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Oral bioavailability of heavy metals and organic compounds from soil; too complicated to absorb ? An inventarisation of factors affecting bioavailability of environmental contaminants from soil.

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SAMENVATTING

Biologische beschikbaarheid is een belangrijke factor in de risicobepaling van milieucontaminanten uit de bodem. Het is één van de determinanten in de bepaling van interventiewaarden. Tot op heden wordt door gebrek aan betrouwbare informatie in PBPK-modellen de biologische beschikbaarheid uit bodem op 100% gesteld. Als blijkt dat de biologische beschikbaarheid in deze benadering met een factor 2 of meer wordt overschat, betekent dit dat de huidige interventiewaarden 50% of meer te laag zijn gesteld. Gevolg hiervan is dat te snel tot het advies bodemsanering wordt overgegaan.

In dit rapport is een overzicht gegeven van de literatuurgegevens over de biologische beschikbaarheid van zware metalen (lood, arseen, cadmium) en organische verbindingen (TCDD's, TCDF's en PCB's) uit bodem. Naast algemene achtergrondinformatie over de verbindingen, is ook aandacht besteed aan de problematiek rondom het definiëren van het begrip "biologische beschikbaarheid", inclusief bepalingsmethoden en factoren die de biologische beschikbaarheid uit bodem kunnen beïnvloeden.

Op basis van de literatuur lijkt de conclusie gerechtvaardigd dat de biologische beschikbaarheid van milieucontaminanten vanuit een bodemmatrix inderdaad een factor 2 tot 10 lager kan zijn dan 100%. Het rapport toont echter aan dat de in de literatuur beschreven data te beperkt zijn voor directe toepassing in de berekening van interventiewaarden. Aanvullend onderzoek lijkt vereist, waarbij de aandacht met name gericht zou moeten worden op (zie Figuur 1 op blz. 20): 1) het definiëren van het begrip "biologische beschikbaarheid", 2) het verkrijgen van informatie over extractie van contaminanten uit de bodemmatrix door digestiesappen, en 3) het verkrijgen van informatie over absorptie van contaminanten vanuit de 'digestiesappen-matrix', waarbij vooral aandacht aan de speciatie van zware metalen in die matrix moet worden besteed.

SUMMARY

Bioavailability plays an important role in risk assessment of environmental contaminants from soil. It is one of the determinants in the assessment of intervention values. In present risk assessment, bioavailability from soil is supposed to be 100% due to a paucity of reliable information. However, when it should appear that in this model bioavailability is overestimated by a factor 2 or more, this would imply that the present intervention values are 50% or more too low.

This report gives an overview of the data available on bioavailability of the heavy metals lead (Pb), arsenic (As), cadmium (Cd), and toxic organic compounds like PCDD's/PCDF's and PCB's from soil. Beside background information on these compounds, attention is paid to the issue of defining the concept of "bioavailability" properly, including methodologies for determining bioavailability and to factors influencing bioavailability.

A critical survey of all these items leads to the conclusion that the soil matrix can reduce the bioavailability of environmental contaminants with factors up to 10, compared to administration in a solution. However, scarcity of data hampers straightforward application such as the calculation of levels that would necessitate intervention. To our opinion, future research is required. It should focus on (see Figure 1 on page 20): 1) defining the concept of bioavailability, 2) obtaining information about the extraction process of contaminants from the soil matrix by digestion juices, and on 3) absorption processes out of digestion juices into systemic blood, in which especially attention should be paid to speciation of heavy metals in these juices.

1. INTRODUCTION

Systemic exposure and associated toxic effects of heavy metals and toxic organic compounds in various populations at risk are governed by the kinetics of these compounds. One of the determinants in kinetics is bioavailability. It is a variable which is of great influence in risk assessment[1]. Generally, bioavailability from environmental matrices, e.g. soil, is assumed to be equal to 100% or, in the most sophisticated approach, is assumed to be equal to bioavailability from a solution. These assumptions are due to the paucity of reliable data on effects of the soil matrix on bioavailability[2]. The "worst-case" approach may have significant financial consequences, since it may result in overestimation of public health risks.

In studying bioavailability of a pollutant from soil, various problems can arise. The first problem is already located in defining the concept of "bioavailability". In literature on environmental topics, bioavailability often is interpreted as being equal to the amount ingested. However, many compounds are not fully absorbed in the gastro-intestinal tract. Therefore, a pharmacological definition, in which the fraction absorbed is taken into account, is more relevant to a toxicologically meaningful assessment of intervention values in soil.

Another problem can be found in the complexicity of the soil matrix. Extraction of the contaminant from the matrix by digestion plays a crucial role in bioavailability, because this step determines which fraction of the ingested amount is potentially available for systemic absorption. Unfortunately this step is paid little attention to in literature. Recently Owen *et al.*[3] published an overview of so-called absorption coefficients of 39 environmental contaminants, without giving relevant background information on the matrices in which the contaminant was administered. To our opinion such lists should be interpreted with caution, because this background information is of utmost importance. First when data on the extraction step are known, it is possible to take, literature data on absorption from a solution, into account in determining bioavailability from soil.

A third problem is determining the amount of soil ingested. Stanek *et al.*[4] conclude from the data available in literature that current estimates of soil ingestion are trace-element specific and vary widely among elements. This conclusion is to our opinion a misinterpretation of the data. In most studies on heavy metals soil ingestion is back-calculated from blood or urine lead levels on the assumption of 100% bioavailability interpreted as systemic uptake. In this

calculation, the crucial role of compound-specific and soil-specific characteristics in relation to bioavailability are not taken into account.

Estimations of soil intake in children demonstrate a wide variability. For example Mushak *et al.*[1] calculated a soil intake of approximately 10 mg/day at ages up to 2 years and 25 mg/day at the age of 2-5 years. The U.S. Enviromental Protection Agency (= U.S.-E.P.A.) employs values of 100 to 200 mg/day. In a recent study of the National Institute for Public Health and Environment in the Netherlands a value of 150 mg/day was found[5].

The report presented here gives an overview of the data available on bioavailability of the heavy metals lead (Pb), arsenic (As), cadmium (Cd), and toxic organic compounds, PCDD's, PCDF's and PCB's, from soil. All these compounds have been recognised for years as environmental pollutants, which can enter the body from various sources including air, water, soil and food. The relative importance of each source is a function of several variables, such as the concentration of the compound in the source, the compound's chemical/physical features, its chemical speciation and the particular characteristics of the population of concern[6]. For example, children up to approximately 6 years of age are generally exposed to contaminants via soil to a higher degree than adults, due to physiological reasons, such as their greater ability to absorb certain compounds from the gut than adults, and due to behavioural reasons, such as more frequent hand-to-mouth activities resulting in higher soil intakes.

In chapter 2 of this report some background information of the compounds considered, is introduced. Subsequently, in chapter 3, attention is paid to defining the term "bioavailibility", including methodologies for its estimation and factors influencing it. Chapter 4 discusses several aspects concerning oral bioavailability, while chapter 5 is about risk assessment based on the available data. Finally, in chapter 6 concluding remarks and recommendations for future research are given.

2. GENERAL PROPERTIES OF REPORTED CONTAMINANTS

2.1. Lead

Lead is a typical trace element in both rock and soil but only soil is a major sink for anthropogenic lead. Contamination from industry, coal ash, paint, sewage sludge, mining and smelting, and fallout from airborne gasoline have led to general soil pollution. It has been estimated that the technological sources together probably introduce lead into the environment at about 100 times the natural rate. Undoubtedly industrial exposure is a major source in some areas and enormously high soil lead levels occur in areas of lead mining and near smelters and other lead works.

The important sources of lead vary in different areas of the world. In general, the environmental lead exposure deviates between different geographic regions, e.g., urban regions of high industrial density compared with rural regions without industrial sites. Humans are exposed to natural as well as anthropogenic sources, e.g., drinking water, soils, industrial emission, car exhaust, contaminated food and beverages[7].

In early 1974, evidence of excess lead intoxication in the surroundings of a lead smelter in northern Idaho became apparent[8]. An extensive epidemiological study, the Silver Valley Lead Study, of both inhabitants and environment of the above mentioned affected area was undertaken. During the whole study period soil lead concentrations from approximately 50 to 24,600 ppm were found. Blood lead levels in humans were found to be most influenced by ambient air lead, soil lead, dustiness indoors and occupation. Blood lead levels appeared to be significantly higher in children than in adults. The same study suggested that, although a definite relationship exists between soil levels and blood lead concentrations, soil lead levels up to 10 mg/g would not result in blood lead levels greater than 40 µg/100 ml. This blood level is supposed to be critical for the development of toxicological symptoms.

In the majority of lead poisoning cases, lead enters the body orally. This lead exists in the environment in many different chemical species: lead sulphide or lead chloride in the mining waste soil, lead oxide in soil adjacent to smelters and lead carbonate, lead chromate and other lead salts used as pigments in older paints. Only very limited data are known about the bioavailability of lead from soil. In 1991, Freeman *et al.*[9], published data on a study in rats in which bioavailability of lead administered via contaminated mining waste soil was compared

to lead administered as a lead acetate containing solution. It appeared that bioavailability of soil-ingested lead was only 8% of lead acetate administered with food. The study did not give any information on absolute bioavailability. However, values of 40-50% in children[10,11] and 10% in adults[12,13] have been demonstrated for lead present in a normal diet. These observations substantiate that the soil matrix may reduce bioavailability considerably. Data on bioavailability from soil in humans have not been found in literature.

On the other hand, toxicological effects of lead have been studied extensively. Generally, blood lead levels are considered to represent the *internal* dose which in turn is correlated with the effective *external* dose. Blood levels are also supposed to correlate with adverse health effects, like neurological development disorders. However, the problem of the variability of observed blood lead levels among different communities and individuals with similar soil lead exposure has not been fully addressed yet[14]. To our opinion differences in composition of the soil and the lead concentration in soil may form an explanation for this observation.

Most studies have been focussed on children, because it is assumed that this is the population at major risk. However, concern has also been expressed that increased bone resorption in previously lead-exposed women could release biologically significant amounts of lead into maternal plasma at the time when the foetus is particularly sensitive to teratogenic effects of lead or most able to accumulate persistent stores of lead in newly mineralising bone[15]. This latter observation also implicates that blood lead levels will be a doubtful indicator of recent exposure for pregnant women. To our opinion, also in other populations demonstrating elevated bone resorption, e.g. peri- and postmenopausal women, blood levels will not represent recent lead exposure well.

2.2. Arsenic

Arsenic is a naturally occurring element which may be present in an inorganic or an organic form in environmental media. Most study data focus on inorganic arsenic, because this is the form of primary toxicological concern. Arsenic in its elementary form is nontoxic[16]. Arsenic compounds have been classified by the U.S.-E.P.A. (1988)[17] as known human carcinogens when exposure occurs by inhalation and oral routes. Elevated levels of arsenic in soil are of particular concern for small children who swallow small amounts of soil while playing, because the developing nervous and cardiovascular systems are targets for arsenic toxicity[18].

The estimated total amount of inorganic arsenic ingested daily from environmental sources by the general population ranges from 0.2 to 0.8 μg/kg body weight/day, with the greatest exposure being in children up to 6 years. However, intake of inorganic arsenic may be greater in communities near sources, such as smelters, landfill sites, geological sources and areas with a history of high arsenic pesticide application.

Bioavailability of arsenic from a solution is considered to be approximately 98%[3]. No data on bioavailability of arsenic from soil in humans are known. Recently, Groen *et al.*[19] demonstrated that bioavailability of arsenic from soil in dogs amounted to only 8.3 ± 2.0%.

2.3. Cadmium

Cadmium is an element which is widespread in the environment. Major sources are found in the United States, Mexico, Canada, Australia and Japan. The main sources, responsible for emissions to air, water, and soil, are steel production, nonferrous metal production, mortar manufacture, pigment manufacture, cadmium plating, and battery manufacture. Other anthropogenic emissions to air, water, and land are oil combustion, waste incineration, coal combustion, and phosphate fertilisers[20].

Cadmium poses a threat to human health. Due to its extremely long biological half-life (10 to 40 years in liver and kidneys) the cadmium concentration in the kidneys can increase over the years. At a critical concentration of cadmium, damage of the proximal renal tubular cells will occur. As a result the urinary excretion of low molecular weight proteins increases. These adverse effects are especially related to orally ingested cadmium. On the other hand, inhaled cadmium has been classified as a probable human carcinogen (U.S.-E.P.A., 1987)[21]. Insufficient data are available to deem it carcinogenic by the oral route[22].

In humans the diet is the main source of cadmium exposure. The contamination of cadmium to soil via air pollution, fertilisers and sewage sludge has caused a steady increase of cadmium into the environment during the last decades. The ongoing acidification of soil, due to acid rain and application of fertilisers, may increase the solubility of soil cadmium and thereby plant uptake.

The average gastro-intestinal absorption of cadmium from food is estimated at about 5% in adults and varies considerably between individuals. Epidemiological studies reveal that lower serum iron levels and high-fibre intake are often related to elevated concentrations of cadmium in blood. Moreover, reduced body iron stores seem to be correlated with increased

intestinal absorption of cadmium. No data for animals, nor for humans about bioavailability of cadmium from soil were found in literature.

2.4. Organic compounds

Various anthropogenic organic compounds can be found in the environment. In this report attention is paid to polychlorinated dibenzo-*P*-dioxins (PCDD's), polychlorinated dibenzo-*P*-furans (PCDF's) and polychlorinated and -brominated biphenyls (PCB's).

PCDD's and PCDF's are produced as by-products of i.a. phenoxy acid herbicides and hexachlorophene and during combustion of organic material or municipal waste. PCDD's have been produced accidentally. They were byproducts in "Agent Orange" that was applied as a defoliant during the Vietnam war.

PCB's have been manufactured commercially from 1929 until the 1980's. They have been used as dielectrics in transformers and capacitors, in heat transfer and hydraulic systems and in the production of oils, pesticides and plastics.

The group of PCDD's and PCDF's contains the most toxic man-made compounds with several deleterious effects. It causes chloracne, liver and kidney damage, thymic atrophy and total body weight reduction. Due to microsomal enzyme induction, particularly induction of cytochrome *P*-450 dependent aryl hydrocarbon hydroxylase (AHH) activity, dioxines induce their own metabolism and will accumulate in the liver even more than in fat due to protein binding. Toxicity of the various congeners is expressed in terms of toxic equivalency factors (TEF's) with respect to the most toxic 2,3,7,8-tetrachlorodibenzo-*P*-dioxin (TCDD).

Of the group of PCB congeners, the coplanar ones are the most toxic and show great resemblance with TCDD. Their concentration is about 100,000 times less then total PCB concentration in the environment. The TEF's (in comparison to TCDD) of all PCB congeners, including the most toxic coplanar ones, are much smaller then 1, but the environmental concentrations are much greater resulting in a higher impact than PCDD's.

3. ORAL BIOAVAILABILITY

3.1. Definition

The concept of bioavailability is subject to a large number of interpretations and definitions. Definitions range from very strict descriptions to more vague references. Moreover, literature makes clear that this term is used in a broad range of scientific fields, varying from environmental and chemical to pharmacological sciences. This wide application explains the lack of uniformity of defining the term, but it also underscores the need for a detailed description of the term in every study.

In this report the overview of definitions of *oral* bioavailability is restricted to the biological relevant ones.

1. *Bioavailability is equal to the total amount ingested.*

   In this definition it is assumed that the entire amount ingested is also available for systemic exposure. This is only valid for contaminants which demonstrate 100% absorption from the gastro-intestinal tract into the systemic circulation and are not subject to a first-pass effect in the liver. Neither the heavy metals, nor the organic compounds can fullfill both these conditions.

   To our opinion this definition for bioavailability can only be relevant for risk assessment of compounds which, among others, demonstrate deleterious effects in the gastro-intestinal tract, e.g. radioactive compounds.

2. *Bioavailability is the percentage of the amount ingested (dose) that is absorbed out of the over the gastro- intestinal wall. lumen*

   In this definition the absorption process over the gastro-intestinal wall is taken into account. In Figure 1. this is represented by F_{act}. Generally, absorption is determined by measuring the parent compound excreted in the faeces following oral administration of that compound. Absorption can then be calculated by subtracting the amount recovered in the faeces from the amount ingested.

   This method of estimation has some restrictions in relation to bioavailability of contaminants. The method is not applicable for investigating absorption of heavy metals, because many of them are actively secreted into the intestinal lumen either via bile (e.g.arsenic[23]) or via intestinal juices (e.g. lead[23]). Due to this process absorption will be

underestimated. Also absorption of organic compounds is a problem. Often, the interpretation of data needs additional assumptions. Fries *et al.* (1989) observed ^{14}C recovery of ^{14}C labeled PCB's in feces. 15 to 25% of the recovered amount was not extractable and it was assumed that this fraction contained only metabolites because the parent compound was for 88 to 96% extractable from soil. Also they neglected enterohepatic recycling of the parent compound. In a study of Fries and Marrow (1992) enterohepatic recycling and metabolism of two ^{14}C labeled hexachlorobiphenyls was neglected in interpreting total ^{14}C recovery.

Moreover, to our opinion, collection of the relevant fraction of the feces is difficult, leading to possible unreliable absorption data.

3. *Absolute bioavailability* described as the percentage of the amount ingested, which is systemically available (following oral ingestion). This definition can be rewritten in the following formula:

$$F = \frac{AUC_{\infty}(oral)}{AUC_{\infty}(i.v.)} \cdot \frac{D(i.v.)}{D(oral)}$$

in which F is absolute bioavailability, AUC_{∞} = area under the plasma concentration-time curve calculated over the time interval 0 to infinity following oral or intravenous administration of the contaminant and D is dose administered.

Bioavailability in this interpretation is based on blood concentrations following oral and intravenous administration. It gives information on the absolute bioavailability of a compound. In Figure 1. it is represented by F_{soil}.

The approach described in this definition is only in part applicable to organic compounds, because it does not give information on the first-pass effect in the liver. In this way, especially for contaminants accumulating in the liver, absorption out of the gastro-intestinal tract will be underestimated.

For heavy metals the above mentioned approach gives the most relevant information about bioavailability. However, experiments for estimating bioavailability in this way are hampered by the long terminal elimination half-life (4 to 5 weeks) for bone-seeking metals which necessitate a long sampling period (at least 12 to 15 weeks).

4. *Relative bioavailability*

Bioavailability expressed as percentage of the amount absorbed from a reference matrix. In

this interpretation, no information is derived on the absolute bioavailability, unless the absolute bioavailability of the control is known.

5. *Bioavailability expressed as a function of toxicodynamic effects observed.*

   In this approach, bioavailability is described on the basis of the observation of certain toxicological or carcinogenic effects (e.g. induction of the P-450 system),. This approach is mainly qualitative and is not applicable for studying bioavailability of contaminants from soil in a quantitative manner.

   Although the relevance of this interpretation of bioavailability is questionable, various studies using this approach have been described in literature (especially for organic compounds). McConnell *et al.*[24] consider effects as acute toxicity and induction of hepatic AHH activity and concludes to bioavailability. Lucier *et al.*[25] even considered more effects, such as body weight or liver weight. But they also considered TCDD liver concentrations as a more quantitative measure for bioavailability. Umbreit *et al.*[26] regard liver concentration and toxicity of TCDD in guinea pigs, while they measure cytochrome *P*-450 content and AHH activity in rats in the same study. They observe seemingly conflicting results from the quantitative measures, because two different soils show about the same enzyme induction, although they differed markedly in toxicity. A possible explanation is found in the existence of other potent inducing compounds than TCDD or compounds antagonising TCDD toxicity.

3.2. Determination

The definition of bioavailability and its determination are closely related. The above mentioned approaches reveal already the method for assessing bioavailability to a great extent.

3.3. Absorption kinetics and absorption processes

With respect to a quantitative analysis of bioavailability different phases can be distinguished: 1) extraction of the contaminant in the GI-tract juices from the ingested soil, 2) absorption of the extracted compound through the GI-tract wall and 3), in case of a strict definition of bioavailability, first passage through the liver. All these phases take place simultaneously. They are represented in Figure 1.

and intestinal juices including bile, and c) physiological aspects like motility and bile secretion in the gastro-intestinal tract.

In literature the terms "*bio-accessible* " [27] and "*availability*" [28] have been posed to describe the fraction of the dose ingested that is, by its mere extractability, *potentially* available (F_{pot}).

To our opinion these terms cannot be used as a replacement for (absolute) bioavailability, because they only describe a part of the absorption process. Moreover, absorption and extraction are mutually dependent, implicating that knowledge about both processes and their interactions is necessary to describe bioavailability from soil completely.

Another restriction in this definition might be found in the term "fraction of dose". This expression presumes knowledge about the contaminant's amount in soil. In experimental settings in which known amounts of contaminant are added to soil, the ingested amount can be determined quite precisely, but in soil samples from contaminated areas the analytical method for determining the content of the contaminant in soil forms the critical step.

One of the few investigators who adress the problem of extractability with respect to bioavailability is Rotard *et al.*[29]. They have described a so-called '*in vitro*' digestion model in which the extractability of dioxines from red slag by digestion juices was studied. In this article they compare three different mixture recipes that are supposed to model saliva, gastric and intestinal juice and bile for their extraction potency. Extractability was expressed as percentage of dose and appeared to depend on mixture recipe of digestion juices, temperature, soil grain size and gastric content. Extractability decreases from 0.8 to 0.6% when temperature increases from 20°C to 37°C. Gastric content increased extractability from 0.9% without to 1.3% with gastric content (salad oil and alcohol). These findings indicate that in determining extractability, one should carefully interprete data with respect to body temperature and feed habit.

Extractability of heavy metals from soil is very poorly described in literature. The only remarks that can be found refer to suggestions to estimate extractability by means of models, such as MINEQL[30], which can calculate the speciation of non-organic compounds in solution.

In vitro studies demonstrated that the availability of lead from a lead-containing solution resulted in 70 times higher absorption in the stomach and 5 times higher absorption in the small intestine when compared to lead derived from mining waste [29]. Although absorption of

lead in the stomach is a questionable observation, the results of this study underscore the inhibiting effect of the soil matrix on bioavailability

It can be concluded that in literature the problem of lacking information about the process of extractability is recognised, but reliable experimental data are still limited. The *in vitro* digestion model of Rotard *et al.*[30] needs extensive optimalisation and validation, but is to our opinion a necessary tool in studying extractability and thus in studying bioavailability of contaminants from soil.

Following extraction from soil, the contaminant is available for absorption into the systemic blood circulation. The absorption kinetics of a contaminant are mainly determined by the mechanism of absorption, both passive and active absorption, the concentration of the contaminant, and the residence time in the relevant gastro-intestinal-absorption compartments.

The mechanisms of absorption of both heavy metals and organic compounds are extensively described in literature, although gaps of reliable and experiment-based information remain. For example, the toxic effects of lead are linked to blood lead concentrations, and thus may be predictable to some extent based on blood lead levels. However, Aungst et al. 31 demonstrated that blood lead concentrations are not linearly related to oral doses that were administered in a solution. This observation underscores the handicaps in extrapolating high dose kinetics to low dose kinetics. Studies in which oral doses are administered in a solution mainly refer to high dose kinetics, whereas low dose kinetics are more appropriate for studying bioavailability of contaminants from soil.

Again, it is tempting to assign a fraction, F_{abs} , to that part of the dose that was available for absorption following extraction from the soil matrix. This fraction would be based solely on absorption kinetics. However, if absorption and extraction are mutually dependent, or absorption is influenced by the kinetics of a contaminant, this may be a fruitless approach.

If the concept is valid that extraction and absorption both are independent processes or at least so in a reasonable approach, then the actual bioavailability can be described as the product $F_{act} = F_{abs} \cdot F_{pot}$. In this formula F_{act} represents the bioavailability of a contaminant from soil, expressed as fraction of the dose administered, whereas F_{pot} represents the fraction of the ingested dose that is extracted from the soil matrix by digestion juices and F_{abs} represents the fraction which is absorbed in case the contaminant is administered in a

solution. It is very misleading that this latter fraction also often is called absolute bioavailability regardless of the matrix in which the contaminant is administered. In case no correction is made for F_{pot} , the term absolute bioavailability is to our opinion only valid for contaminants administered in a liquid matrix. No reference was found adressing the above mentioned points of discussion.

In case data on the absorption of the contaminant over the intestinal wall are required, values of absolute bioavailability have to be corrected for the first pass effect in the liver F_{met}. This first pass effect is especially of interest for organic compounds, because these compounds are, unlike most heavy metals, metabolised in the liver. In case of non-linear metabolism kinetics, for instance Michaelis-Menten like, or inducible metabolism, e.g., benzo(a)pyrene inducing its own metabolism, or induction by co-contaminants, this can be an impracticable concept because F_{met} will be dose dependent or dependent on co-contaminant concentrations. Again, under the assumption of independence of processes, the strictly available fraction is $F_{soil} = (1 - F_{met}) \cdot F_{pot} \cdot F_{abs}$.

Literature makes clear that most researchers think of F_{act} as **the** bioavailability of a compound. However incorrect interpretion of experimental data leads to a misleading description of the various phases of bioavailability. PBPK-modelling can be a useful tool in assessing F_{soil} providing the above mentioned phases are applied correctly. Conversely, PBPK-modelling may contribute to a good understanding of experiments on bioavailability. Such an approach was initiated by O'Flaherty *et al.*[28], who explicitely used a PBPK-model to estimate the actual bioavailability from blood lead concentration data.

We conclude that the concept of bioavailability also is used outside of our scope. Not surprisingly, this confuses the communication in research. Moreover, it results in various interpretations of the same term. Therefore, we strongly suggest that in each study "bioavailability" should be defined as detailed as possible. The aim of the study, the compound under investigation, and the matrix in which the compound is administered will determine whether F_{soil}, F_{met}, or F_{abs} has to be assessed.

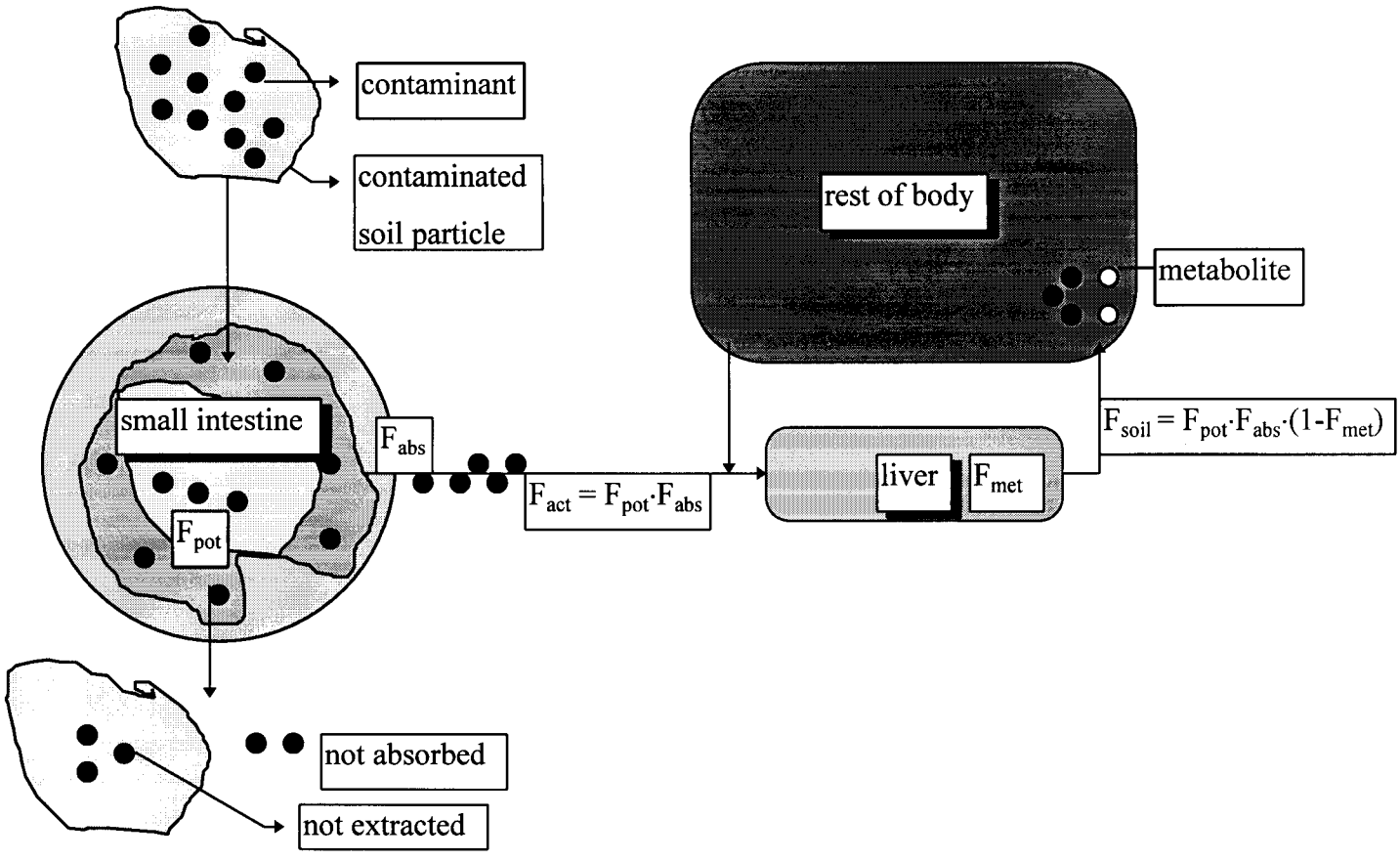

Figure 1: a contaminated soil particle is ingested and 7/10 of the amount of contaminant adsorbed on it is extracted ($F_{pot} = \frac{7}{10}$); from the amount extracted 5/7 is absorbed ($F_{abs} = \frac{5}{7}$ and $F_{act} = \frac{1}{2}$); during first passage of the liver 2/5 of the amount actually absorbed is metabolised ($F_{met} = \frac{2}{5}$) resulting in a systemic availability $F_{soil} = \frac{3}{10}$.

3.4. Experimental results

As stated earlier in this report, the number of experimental results on the bioavailability (F_{soil}) of environmental contaminants from soil are limited. In this section the data found in literature are summarised. The data on the heavy metals indicate that intervention values calculated by means of the conventional approach using 100% bioavailability or bioavailability without correction for extractability from the soil matrix (only F_{abs} is then taken into account) may be overestimated by a factor 2 or even more. The data on the organic compounds make clear that F_{met} is an important factor which has to be taken into account.

3.4.1. Heavy metals

It is generally accepted that 100% of ingested arsenic and 30% or more of ingested lead is assumed to be bioavailable. These data are mainly based on toxicological studies using pure arsenic and lead salts. Davis *et al.*[33] pointed at the problem that this approach is flawed if the objective is to evaluate the bioavailability of metals from soils. In their metallic form, metals in soil are typically much less soluble than metal salts [32].

In a study in rats, O'Flaherty *et al.*[28] demonstrated that bioavailability of lead from mining waste-contaminated soils is only around 1% of the dose administered. Application of the obtained data into a physiologically based PBPK model demonstrated that at increasing dietary lead intake bioavailability systematically decreased from 1.6% to 0.3% in the male rats and from 0.90% to 0.24% in the female rats. At all concentrations tested, females absorbed a smaller percentage of lead than males. However, when lead uptake from the gastrointestinal tract was expressed in terms of mg/kg bodyweight/day, fractional uptake was not demonstrably different between males and females.

Also in great contrast, Davis *et al.*[33] presents data that compare the representativeness of soluble salts and natural soil as estimators of metal availability. When a mine waste impacted soil was fed to New Zealand White rabbits, only 11% of the total arsenic and 6% of total lead were solubilised in the small intestine.

3.4.2. Organic compounds

Poiger and Schlatter[33] found an amount of 24% of the dose TCDD in the livers of Sprague-Dawley rats, 24 hours after administration. The TCDD contaminated soil had been stored 10-15 hours before administration. Soil samples free from TCDD contamination were taken from the Seveso region. When the soil storage period was 8 days at a temperature of 30-40°C only 16% retention was observed. No absorption was detected when also activated carbon was added to the soil.

Van den Berg[34] found liver retention of PCDD's and PCDF's on fly ash administered to male Wistar rats less than 5%, with a TCDD retention of less than 0.4%. Administration with an oily vehicle increased retention about ten-fold.

In a field experiment with cattle, Fries[27] found a PBB fat concentration/soil concentration ratio of about 30%. In a controlled experiment on lambs, they found bioavailability ranging from about 40 to 70%. A silt loam that had an experimental PBB application in the past was

used. In contrast to the findings of Poiger and Schlatter[34] bioavailability was only slightly reduced adding 0.3 or 0.6% of activated carbon to the experimental soil. Fries *et al.*[35] reported a bioavailaibility of 80 to 90% for three PCB's for male Sprague-Dawley rats. The PCB spiked soils were from another study in 1981[36]. In an experiment with male Sprague-Dawley rats, to which were administered ^{14}C labeled HCB's on a clay and a sandy soil Fries and Marrow[37] found a bioavailability fraction of 75 to 80%. This number was not influenced by "ageing" the soil, after spiking it, for 6 months.

In a comparison of bioavailability between grain and soil-borne tritiated TCDD, administered to lactating Holstein cows, Jones *et al.*[38] found almost the same results for both treatments. The soil, Geralt silt loam, was aged for four weeks after addition of ^{14}C labeled TCDD.

Lucier *et al.*[25] administered TCDD contaminated soil from the Minker site in Missouri to female Sprague-Dawley rats. The soil was intentionally sprayed with waste oil to control dust. The oil contained a wide variety of hydrocarbon contaminants. Their results, comparing with administration of TCDD in corn oil, suggest a relative bioavailability of approximately 50%.

Shu *et al.*[39] administered soils sampled from areas of Times Beach sprayed with a mixture of oils containing trace amounts of TCDD of various concentrations to male Sprague-Dawley rats. They compared bioavailability with respect to corn oil but, in contrast to Lucier[25], corrected for an assumed bioavailability of 70% for corn oil (Piper *et al.*[40]). Their result is an approximate absolute bioavailability of 43%.

Rotard *et al.*[30] found a potential availability of PCDD's and PCDF's from red slag of about 2% TEQ. The red slag originated from copper mining between 1937 and 1945. It was heated during copper recovery to 600 °C.

Umbreit *et al.*[45] reports a TCDD-bioavailability in guinea-pigs of 29.5% for a Times Beach soil and only 1.6% for soil from the vicinity of a Newark phenol products plant. These figures are relative to the same, but decontaminated, Newark soil, which was recontaminated immediately before use with pure TCDD. In rats they found the Times Beach and Newark soils to be equally potent in inducing AHH and total cytochrome *P*-450 activity, but Newark soil to be considerably less potent in lethal toxicity.

4. FACTORS INFLUENCING BIOAVAILABILITY

4.1. Chemical compound aspects

4.1.1. Classification

Environmental contaminants can be distinguished into two main groups, namely heavy metals (like cadmium, lead, mercury) and organic compounds (like PCB's, PCDD's and PCDF's). Beside this classification, a classification with respect to toxicity or carcinogenicity could be of interest. For such classifications information about the toxicokinetics of a contaminant is essential. Toxicokinetics is among others determined by bioavailability, which in turn depends on the physico-chemical characteristics of both the soil and the contaminant, on the interaction between soil and the contaminants, and on the absorption kinetics of the contaminant.

4.1.2. Speciation

The chemical form of metals (= speciation) is a factor in bioavailability research which deserves special attention, because it may affect bioavailability to a great extent. For example, lead administered as lead acetate is better absorbed than lead administered as lead nitrate. In soil, lead is present in the form of lead salts[41]. Its speciation depends on the pH of the soil, the content of organic material, the presence of inorganic colloids and ferro-oxides, the possibility of ion interactions. On the other hand, we think that knowledge about the speciation in the digestion juice at the moment of availability for absorption will be of greater interest than the speciation in soil, because bioavailability will be more affected by speciation in that step of the absorption process. Unfortunately, no data are available on speciation of metals in digestion juices. The equilibrium speciation could be obtained from Westall *et al.*[31] if the right composition of the gastro-intestinal juices were known.

4.2. Soil aspects

4.2.1. Classification

As for the contaminants, soils can be classified in various ways. Classification can be made with respect to the soil's physico-chemical characteristics, its interaction with (classes of)

contaminants of interest or its transport through the gastro-intestinal tract. In literature, the most generally applied classification is based on the soil's mineral composition, i.e., its sand, silt and clay content and its organic matter content. Not only the soil's bulk content, but also its level of contamination could lead to a classification. This does not only regard the compound of interest: co-contaminants can influence the potential and actual availability and also the kinetics of the contaminant of interest, e.g. by inducing metabolism or blocking toxicity [26]. This may lead to a new classification or a subclassification of soils.

The data found in literature suggest that bioavailability of heavy metals is much more influenced by the composition of the soil matrix than the bioavailability of organic compounds. This is not surprising as metals are much more sensitive to pH, complexing agents, ion strength of the soil, etc.[42] The geochemical factors that control lead availability (mineralogy, encapsulation, grinding, and particle size) in mining waste combine to decrease the potential bioavailability of lead when compared to urban and smelter-derived soils. Consequently, the blood lead level noted in the different areas reflects the difference in lead availability based on lead geochemistry at different sites[29].

In studying bioavailability of organic compounds from soil, some authors explicitely mention soil composition (Fries[27,37,38] and Jones[39]). In a study of Fries *et al.*[37] three different types of soil, one sandy, one clay-type and the last with 14% organic matter were used and compared with respect to hexachlorobiphenyl bioavailability. No significant differences could be demonstrated between the three types of soil. Umbreit *et al.*[28] found about twentyfold less bioavailability in soil samples from Newark (New Jersey) compared to soil samples from Times Beach (Missouri), while both soils were equally potent in inducing cytochrome *P-450* activity. Again, this indicates that such qualitative measures need careful interpretation before deciding on bioavailability.

4.2.2. Contamination history

Various studies have demonstrated that the contamination history may play an important role in bioavailability. The duration and level of contamination, i.e. was the soil contaminated at a low concentration level but during a long period, or was it contaminated during a short period at a high concentration level, are of interest, because these factors may influence the contaminants transport and sorption behaviour in the soil. Another factor is the question whether

contamination was recent or from the past. In this respect weathering in combination with adsorption and desorption to soil or absorption kinetics (e.g. intra-particle diffusion) may influence the extractability and thus the potential available fraction. This is of importance when a soil is contaminated intentionally for an experiment, thus knowing the soils contaminant content exactly (Poiger and Schlatter[34], Fries[27] , Jones *et al.*[39], Fries *et al.*[37]). However, then the soil is not contaminated under natural conditions, not weathered or thouroughly "aged", which may result in an overestimation of the bioavailaiblity from the soil of interest.

The difference in bioavailability from Times Beach and Newark soil was attributed partly to weathering and partly to the Newark soil relative large content of carbonaceous materials like asphalt and tar[43]. Poiger and Schlatter[34] performed an experiment studying the effects of temperature and history. They exposed soil samples to dioxin either I) for 10-15 hours at room temperature or II) for 8 days at about 40°C. During the storage period the samples were dried and moistened several times in an attempt to simulate natural conditions. The soil samples of group II demonstrated a reduced bioavailability, i.e. only 65% of group I. These results underscore the significance of weathering in bioavailability assessment. In a study of Jones *et al.*[39] soil was "aged" for 4 weeks in a sealed glass container in the dark. However, these conditions did not favour weathering. Fries and Marrow[38] demonstrated that "ageing" soils for 5 days or 6 months respectively did not result in significant differences in bioavailability of three different soil types, but their method of ageing was not reported.

In soil literature attention has been paid to sorption/desorption of hydrophobic organic contaminants like PCDD's, PCDF's and PCB's. It has been described as a biphasic process: a fast phase, in the order of minutes to hours, of surface sorption and a much slower phase [44]. The slow phase has been ascribed to intraparticle pore diffusion[45] or to diffusion into organic matter[46]. Pedit *et al.*[47] developed a multiple-particle class pore diffusion model that accounts for variations in physical and sorptive properties.

4.2.3. Relevance of soil depth

Is only the upper soil layer of a few millimetres of relevance, or should an upper layer of a few centimetres be considered, or the root-layer. This is of importance if the contaminant's soil distribution versus soil depth is quite variable. If one considers bioavailability in ruminants, then the "root"-layer will be of importance. For children, the fact whether they are digging in ground

or just playing at the soil surface can make a big difference. Only Shu *et al.*[40] reports on the depth from which his soil samples were obtained.

4.2.4. Grain size fraction

The contamination level can vary quite drastically with the soil grain size fraction, favouring a much higher contaminant level per gram of soil for the smaller sized soil particles due to a higher area per m^3. For instance, Rotard *et al.*[30] found a contamination level of TEQ's in a fine grain fraction of red slag ($\leq$ 20 μm) that was fivefold as high as in the total fraction. The potential availability as percentage of dose was the same for both the fine and total grain size fraction. So, the amount uptaken from the same amounts of soil will depend on the grain size fractions that were ingested.

Another aspect concerning grain size is persorption, i.e. the absorption of very small soil particles through the intestinal wall. Rotard *et al.*[30] mention a fraction of 1% of the total grain fraction to be persorbed. Their assumptions are based on experience. The persorbed particles would have a diameter smaller than 100 μm.

5. RISK ASSESSMENT

The ultimate aim of obtaining bioavailability data is the risk assessment for a particular (class of) soil(s) with respect to a specific (class of) environmental contaminant(s) for man. Human absorption data are rare and often only animal data are available. Extrapolation of these animal data is a necessary step towards the goal. In extrapolating the data not only the kinetic and metabolic characteristics should be taken into account, but also the amount and type of soil ingested. As stated before, ruminants for example ingest relatively large amounts of soil from the root-layer, whereas children on playing grounds also may ingest soil from deeper layers caused by digging.

5.1. Interpretation of animal data

Because of the interaction of extraction and absorption, data obtained are difficult to interpret. Some variables are measured in time, other variables only *post mortem*, which makes the interpretation task even more difficult. Another complication arises when labeled organic compounds are used and total activity measurements have been carried out, which do not discriminate between parent and metabolites. PBPK modelling seems to be an indispensable tool for a correct interpretation of the data. Modelling may also help in quantifying bioavailability. e.g., when fitting F_{act} to the data[27].

5.2. Extrapolation to humans

Bioavailability data from literature concern mainly data about rodents, like rats, mice and guinea pigs, or ruminants, like cows or sheep. However, physiological aspects determining the contaminant distribution can widely differ between species, as do physiological aspects determining elimination. For data extrapolation to humans, it seems that only detailed modelling of the relevant processes, for instance with a PBPK model, will result in a more reliable bioavailability estimation.

In extrapolating bioavailability to humans use of the animal model can be made by substituting the right parameters.The substitution of physiological parameters, such as organ volumes and blood flows, will not be difficult. However, with respect to bio-physical aspects severe problems may arise. For instance, one should know if metabolism level is induced in the species serving for extrapolation and not in humans, or if metabolising enzymes exist in the species

serving for extrapolation and not in humans. Another important factor, concerning potential (F_{pot}) and actual (F_{act}) absorption, that should not be overlooked, is the physiology of the gastro-intestinal tract. Fries[27] comments on his data that they were derived from ruminant species and that conclusions may not be applicable to monogastric mammalian species: e.g., anaerobic fermentation in the rumen and long residence times of soil particles in the gastro-intestinal-tract probably will prohibit a straightforward extrapolation.

Nevertheless, a careful selection of both animal specific and humane parameters should lead to a realistic estimation of humane bioavailability.

6. CONCLUSIONS AND RECOMMENDATIONS

In reviewing the literature on bioavailability of environmental contaminants from soil, it is demonstrated that despite the considerable public health and socioeconomic relevance of information on this topic, reliable data are still lacking. To our opinion this is mainly due to the complexity of the questions that arise and the different existing perceptions of the problem within the environmental research field.

Future aspects in research should be focussed on various topics, i.e.:

1. *The term bioavailability should be well defined.*

   Bioavailibility is a term that is widely applied in various fields of science. However, it seems that the definitions used are not only field-specific but also experiment-specific; even within a certain field of science (for example medical research) many interpretations are applied. The risk of such lack of uniformity is misinterpretation of data on bioavailability which is especially relevant for multidisciplinary studies. Risk assessment of environmental contaminants in soil is a good example of research using data from various disciplines. In this type of research bioavailability is an important determinant for assessing intervention values, which underscores the importance of reliable bioavailability data. The list of absorption coefficients published by Owen *et al.*[3] is already accepted by investigators who focus their interest on soil. However, for investigators who are interested in bioavailability from soil from a biological/toxicological point of view, these figures are still questionable.

   We suggest that a thourough overview of definitions of bioavailability applied in all kinds of disciplines should be made. Moreover, the review should contain a list of methods available for assessing bioavailabililty and should include advantages and restrictions of the methods. In this way misinterpretation of bioavailability data can be minimised and comparison of data obtained in different disciplines can be simplified. Regarding scientific as well as financial implications related to reliable bioavailability data, such a study will be worth the effort.

2. *More information should be obtained about extractability of contaminants from soil*

   As pointed out in section 3.3, extractability of the contaminant from the soil matrix is a major determinant for bioavailability from soil. The limited data available indicate that under favourable circumstances bioavailability from soil is substantially lower than from a

liquid-based matrix, implicating that the recently used intervention values could be overestimated. However, all these studies lack a structural approach for investigating the effect of the soil matrix on extractability. The Laboratory for Exposure Assessment (LBO/RIVM) recently started a project to work on this problem. In an *in* vitro digestion model the extractability of various environmental contaminants in various types of soil will be investigated. However, the model first needs to be optimised and validated. The results of this project can provide important information for assessing intervention values.

3. *More information should be obtained about gastro-intestinal absorption of contaminants from digestion juices.*

   Another topic which needs further investigation is absorption from the gastro-intestinal tract into the blood circulation, which is especially relevant for heavy metals. For this group of environmental contaminants still a substantial amount of research is required. Literature makes clear that absolute bioavailability of metals is almost entirely assessed in studies using a liquid-based matrix and in which relatively high doses are administered. Nonlinear absorption kinetics may hamper an adequate extrapolation to low dose exposure. Moreover, research should be focussed on the speciation of metals in digestion juices, because the speciation of a metal may also contribute substantially to the amount absorbed. Especially on this latter topic literature data are limited; only data on various speciations of a metal administered in a liquid-based matrix can be found, but no data exist on the speciation in digestion juices.

   To our opinion furture research, should be focussed on the above mentioned issue. An extensive literature study on effects of speciation of the contaminants, methods applied for assessment of bioavailability, physiological conditions, such as age-dependent absorption, should form the start of such an investigation. Subsequently, experiments will be carried out in a more structural approach in order to fill the gaps of information required for 'building' a risk model.

4. *PBPK modelling.*

   Application of PBPK models, in which the mutual influence of the separate processes are taken into account, may contribute to refinement of intervention values.

   As has been argued before, modelling could be of great use for data interpretation. Models could be used for estimating total body burden based on concentration levels as measured in liver or fat. O'Flaherty *et al.*[28] employed a model to estimate lead bioavailability. In

extrapolating from animal data to man, modelling seems to be an indispensable tool, as well as in extrapolating from high dosis to more realistic low dosis levels. Moreover, models could be valuable instruments in designing experiments.

The risk assessment community should stimulate a close cooperation between modellers and experimenters.

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