	: 10 ml

3.25.6 **Pancreatic juice, "final",** pH 1,07 ± 0,07.

3.25.6.1 Mix 500 ml anorganic solution (3.25.4) and organic solution (3.25.5) in an erlenmeyer flask on a stirrer with a stirring rod.

3.25.6.2 Add 1 g BSA (3.21) and dissolve

3.25.6.3 Add 1 g pepsine (3.20) and dissolve.

3.25.6.4 Add 3 g mucine (3.19) and dissolve.

3.25.6.5 Check the pH. If there is a deviation from pH 1,07 ± 0,07 add sodiumhydroxide solution of 1 M (3.11.1) or concentrated HCl (3.12) until the right pH is reached.

3.25.7 **Duodenal juice, anorganic.**
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.7.1	Sodiumchloride solution (3.1.1)	: 80 ml
3.25.7.2	Sodiumhydrogencarbonate solution (3.8.1)	: 80 ml
3.25.7.3	Potassiumdihydrogenphosphate solution (3.9.1)	: 20 ml
3.25.7.4	Potassiumchloride solution (3.5.1)	: 12,6 ml
3.25.7.5	Magnesiumchloridesolution (3.10.1)	: 20 ml
3.25.7.6	Hydrochloric acid 37 % g/g (3.12)	: 360 µl

3.25.8 **Duodenal juice, organic.**
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.8.1	Urea solution (3.13.1)	: 8 ml

3.25.9 **Duodenal juice "final",** pH 7,8 ± 0,2.

3.25.9.1 Mix 1000 ml anorganic solution (3.25.7) and organic solution (3.25.8) in an erlenmeyer flask on a stirrer with a stirring rod.

3.25.9.2 Add 18 ml calciumchloride solution (3.6.1) and mix.

3.25.9.3 Add 2 g BSA (3.21) and dissolve.
3.25.9.4 Add 6 g pancreatine (3.22) and dissolve.
3.25.9.5 Add 1 g lipase (3.23) and dissolve.
3.25.9.6 Check the pH. If there is a deviation from pH 7,8 ± 0,2 add sodiumhydroxide solution of 1 M (3.11.1) or concentrated HCl (3.12) until the right pH is reached.

3.25.10 **Bile, anorganic.**
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.10.1	Sodiumchloride solution (3.1.1)	: 30 ml
3.25.10.2	Sodiumhydrogencarbonate solution (3.8.1)	: 68,3 ml
3.25.10.3	Potassiumchloride solution (3.5.1)	: 4,2 ml
3.25.10.4	Hydrochloric acid 37 % g/g (3.12)	: 200 µl

3.25.11 **Bile, organisch.**
Add the following amounts to a 500 ml volumetric flask and fill up with destilled water.

3.25.11.1	Urea solution (3.13.1)	: 10 ml

3.25.12 **Bile "final",** pH 8,0 ± 0,2.
3.25.12.1 Mix 500 ml anorganic solution (3.25.10) and organic solution (3.25.11) in an erlenmeyer flask on a stirrer with a stirring rod.
3.25.12.2 Add 10 ml calciumchloride solution (3.6.1) and mix.
3.25.12.3 Add 1,8 g BSA (3.21) and dissolve.
3.25.12.4 Add 6 g bile (3.24) and dissolve.
3.25.12.5 Check the pH. If there is a deviation from pH 8,0 ± 0,2 add sodiumhydroxide solution of 1 M (3.11.1) or concentrated HCl (3.12) until the right pH is reached.

4 PROCEDURE

4.1 Put the rotators in the stove and turn on the stove. Make sure the stove is at the right temperature (37 ± 2 ° C).
4.2 Turn on the waterbath and set up the temperature at 37 ± 0.5 ° C. Put the 'final' saliva; 'final' gastric juice; 'final' duodenal juice and 'final' bile in the waterbath to reach a temperature of 37 ° C.
4.3 Check the pH of the 'final' digestion juice. If the pH is lower than 5, than check all juices seperately and if necessary prepare them freshly.
4.4 Put 0.6 g dry weight of soil in a tube
4.5 Add 9 ml saliva and incubate for 5 min. at 37 ± 2 ° C.
4.6 Add 13.5 ml gastric juice and incubate for 2 hrs at 37 ± 2 ° C
4.7 Add 27 ml duodenal juice and 9 ml bile. Determine the pH in the tube and check whether the pH has changed to pH = 5. Incubate for 2 hrs at 37 ± 2 ° C
4.8 Tubes are centrifuged for 5 min. at 2750 g.
4.9 In the chyme the contaminant concentration is determined.
4.10 The pellet can be used to determine the mass balance.
4.11 Determine the pH of the separate final juices at room temperature as a final check of the quality of the juices.

Appendix II Bioaccessibility of $Pb(NO_3)_2$

Bioaccessibility of Pb from eight soil types, spiked with **$Pb(NO_3)_2$** at four concentration levels (n=3).

Soil location and texture	Pb added to soil		Bioaccessibility	
	Pb/I.V.	µg Pb/g dry matter soil	Mean %	CV %
OECD-medium	0	n.d.	n.d.	n.d.
	0.5	275	17	6
	1.1	559	25	9
	2.9	1548	20	11
	5.1	2709	27	7
Appelscha	0	8 [a)]	60	26
sand	0.5	274	64	6
	1.0	537	45	4
	3.0	1600	51	29
	5.0	2660	64	15
Hulshorst A	0	n.d.	n.d.	n.d.
sand	0.5	266	72	19
	1.0	531	20	31
	3.0	1593	23	18
	5.0	2658	61	3
Hulshorst B	0	7 [a)]	56	66
sand	0.5	272	53	5
	1.0	537	25	27
	3.0	1599	18	20
	5.0	2662	61	16
Bemelen	0	14 [a)]	n.d.	n.d.
silt	0.5	279	50	3
	1.0	542	7	20
	3.0	1606	7	7
	5.0	2666	61	5
Boskoop	0.6	294 [a)]	40	6
silt loam	1.1	560	45	10
	1.6	824	33	6
	3.6	1887	8	11
	5.6	2946	39	3
Angeren	0.4	188 [a)]	18	24
silty clay loam	0.9	452	24	14
	1.4	719	10	18
	3.4	1780	6	10
	5.4	2842	32	13
Akkrum	0	21 [a)]	20	9
loam	0.5	286	45	11
	1	549	8	16
	3.0	1612	8	7
	5.0	2674	51	3

[a)] present in “blank soil samples”
n.d. = not detectable

Appendix III Bioaccessibility of $PbSO_4$

Bioaccessibility of Pb from eight soil types, spiked with **$PbSO_4$** at four concentration levels (n=3).

Soil location and texture	**Pb added to soil**		**Bioaccessibility**	
	Pb/I.V.	µg Pb/g dry matter soil	Mean %	CV %
OECD-medium	0	n.d.	n.d.	n.d.
	0.5	275	14	5
	1.1	559	20	8
	2.9	1548	15	5
	5.1	2709	7	7
Appelscha	0	8 [a)]	71	13
sand	0.5	274	61	1
	1.0	537	46	3
	3.0	1600	39	10
	5.0	2660	47	15
Hulshorst A	0	n.d.	n.d.	n.d.
sand	0.5	266	41	10
	1.0	531	30	23
	3.0	1593	28	9
	5.0	2658	34	27
Hulshorst B	0	7 [a)]	39	22
sand	0.5	272	43	12
	1.0	537	25	13
	3.0	1599	29	5
	5.0	2662	49	10
Bemelen	0	14 [a)]	n.d.	n.d.
silt	0.5	279	43	8
	1.0	542	9	9
	3.0	1606	28	60
	5.0	2666	43	6
Boskoop	0.6	294 [a)]	35	4
silt loam	1.1	560	33	5
	1.6	824	22	5
	3.6	1887	19	8
	5.6	2946	20	3
Angeren	0.4	188 [a)]	11	40
silty clay loam	0.9	452	8	13
	1.4	719	10	21
	3.4	1780	6	25
	5.4	2842	9	22
Akkrum	0	21 [a)]	38	4
loam	0.5	286	45	15
	1	549	12	6
	3.0	1612	12	2
	5.0	2674	52	9

[a)] present in “blank soil samples”
n.d. = not detectable

Appendix IV Ageing of soil

Bioaccessibility of four lead species in OECD-medium at two contamination levels (1× and 3×I.V.) at t = 0 and t =1½ year after spiking.

		t = 0 (n = 3)				t = 1½ year (n = 6)			
Pb form	I.V.	Bioaccessibility µg Pb/ g dry matter	SD	VC	Bioacces-sibility %	Bioaccessibility µg Pb/ g dry matter	SD	VC	Bioacces-sibility %
$PbSO_4$	1	111	9	8	21	150	34	23	28
	3	237	13	5	15	593	75	13	37
$Pb(NO_3)_2$	1	140	13	10	25	161	7	5	29
	3	305	33	11	20	630	75	12	41
$PbCl_2$	1	77	2	2	14	139	5	4	25
	3	322	4	1	20	609	73	12	38
$PbAc_2$	1	78	4	5	17	119	2	2	26
	3	315	15	5	23	557	40	7	41

*Bioaccessibility of **$PbSO_4$** spiked to various Dutch soil types at two contamination levels (1× and 3×I.V.) at t = 0 and t =1½ year after spiking.*

		t = 0 (n = 3)				t = 1½ year (n = 3)			
Location and soil type	I.V.	Average µg Pb/ g dry matter	SD	VC	Bioacc.	Average µg Pb/ g dry matter	SD	VC	Bioacc.
Appelscha	1	248	8	3	47	345	48	14	65
Sand	3	618	63	10	39	1068	169	16	67
Hulshorst A	1	158	37	23	30	232	39	17	44
Sand	3	448	42	6	28	710	281	40	45
Hulshorst B	1	135	18	13	26	163	15	9	31
Sand	3	459	25	5	29	536	37	7	34
Bemelen	1	47	4	9	9	82	13	16	16
Silt	3	456	272	60	29	282	100	35	18
Boskoop	1	178	10	5	34	227	10	4	43
silt loam	3	355	29	8	22	442	29	7	28
Angeren	1	69	14	21	13	46	16	36	9
silty clay loam	3	111	28	25	7	110	40	37	7
Akkrum	1	64	4.0	6	12	80	6	8	15
Loam	3	193	4	2	12	280	66	24	18

*Bioaccessibility of **$Pb(NO_3)_2$** spiked to various Dutch soil types at two contamination levels (1× and 3× I.V.) at t = 0 and t =1½ year after spiking.*

		t = 0 (n = 3)				t = 1½ year (n = 3)			
Location and soil type	I.V.	Average µg Pb/ g dry matter	SD	VC	Bioacc.	Average µg Pb/ g dry matter	SD	VC	Bioacc.
Appelscha	1	241	9	4	46	332	85	26	63
Sand	3	821	236	29	52	789	173	22	50
Hulshorst A	1	108	33	31	20	143	23	16	27
Sand	3	365	64	18	23	459	131	28	29
Hulshorst B	1	135	36	27	25	115	32	28	22
Sand	3	294	58	20	19	237	14	6.0	15
Bemelen	1	37	7	20	7	107	50	47	20
Silt	3	106	7	7	7	209	86	41	13
Boskoop	1	276	17	6	52	263	15	6	50
silt loam	3	148	16	11	9	144	13	9	9
Angeren	1	69	13	18	13	32	10	32	9
silty clay loam	3	111	11	10	7	65	27	41	4
Akkrum	1	42	7	16	8	67	15	23	13
Loam	3	128	9	7	8	200	21	11	13

Appendix V Bioaccessibility of benzo[a]pyrene

Bioaccessibility of **benzo[a]pyrene** from eight soil types at four concentration levels (n=3).

Soil location and texture	B(a)P added to soil		Bioaccessibility	
	B(a)P/I.V.	µg B(a)P/g dry matter soil	mean %	CV %
OECD-medium	0	n.d.	n.d.	n.d.
	0.5	20	25	2
	1.0	40	23	1
	3.0	120	18	3
	5.0	200	15	1
Appelscha	0	n.d.	n.d.	n.d.
Sand	0.5	20	44	6
	1.0	40	36	3
	3.0	120	21	6
	5.0	200	13	1
Hulshorst	0	n.d.	n.d.	n.d.
sand A	0.5	20	50	9
	1.0	40	44	0
	3.0	120	27	4
	5.0	200	14	4
Hulshorst	0	n.d.	n.d.	n.d.
sand B	0.5	20	34	4
	1.0	40	31	13
	3.0	120	15	1
	5.0	200	11	10
Bemelen	0	n.d.	n.d.	n.d.
Silt	0.5	20	42	3
	1.0	40	41	11
	3.0	120	40	21
	5.0	200	34	10
Boskoop	0	n.d.	n.d.	n.d.
silt loam	0.5	20	6	7
	1.0	40	10	7
	3.0	120	7	6
	5.0	200	5	9
Angeren	0	n.d.	n.d.	n.d.
silty clay loam	0.5	20	2	75
	1.0	40	3	36
	3.0	120	7	17
	5.0	200	7	7
Akkrum	0	n.d.	n.d.	n.d.
Loam	0.5	20	7	4
	1.0	40	8	15
	3.0	120	15	14
	5.0	200	10	4

n.d. = not detectable

Appendix VI Mailing list

1-6. Directeur DGM/BWL-directeur, Drs. J.A. Suurland
7. Directeur-generaal Milieubeheer, Ir. J. van der Vlist
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