ENVIRONMENTAL RESEARCH OF THE
FEDERAL MINISTRY OF THE ENVIRONMENT,
NATURE CONSERVATION AND NUCLEAR SAFETY

Research Report 201 64 214
UBA-FB 000405

Texte
66
03

ISSN
0722-186X

Assessing the bioavailability of contaminants in soils: a review on recent concepts

by

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On behalf of the Federal Environmental Agency

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Publisher: Federal Environmental Agency (Umweltbundesamt)
Postfach 33 00 22
14191 Berlin
Tel.: +49/30/8903-0
Telex: 183 756
Telefax: +49/30/8903 2285
Internet: http://www.umweltbundesamt.de

Edited by: Section IV 2.3
Carsta Hufenbach

Berlin, September 2003

Berichts – Kennblatt

Berichtsnummer
1. UBA-FB 201 64 214
2.
3.

4. **Titel des Berichts**
Erfassung der Bioverfügbarkeit von Schadstoffen in Böden: Literaturstudie zu aktuellen Konzepten

5. **Autor(en), Name(n), Vorname(n)**
Frische, Tobias
Mebes, Klaus-Holger
Filser, Juliane

8. **Abschlussdatum**
30.09.2002

9. **Veröffentlichungsdatum**

6. **Durchführende Institution (Name, Anschrift)**
Universität Bremen, UFT, Abt.10
Leobener Strasse, D-28359 Bremen

10. **UFOPLAN - Nr.** 2001

11. **Seitenzahl** 103 + 12

7. **Fördernde Institution (Name, Anschrift)**
Umweltbundesamt, Bismarckplatz 1, D-14191 Berlin

12. **Literaturangaben** 149

13. **Tabellen** 4 + 53

14. **Abbildungen** 13

15. **Zusätzliche Angaben**

16. **Kurzfassung**
In einer Literaturstudie wurden am Beispiel polyzyklischer aromatischer Kohlenwasserstoffe und Schwermetalle aktuelle Konzepte und methodische Ansätze zur Abschätzung der Bioverfügbarkeit von Schadstoffen überprüft. Gegenwärtig gibt es kein Konzept, in dem die verschiedenen theoretischen und experimentellen Ansätze vereinbar wären. Chemie und Bodenkunde dominieren die Arbeiten, die biologische Komponente ist unterrepräsentiert. Hierbei werden Bioabbau (Mikroorganismen), Bioakkumulation und Toxizität (Pflanzen und Tiere, dominiert von den wenigen für Standardtests empfohlenen Arten) untersucht. Diese Parameter werden in Bezug zu “sanft” extrahierten Schadstoffgehalten gesetzt, oder zu mit Hilfe von Modellen (z.B. equilibrium partitioning theory, EPT) berechneten Werten. Grundsätzlich werden die räumliche und zeitliche Komponente des Prozesses Bioverfügbarkeit kaum berücksichtigt. Nach gegenwärtigem Kenntnisstand kann Bioverfügbarkeit nicht in die Risikoabschätzung für Chemikalien einbezogen werden: weitere Forschung, sowohl theoretische (Konzepte, Modelle) wie experimentelle (Standardisierung), ist vonnöten.

17. **Schlagwörter**
Bioverfügbarkeit, Metalle, PAK, Polyzyklische Aromatische Kohlenwasserstoffe, Boden, Tiere, Pflanzen, Mikroorganismen, Konzepte, Technical Guidance Document, Critical Load

18. **Preis**
19.
20.

UBA-F+E-Berichtsmerkblatt (6.2000)

Report - Data Sheet

1. **Report No.:**
1. UBA-FB 201 64 214

2.

3.

4. **Report Title**

Assessing the bioavailability of contaminants in soils: a review on recent concepts

5. **Author(s), Family Name(s), First Name(s)**

Frische, Tobias
Mebes, Klaus-Holger
Filser, Juliane

8. **Report Date**
30.09.2002

9. **Publication Date**

6. **Performing Organisation (Name, Address)**

Universität Bremen, UFT, Abt.10
Leobener Strasse, D-28359 Bremen

10. **UFOPLAN – No.** 2001

11. **No. of Pages** 103 + 12

7. **Sponsoring Agency (Name, Address)** Umweltbundesamt, Bismarckplatz 1, D-14191 Berlin

12. **No. of References** 149

13. **Tables** 4 + 53

14. **Figures** 13

15. **Supplementary Notes**

16. **Abstract**

This literature study examines recent theoretical concepts and methodological approaches for assessing the bioavailability of contaminants in soils, exemplified by polycyclic aromatic hydrocarbons and heavy metals. At present there is no general concept that could unify the various theoretical and experimental methods for assessing bioavailability. Studies are biased towards chemistry and soil science whereas the biological component is underrepresented. The latter is studied by biodegradation (microorganisms), bioaccumulation and toxicity (plants and animals, dominated by the few species recommended for standard ecotoxicological tests). The biotic parameters are related to contaminant concentrations after non-exhaustive chemical extractions, alternatively to values calculated by theoretical models such as the equilibrium partitioning theory. A general problem of all methods is that the spatial and temporal component of the bioavailability concept are hardly taken into account. According to current knowledge, bioavailability cannot be included in risk assessment schemes but requires extensive further work, both in theory (concepts, models) and in practice (standardisation).

17. **Key Words**

Biovavailability, metals, PAH, polycyclic aromatic hydrocarbons, soil, animals, plant, microorganisms, concepts, Technical Guidance Document, Critical Load

18. **Preis**

19.

20.

UBA-F+E-Berichtsmerkblatt (6.80

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List of abbreviations

ASTN	American Society for Testing and Chemicals
BAF	bioaccumulation factors
BCF	bioconcentration factors
BSAF	biota-to-soil accumulation factors
CEC	catione exchange capacity
cf.	confer
C_{mic}	microbial carbon
C_{tot}	total soil concentration
DGT	diffusive gradients in thin-films
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
d.s.	dry substance
DTPA	diethylenetriamine pentaacetic acid
e.g.	example given
EC	effective concentration
EC_{50}	median effective concentration
EPT	equilibrium partitioning theory
G-AAS / GFAAS	graphite furnace atomic absorption spectrometry
HPCD	hydroxypropyl-ß-cyclodextrine (solvent)
LUFA	Landwirtschaftliche Untersuchungs- und Forschungs -Anstalt Speyer
K_D	soil-water partitioning coefficients .
Koc	soil-water partitioning coefficients corrected for organic carbon content
K_{ow}	octanol/water partition coefficient
LC_{50}	median lethal concentration
NOEC	no-observed-effect concentration
OECD	Organisation for Economic Co-operation and Development
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
pk	dissociation constants of the substances
PSD	passive sampling devices
PTD	polyethylene tube analysis
QSAR	quantitative structure-activity relationship
SFE	supercritical fluid extraction
SPMDs	semi permeable membrane devices
SSFE	selective supercritical fluid extraction
TOC	total organic carbon
US-EPA	Environmental Protection Agency (USA)

1. Introduction

This review lists and comparatively evaluates recent concepts on bioavailability of contaminants in soils, thus focussing a central concept in environmental chemistry and ecotoxicology (Fent, 1998; Hamelink et al., 1994) which aims at integrating concentration, fate and behaviour as well as effects of contaminants in the environment (Fig. 1a).

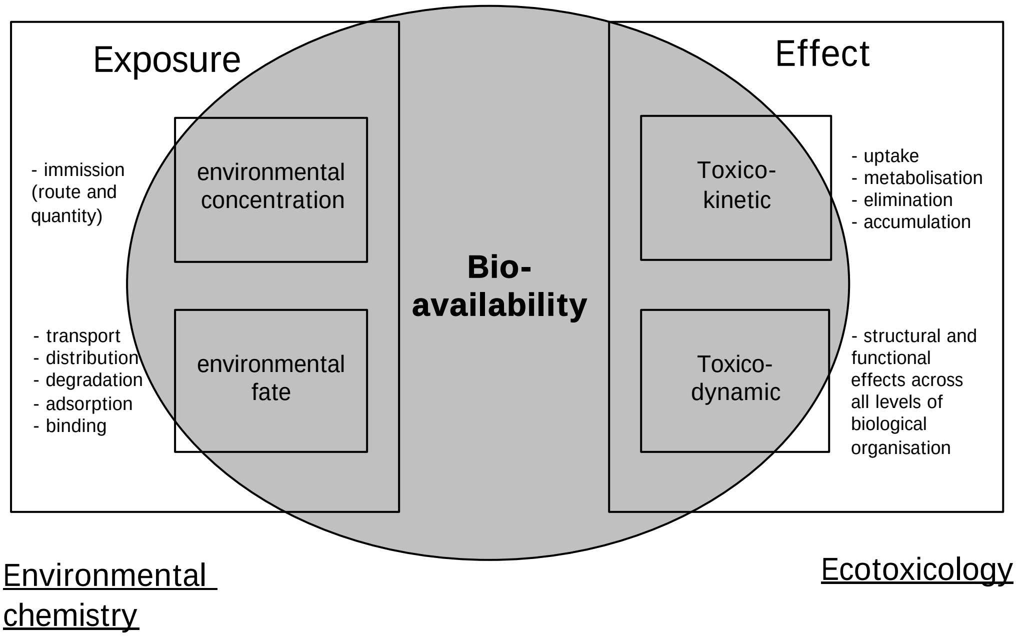

Fig. 1a The integrative idea of the bioavailability concept

The bioavailability concepts originates from the fact that detrimental effects in exposed organisms and ecosystems are not caused by the entire amount of chemical compounds released to the environment but only by a certain, the bioavailable part of them. A main goal of bioavailability research is thus a description as exact and realistic as possible of organism exposure in a contaminated environmental matrix.

In particular for the soil environment this is a necessary and difficult task, for soils are characterised by a highly complex, three-phase reaction system (soil matrix, soil water and soil pore space), manifold substance processes (distribution, sorption, transformation and breakdown) and a high variability in space and time (Gisi et al., 1990; Koehler et al., 1999; Scheffer & Schachtschabel, 1989). Consequently, first hints on the influence of different soil properties and of ageing processes on the bioavailability of contaminants in soils have already been published some years ago (Alexander, 1995; Alexander, 2000). Irrespective of this until now there is no uniform strategy for the assessment and evaluation of bioavailability for soil organisms, instead a multitude of different approaches, concepts and methods exists, as we will show in this study. However, it is generally doubtful if it will be possible at all to develop a scientifically sound uniform strategy (Fig. 1b): the multitude of different soils, their enormous (and to a great deal still unexplored) biodiversity and the large number of environmentally relevant chemicals with different substance properties result in a complexity of the systems that will be very hard to survey, let alone to conceptualise.

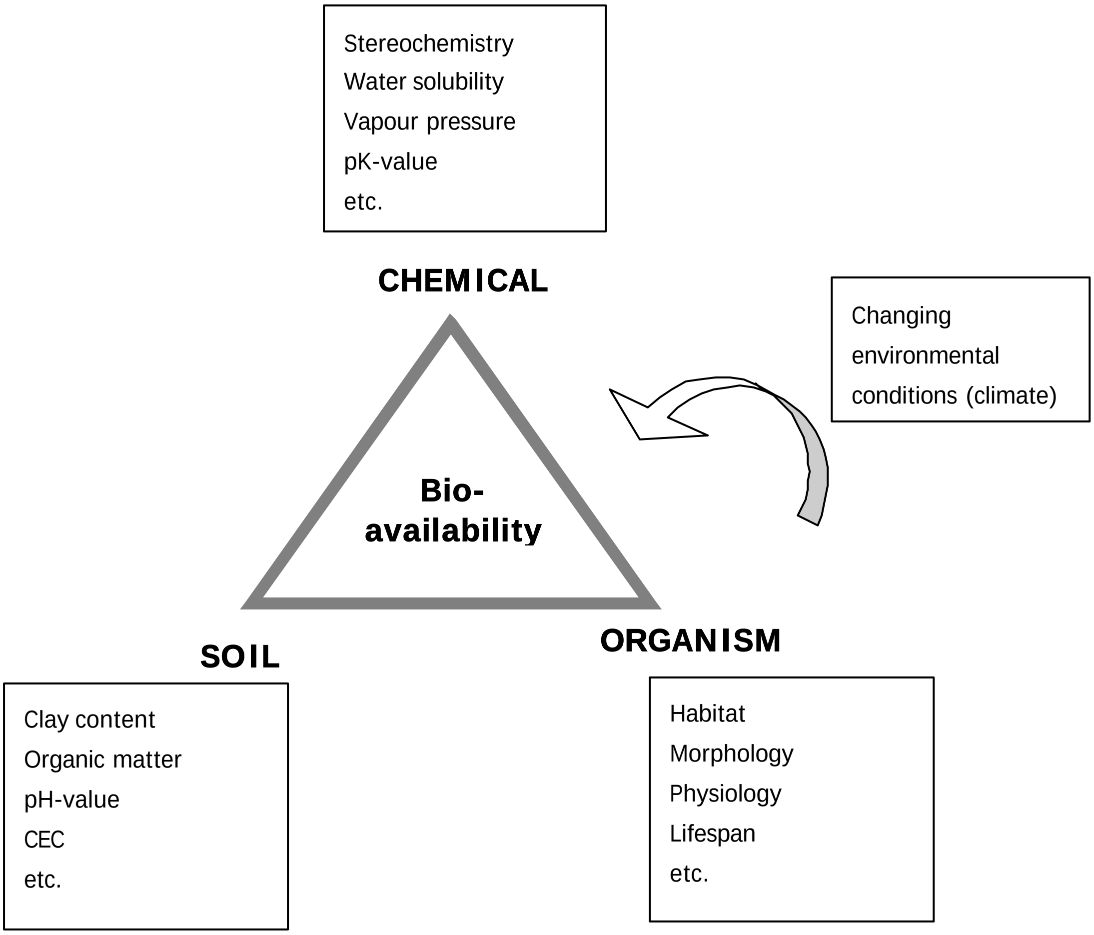

Fig. 1b Bioavailability – complexity of parameters and processes

The insecurity among scientists (resulting from non-existing knowledge) concerning bioavailability has direct consequences for the regulation of chemicals and for soil protection. Risk assessments and subsequent regulatory measures (permission of chemicals, threshold values and redemption goals in soils) are particularly subject to considerable uncertainties as long as nominal and measured total concentrations are the basis of exposure assessment (Peijnenburg et al., 1999b). Due to necessary extrapolations, the present non-consideration of bioavailability in practice can result both in over- and underestimation of risks of chemicals for soils, which is why in recent years bioavailability in soils has been explored and discussed in increasing detail (Alexander, 1995; Alexander, 2000; Eijsackers et al., 1997; Reid et al., 2000a; Sijm et al., 2000).

Nevertheless there is still ample research need with respect to theoretical (definition, concepts, models) and methodological implementation (experimental approaches). The critical documentation and integration of current knowledge is goal of this study, with special respect to

(1) defining “bioavailability for soil organisms“
(2) documentation of approaches, concepts and methods discussed in the literature
(3) systematic comparison and critical evaluation of relevant concepts
(4) suggestions on their applicability in regulation of chemicals and soil protection
(5) future research needs.

We emphasise at this point that this study is focussed on theoretical, conceptual and methodological aspects but does *not* aim at exhaustively compiling substance data with respect to their bioavailability in soils.

2. Bioavailability in soil – definition

In ecotoxicological literature, “bioavailability“ has often been used relatively unspecifically, most probably because no single and clear definition exists. Instead there are a multitude of descriptions and interpretations, each of them focussing on different aspects. The approaches range from restriction to a specific context (e.g., biodegradation) over more integrating definitions (see below) up to conceptionally splitting bioavailability in single processes (Hamelink et al., 1994; Peijnenburg et al., 1997). The latter sub-divide bioavailability into physical-chemical desorption processes in the environment (environmental availability), physiological uptake processes into organisms (environmental bioavailability) and in internal distribution and metabolisation processes (toxicological bioavailability). Although this differentiation might be helpful for understanding bioavailability as a complex, dynamic process we prefer integrating definitions which were formulated by:

- Belfroid et al. (1996): „..., we will define bioavailability as the fraction of the bulk amount of the chemical present in soil/sediment and (interstitial) water that can potentially be taken up during the organism‘s life-time into the organism tissue (excluding the digestive tract).“
- Eijsackers et al. (1997): „The amount/percentage of a compound that is actually taken up by an organism as the outcome of a dynamic equilibration of organism-bound uptake processes, and soil particle-related exchange processes, all in relation to a dynamic set of environmental conditions.“
- Sijm et al. (2000): „Bioavailability is thus a complex process which includes all kinds of relationships between the concentration in the ambient environment and the portion of that concentration an organism experiences with regards to uptake.“

As an integration of these, and as a result of our literature survey and the lively discussions during our international workshop („Bioavailability of Soil Contaminants: Problems and Perspectives“, July 18-19, 2002, University of Bremen), we suggest the following definition:

Bioavailability describes the complex processes of mass transfer and uptake of contaminants into soil-living organisms which are determined by substance properties, soil properties, the biology of organisms and climatic influences. The bioavailable contaminant fraction in soil represents the relevant exposure concentration for soil organisms.

This definition explicitly points out that bioavailability is a set of processes, from which results that a scientific understanding is only possible when adequately addressing the dimension *time*. The second sentence leads to the context of application, risk analysis and hazard assessment, expressing the need for quantifying assessment methods for bioavailable contaminant fractions in soil. Resulting from this, the "chemical perspective", discriminating several fractions in contaminated soils, becomes more emphasised (Fig. 2.a). The "bioavailable" fraction derived from this should thus allow for better describing and perceptively assessing effects on organisms than the thus far mostly used total concentrations.

However, the „chemical perspective" of bioavailability suffers from two principal problems. First, the assessment or identification of a bioavailable concentration in soil is a "point measurement", neglecting (or only partially being able to methodologically integrate) the factor time. This holds both with respect to ageing processes of chemicals in soil, which result in decrease of bioavailability, and for the varying periods of exposure time for different soil organisms and biological tests, respectively. The latter aspect was extensively discussed during our workshop (see attachment: workshop conclusions). Here, a further discrimination into "actual bioavailability" and "potential bioavailability" was suggested. The former expression represents the actual exposure concentration for an organism, whereas the second aims at the potentially available contaminant concentration over a longer period of time. For a methodological implementation of both, especially different physical-chemical extraction procedures were discussed for both organic contaminants and heavy metals.

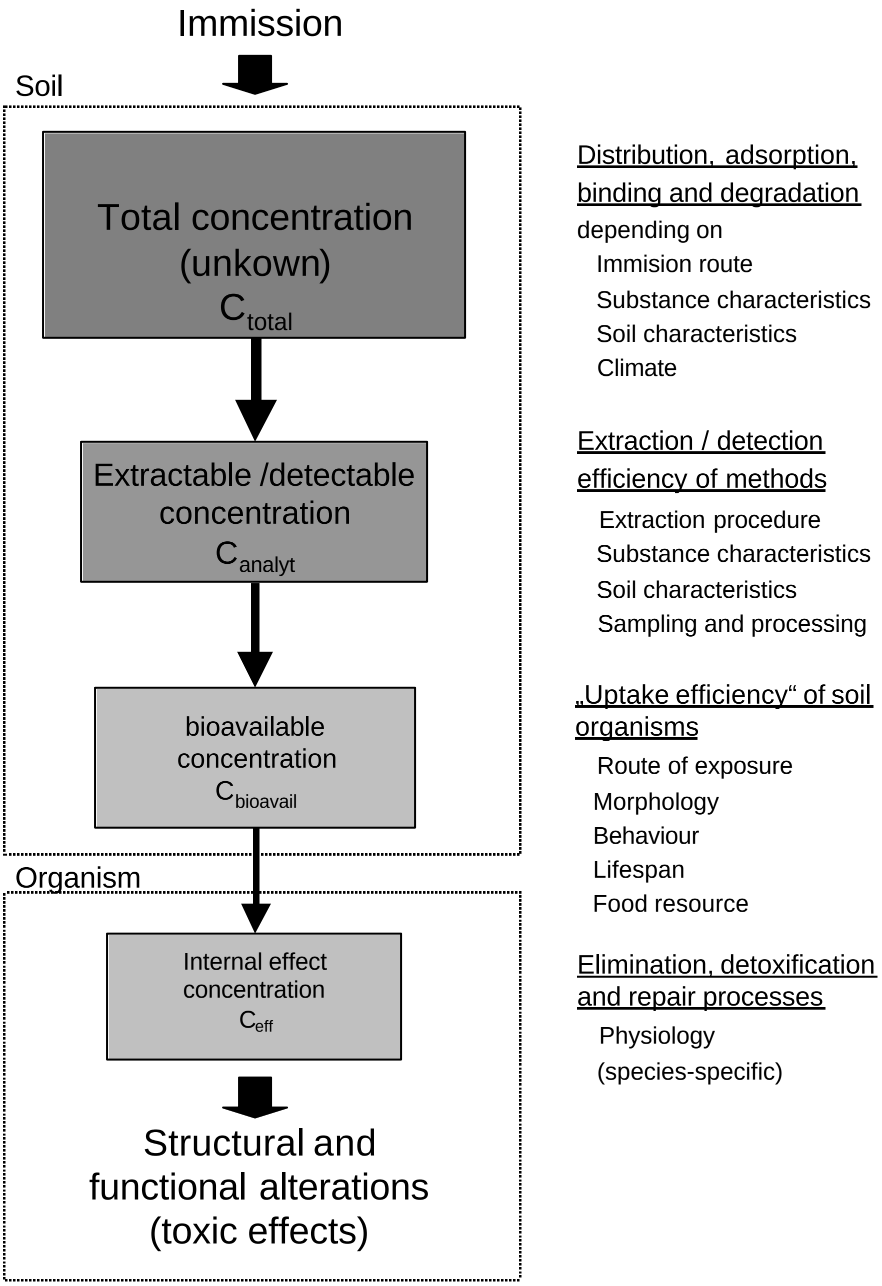

Fig. 2a Theoretically specifiable contaminant fractions in soil, including relevant influence factors and processes (modified from: Hammel & Herrchen, 1999)

The second relevant criticism of the “environmental chemistry quantifying perspective“ relates to the problem of neglecting the biology of soil organisms, which is an important - but mostly not yet understood - determinant of the bioavailability process. This is of uttermost importance since the soil organisms (and the ecological functions performed by them) are the central goal of effect assessment and soil protection. In this study, we refer to soil organisms as all those being in direct contact with soil permanently, only during certain life stages (e.g., larvae) or only with parts of their tissue (e.g., plant roots). Exclusively above-ground living organisms or organisms which come into intensive contact with soil only under certain circumstances (e.g., birds picking worms or digging mammals), are excluded. In these cases soil as a exposure route is of minor importance, or other uptake routes (e.g., food) are relevant. Groundwater organisms are excluded as well. The main focus is thus on “true soil organisms”, namely microorganisms, invertebrates and plant roots.

To adequately consider the organism perspective, Hammel & Herrchen (1999) demand a more differentiated use of the expression “bioavailability“: “The term ‘(bio)available’ should always be given with respect to the exposure route that has to be evaluated and the considered protection goal (‘available for...’).“ [translated from German]. Here we object that there are still considerable ambiguities concerning the relevant exposure routes, which again differ between contaminant classes. The relevant exposure routes in soil are inorganic and organic matrix, air and water, for predacious organisms in addition their prey (secondary poisoning) (Fig. 2.b).

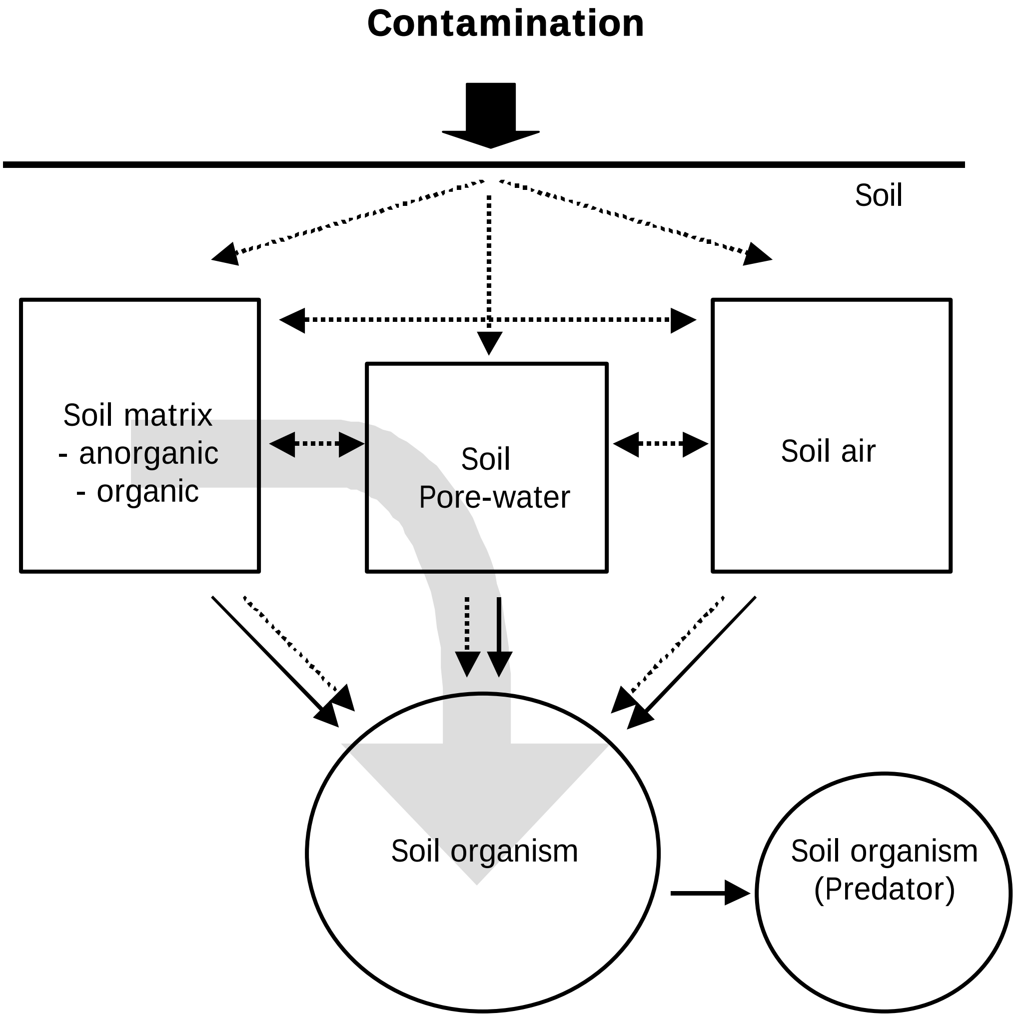

Fig. 2b Compartment model of mass transfer processes in soil and exposure routes for soil-living organisms (dotted arrows: thermo-dynamic equilibrium distribution, solid arrows: active uptake, grey arrow: Equilibrium Partitioning Theory (EPT) / pore-water hypothesis)

Each of these exposure routes can be considered a microhabitat with specific properties and is inhabited by a specialised organism community (Beare et al., 1995). Consequently, contaminant availability depends on the biology (especially microhabitat and morphology) of the organism under consideration. Although many soil organisms integrate over several of them, usually a main exposure route can be identified [or assumed - the knowledge of soil organism biology is in most cases very poor]. Hammel (1999) presented a first compilation (Tab. 2). To what extent these assignments are

(i) supported by scientific evidence, (ii) allow for generalisation for a whole taxonomic group as well as species living in the field and (iii) are considered in recent concepts on assessment of bioavailable contaminants will partly be addressed in the following or is subject to future research needs. However, the central significance of the exposure route soil water for bioavailability resulting from Tab. 2 reflects the consensus within the current scientific debate (see also workshop conclusions).

Tab. 2 Exposure routes for organisms in terrestrial test systems (translated from Hammel 1999).

Taxonomic group		Species	Soil solution	Soil air	Air near surface	Organic matter dead	Inorganic matter	Organic matter alive
Plants	Monocotyl	Avena sativa	++	(+)	(+)	-	-	-
	Dicotyl	Brassica rapa*						
Micro-organisms	Bacteria	mixed populations	++	-	-	(+)	(+)	-
	Fungi	mixed populations	++	-	-	(+)	(+)	-
	Protozoa	mixed populations	++	-	-	(+)	(+)	+
Fauna	Nematoda	Caenorhabditis elegans	++	-	-	-	-	+
	Enchytraeidae	Enchytraeus albidus	++	-	-	+	-	+
	Lumbricidae	Eisenia fetida	++	(+)	-	+	+	-
	Oribatidae	Plathynothrus peltifer	+	+	-	++	-	-
	Gamasina	Hypoaspis aculeifer	+	+	-	-	-	++
	Isopoda	Porcellio scaber	-	+	-	++	-	-
	Collembola	Folsomia candida	+	+	+	++	-	-
	Carabidae	Poecilus cupreus	-	+	+	-	-	++
	Staphylinidae	Aleochara bilineata	-	+	+	-	-	++

++ = main exposure, + = exposure, (+) = subordinate significance, - = probably no significance; *data on *B. rapa* are missing in the original table.

This exposure route does not only affect organisms living directly in the soil pore-water (mainly microorganisms, protozoa, nematodes), or those actively uptaking it (a.o. plants), but also annelids and representatives of the mesofauna living in the airfilled soil pores. Therefore both theoretical (Equilibrium Partitioning Theory, see Fig. 2b and chapter 4.3), and several methodological approaches on exposure assessment focus on soil pore-water.

As a final introductory remark, we want to point out another reason for the present differences and ambiguities of the bioavailability concept. Bioavailable contaminants in soil can have three principal consequences for organisms:

(i) biodegradation (valid only for organic contaminants)
(ii) bioaccumulation and
(iii) toxicity.

In particular biodegradation is a relatively independent field of research, interpreting bioavailability mainly with respect to the soil microorganisms of interest, ageing pro??cesses of contaminants and potential bioremediation of contaminated sites (cf. chapter 4.5.1.4). Toxic effects on soil organisms and their hazard or risk assessment are subordinate in this context. In contrast to this, bioaccumulation and toxicity are two main hazard or risk indicators in ecotoxicology, being investigated both separately and in combination. There is a comparably large overlap of the expressions bioavailability and bioaccumulation, the latter being defined as (Streit, 1992): “enrichment of a substance in an organism exceeding the concentration in the surrounding medium. Bioaccumulation integrates the uptake from the surrounding medium (bioconcentration) and via the food source (biomagnification).” [translated from German]. It has to be kept in mind that bioavailability is a necessary precondition for bioaccumulation, yet the latter is not a precondition for biodegradation or toxic effects. Both phenomena are also possible without uptake of contaminants into the organism, e.g., when considering processes bound to external biomembranes. Historically, bioaccumulation seems more focussed on organism biology with respect to their role in the process (cf. calculation of bioaccumulation potential on the basis of the n-octanol/water partitioning coefficient, K_{ow}). Since also the K_{ow} refers to chemical parameters, the confounding of the concepts becomes obvious: A higher K_{ow} can signify sorption to both membranes of living organisms (increased bioavailability) and to dead organic matter (reduced bioavailability). Therefore, „..., limited knowledge on bioavailability hinders the predictability of bioaccumulation.“ (Sijm & Hermens, 2000).

3. Methods

The study is based on a systematic literature review from the following sources:

- literature provided by the funding agency (UBA)
- literature data bases Biological Abstracts, ISI –Web of Science, Science Direct and ULIDAT (UBA)
- relevant abstracts of the 11th SETAC-Europe (Society of Environmental Toxicology and Chemistry) conference, May 6-10, 2001, Madrid (Spain)
- relevant abstracts from the proceedings of the Consoil 2000 conference September 18-22, 2000, Leipzig (Germany).

The data base surveys were conducted during the fourth quarter of 2001 and the first quarter of 2002 with the following search words in title, keywords or abstract:

- bioavail* AND soil*
- biolog* AND avail* AND soil*
- Bioverfügbarkeit AND Boden

In a first step the publications were sighted and summarised in a numerical overview with respect to contaminants, organisms and soils (chapter 4.1), and to research questions, biological parameters (endpoints) and methods for exposure assessment (chapter 4.2). This compilation is mainly based on the available information in title, keywords and abstract. Simultaneously relevant publications were identified for more detailed evaluation with respect to relevant theoretical concepts on bioavailability. A systematic survey of this (chapter 4.3) and approaches for experimental assessment are summarised in chapter 4.4. Chapter 4.5 exemplarily describes the state of the art for two prioritary contaminant classes (PAH and cadmium), compares the applied concepts on bioavailability and evaluates them with respect to their general relevance. Finally the reviewed recent concepts on bioavailability were comparatively evaluated and discussed (chapter 5). Especially this chapter includes relevant results of the discussion during our workshop (see attachment).

4. Results

4.1 Literature review: contaminants, soils and organisms

The following sub-chapters 4.1.1 - 4.1.3 document the results of the systematic evaluation of 303 references on bioavailability of contaminants in soil, numerically differentiated between contaminants, organisms and soils. This is based on titles, keywords and abstracts, only in single cases the articles were analysed more detailed. For reviews, proceedings and guidelines a definite assignment was mostly not possible, due to overlapping or not concretely given categories. These studies were summarised as „no data or review“. An overlap of categories in many studies (e.g. investigation of several soils, organisms or contaminants) resulted also in the fact that the sum of studies in single categories is greater than the total sum of reviewed literature.

4.1.1 Contaminants

Fig. 4.1.1a shows the distribution of articles among different contaminants. Most studies deal with the bioavailability of metals (almost exclusively heavy metals) and PAH. The next two more frequently addressed contaminant classes (pesticides and other organic chemicals) are chemically very heterogeneous groups. For the remaining contaminant classes not at most 16 references each were found. The best investigated group, metals, are subdivided into elements in Fig. 4.1.1b. Cadmium, zinc, copper and lead were investigated in more than 60 articles each, nickel, chromium, arsenic and mercury were investigated in less than 40 studies.

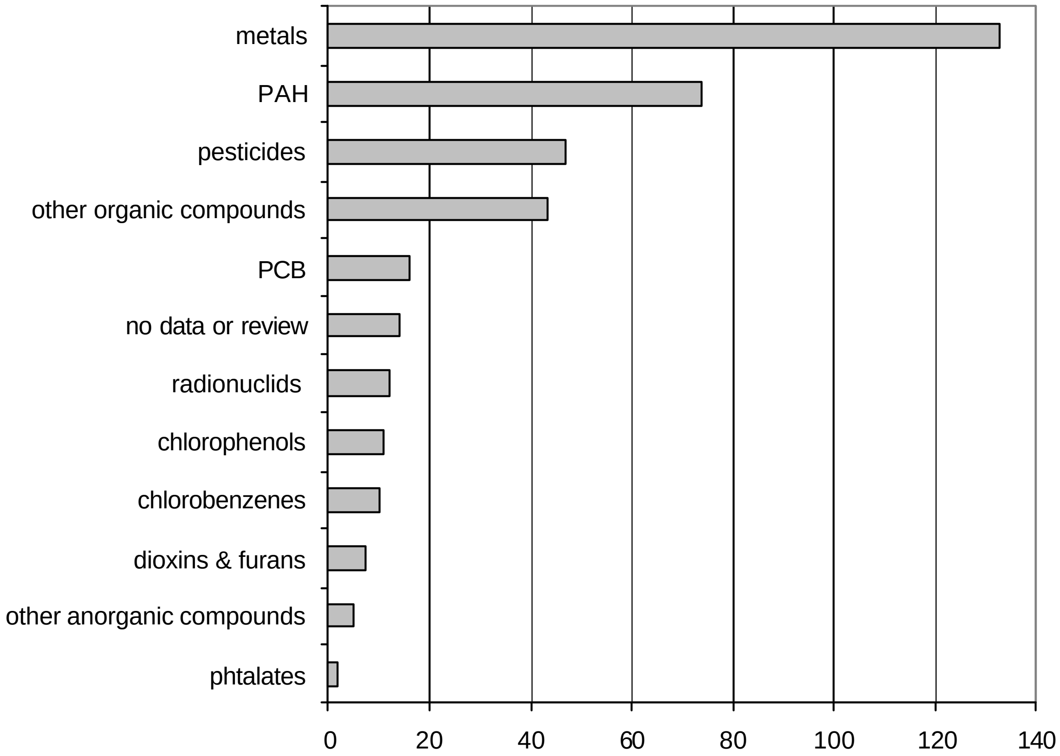

Fig. 4.1.1a Literature review on bioavailability in soil (? 303 references): chemicals studied (PAH = polycyclic aromatic hydrocarbons, PCB = polychlorinated biphenyls).

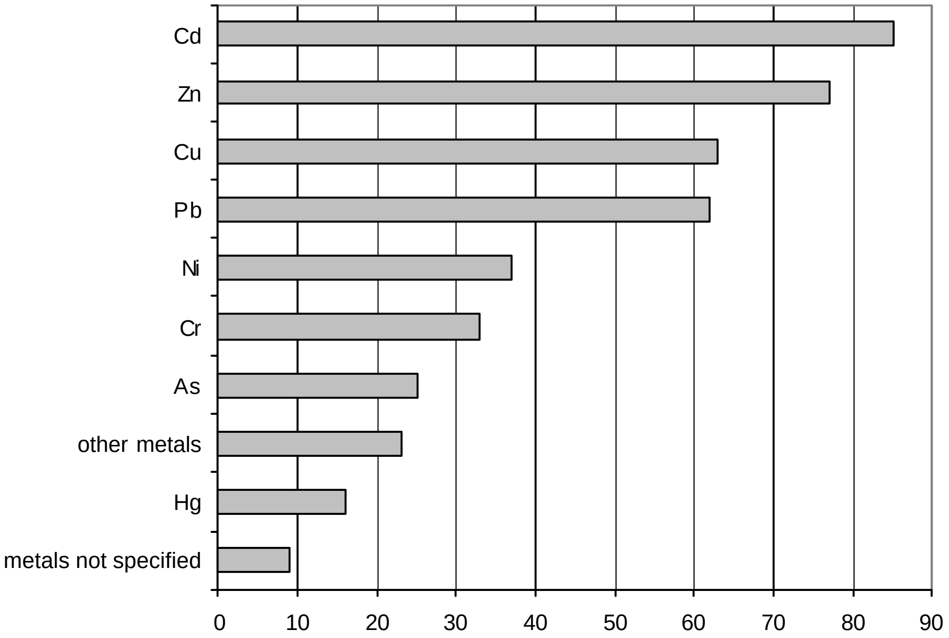

Fig. 4.1.1b Literature review on bioavailability in soil (? 303 references): chemicals studied (metals in detail).

4.1.2 Organisms

Fig. 4.1.2a shows the distribution of studies among organisms. Most frequently microorganisms were investigated, followed by lumbricids and several plant taxa (note: the sum of the latter is in the same order of magnitude as studies on microorganisms). Comparing this with the exposure routes suggested in Tab. 2 shows that the majority of studies deal with the exposure route pore-water (microorganisms, plants, enchytraeids, lumbricids). Only few studies consider organisms that are mainly exposed via soil air and dead organic matter (Collembola and isopods), and only one of them dealt with a predacious species (main pathway: living organic substance).

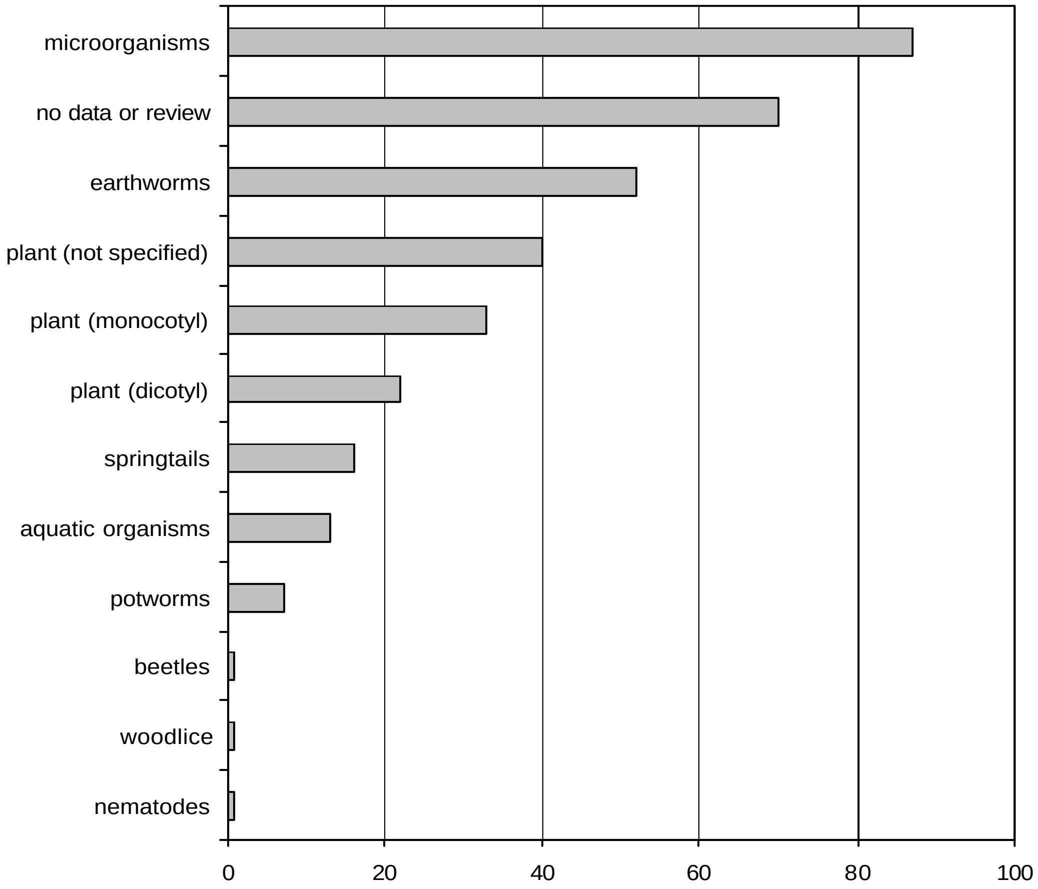

Fig. 4.1.2a: Literature review on bioavailability in soil (? 303 references): organisms studied.

Fig. 4.1.2b shows a more detailed distribution, sorted by animal and plant species. *Eisenia fetida* (15) was most abundant, followed by *Folsomia candida*, *Zea mays* and *Triticum sativum* (> 10 each) and *Eisenia andrei*, *Lactuca sativa* and *Lolium perenne* (more than 5 each). The dominance of organisms recommended for internationally standardised laboratory tests (OECD, ISO) is obvious.

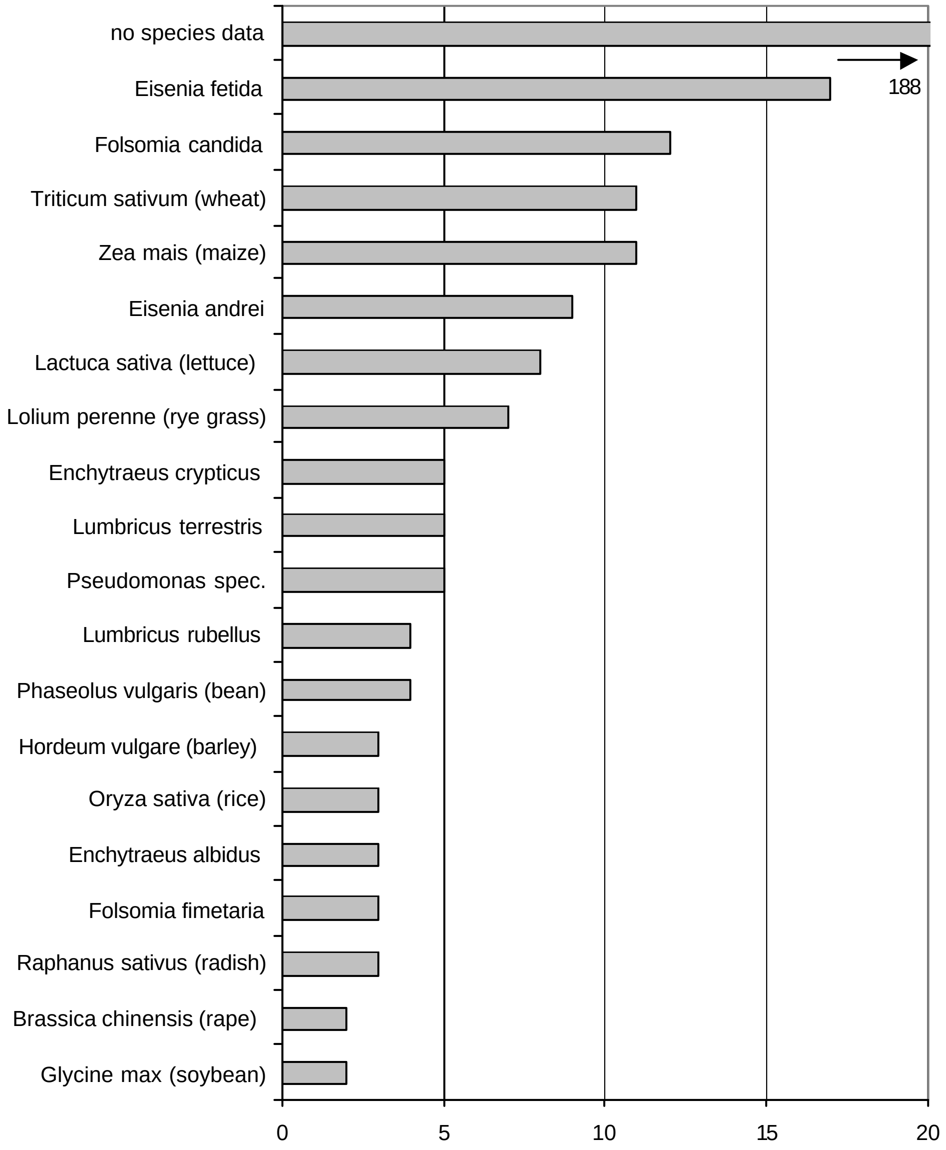

Fig. 4.1.2b: Literature review on bioavailability in soil (? 303 references): organisms studied (species in detail). Note: species only were displayed, if named more than one times in the literature.

Tab. 4.1.2 shows which organism groups were investigated with respect to bioavailability for the most frequently studied contaminants. Among organic chemicals, only the combinations of PAH (PCB's) with oligochaeta revealed a data base sufficient for comparative studies. Metals (as one group) reveal more than 10 studies for each of the three organism groups, allowing for comparisons of results under different conditions (e.g., soil properties).

Tab 4.1.2 Literature review on bioavailability in soil: combination of chemicals and organisms (selection).

	Oligochaeta	Collembola	Plants
PAH	18	2	4
PCB	9	0	1
Chlorophenols	5	0	2
Chlorobenzenes	6	0	1
Metals (sum)	26	11	62
Cadmium	22	7	41
Zinc	17	7	37
Copper	14	4	29
Lead	14	2	31

4.1.3 Soils

Fig. 4.1.3 gives an overview on the soils investigated. Only the black bars represent studies with more detailed descriptions of soil properties (especially texture, organic substance and pH). The category „no data or review“ includes (i) all studies without any reference to soil types, (ii) studies addressing only “soil properties” (or similar) in the research topic, without further details (remaining grey bars) and (iii) review articles. It is obvious that within the selected degree of detail of evaluation - mostly title, keywords and abstracts - only few references gave detailed information on soil properties. Mostly sandy soils (i.e. „worst case“ scenarios with respect to bioavailability) were investigated, frequently including the influence of pH and organic matter content on bioavailability.

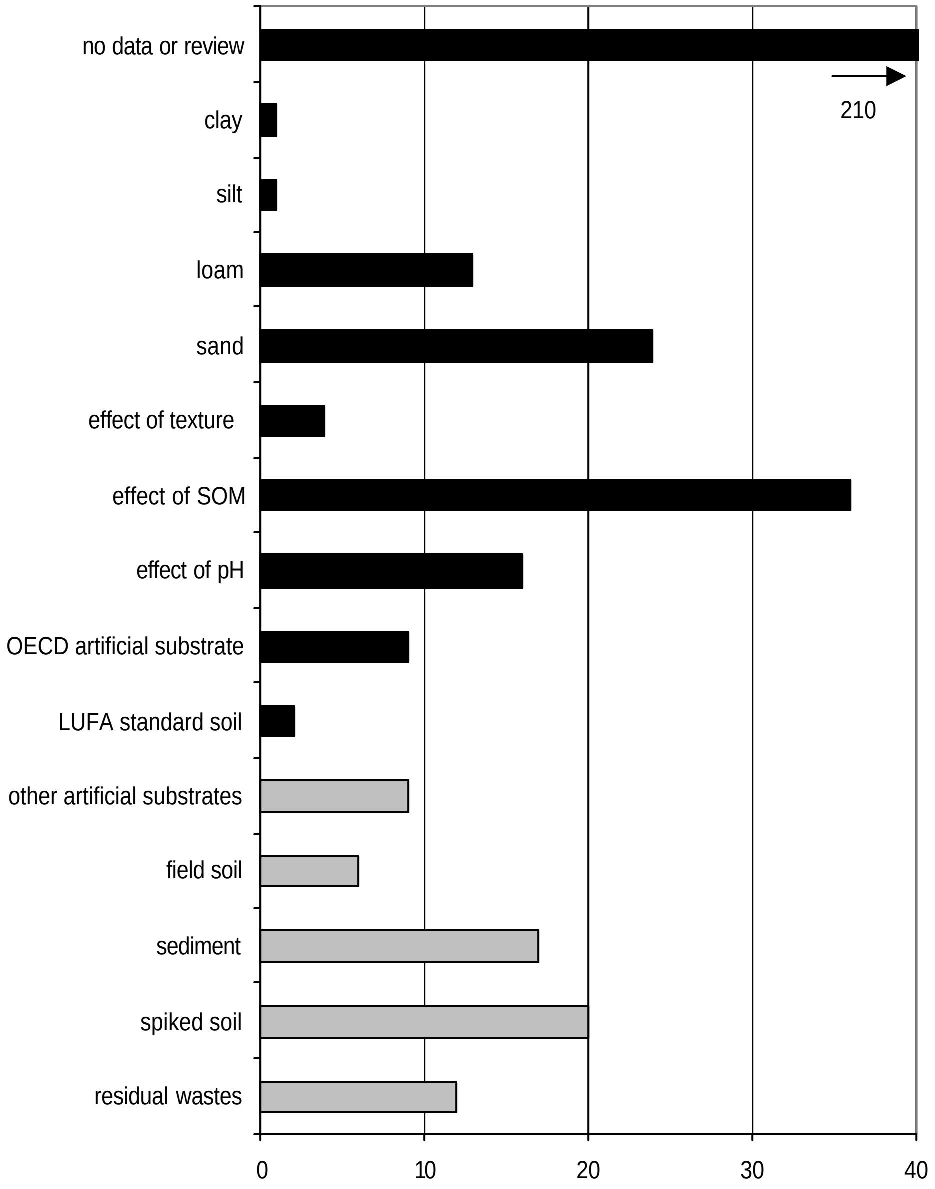

Fig. 4.1.3 Literature review on bioavailability in soil (? 303 references): soils studied, for further information see text.

4.2 Literature review: Topics, endpoints and methods

The general approach of recent studies on bioavailability can be described as follows (Fig. 4.2): Experimental studies on (i) effects of soil contaminants on, (ii) uptake of soil contaminants into, and (iii) degradation of soil contaminants by selected soil organisms are correlated with theoretically estimated and/or experimentally determined bioavailable contaminant concentrations in soil. This is usually compared to nominal concentrations (in case of spiking experiments) or total concentrations as revealed by exhaustive extraction of the contaminant.

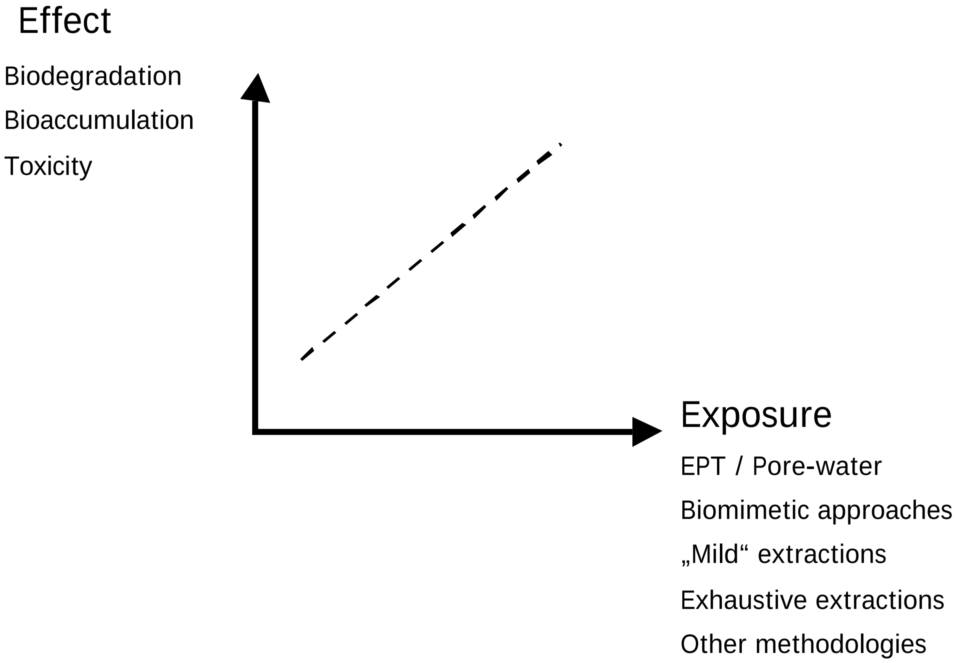

Fig. 4.2 Bioavailability research in soils – experimental design

Based on this general design, most studies on the bioavailability of contaminants in soils focus on one of the four following topics:

- Influence of soil properties
- Influence of ageing processes (bioavailability with respect to time of storage)
- Influence of substance properties within one soil type
- Differences between organisms

The following sub-chapters comment the results of the literature study with respect to central topics (4.2.1), the biological parameters (4.2.2) and the method of exposure assessment (4.2.3). For the reasons mentioned above, also here in some cases no or no clear information was given (category „no data or review“), or topics were overlapping (e.g.: ageing of substance xy in two different soils), the latter resulting in multiple counts. Sub-chapter 4.2.4 documents some combinations of the single evaluations: contaminants (organic compounds and heavy metals) AND topics / endpoints / methods, and organisms (microorganisms/plants/fauna) AND topics / endpoints / methods.

4.2.1 Topics

The majority of studies is mainly focussed on the influence of soil or substance properties on bioavailability in soil (Fig. 4.2.1), both aspects being often investigated in parallel. Studies on the influence of ageing of contaminants on bioavailability are ranking third, whereas only few investigations (approx. 30) have dealt with differences of bioavailability between various organisms.

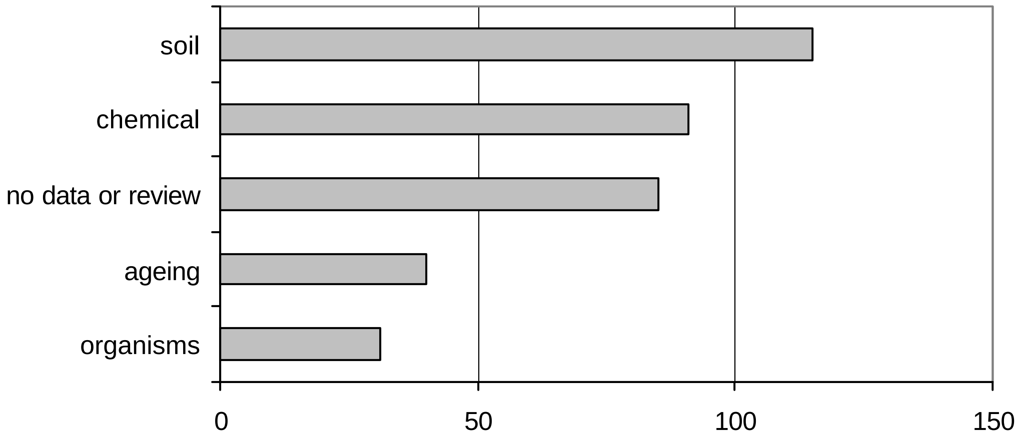

Fig. 4.2.1: Literature review on bioavailability in soil (? 303 references): main research focus.

4.2.2 Endpoints (biological parameters)

Fig. 4.2.2 shows the distribution of studies with respect to biological parameters. Investigations on bioaccumulation prevail, followed by studies on bioavailability related to toxic effects or to biological degradability of organic compounds. Many of them consider both bioaccumulation and toxicity. Besides the aforementioned restriction concerning our degree of detail, the high number of studies in the category „no data or review" is remarkable. This might be a consequence of the widespread, very unspecific use of the expression „bioavailability".

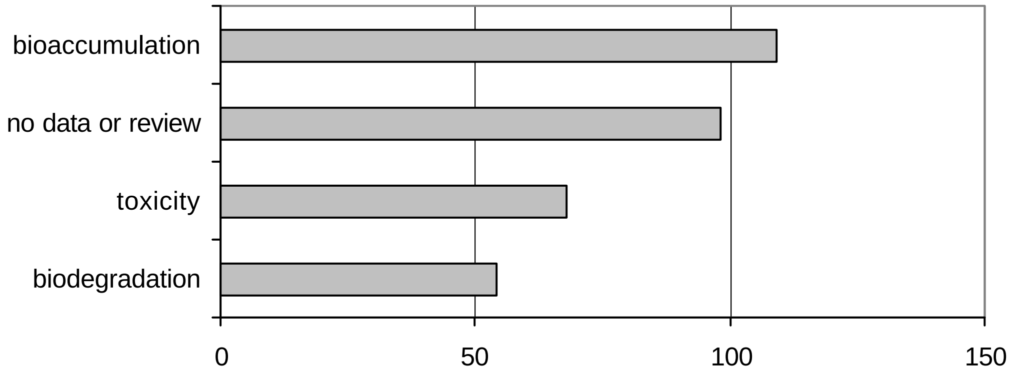

Fig. 4.2.2 Literature review on bioavailability in soil (? 303 references): endpoints studied.

4.2.3 Methods for exposure assessment

The methods used for exposure assessment were assigned to six categories. While the category „EPT / pore-water" focuses theoretical assessment and/or the experimental determination of contaminant concentrations in soil pore-water, the remaining five cover purely experimental approaches. Different solvents (water, salt solutions, organic solvents) or procedures such as special solid phase extractions (biomimetic approaches) are used for quantifying bioavailability. The category "other procedures" summarises all experimental approaches that could not be assigned to the above categories. Chapters 4.3 and 4.4. give detailed descriptions and an overview on concepts and methods.

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From title, keywords and abstracts of the majority of the studies it could not clearly extracted in which way the exposure was assessed. Also here many articles used the expression „bioavailability“ without experimental or theoretical approaches clearly aiming at it. The exposure was most frequently assessed via EPT/pore-water and organic solvents or inorganic salt solutions as extractants. Less than 20 studies used water extractions, even less the relatively new “biomimetic approaches“. Frequently several extraction procedures were used simultaneously, comparing the respective correlations with the biological parameters investigated.

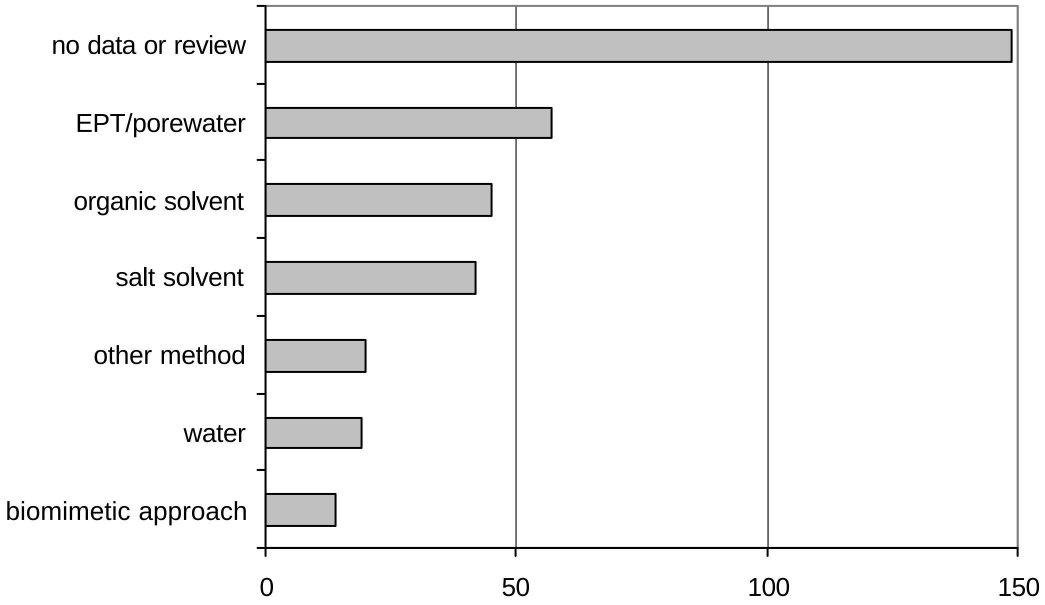

Fig. 4.2.3 Literature review on bioavailability in soil (? 303 references): methods for exposure assessment.

4.2.4 Combinations of review categories

Combining contaminant group with endpoint, topics and methods (Tab. 4.2.4a) reveals distinct differences: biodegradation is only relevant for organic compounds whereas the endpoints toxicity and bioaccumulation were distributed more or less evenly over both organic contaminants and heavy metals. Ageing was much more often addressed for organic compounds, although this process is very relevant for heavy metal bioavailability as well. On the other hand the impact of soil properties on contaminant bioavailability was more frequently investigated for metals. Among experimental methods for exposure assessment, water and organic solvents as extractants were similarly frequent for both groups of contaminants, but EPT / pore-water were dominated by studies on organic compounds and salt solutions by studies dealing with heavy metals. „Biomimetic approaches“ had been developed especially for hydrophobic organic compounds.

Tab. 4.2.4a Literature review on bioavailability in soil (? 303 references): combination of chemicals AND endpoints / research focus / methods for exposure assessment.

		Organic compounds	Metals
Endpoints	Biodegradation	54	0
	Toxicity	30	37
	No data or review	37	48
	Bioaccumulation	41	62
Research focus	Organisms	14	18
	Ageing	32	7
	No data or review	29	40
	Chemical	51	40
	Soil	48	62
Methods exposure	Biomimetic approach	14	0
	Water	7	11
	other methods	8	10
	salt solvent	6	35
	organic solvent	24	18
	EPT/pore-water	39	18
	No data or review	64	68

The combination of organisms with topics/endpoints/methods (Tab. 4.2.4b) shows that studies dealing with bioavailability for microorganisms are dominated by degradation of organic contaminants whereas toxicity and bioaccumulation are only subordinate. In contrast, the latter two endpoints prevail studies on bioavailability for plants and soil fauna. The few studies covering both plants / soil fauna and biodegradation are an “evaluation-made artefact“: these do not investigate biodegradation of organic contaminants by plants or soil fauna, but their impact on microbial degradation (or bioavailability thereafter). Primary topics do not differ remarkably between organism groups, with the exception of ageing / plants which was covered by only few studies (8) as compared to microorganisms (19) and soil fauna (15).

Tab. 4.2.4b Literature review on bioavailability in soil (? 303 references): combination of organisms AND endpoints / research focus.

		Micro-organisms	Plants	Fauna
Endpoints	Biodegradation	50	4	8
	Toxicity	23	12	29
	No data or review	13	23	8
	Bioaccumulation	12	59	45
Research focus	Organisms	9	11	14
	Ageing	19	8	15
	No data or review	10	29	9
	Chemical	26	22	27
	Soil	39	33	30

4.3 Approaches for theoretical assessment of bioavailability

These approaches aim at the theoretical assessment of bioavailability by means of mathematical distribution models using measured or nominal contaminant concentrations of soils to calculate exposure concentrations. The quality of the resulting assessment depends on the degree of differentiation of the models for distribution [of the chemical] and exposure [route] and on data quantity and quality for contaminant properties, soil properties and soil organisms.

Most studies are based on the “equilibrium partitioning theory (EPT)“ which originally had been developed for sediments and organic contaminants (DiToro et al., 1991). The theory basically assumes a thermodynamic equilibrium distribution of contaminants between soil particles, soil water and soil organisms. This approach - also called “pore-water hypothesis“ - postulates the (passive) uptake of the contaminants dissolved in soil pore-water as the main exposure route for soil organisms (cf. Fig. 2b). To prove this hypothesis, usually contaminant concentrations in soil pore-water are calculated by means of soil-water partitioning coefficients (K_D). These pore-water-concentrations are related to (toxic) effects observed in bioassays and compared with nominal or measured contaminant concentrations. For bioaccumulation tests, the uptake of organic contaminants from pore-water into selected soil organisms is calculated using bioconcentration factors (BCF) and compared with values directly measured in the organisms. Important input parameters are two substance-specific measures: the soil-water partitioning coefficients (usually corrected for organic carbon content, Koc) and (for bioaccumulation) the BCF, usually corrected for lipid content of the organisms. For neutral organic compounds, both measures are highly dependent on the octanol-water partitioning coefficient (K_{ow}) or can be estimated on the basis of K_{ow} in case the necessary data are not available. For ionic organic compounds, in addition soil pH and dissociation constants of the substances (pK value) have to be considered in order to assess (de-)sorption to soil matrix and pore-water concentrations. Detailed listings of mathematical distribution models are provided by Hammel & Herrchen (1999) and BUA (2001).

A number of studies dealt with the validation of this approach, using different organisms (mainly plants and soil fauna), organic contaminants (chlorobenzoles, -phenoles, -anilines, PCB, PAH, some pesticides) and exposure scenarios (Belfroid et al., 1995a; Belfroid et al., 1995b; Belfroid et al., 1996; Boesten, 1993; Houx & Aben, 1993; Ma et al., 1998; Ronday et al., 1997; Sijm et al., 2000; Spurgeon, 1997; Van Gestel et al., 1996).

Despite the more complex physical-chemical interdependencies (speciation), the general applicability of the EPT has been discussed also for heavy metal accumulation (As, Cd, Cr, Cu, Ni, Pb, Zn) in oligochaetes exposed to contaminated soils (Janssen et al., 1997a; Peijnenburg et al., 1999a, b). Also here the freely dissociated ions in pore-water are regarded as the primary bioavailable fraction in soils, at least for oligochaetes. Currently an refinement of the EPT / pore-water approach, the “Biotic Ligand Model“ - originating from aquatic ecotoxicology - is under discussion, aiming at a more pronounced consideration of water chemistry. This model integrated pH and competition of metal ions with non-toxic ions (e.g., calcium) for receptors both in organisms and in other potential ligands, especially dissolved organic carbon (De Schamphelaere & Janssen, 2002; DiToro et al., 2001; Santore et al., 2001).

4.4 Approaches for experimental assessment of bioavailability

These approaches are mainly based on special physical-chemical extraction procedures for determining bioavailable contaminant concentrations in soils. The power and quality of the assessment depends on experimental conditions and on the plausibility of the extraction procedures used (comparability to the real exposure situation for the organism under study).

Different extractants (inorganic or organic solvents, mostly specific for groups of organisms or substances) are used at varying extraction conditions:

a) Water: the water-extractable contaminant fraction (of both organic and inorganic contaminants) is regarded bioavailable. The soil extracts or eluates, respectively, are used in biological tests, and their effects are correlated with the measured contaminant concentrations in the extracts (Frische, 2002; Kelsey et al., 1997; Posthuma & Notenboom, 1996; Schuler & Lydy, 2001; Wahle & Kördel, 1997). This experimental procedure partly overlaps with the pore-water approach. It has to be kept in mind, however, that water extracts are not identical with pore-water sampled by suction caps or centrifugation.

b) Salt solutions: Some studies investigated the appropriateness of neutral salt solutions as extractants for revealing heavy metals bioavailable for plants

(Lebourg et al., 1996; McLaughlin et al., 2000; Qian et al., 1996) and for oligochaetes (Belotti, 1998; Conder & Lanno, 2000).

c) Organic solvents: So-called “mild“ extractions with various organic solvents (alcohols, organic acids, detergents, tetrahydrofuran, cyclodextrin etc.) or mixtures of them, respectively, have often been used for identifying the bioavailability of organic contaminants for a number of organisms. Most studies relate extraction efficiency of the procedure or the extractant to bioaccumulation of the contaminants within the organisms (Kelsey et al., 1997; Krauss et al., 2000; Liste & Alexander, 2002; Schuler & Lydy, 2001; Tang & Alexander, 1999; Tang et al., 1999;) or to biodegradation (Chung & Alexander, 1998; Cuypers et al, 2000; Cuypers et al, 2002; Johnson et al, 1999; Kelsey et al., 1997; Reid et al., 2000b; Tang & Alexander, 1999).

d) Biomimetic approaches stands for extraction procedures trying to mimic the uptake of dissolved organic contaminants from soil pore-water into organisms, simulating interactions of the contaminant with an organic matrix (or the uptake via a biomembrane) and then measuring this concentration. Parallel experiments on contaminant uptake by selected organisms (bioaccumulation) allow for validation of the method. Currently different materials and techniques are being used, e.g. solid phase extraction (Krauss & Wilcke, 2001; Mayer et al., 2000a, b; Morrison et al., 2000; Sijm et al., 2000; Tang et al., 1999) or semipermeable membrane devices (SPMD) (Macrae & Hall, 1998). Like in the EPT approach (see above), also here the soil pore-water (or soil suspensions, as a scenario) is assumed to be the main exposure route for the soil organisms under study. A comparable biomimetic approach is the DGT (diffusive gradients in thin-films) technique for quantifying bioavailable heavy metals in soils. This technique measures not only metals in the soil solutions but also metals labile bound to the soil matrix. It has been successfully used for predicting heavy metal uptake into plants (Zhang et al., 2001).

e) Other approaches: For determining the bioavailability of organic compounds (PAH, PCB), recently some new approaches were suggested: the „selective supercritical fluid extraction“ (SFE) which aims at simulating desorption pro??cesses in contaminated soils or sediments by varying extraction conditions (Björklund et al., 2000; Hawthorne & Grabanski, 2000) and the oxidation of organic contaminants adsorbed to the soil organic substance (Cuypers et al., 2000).

4.5 Bioavailability - examples

This chapter documents the current knowledge on bioavailability of PAH and cadmium by selected studies. The substances were selected because of their relevance in the soil environment (Curlík et al., 2000; Wilcke, 2000) and due to data availability revealed by our study (cf. Tab. 4.1). We collected studies on bioavailability of both contaminants for oligochaetes as soft-bodied organisms and for Collembola as soil animals with a „hard“ exoskeleton, and studies on bioavailability for plants and soil microorganisms. Data were evaluated and summarised in tables according to the criteria species, substances, soils, experimental design, bioavailability approach and conclusion. The documentation in tables is followed by a short summary of current knowledge.

4.5.1 Polycyclic aromatic hydrocarbons (PAH)

4.5.1.2 Availability of PAH for oligochaeta

Van Brummelen et al. (1996)	
Species	*Lumbricus rubellus*
Chemicals	8 PAH (phenantrene, pyrene, fluoranthene, benzo(b)fluoranthene, chrysene, benzo(a)anthracene, benzo(k)fluoranthene, benzo(a)pyrene)
Soils	10 sampling sites along a gradient of decreasing contamination (1-10km distance from a waste incineration plant); litter, humus and mineral soil
Experimental design	Measurement of *in-situ* bioaccumulation in *L. rubellus* (handsorted from the 10 soils)
Approach bioavailability	Comparison of PAH-concentrations in *L. rubellus* with the concentrations in the different soil layers. Biota-to-soil accumulation factors (BSAF) compared to the octanol-water-partitioning coefficient (K_{ow}) of the substances.
Conclusions	PAH-concentrations in *L. rubellus* were most closely correlated with the concentrations in the humus layer (in accordance with the biology of the species). For the tested PAH the *in-situ* biota-to-soil accumulation factors (BSAF) indicated an equilibrium distribution between the organic substance of the humus layer and the lipids of the organisms. They were independent of the K_{ow} of the substances (in coincidence with EPT).

Kelsey et al. (1997)	
Species	*Eisenia fetida*
Chemicals	Phenantrene
Soils	Loam (pH 7.1 / Corg 8.7%)
Experimental design	Bioaccumulation experiment under controlled laboratory conditions with sterilised and spiked soil, artificial ageing (up to 124 days)
Approach bioavailability	Correlation of the accumulated phenantrene concentrations with different solvent-extractable fractions (methanol-water, n-butanol, ethanol, hexane, methanol, toluene, water)
Conclusions	Some of the solvent extracted amounts of phenantrene showed a correspondence with measured concentrations in the worms (in particular n-butanol). A tendency to overestimate the accumulation in the worms by solvent extraction was observed. After ageing the soils showed a clear decrease of the accumulated and extractable contents. Objection: very artificial exposure scenario (6 worms in 10 g soil).

Ma et al. (1998)	
Species	*Lumbricus rubellus*
Chemicals	11 PAH (anthracene, phenantrene, pyrene, fluoranthene, benzo(b)fluoranthene, chrysen, benzo(a)anthracene, benzo(k-fluor-anthene, benzo(a)pyrene, benzo(ghi)perylene, dibenzo(a)anthracene)
Soils	12 flood plain soils with variable soil characteristics (sandy loam to silty clay, pH (KCl) 7.1-7.6 / organic material 2.1-10.7%) and variable contamination
Experimental design	Measurement of the *in-situ* bioaccumulation in *L. rubellus* (handsorted out of the 12 soils) and bioaccumulation-experiments with phenanthrene, fluoranthene, pyrene and benzo(a)pyrene in spiked water with uncontaminated *L. rubellus.*
Approach bioavailability	Comparison of the measured uptake factors with the estimated (EPT) or the values derived in water experiments for the *in-situ* bioaccumulation.
Conclusions	*In-situ* biota-to-soil accumulation factors (BSAF) showed for the tested PAH equilibrium with the organic matter of the soils and the lipid of the test organisms and were independent of the K_{ow} of the substances (in accordance with EPT). The bioaccumulation factors (BAF) estimated from the concentrations in the soil pore-water for phenanthrene, fluoranthene and pyrene in the field soils were within the same range as the bioconcentration factors (BCFs) derived from the water experiments. The exposure of worms to these substances is primarily via the pore-water, which is not valid for the more hydrophobic PAH (benzo(a)pyrene), for which the accumulation by EPT was underestimated.

Leppänen & Kukkonen (1998)	
Species	*Lumbriculus variegatus*
Chemicals	Pyrene
Soils	Natural fine sediment (Corg. 3.6%)
Experimental design	Bioaccumulation test (28 days) with spiked sediment (radiolabelled pyrene)
Approach bioavailability	Differences in bioaccumulation between test-organisms feeding or not feeding (due to the reproductive phase) in the sediment but otherwise identical exposure.
Conclusions	The major uptake route for pyrene in this experiment was definitely via the digested sediment and not via the pore-water. This is a clear indication for the significance of exposure via „food“ or the impact of the feeding behaviour of organisms on bioavailability. Restriction: not a terrestrial work.

Krauss et al. (2000)	
Species	*Lumbricus terrestris*
Chemicals	20 PAH (16 US-EPA PAH and triphenylene, benzo(j)fluoranthene, benzo(e)pyrene, perylene)
Soils	25 urban soils of different use with variable soil characteristics (mainly loamy soils, pH (KCl) 3.5-7.2 / Corg 0.6-10.6%) and variable contamination
Experimental design	Bioaccumulation experiment under controlled laboratory conditions (15 days of exposure)
Approach bioavailability	Comparison of the uptake into test organisms with mild extraction solvents (methanol-water-extractions (1:1) and 0.5M NaOH). Estimation of the bioaccumulation on the basis of EPT and comparison with measured values.
Conclusions	The estimation of the bioaccumulation on the basis of EPT was possible for the tested soils and PAH. The total PAH contents in the soils exhibited better correlation with the PAH concentrations in the test organisms than those extracted by mild solvents. This study presents a rather realistic exposure scenario and a promising method.

Jager et al. (2000)	
Species	*Eisenia andrei*
Chemicals	Phenantrene, pyrene, fluoranthene, benzo(a)pyrene
Soils	OECD artificial soil material OECD, 1984a (pH (KCl) 5.5 / Corg 10%)
Experimental design	Bioaccumulation experiment (dynamic) under controlled laboratory conditions (21 days of exposure)
Approach bioavailability	Comparison of the uptake in test organisms with mild extractions (methanol-water-extraction (1:1) and 0.5M NaOH). Estimation of the bioaccumulation on the basis of EPT and comparison with the measured values.
Conclusions	The tested PAH showed similar uptake rates based on the concentrations measured in the pore-water despite differing lipophilic properties, thus supporting the equilibrium-partitioning theory (EPT). Objection: very artificial design, the transfer to natural soils and aged contamination is not clear.

Krauss & Wilcke (2001)	
Species	*Lumbricus terrestris* L.
Chemicals	20 PAH (16 US-EPA PAH and triphenylene, benzo(j)fluoranthene, benzo(e)pyrene, perylene)
Soils	25 urban soils of different use with variable soil characteristics (mainly loamy soils, pH (KCl) 3.5-7.2 / Corg 0.6-10.6%) and variable contamination; divided in 2 groups with 11 (experiments for the model development) and 14 soils (independent validation)
Experimental design	Bioaccumulation experiment under controlled laboratory conditions (15 days of exposure)
Approach bioavailability	Comparison of the uptake of PAH in test-organisms with solid phase extractions (C_{18}-disk) and methanol-water-extractions (1:1)
Conclusions	The prediction of measured biota-to-soil accumulation factors (BSAF) was possible based on the C_{18}-extractions (but slight overestimation with the model validation). A slight underestimation is described for the higher molecular PAH - possible causes: additional uptake routes. (release by passage through the gut) and/or metabolisation of lower molecular PAH. The methanol-water-extraction had a lower predictive power. This study presents a rather realistic exposure scenario.

Schuler & Lydy (2001)	
Species	*Lumbriculus variegatus*
Chemicals	Benzo(a)pyrene
Soils	Natural sediment (silty loam, Corg. 1%)
Experimental design	Bioaccumulation test (7 days) with spiked sediment (radiolabelled benzo(a)pyrene) after different ageing periods (1-120 days).
Approach bioavailability	Comparison of the bioaccumulation with 4 solvent extractions (water, methanol, octanol, acetonitril) and the extraction with passive sampling devices (PSD)
Conclusions	The extraction with water gave the best correlation with the concentrations determined in exposed organisms; all other methods overestimated the bioavailable contents. Hardly any change of the chemical availability with the ageing, but a decrease of the bioavailable contents as well as of the free pore-water concentrations (PSD). Objection: artificial and not a terrestrial study!

Liste & Alexander (2002)	
Species	*Eisenia fetida*
Chemicals	Chrysene, pyrene
Soils	Silty loam (pH 6.3 / Corg 2.2%)
Experimental design	Bioaccumulation experiments under controlled laboratory conditions with spiked soil (sequential exposure), effect of „ageing“
Approach bioavailability	Comparison of the uptake in test organisms with butanol- and solid phase extraction (C_{18}-disk)
Conclusions	A clear correlation between the butanol-extracted amount of chrysene and pyrene from the test soils with the concentrations measured in the worms was shown (even after ageing of the soils). With solid phase extraction (C_{18}) the uptake was underestimated. Due to the very artificial exposure scenario, further experiments for validation seem to be necessary.

The majority of the studies focused on soil pore-water as primary exposure route for oligochaetes and PAH, both under field conditions and in laboratory experiments. However, this holds only partly for higher-molecular (and more lipophilic, log $K_{ow} > \sim 5$) PAH, for which a bioavailability assessment (esp. bioaccumulation) based on a dynamic equilibrium between the solid organic phase of the soil, pore-water and the lipid pool of the organisms (according to the EPT) appears not plausible. Here, additional uptake routes (release of particle-bound PAH during gut passage) have been suggested or, in single studies with sediments, already shown. Most of the investigated “mild” extractions (using methanol, octanol, acetonitril, toluol, hexane, NaOH solutions etc.) are not appropriate for predicting bioavailable contaminant concentrations (mostly overestimation of bioavailability). Butanol, however, in two studies was a good predictor for bioavailable soil concentrations. Due to the very artificial exposure conditions of these bioaccumulation experiments, their general applicability and validity must be questioned, though. Here, further validation studies under varying conditions (different soils, contaminated land, organism density etc.) are required. Two studies that used C_{18} solid phase extractions as “biomimetic approaches“ in one case coincided well with accumulated PAH contents and underestimated them (for higher-molecular compounds), respectively. In the second case the uptake by earthworms was underestimated by this procedure. Ageing of PAH contamination resulted in a decrease of bioavailability, considerably restricting the transferabilities of spiking experiments to the exposure under filed conditions.

4.5.1.2 Availability of PAH for plants

Comparably few studies specifically addressed the bioavailability of PAH in the system soil-plant. Based on some reviews and specific studies on that topic (Beck et al., 1996; Böhme et al., 1999; Crößmann, 1992; Harvey et al., 2002; McLachlan, 1999; Werkbüro für Boden und Bodenschutz, Beuren und Plochingen, 1998; Wilcke, 2000), the current knowledge can be summarised as follows:

- The uptake of PAH from soil into plants can generally be regarded as low (transfer factors plant-soil mostly < 0.01).
- The uptake of PAH via the root and their distribution within the plant is low, due to the low water solubility and the strong sorption of hydrophobic substances to particularly the organic fraction of the soil. While an uptake into plant roots is possible for 3- and 4-ring-PAH, this transfer route is rather improbable for higher-condensed PAH (log $K_{ow} > 5$).
- Often an absorption of PAH to the root surface without a transition into the root tissue or a further distribution within the plant was described.
- The main uptake of PAH is via the atmosphere (gas phase, dry and wet deposition of particle-bound PAH) into the above-ground plant organs. Consequently, the atmospheric uptake “overrules” the uptake from soil, which is often a methodological problem.
- Many experimental studies on the transfer of PAH into plants were conducted with spiked soils, which makes the transferability for historically aged contaminations doubtful.

The following five studies more or less specifically addressed the availability of PAH for plants in soils:

Hulzebos et al. (1993)	
Species	*Lactuca sativa* (lettuce)
Chemicals	Naphtalene, Acenaphtene (together with a set of further 74 „priority organic contaminants“)
Soils	Loam from arable land (pH 6.3 (KCl) / organic matter 1.4% / clay 12%)
Experimental design	Phytotoxicity test according to OECD (1984b) Guideline 208 („early seedling growth test“) performed with spiked soil and standardised incubation conditions, toxicity parameter: EC_{50} growth inhibition (aboveground phyto-mass

	after 14 days on a fresh weight basis)
Approach bioavailability	Comparison of the calculated pore-water EC$_{50}$-values gained from the adsorption coefficients with the results of growth experiments in spiked aqueous nutrient solution performed in parallel.
Conclusions	No special statement for the tested PAH is given in this study, but a general conclusion for the investigated organic compounds. The observed agreement of calculated pore-water effect concentrations with the experimental effect-concentrations from nutrient solution experiments indicates the importance of the soil pore-water for phyto-toxicity. This study represents a very artificial test design, especially due to the freshly spiked soils.

Thiele & Brümmer (1997)	
Species	Spinach
Chemicals	15 US-EPA PAH
Soils	Different PAH-contaminated soils from residual wastes exhibiting different soil texture (sandy- and silty soils/ Corg. 2.2-16.6%)
Experimental design	PAH-uptake in plants, details described in Thiele (1997)
Approach bioavailability	Comparison of the PAH-contents in the plants with NH$_4$NO$_3$-extractable soil concentrations.
Conclusions	A correlation of detectable PAH-contents in plants with the NH$_4$NO$_3$-extractable soil concentrations for several soil samples was shown. The NH$_4$NO$_3$-extractable soil concentration corresponds on average to about 0.2% of the total concentration (soxhlet-extraction wih toluene/acetone).

Tang & Alexander (1999)	
Species	Wheat and barley
Chemicals	Fluoranthene, pyrene, phenantrene
Soils	Loamy soil / pH 7.1 / Corg. 11.4%
Experimental design	Growth and accumulation experiment with spiked soil under standard conditions, influence of artificial ageing
Approach bioavailability	Comparison of the PAH-contents taken up by the plants with the concentrations extractable by several organic solvents (n-butanol, propanol, ethyl-acetic acid, methanol)
Conclusions	A clear decrease of the biological (uptake in plants) and chemical availability (solvent extracted soil concentrations) was shown after the ageing of the spiked soils. The solvent extractable soil fractions correlated with the availability to plants. Comparing the availability of the 3 PAH in the soil for different organisms indicates the following order (decreasing availability): bacteria (mineralisation) > uptake in compost worms > uptake in plants.

Kipopoulou et al. (1999)	
Species	Cabbage (*Brassica*), carrot (*Daucus*), lettuce (*Lactuca*), onion (*Allium*) and endive (*Chichorium*)
Chemicals	16 US-EPA PAH
Soils	Soil from arable land in an industrial region (sandy and silty loam/ slightly acidic to alkaline / organic matter content 1.1-2.0%)
Experimental design	Measurement of the PAH-concentrations of the above and underground plant organs, soil and atmosphere at the end and during two vegetation periods
Approach bioavailability	Calculation of transfer factors, comparison of PAH-patterns, comparison of bioconcentration factors with the octanol-water partitioning coefficient (Kow) of the substances
Conclusions	In general a low plant uptake was observed. The study indicated differences between the species. In the above and underground parts of the plants the lower molecular PAH dominated, the PAH-pattern indicated a major significance of the uptake from the atmosphere than from soil. The Kow respectively the solubility of PAH were good predictors for the soil-plant transfer (liquid phase).

Sverdrup (2001)	
Species	*Sinapis alba* (mustard), *Trifolium pratense* (clover), *Lolium perenne* (rye grass)
Chemicals	Fluoranthene, pyrene, phenantrene, fluorene and carbazole, dibenzothiophene, dibenzofurane, acridine (heterocycles: N-, S-, and O-substituted analogous of fluorene)
Soils	Soil from arable land (sandy loam / pH 6.2 (H_2O) / Corg. 1.6%)
Experimental design	Phytotoxicity test according to OECD, 1984b („early seedling growth test“) with spiked soil and standard incubation conditions, toxicity parameter: $EC_{20/50}$ growth inhibition (aboveground phyto-mass after 21 days on a fresh and dry weight basis)
Approach bioavailability	Comparison of pore-water $EC_{20/50}$-values calculated from the adsorption coefficients with the octanol-water partitioning coefficient (Kow) of the tested substances
Conclusions	In the experiments differences in sensitivity between the plant species were visible, but just small differences in toxicity between substances. Only a few of the more lipophilic substances exhibited a higher phytotoxicity. This is a very artificial test design especially due to the freshly spiked soils.

These studies partly underline the above summarised state of the art, yet also hint at contradictions and open questions. For example, the relative significance of the PAH uptake via soil water, the appropriateness of different solvents for predicting plant-available PAH concentration in soil or the availability of aged contamination at present cannot be conclusively judged.

Wilcke’s (2000) review points out the research need on availability of PAH for plants: „.. there are still no reliable methods to characterise PAH pools differing with respect to their bioavailability.“

4.5.1.3 Availability of PAH for Collembola

Erstfeld & Snow-Ashbrook (1999)	
Species	Total abundance of springtails
Chemicals	16 US-EPA PAH in soil samples of a residual waste: 6 locations within a gradient of increasing PAH-concentrations (sum of PAH: 5.2 – 80.4 mg/kg d.s.)
Soils	Taken from the area of a former production plant of wood distillation products and charcoal (out of action since about 30 years): loamy sand or sandy loam / pH 6.7-8.6 (solvents not described) / Corg. 3.3-38.7%)
Experimental design	Single sampling of 5 samples from each location, extraction of microarthropodes with a Tullgren-apparatus, counting of collembola (total abundance in individuals/g d.s.)
Approach bioavailability	Multiple regression models of the monitored data with consideration of the PAH-contents in soil was well as of different soil characteristics relevant for the habitat of soil organisms (pH, water content, Corg., clay and sand fraction)
Conclusions	A positive correlation of the total abundance of collembola with the PAH-concentrations of the soil samples was described (also valid for data on nematodes and earthworm tests performed in parallel). A stimulant effect of the contamination is suggested possibly from activation of PAH-metabolising soil microorganisms (increase in biomass and activity).

Sverdrup et al. (2001)	
Species	*Folsomia fimetaria*
Chemicals	Fluoranthene, pyrene, phenantrene, fluorene and carbazole, dibenzothiophene, dibenzofurane, acridine (heterocycles: N-, S-, and O-substituted analogous of fluorene); nominal concentrations up to 3000 mg/kg d.s.
Soils	Soil from arable land (sandy loam / pH 6.2 (H_2O) / Corg. 1.6%)
Experimental design	Reproduction test with spiked soil and standard incubation conditions, toxicity parameter: EC_{10} and EC_{50} of the inhibition of reproduction (number of juveniles after 21 days)
Approach bioavailability	Comparison of the pore-water $EC_{10/50}$-values calculated from the adsorption coefficients with the octanol-water-partitioning coefficients (Kow) of the tested substances
Conclusions	Only minor differences in toxicity between the substances tested were apparent on the basis of total soil concentrations (EC-values). A significant correlation of the calculated pore-water EC_{10}-values with the log Kow-values of the tested substances and a good agreement with QSAR-estimated NOEC-values for *Daphnia magna* indicates an narcotic mode of toxic action of the tested PAH. Objection: very artificial test design especially due to the freshly spiked soils.

Sverdrup et al. (2002a)	
Species	*Folsomia fimetaria*
Chemicals	Pyrene and phenantrene; nominal test concentrations: 25-400 mg/kg d.s. (phenantrene) and 7.5-480 mg/kg d.s. (pyrene)
Soils	Soil from arable land (sandy loam / pH 6.2 (H_2O) / Corg. 1.6%)
Experimental design	Reproduction test with freshly spiked soil and following different preincubation periods (0-120 days). Toxicity test with standard incubation conditions, toxicity parameter: Survival of adults (LC_{50}) and inhibition of reproduction after 21 days (EC_{10})
Approach bioavailability	Comparison of the effect concentrations after different ageing periods of the spiked soils
Conclusions	No clear decrease of the toxicity with time was visible (only a slight decrease for phenantrene). The observed toxicity is explained as a mixture effect of the spiked substances and the metabolites produced during (pre)incubation of the non sterile soils. The relatively low Corg-content of the soil and the rather high concentrations of the substances are discussed as possible explanations for the missing influence of the ageing on bioavailability and toxicity, respectively. Objection: very artificial test design.

Sverdrup et al. (2002b)	
Species	*Folsomia fimetaria*
Chemicals	16 US-EPA PAH; nominal concentrations: 3-1000 mg/kg
Soils	Soil from arable land (sandy loam / pH 6.2 (H_2O) / Corg. 1.6%)
Experimental design	Reproduction test with freshly spiked soil and standard incubation conditions, toxicity parameter: Survival of adults (LC_{50}) and inhibition of reproduction after 21 days (EC_{10})
Approach bioavailability	Comparison of the pore-water LC_{50}/EC_{10}-values calculated from the adsorption coefficients with the octanol-water-partitioning coefficients (Kow) as well as the water solubility of the tested substances
Conclusions	The significant correlation of the calculated pore-water effect concentrations with the log Kow-values (exception: anthracene) indicates a non-specific mode of toxic action (narcosis). The more lipophilic PAH (log Kow of about > 5.5) were not toxic in the tested concentration range due to their lower water solubility. Thus the importance of pore-water exposure was supported by experimental data. Objection: The extrapolation of results to aged contamination is questionable.

Sverdrup (2001)	
Species	*Folsomia fimetaria* (Collembola), *Enchytraeus crypticus* (Enchytraeidae), *Eisenia veneta* (Lumbricidae)
Chemicals	Fluoranthene, pyrene, phenantrene, fluorene and carbazole, dibenzothiophene, dibenzofurane, acridine (heterocycles: N-, S-, and O-substituted analogous of fluorene); five experimental concentrations: 10-1000 mg/kg d.s.
Soils	Soil from arable land (sandy loam / pH 6.2 (H_2O) / Corg. 1.6%)
Experimental design	Reproduction test with freshly spiked soil and standard incubation conditions, toxicity parameter: Survival of adults (LC_{50}) and inhibition of reproduction after 21 days (EC_{10})
Approach bioavailability	Comparison of the acute and chronic toxicity data (LC_{50} and EC_{xx} respectively NOEC-values) for the three test species and eight substances
Conclusions	Clear differences in sensitivity between the investigated test species were visible with *F. fimetaria* being the most sensitive organism (exception: carbazole and acridine).

Up to now only few studies (mostly by Sverdrup and co-workers, see above) specifically investigated the bioavailability of PAH for Collembola. For methodological reasons (small size of these organisms), research has focussed on toxic effects rather than on bioaccumulation. Experiments with both spiked soils and standardised tests render the soil pore-water as primary exposure route, in analogy to the oligochaetes. Furthermore these studies hint at an narcotic mode of toxic action of most PAH and at relatively low toxicity differences between the 2- to 4-ring PAH. Higher condensed (and more lipophilic, log K_{ow} > ~5.5) PAH were considerably less toxic, obviously due to their low water solubility. Compared to one species each of lumbricids and enchytraeids, the collembolan species *Folsomia fimetaria* seems to react most sensitive towards the PAH investigated. Interestingly, experimental ageing (up to 120 days) of PAH-spiked soils did not result in reduced toxicity (and thus bioavailability), despite otherwise comparable experimental conditions. Main objection against these painstaking and systematic laboratory studies is the rather artificial design (only one soil, spiking, one test species), which restricts their transferability to other (field, soil, organisms) conditions. This - and the resulting research need what comes to the bioavailability of PAH for “hard-bodied” soil animals in general - is underlined by the monitoring study by Erstfeld & Snow-Ashbrook (1999), who found a positive (!) correlation of Collembola abundance with PAH contents in soil samples of residual wastes.

4.5.1.4 Availability of PAH for microorganisms

A lot of experimental studies have dealt with the availability of PAH for soil microorganisms, most of them using the biodegradation of the substances under controlled laboratory conditions as an indicator of bioavailability. For that purpose, soils spiked with selected PAH or PAH-contaminated field soils are inoculated with (pure or mixed) cultures of PAH-degrading bacteria, or - without inoculation - the degradation by the autochthonous microflora is studied. Typically, these experiments are associated with investigations aiming at the sorption/desorption behaviour of the chemicals (various extraction methods, desorption experiments etc.). These works are mostly focused on the environmental fate of the substances or the remediation potential for contaminated sites, yet not on environmental exposure or risk assessment. Some general findings on the availability of PAH (and of further hydrophobic xenobiotics) for microorganisms are summarised in the following.

The current concept on microbial degradation of contaminants is based on the assumption that hydrophobic organic xenobiotics are taken up into the bacterial cell exclusively via the soil water phase (Mahro, 2000). Complex processes of sorption and desorption (Marschner, 1999) at and inside the inorganic and organic soil matrix and distribution and diffusion processes in the water phase thus determine bioavailability and biodegradation (Bosma et al., 1997). The microbial degradation typically is divided into two phases, with a high initial degradation rate, followed by an increasing deceleration, illustrated by a so-called „hockey-stick“ degradation curve (Alexander, 2000). These kinetics reflect the mass transfer of the substance subject to degradation: first, the water-dissolved and easily “deliverable” (i.e. only weakly sorbed to the soil matrix) fractions are available for microbial degradation, whereas the strongly sorbed fraction is subject to a remarkably reduced or delayed biological breakdown. Numerous experimental studies (particularly by Alexander and co-workers) identified the factors relevant for microbial availability and thus for PAH degradation in the soil system:

- Bioavailability depends on the desorption of the substances (White & Alexander, 1996; White et al., 1999; Zhao & Voice, 2000).
- The desorption (and thus bioavailability) is particularly determined by soil organic carbon content, the desorption decreasing with increasing carbon content. Higher condensed, more lipophilic PAH are sorbed more strongly than less condensed ones (Smith et al., 1997; White & Alexander, 1996).
- Increasing contact times of PAH with the soil reduce the desorption processes, without any structural change of the substances (called sequestration or „ageing“) (Alexander & Alexander, 1999; Alexander & Alexander, 2000; Hatzinger & Alexander, 1995; White et al., 1999). This phenomenon is explained by the slow diffusion of hydrophobic organic compounds into, or hydrophobic adsorption to organic macro-molecules of the humus fraction (Marschner, 1999). The degree of the ageing effect depends on the soil organic carbon content (low in soils with Corg < 2%) (Nam et al., 1998).
- Further factors influencing the ageing effect are clay content, porosity, soil water content (White at al., 1997) and PAH concentration (Chung & Alexander, 1999).

In the following, studies on availability of PAH for microorganisms in soils are summarised, with emphasis on the methodological spectrum.

Kelsey et al. (1997)	
Species	PAH-degrading *Pseudomonas* strain (pure culture)
Chemicals	Phenantrene
Soils	loamy soil (pH 7.1 / Corg 8.7%)
Experimental design	Degradation experiment under controlled laboratory conditions, sterilised and spiked soil with an inoculum of the *Pseudomonas* strain, artificial ageing of the spiked soil (up to 124 days)
Approach bioavailability	Comparison of the mineralised amounts of phenantrene in degradation experiments with the extractable fraction of various „mild“ solvents (methanol-water, n-butanol, ethanol, hexane, methanol, toluene, water)
Conclusions	Occasionally the amounts of phenantrene extracted with solvents from the tested soils correlated with the observed microbial degradation. However, no clear preference for one method, as either overestimation as well as underestimation of the availability to microorganisms were observed. A clear decrease of both chemically and biologically available concentrations was apparent as a consequence of ageing.

Macrae & Hall (1998)	
Species	no data
Chemicals	Complex contamination with PAH (16 US-EPA-PAH analysed)
Soils	PAH-spiked sediment and contaminated river sediment (Corg. 11.4-13.8)
Experimental design	Degradation experiments (not described in detail)
Approach bioavailability	Comparison of the microbial PAH degradation in the sediments with the chemically available concentrations derived from desorption / extraction experiments („biomimetic approaches“): Semi Permeable Membrane Devices (SPMDs), solid phase extraction (Tenax-TA) and polyethylene tube analysis (PTD)
Conclusions	The PTD results showed a correlation with biologically degradable PAH in the sediment; PTD was suggested as a suitable method for the exposure assessment. According to the authors opinion SPMD and Tenax-TA are suitable for the assessment and prediction of easily desorbing PAH-fractions respectively.

Tang & Alexander (1999)	
Species	mixed culture of PAH-degrading bacteria (not specified)
Chemicals	Phenantrene, pyrene, fluoranthene
Soils	loamy soil/ pH 7.1 / Corg 11.4%
Experimental design	Degradation experiment under standard conditions after addition of an bacterial inoculum to the sterilised and PAH-spiked soil (artificial ageing after the spiking)
Approach bioavailability	Comparison of the PAH-amounts degraded by microorganisms (by determination of the decrease in the total contents using a soxhlet-extraction protocol) with the soil contents extracted by „mild“ extractions (solvents: n-butanol, methanol, n-propanol, ethyl-acetic acid)
Conclusions	A significant decrease of the biological and chemical availability was apparent after the ageing of the spiked soils. A clear correlation between all of the extractable soil fractions (4 solvents) and the biodegradation was visible.

Cuypers et al. (2000)	
Species	mixed culture of PAH-degrading bacteria
Chemicals	Complex contamination with PAH (sum of the 16 US-EPA PAH: 28-2036 mg/kg d.s.)
Soils	7 residual waste soils and 7 contaminated sediments of different texture, organic matter 1.7-33.8%
Experimental design	Degradation experiment under standardised conditions (21 days) following the inoculation of the soils and sediments with bacteria
Approach bioavailability	Comparison of the mineralised PAH portions as well as the remaining PAH-contents after degradation via persulfate-oxidation (oxidises amorphous organic matter including sorbed PAH); at the same time determination of total concentrations as well as solid phase extraction (Tenax–TA)
Conclusions	A clear correlation between the oxidised PAH and the mineralised PAH for 2-, 3- and 4-ring-PAH as well as the sum of all PAH was observed. This was not true for 5- and 6-ring-PAH (degradation is overestimated by persulfate-oxidation). This correlation (also for 5- and 6-ring-PAH) was given for the remaining contents after the degradation experiments, too. There was no correlation between the brief solid phase extraction of PAH with long-term degradation experiments; thus longer extraction times seem to be necessary.

Hawthorne & Grabanski (2000)	
Species	Site-specific microorganisms (not specified)
Chemicals	Complex contamination (analysed among others naphtalene, 2-methylnaphtalene, pyrene, acenaphtene, benzo(a)pyrene, benzo(ghi)perylene)
Soils	Soils from a large-scale bioremediation project (sandy soil, C-content 4.6%)
Experimental design	Sequential SSFE-extractions (Selective Supercritical Fluid Extraction) with liquid CO_2 under varying conditions (temperature/ pressure/time), to extract and distinguish different contaminant fractions („fast-moderate-slow-very slow desorbing fraction“)
Approach bioavailability	Comparison of the PAH-contents of the untreated soils as extracted by SSFE-extraction with analysis data after 6 / 12 months of remediation (landfarming), as well as with the corresponding total contents (exhaustive extraction)
Conclusions	After 6 months the easily desorbing PAH-fractions were primarily degraded (2-3 rings). The 4-ring-PAH only showed limited degradation, whereas the 5- and 6-Ring-PAH had not been degraded at all after one year. A defined SSFE-extraction correlated with biological degradation. Thus this method seems to be suitable for prediction of the bioavailability of PAH to micro-organisms.

Reid et al. (2000b)	
Species	PAH-degrading *Pseudomonas*-strain (pure culture)
Chemicals	Phenantrene
Soils	Soil from a ruderal site (pH 5.1 / Corg 3.7%)
Experimental design	Degradation experiments (respiration measurements) under controlled laboratory conditions of spiked soils inoculated with bacteria
Approach bioavailability	Comparison of the mineralised amounts of phenantrene with the fractions extractable by several solvents (n-butanol and aqueous solutions of hydroxy-propyl-ß-cyclodextrine (HPCD) for the shake extraction and dichloromethane for the soxhlet-method)
Conclusions	The best correlation with the observed mineralisation was given by the amount of phenantrene extracted by HPCD (even after different ageing periods of the spiked soils). The extractions with dichloromethane and n-butanol revealed a tendency to overestimate the availability of PAH to microorganisms.

Sverdrup et al. (2002c)	
Species	Site-specific microorganisms and protozoa
Chemicals	Fluoranthene, pyrene, phenantrene, fluorene and carbazole, dibenzothiophene, dibenzofurane, acridine (heterocycles: N-, S-, and O-substituted analogous of fluorene)
Soils	Soil from arable land (sandy loam/ pH 6.2 (H_2O) / Corg. 2.8%)
Experimental design	Spiking experiment (1-3000 mg/kg d.s.), after 21 days incubation under standard conditions the inhibition of nitrification was determined as well as the microbial DNA diversity with DGGE (denaturant gradient gel electrophoresis) and the abundance of protozoa (total)
Approach bioavailability	Comparison of the calculated pore-water effect concentration (using adsorption coefficients) with the octanol-water-partitioning coefficients (Kow) and the water solubility of the substances
Conclusions	The tested substances clearly inhibited the nitrification above 20 mg/kg d.s., but only slight adverse effects on the total abundance of protozoa and no effects on the microbial DNA diversity were observed (possibly both last mentioned methods are not suitable toxicity parameter). The significant correlation of the calculated pore-water effect concentrations (nitrification data) with the Kow of the substances indicates a rather unspecific mode of toxic action (narcosis). The observation of a „plateau“ in the dose-effect-curves reveals that the restricted water solubility of some substances is the toxicity limiting factor at higher concentrations.

Cuypers et al. (2002)	
Species	Mixed culture of PAH-degrading bacteria (not specified)
Chemicals	Complex Contamination of PAH (sum of the 16 US-EPA PAH 122 mg/kg d.s. and 2036 mg/kg d.s. plus mineral oils)
Soils	2 contaminated harbour sediments (Corg. 13.3% / 9.7%, pH 7.1 / 7.8)
Experimental design	Degradation experiments under standard conditions (21 days) after inoculation of the soils and sediments with bacteria
Approach bioavailability	Comparison of the degraded PAH amounts with the fractions extractable by different methods: aqueous solutions of hydroxypropyl-ß-cyclodextrine (HPCD) and Triton X-100™ (detergent), solid phase extraction (Tenax –TA) and total concentrations (acetone/water)
Conclusions	A correlation between the HPCD-extractable and the mineralised PAH-fractions („readily bioavailable fraction“) could be seen. The solid phase extraction, too, was a suitable approach to predict the biodegradation, but not the extraction with Triton X-100™.

Liste & Alexander (2002)	
Species	Mixed culture of PAH-degrading bacteria (different isolates of *Pseudomonas*)
Chemicals	Phenantrene, pyrene
Soils	Silty loam (pH 6.3 / Corg 2.2%)
Experimental design	Degradation experiments after the addition of an bacterial inoculum to non sterile, spiked soil (artificial ageing following the spiking)
Approach bioavailability	Comparison of the microbially degraded PAH-contents (determination of the decrease in total contents by soxhlet-extraction) with the amounts extracted with n-butanol
Conclusions	The n-butanol extractable amounts of phenantrene and pyrene clearly correlate with the PAH-concentrations available for microbial degradation.

A multitude of (competing) chemical-analytical methods were used for assessing or predicting the availability of PAH for microorganisms in soil. Most procedures aim at the extraction and identification of the “maximum” or “potentially” bioavailable fraction. This fraction is thus operationally defined and hypothetically correlates with the maximum degraded amount of the contaminant during the course of an experiment. This means that

(a) this potentially bioavailable fraction depends on the methods (with respect to experimental design and extraction / determination procedure) and

(b) the exposure route that is considered to be the relevant one – mainly soil water, see above - and the fraction dissolved therein (representing the “actual“ exposure concentration) plays no role in most cases.

The latter especially holds for the various “mild“ extractions, whereas in desorption experiments and in the “biomimetic approaches“ processes of partitioning into and uptake from the watery phase do matter. The latter procedures are thus possibly appropriate for specifically describing the (acute) microbial toxicity of PAH in soils; however, studies on this comparison are lacking. Sverdrup et al. (2002c) showed that in PAH-spiked soils toxic effects (nitrification inhibition) are determined by the fraction dissolved in pore-water. Generally, only few studies on PAH availability for soil microorganisms consider toxicity as an endpoint. Due to the intrinsic toxic potential of PAH and the clear effects described by Sverdrup et al. (2002c) there is obvious research need in ecotoxicological risk assessment.

Comparing „mild“ extractions with various organic solvents (or mixtures of them), there is no clear trend concerning their power for predicting „potentially“ bioavailable PAH contents. Although some experiments showed good coincidence between concentrations obtained by certain extracts (e.g., n-Butanol) and the microbial-degradable fraction (Liste & Alexander, 2002; Tang & Alexander, 1999), others both over- and underestimated bioavailability (Kelsey et al., 1997; Reid et al., 2000a). Ditto contradicting are results from solid phase extractions, which have been shown to be both appropriate and less appropriate for predicting the potential bioavailability (Cuypers et al. 2002; Cuypers et al., 2000). A good predictive power for this fraction revealed a study using the PTD technology (Macrae & Hall, 1998) and two using HPCD (hydroxypropyl-ß-cyclodextrine) solutions (Cuypers et al., 2002; Reid et al., 2000b), whereas one detergent proved to be an inappropriate extractant (Cuypers et al., 2002). Persulfate oxidations (Cuypers et al., 2000) and SSFE (Selective Supercritical Fluid Extractions, Hawthorne & Grabanski, 2000) appear to be novel and promising indirect methods for assessing bioavailability.

In summary, despite extensive research on PAH availability for soil microorganisms, up to now there is no common, prevailing method for the whole substance group and the various experimental conditions (spiked versus historically contaminated, various soils and sediments, autochthonous microorganisms and inoculated cultures etc.). Still, new approaches and methods are being suggested - which are hard to evaluate on the present data base: further, systematic investigations are needed.

In general, extrapolation of the presented results to realistic field conditions is restricted, due to the typically very artificial conditions of standardised laboratory degradation experiments. Also for microorganisms extrapolation from an investigated strain to other strains are not generally possible (Smith et al. 1997). Last not least we have to stress that microorganisms comprise not only bacteria but also soil fungi, which play a very important role in the soil ecosystem but have not been considered (or only indirectly - “autochthonous microflora”) in present studies on bioavailability: also here is obvious research need.

4.5.2 Cadmium

4.5.2.2 Availability of cadmium for Oligochaeta

Posthuma & Notenboom (1996)	
Species	*Eisenia andrei, Enchytraeus albidus* and *E. crypticus*
Chemicals	Cd, Cu, Pb, Zn
Soils	11 soils with different metal contamination, clay: 1.2-2.0%, pH (KCl) 2.9-4.9, Corg 1.1-4.3% and spiked OECD artificial soil substrate (OECD, 1984a)
Experimental design	Correlation between toxic effects and bioavailability
Approach bioavailability	The metal concentrations were determined by total, $CaCl_2$ and H_2O extractions. Concentration-response-curves were calculated for the dominant Zn alone and for the mixture of metals at similar pH-values.
Conclusions	*E. albidus* did not perform well in the soils, and was not used further. If the toxicity was calculated on the basis of the total concentrations, the field and laboratory data differed markedly: Toxic effects in field soils were less pronounced than in artificial soil for *E. andrei*. For *E. crypticus* the opposite was found. The predictability of effects in field soil from laboratory toxicity data improved when differences in metal extractability ($CaCl_2$) and joined effects of metals were taken into account. The concentrations determined by the $CaCl_2$ extraction were better correlated with the bioavailability than the total concentrations.

Janssen et al. (1997 a, b)	
Species	*Eisenia andrei*
Chemicals	As, Cd, Cr, Cu, Ni, Pb, Zn
Soils	20 soils with different metal contamination, clay: 0.8-33.8%, pH (KCl) 2.8-7.0, organic matter 2.0-21.8%
Experimental design	Validation of the equilibrium portioning theory (EPT) for the prediction of bioaccumulation
Approach bioavailability	Multivariate regressions to determine the correlation between total metal concentration in the soil and the worms (= BSAF = biota-to-soil-accumulation factor) under the influence of some easily measurable soil parameters
Conclusions	For the metals As, Cd, Cu, Zn the BSAFs were dependent on soil characteristics which also affected the partition coefficients between soil and pore-water. This suggests that uptake of metals is either direct from pore-water or indirect to an uptake route closely related to pore-water.

Peijnenburg et al. (1999a)	
Species	*Enchytraeus crypticus*
Chemicals	Cd, Cu, Pb, Zn
Soils	20 soils with different metal contamination, clay: 0.8-33.8%, pH (KCl) 2.8-7.0, organic matter 2.0-21.8%
Experimental design	Uptake of metals by oligochaeta in field soils. Measurement of the metal concentration in different soil phases
Approach bioavailability	Multivariate regressions to determine the most important soil parameters regulating the metal uptake and elimination / bioaccumulation factors for oligochaeta
Conclusions	$CaCl_2$-extraction could be used to discriminate the 9 from in total 11 soils in which accumulation of Pb in worms was observable. pH-value and cation exchange capacity were the most important soil parameters which determined the uptake rate constants and the bioaccumulation factors.

Peijnenburg et al. (1999b)	
Species	*Eisenia andrei*
Chemicals	As, Cd, Cr, Cu, Ni, Pb, Zn
Soils	20 soils with different metal contamination, clay: 0.8-33.8%, pH (KCl) 2.8-7.0, organic matter 2.0-21.8%
Experimental design	Uptake of metals by oligochaeta in field soils. Measurement of the metal concentration in different soil phases
Approach bioavailability	Multivariate regressions to determine the most important soil parameters regulating the metal uptake and elimination and the bioaccumulation factors for oligochaeta.
Conclusions	For Cr, Cu, Ni, Zn the equilibrium between the concentrations in the soil and in the worms was reached rapidly. The authors therefore suggested, that the internal concentrations of these metals are primarily linked to total concentrations in the solid phase and the influence of soil parameters is small. For the essential metals Cu and Zn this could partly be due to regulation of the internal body concentrations by the worms. For As, Cd, Pb a significant influence of some soil characteristics on metal uptake of *E. andrei* was determined. The main factors for the prediction of the BSAF were pH value, DOC (dissolved organic carbon), clay content and the concentration of amorphous aluminium-oxide.

Conder & Lanno (2000)	
Species	*Eisenia fetida*
Chemicals	Cd, Pb, Zn
Soils	spiked artificial soil ASTM (1997)
Experimental design	Chemical extraction methods and biomimetic approaches as surrogate measures for direct determination of internal body concentrations
Approach bioavailability	Evaluation of weak-electrolyte soil extractions (0.1 M $Ca(NO_3)_2$) and ion-exchange membranes coated with a metal chelator (Plant Root Simulators$^{®}$ = PRS$^{®}$) as measures of metal bioavailability compared to acute lethal toxicity
Conclusions	The uptake of metals by the PRS was very variable and not well related to toxicity, while the weak-electrolyte extraction was closely correlated to the acute lethal toxicity and showed promise as precise, inexpensive surrogate measure of metal bioavailability in soil.

Lock et al. (2000)	
Species	*Enchytraeus albidus*
Chemicals	Cd, Zn
Soils	2 field soils, OECD-substrate clay 0-20%, pH 4-7, org. matter 0-10% (OECD, 1984a)
Experimental design	In order to minimise the number of treatments while studying the effect of pH, organic matter and cation exchange capacity (CEC) on toxicity a fractional factorial design and a central composite design was used
Approach bioavailability	Toxicity of metals under the influence of soil parameters
Conclusions	The toxicity of Cd and Zn could be predicted by pH and CEC values. The toxicity decreased with increasing pH and CEC.

Oste et al. (2001)	
Species	*Lumbricus rubellus*
Chemicals	Cd
Soils	spiked sandy soil, pH 4.4-6.2; Corg 2.0%
Experimental design	Validation of the equilibrium partitioning theory (EPT) for the prediction of the metal uptake of earthworms
Approach bioavailability	The Cd concentration in the pore-water was varied by increasing the soil pH by the addition of lime ($Ca(OH)_2$) and by adding manganese oxide MnO_2 which has a metal binding capacity in a factorial design

Conclusions	Both lime and manganese oxide reduced the Cd concentration in the pore-water by a factor of 25, while the internal Cd concentrations in the worms was reduced by a factor of 1.3 in lime-treated soils and 2.5 in MnO_2-treated soils. Cadmium uptake was weakly related to the free metal concentration $R^2_{adj} = 0.66$. Adding pH as an explanatory variable increased R^2_{adj} to 0.89, indicating that Cd uptake from pore-water is pH dependent, which might be attributed to competition of protons and Cd on the surface of the earthworm body.

van Straalen et al. (2001)	
Species	*Lumbricus castaneus, L. rubellus, L. terrestris, Aporrectodea caliginosa, A. rosea* and some arthropod taxa
Chemicals	Cu, Cd, Fe, Ni, Pb, Zn,
Soils	alluvial soils in a flood plain along a gradient of contamination, clay 20-32 %, pH 5.0-6.9, organic matter 8.9-10.4 %
Experimental design	Bioaccumulation experiment with contaminated soils (metallurgical factory)
Approach bioavailability	Evaluation of total, exchangeable ($CaCl_2$-extraction) and pore-water concentrations as predictors for metal uptake by different invertebrate taxa
Conclusions	Exchangeable metal concentrations were very low and were not correlated with the total concentrations in the soil, indicating low bioavailability of the pollution. Apart from the high species-specific variability the following general patterns were identified. Concentrations were high in earthworms, oribatid mites, and carabid beetles, and low in springtails, centipedes and spiders. There was no relationship between the trophic position of a species and its metal accumulating ability. The prediction of metal concentrations in invertebrates from total soil concentrations and soil properties seems to be limited to invertebrates that take up metals mainly from the pore-water such as earthworms and maybe small Collembola. For most saprophagous and predatory arthropods studied neither total concentrations nor exchangeable concentrations in soil are good predictors. Species-specific feeding mechanisms and metal physiology seem to be the main determinants.

The following conclusions can be drawn from the above summarised investigations on cadmium bioavailability (“biased” towards oligochaetes, for the reasons stated in chapters 3, 4.1.2 and 4.2.4):

The available heavy metal concentration for oligochaetes (as determined by mild electrolyte extractions, $CaCl_2$ and $Ca(NO_3)_2$) is more closely related to internal body concentrations than total concentrations in soil. This good coincidence holds only for animals that are closely related to the pore-water (worms and small Collembola), not for "hard-bodied" arthropods that are isolated from the pore-water by their hydrophobic cuticle. For these, neither total nor electrolyte extracts are good predictors for bioaccumulation. Ion exchange membranes coated with metal chelators as a "biomimetic approach" revealed a less good relation to bioavailability than electrolyte extracts.

The EPT (see previous chapters) is a frequently used and promising concept for predicting metal bioavailability, which means that also for heavy metals the exposure via the pore-water is regarded the essential pathway. However, for some metals (in particular those that become toxic only at relatively high concentrations and are essential for organism biochemistry: Cu, Zn), soil parameters have no influence on the equilibrium between the compartments soil matrix, pore-water and internal body concentration, for here regulatory mechanisms by the organisms counteract. In these cases bioavailability (esp. bioaccumulation) must not be considered without toxicity; combined experiments are necessary.

The calculation of bioaccumulation factors (BSAF: ratio of internal body concentration / total soil concentration) provides a possibility for the prediction of metal uptake / toxicity. These factors are calculated by means of multivariate equations based on various easily measurable soil parameters such as pH, cation exchange capacity (CEC), dissolved and solid organic matter content in soil, clay content and metal oxides, the first two being most important.

4.5.2.2 Availability of cadmium for plants

Availability of heavy metals for plants has been investigated mainly with respect to "human health". The accumulation of heavy metals such as arsenic (As), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), zinc (Zn) into food products or the transfer within the system soil-food plant-man is the focus of interest (McLaughlin et al., 2000). Special attention is drawn to cadmium as the only metal that can, via the food chain, result in health risks for humans and animals without acting toxic in the plant tissue (Peijnenburg et al., 2000).

Compared to other contaminants or exposed organisms, respectively, the standardisation of the determination of plant-available metal concentrations is relatively advanced.

The handbook for soil protection (Prüeß, 1994) propagates the extraction with 1M ammonium nitrate solution. From a literature review, the author concludes that the choice of the salt has no general influence on a method for assessing accumulated concentrations in plants, yet $CaCl_2$ and $NaNO_3$ solutions can be problematic in alkaline soils. Furthermore, the ammonium nitrate extraction allows for less disturbed measurements in the routinely used graphite furnace atom absorption spectrometry (G-AAS), because of more complete burning during flame ionisation as compared to many other solutions. Details on the extraction procedure are given by Prüeß (1994). In contrast to this, Jackson & Alloway (1991), Houba et al. (1996) and Peijnenburg et al. (2000) conclude that $CaCl_2$ extractions are the best method for predicting plant-available metals (see Tables below), although also these authors used G-AAS.

Welp et al. (1999) recommend 1M NH_4NO_3 solutions as best extractant for determining the *actual soluble* heavy metal fraction. Metals extracted with this solution (As, Pb, Cd, Cu, Mn, Ni, Tl, V, Zn) significantly correlated with metal concentrations in wheat and other plants. The authors propagate regression equations for the prediction of heavy metal concentration in plants, the derivation of threshold / trigger values for heavy metals in soils and for the evaluation of (de-)contamination of soils. For the identification of the potentially available metal pool they recommend complexing solutions such as DPTA or EDTA. Whether actual or potential available concentrations should be used (cf. workshop discussion, attachment) depends on the question under study and has to be decided for each case individually by the responsible legislative authorities (McLaughlin et al., 2000). Although the authors regard the description of the heavy metal transfer from soil to plant based on the mobile fraction a principal progress as compared to total soil concentrations they point out gaps in current knowledge, especially concerning reaction kinetics of further soil and plant parameters, ion concentrations, speciation in the dissolved phase and antagonistic or synergistic effects during plant uptake.

Whereas in Germany ammonium nitrate extractions are a fixed standard for predicting plant-available metal concentrations (DIN 19730) other countries have different standards (neutral salt solutions). Jackson & Alloway (1991) prefer $CaCl_2$ extractions due to their strong correlation with internal concentrations in plants. The (dis-)advantages of the various neutral salt solutions have been extensively reviewed by McLaughlin et al. (2000). Although the author mentions problems of ammonium nitrate extractions for determining cadmium in alkaline soils in Canada he suggests no alternatives that would not be accompanied by other problems (see discussion above and Prüeß, 1994).

The use of ion exchange resins as extraction method is less common and still far from commercial use (McLaughlin et al., 2000). In a study cited therein (Jing and Logan, 1991) Chelex^{TM} resin pellets were packed in dialyse bags for analysing the plant availability of cadmium in 17 different sewage sludges spread on soil, revealing no significant advantages as compared to classical extraction methods. Also variations of this method (like pre-treatment of the resin membranes with DPTA or the use of DGT) either no relations between extract concentrations and plant concentrations were found, or field studies with a large number of "truly" contaminated soils were missing. Becquer et al. (2002) tested ion exchange resin bags in field studies and compared the Ni extracts with the Ni concentrations in five different plant species. In contrast to McLaughlin et al. (2000) they judged their method appropriate for routine measurements. However, the summary (the article itself was not available yet) does not mention how many soils were tested and if they were aged or freshly spiked. Also the studies by Zhang et al. (2001, details see Table) and Ernstberger et al. (2002) revealed positive results for the DGT method. Besides better correlation of the plant extracts with the DGT extracts as compared with free ion concentrations in the soil solution and in EDTA extracts the authors saw two more advantages of their method: 1. minimal disturbance of the soil structure and 2. a mechanism similar to the uptake of contaminants via the plant root, associated with a dynamic desorption of metals from the solid phase due to decreasing concentrations in the soil solution in the surrounding of the DGT units.

Zhang et al. (2001)	
Species	*Lepidium heterophyllum*
Chemicals	Cu
Soils	21 soils differing in metal contamination and 8 (six years before the study) spiked soils from liming experiments; pH 4.2-8.0, total carbon 9.8-69.8 mg/g
Experimental design	Evaluation of the DGT technique (diffusive gradient in thin films) and of the effective concentration concept
Approach bioavailability	Comparison of effective concentration to EDTA extraction (free ion activity) and pore-water concentration for the prediction of uptake by plants
Conclusions	The effective concentration values provided by the DGT technique shows a closer correlation with internal plant concentrations than the conventional extraction methods. The uptake mechanism is obviously similar to the uptake by the plant roots. The DGT technique takes into account the ability of the soil to sustain the pore-water concentration following depletion by uptake, a phenomenon which is neglected by measurements of the metals in the soil solution. The DGT technique is an *in situ* method with minimal soil disturbance.

Peijnenburg et al. (2000)	
Species	*Lactuca sativa*
Chemicals	As, Cd, Cr, Cu, Ni, Pb, Zn
Soils	17 field soils differing in metal contamination (wide range of soil characteristics but no data) and 2 spiked artificial soils
Experimental design	Suitability of the $CaCl_2$-extraction for the prediction of metal concentrations in plants
Approach bioavailability	Evaluation of a neutral salt extraction for the prediction of the plant available metal fraction
Conclusions	The $CaCl_2$-extraction is recommended as the best method for the prediction of the plant available metal fraction, but the method is not compared to other extraction techniques.

Jackson & Alloway (1991)	
Species	*Lactuca sativa, Brassica oleracea*
Chemicals	Cd
Soils	20 soils manured with sewage sludge differing in metal contamination; pH (H_2O) 6.5, organic matter 16.9% (averages)
Experimental design	Evaluation of three solvents ($CaCl_2$ or NH_4NO_3, EDTA-Na_2) for the prediction of plant available cadmium and comparison of limed and control treatments
Approach bioavailability	Extraction methods to predict plant availability
Conclusions	The $CaCl_2$-extraction was the most suitable method to predict cadmium uptake by lettuce and cabbage. Liming always reduced Cd availability to plants.

While any salt or solvent extracts represent only the actually available metal concentration and are technically very artificial compared to plant uptake, ion exchange resin techniques (e.g. DGT) integrate over a longer period of time and provide the possibility of releasing metals from the solid phase, thus simulating plant uptake much better. The opinions on the development stage of the method, aiming at commercial use, are divergent: Whereas McLaughlin et al. (2000) demands a validation in comparison with other procedures and a multitude of “naturally” contaminated soils, Becquer (2002) regards his method as “appropriate for use in routine analyses”.

4.5.2.3. Availability of cadmium for Collembola

Crommentuijn et al. (1997)	
Species	*Folsomia candida*
Chemicals	Cd
Soils	Spiked artificial substrate (OECD, 1984a); pH (KCl) 3.1-7.0, organic matter 2.0-10.0%
Experimental design	Complete three factorial design (pH, organic matter, Cd concentration) with 6 treatments per factor
Approach bioavailability	Bioaccumulation, Validity of the pore-water hypothesis (van Gestel, 1997)
Conclusions	Water soluble Cd concentrations increased with decreasing pH and decreasing organic matter content for the artificial soils studied. Accumulation of Cd by *F. candida* was not related to pH and organic matter content. So water soluble Cd does not reflect bioavailability. This hampers a straight-forward prediction of metal toxicity using the pore-water hypothesis. The interpretation of the data is complicated by the fact that soil parameters affecting Cd pore-water concentrations also acted as stressors themselves (reproduction of *F. candida* decreases at pH > 6). The toxicity of Cd is not only determined by the internal Cd concentrations but also by differences in soil characteristics.

van Gestel & van Diepen (1997)	
Species	*Folsomia candida*
Chemicals	Cd
Soils	(OECD, 1984a) substrate
Experimental design	8 different treatments with soil moistures of 25-162% of the field capacity, spiked with 7 Cd concentrations of 0-2560 µg/g d.s.
Approach bioavailability	Influence of soil moisture on the toxicity and bioavailability of cadmium; measurement of cadmium concentrations in soil, pore-water, ammonium acetate extract, and in collembola
Conclusions	Cadmium sorption to the soils, estimated from water-soluble concentrations, was not significantly affected by soil moisture. Significant effects of soil moisture were found for the toxicity of cadmium on body mass of *F. candida*. EC_{50} did, however, not differ by more than a factor of 2. Reproduction in the controls was strongly affected by soil moisture but EC_{50}s did not differ for the different moisture levels. Bioaccumulation of Cd was not significantly affected by soil moisture. Summarising it can be concluded that the soil moisture does not have a great influence on the bioavailability and toxicity of cadmium for *F. candida*.

van Gestel & Hensbergen (1997)	
Species	*Folsomia candida*
Chemicals	Cd, Zn
Soils	(OECD, 1984a) substrate
Experimental design	Influence of different Cd and Zn concentrations (alone or in combination) on survival, growth and reproduction of *F. candida*
Approach bioavailability	Interaction of Cd and Zn toxicity
Conclusions	The water solubility of Cd in the soil was significantly increased by the presence of Zn, whereas Cd did not affect the water solubility of Zn. In spite of this, uptake of Cd or Zn in the animals was not affected by the presence of the other metal, suggesting that water solubility does not determine the uptake of these metals in *F. candida*. The combined toxic effects of Cd and Zn on the growth of the springtails were antagonistic, while the effects on reproduction were additive. Whether the EC_{50} (or EC_{10} values) were calculated on the basis of the total, water-soluble or internal body concentrations does not make any difference for the conclusions.

Smit & van Gestel (1996)	
Species	*Folsomia candida*
Chemicals	Zn
Soils	11 contaminated and 1 control field soil and standard soil (LUFA); clay 1.1-3.3%, pH (KCl, adjusted) 5.5-6.5, organic matter 1.9-6.4% ; both control and standard soil were spiked with $ZnCl_2$
Experimental design	Comparison of spiked and contaminated field soils subjected to ageing
Approach bioavailability	Ageing effects and predictive value of a standardised laboratory test with artificial contaminated soils for effects in the field
Conclusions	In the spiked soils $ZnCl_2$ had a strong effect on reproduction, whereas the effects on growth were not conclusive. In the polluted field soils however, no relationship was observed between zinc concentrations and growth or reproduction. The water soluble Zn concentrations were higher in the artificially contaminated soils than in the polluted field soil. However, differences in water soluble Zn concentrations could not fully explain the differences in effects among the soils. Artificially contaminated soils should be used with caution in ecotoxicological risk assessment. It is recommended to calculate ecotoxicological data on the basis of pore-water concentrations.

Smit & van Gestel (1998)	
Species	*Folsomia candida*
Chemicals	Zn
Soils	Spiked field soil and OECD substrate (OECD, 1984a); clay 1.9-20%, pH (KCl, adjusted) 5.5-6.5, organic matter 2.4-10%
Experimental design	Comparison of the spiked OECD substrate and a spiked field soil subjected to 3 treatments: 1. control, 2. percolated with an amount of deionized water equal to four times the pore volume 3. ageing under field conditions for 1.5 years.
Approach bioavailability	Ageing effects and predictive value of a standardised laboratory test with artificial contaminated soils for effects in the field
Conclusions	Ageing processes and soil related factors, such as organic matter or clay content play an important role for the toxicity of Zn for *F. candida*. Effects of Zn on *F. candida* cannot be fully explained by accumulation, which indicates that the internal threshold concentration of Zn above which physiological functions are impaired is also dependent on the test soil.

Vijver et al. (2001)	
Species	*Folsomia candida*
Chemicals	As, Cd, Cr, Cu, Ni, Pb, Zn
Soils	16 field soils with different metal contamination clay 0.2-46.7%, pH ($CaCl_2$) 3.1-7.3, organic matter 0.3-35.0%, and a spiked OECD-soil (OECD, 1984a)
Experimental design	Determination of total concentrations, $CaCl_2$-extracts, pore-water and internal concentrations of metals in soils with a broad range of soil characteristics
Approach bioavailability	Correlation between bioaccumulation and the metal concentrations in different soil phases, multivariate regressions for the estimation of the influence of easily measurable soil parameters
Conclusions	The metals were assigned to three groups: 1. Essential metals (Cu, Zn), which are accumulated up to a fixed level. This level is maintained independent of the soil concentrations. 2. Metals, which were below the detection limit as body concentrations (As, Cr, Ni). 3. Metals showing a linear relationship between internal body concentrations and total concentrations in the soil (Cd, Pb). Solid, extractable, and dissolved metal pools as well as various sorbing soil phases contribute to the metal uptake by the springtails. In general terms the uptake by *F. candida* is statistically associated strongest to total metal pools and metal binding phases in the soils like carbonates, clay and oxyhydroxides. Finally it was shown that Cd accumulation in springtails exposed in spiked OECD soil differs in an absolute sense from accumulation in field soils. The extrapolation from OECD to field soils should be based on internal body concentrations

Lock & Janssen (2001)	
Species	*Folsomia candida, Eisenia fetida, Enchytraeus albidus*
Chemicals	Cd
Soils	A sandy and a loamy field soil and an (OECD, 1984a) substrate were spiked with $CdCl_2$; clay 1.0-20%, pH (KCl, adjusted) 4.4-6.3, organic matter 1.5-10%
Experimental design	Comparison of the spiked OECD substrate with spiked field soils (OECD, 1984a)
Approach bioavailability	Influence of soil characteristics on the toxicity (Although the title deals with ”bioavailability” the toxicity data are exclusively calculated on the basis of total soil contents. Neither concentrations obtained from specific extractions nor internal concentrations were determined).
Conclusions	The authors included literature data in their study because their own data basis (only 3 soils) was to small to evaluate the influence of soil parameters on the bioavailability. These additional data were to heterogeneous to calculate regression models. In contrast to Zn effect concentrations of Cd were always much higher than the background concentration. As the effect of ageing on bioavailability of Cd was never taken into account (because toxicity experiments were always carried out in freshly spiked soils) the effect concentrations may even be regarded as conservative. Furthermore, the zinc-cadmium ratio in soils is usually high, indicating that the risk of zinc toxicity for terrestrial invertebrates is expected to be usually much greater in comparison with cadmium toxicity.

Pedersen et al. (1997)	
Species	*Folsomia candida*
Chemicals	Cu
Soils	Spiked field soil and OECD substrate (OECD, 1984a); clay 1.1-3.3%, pH (KCl, adjusted) 3.8-5.4, organic matter 1.3-1.5%
Experimental design	full factorial design with 3 different Cu concentrations and 4 pH levels (only one pH level with 4 different soil moistures); measurement of Cu concentration in the pore-water (centrifugation), artificial rainwater, $CaCl_2$, HNO_3 extract, and internal body concentrations
Approach bioavailability	Influence of pH levels and soil moisture on the bioaccumulation

Conclusions	No effects of Cu (11-122 mg/kg) on the three fitness parameters (survival, reproduction and growth) of the springtails were found. Total soil copper was the best predictor of copper concentrations in the adult collembola and the inclusion of pH as a second factor improved the predictive value. Soil moisture had a clear effect on fitness parameters and copper accumulation. The pore-water hypothesis in its original form is not valid for copper and collembola, whereas the alternative hypothesis by van Gestel (1997) fits better. The exposure route (food or pore-water) for Collembola is not known. Further work should focus on exposure routes at toxic Cu concentrations and on species specific differences in bioaccumulation and toxicity.

The studies on availability of heavy metals for Collembola give answers to three essential questions. The first deals with the main exposure route for Collembola and which metal species has the best predictive power for bioavailability. Crommentuijn et al. (1997) and van Gestel & Hensbergen (1997) in spiked OECD-soils found no relation between Cd accumulation and pore-water concentrations. This is supported by Vijver et al. (2001) who in 16 field soils contaminated by a variety of metals and an OECD soil identified total soil concentrations and concentrations in metal-binding phases such as carbonates and oxyhydroxides as best predictors for bioaccumulation. Also Pedersen et al. (1997), investigating Cu accumulation in spiked field and OECD soils, found the best correlations with total soil concentrations. The authors identified exposure routes of Cu for Collembola as an important research need.

The second question addresses the transferability of experimental studies with newly spiked soils to aged contamination and field soils. Vijver et al. (2001) found that data from spiked OECD substrates could not directly transferred to field soils and suggests calculations on the base of internal body concentrations. This was also concluded by Smit & van Gestel (1996), who suggest the integration of pore-water concentrations into the calculations. Comparing freshly $ZnCl_2$-spiked soils and soils aged for 19 months, Smit & van Gestel (1998) noted an overestimation of the toxic effects by a factor between 5 and 8. The authors therefore suggest to base toxicity predictions for soils with unknown contamination history on pore-water concentrations.

The third central question aims at the modifying influence of soil properties on bioavailability. For OECD substrates the accumulation of Cd was not influenced by pH or organic matter content (Crommentuijn et al., 1997), nor by water content (van Gestel &

van Diepen, 1997). Pedersen et al. (1997) found that the prediction of Cu bioaccumulation based on total soil concentrations could be improved by integrating the pH. Also the soil water content influenced the accumulation. An extensive literature review by Lock & Janssen (2001) revealed a very heterogeneous data base so that model calculations based on the influence of soil properties were not possible.

Although the assumptions regarding exposure are contradictive, the following conclusion can be drawn from the studies: The main route of exposure for Collembola is - unlike for oligochaetes - not the pore-water, but is distributed among the different phases in soil, including solid, extractable, dissolved and various adsorbing phases (Vijver et al., 2001). There is urgent research need on identifying the relevance of the different soil phases from which metals are taken up by arthropods. This includes the availability for different life forms and species-specific differences within these. Investigations on metal concentrations of various invertebrate taxa in the nearness of metal works (van Straalen et al., 2001) and a literature study on the bioaccumulation of metals in invertebrates (Heikens et al., 2001) exhibit a wide range for the single taxa (factor 2-12). Data from laboratory experiments with spiked substrates can be transferred to field conditions by standardising them with respect to pore-water or internal tissue concentrations. Soil properties that can influence heavy metal bioavailability include pH values and metal-adsorbing phases such as clay, carbonates, oxyhydroxides and organic substance. Their influence with respect to Collembola is however controversial.

4.5.2.4 Availability of cadmium for microorganisms

Welp & Brümmer (1997)	
Species	Site-specific soil microorganisms
Chemicals	Cd, Hg
Soils	18 spiked field soils, clay 2-41%, pH ($CaCl_2$) 3.5-7.8, Corg 0.9-11.4%
Experimental design	The microbial reduction of Fe(III) oxides to Fe^{2+} ions was used as a parameter of microbial activity / toxic effects
Approach bioavailability	The concentration of the metals in the pore-water was deduced by Freundlich adsorption isotherms from the total concentrations. The toxicity of the metals was calculated on the basis of the effective doses (ED_{10}, related to total soil concentrations) and the effective concentration (EC_{10}, related to pore-water concentrations)
Conclusions	For Cd the ED_{10} varies by a factor of 19 and for Hg by a factor of 1000. The EC_{10} for Cd varies by a factor of 113 and for Hg by a factor of > 41. Although the variability of the toxicity of Cd is greater on the basis of pore-water than on the basis of total concentrations, it was concluded that the ecotoxicological risk must not be calculated on the basis of total concentrations. The authors assume, that the influence of soluble soil-borne substances on metal speciation is the main reason for their varying toxic potential in different soil solutions.

Wilke (1991)	
Species	Site-specific soil microorganisms
Chemicals	Cd, Pb
Soils	10 spiked arable soils and 9 spiked soils taken from a nature reserve area; clay 4-41%, pH ($CaCl_2$) 3.8-7.5, organic matter 1.0-13.5%
Experimental design	Determination of the inhibition of dehydrogenate activity and arginine ammonification as a measure of toxicity
Approach bioavailability	Effect of soil properties on the toxicity
Conclusions	Arginine ammonification tests were not suitable to show the microbial toxicity of both metals. The effect of Pb on dehydrogenate activity was mainly influenced by the concentration in soil solution. Thus all soil properties which determined the adsorption of lead correlated significantly with the dehydrogenate activities of the contaminated soils. Most important were pH and cation exchange capacity. The pH-value showed a negative correlation with the microbial toxicity. Therefore, correlation between Cd concentrations of soil solutions or clay content and dehydrogenate activity were only significant if partial correlation analysis was used.

Dar (1996)	
Species	Site-specific soil microorganisms
Chemicals	Cd, and other metals from sewage sludge
Soils	Clay-loam, loam, sandy loam; pH 6.5-8.1, Corg 0.5-8.9%
Experimental design	Determination of the microbial biomass and the inhibition of enzyme activities (dehydrogenate, alkaline phosphatase, and arginine ammonification) as measures of microbial toxicity
Approach bioavailability	Correlation between DPTA (complexing agent) extracts and observed toxicity
Conclusions	A significant negative correlation was observed between DPTA-extractable Cd and microbial biomass. This means, that the DPTA extraction could be a promising measure to estimate the metal content available to microorganism. No correlation existed between microbial biomass and arginine ammonification, suggesting that the arginine ammonification is no suitable indicator for the microbial biomass.

Leita et al. (1999)	
Species	Site-specific soil microorganisms
Chemicals	Cd, Cu, Ni, Pb, Zn
Soils	Sandy loam; clay 28.7%, pH 7.8, Corg 0.8%
Experimental design	Long-term effects (12 years) of municipal compost application on the bioavailability of metals as compared to different manure treatments
Approach bioavailability	Evaluation of the effect of total soil concentration (C_{tot}), total organic carbon (TOC), and microbial biomass (C_{mic}) on the DPTA-extractable “bioavailable” metals
Conclusions	A stepwise multiple regression shows, that the factors (C_{tot}, TOC, C_{mic}) have varying effects on the DPTA-extractable concentrations depending on the metal. Mechanisms, which determine metal bioavailability might be metal specific.

Prokop & Holoubek (2001)	
Species	*Bacillus cereus*
Chemicals	Cd
Soils	4 spiked soils (no soil data)
Experimental design	5 different $CdCl_2$ concentrations of 0.01 – 5.0 mg/kg d.s.
Approach bioavailability	Correlation between distribution coefficient K_p (soil/pore-water) and microbial contact toxicity
Conclusions	A significant correlation between contact toxicity and the partitioning of cadmium between soil and pore-water was found The microbial contact toxicity test might be a suitable tool for detecting cadmium bioavailability.

Although many studies deal with metal toxicity to microorganisms (cf. Giller et al., 1998), bioavailability is usually just addressed as a cause for the high variability in data. Comparative studies including different exposure routes or methods for the assessment of availability for microorganisms are unknown. Giller et al. (1998) note that also metals dissolved in pore-water can be bound as chelates or found in chemical species that cannot be taken up, thus being only partly bioavailable. It is general assumed that free ion activity is the chemical species that is taken up and causes toxicity. This has however been seldom shown, and there is hardly any literature on this field referring to microorganisms (Giller et al., 1998).

Many recent studies deal with genetically modified microorganisms as luminescent biosensors for identifying bioavailable metals in soils (Chaudri et al.,1999, 2000; Leth et al. 2002; Preston et al., 2000; Tibazarwa et al., 2001). These studies will be excluded here since they usually do not cover microorganisms naturally occurring in soils (as a protection goal), but only use microorganisms as – qualitative, not quantitative - bioavailability indicators under rather artificial conditions.

None of the five studies (which were the only ones we could find for microorganisms and metal bioavailability) summarised in the tables tries to compare to which extent the single metal species contribute to toxicity or bioavailability, and there are no comparative studies with respect to extractants. Welp & Brümmer (1997) and Prokop & Holoubek (2001) base on the assumption that pore-water is the main exposure route, for which metal concentrations depend on soil properties that determine their solubility. Dar (1996) and Leita et al. (1999) use DPTA as ion exchange device for measuring free metal ions and use these concentrations as a measure for the microbial availability.

As parameters for identifying toxicity to microorganisms, microbial biomass and different enzyme activities were used and correlated with metal concentrations. The only measured parameter not appropriate for such correlations was the arginine ammonification (Dar, 1996; Wilke, 1991). Another method for measuring enzyme activities is the microbial contact toxicity test (Prokop & Holoubek 2001), in which. dehydrogenate activity is measured photometrically by means of a colour reaction.

5. Discussion

5.1 Comparative evaluation of recent concepts on bioavailability in soils with respect to their applicability in the context of risk assessment and soil protection

For the integration of the present approaches on soil bioavailability into the risk assessment of chemicals they have to fulfil various criteria relevant for science and applications. These are basically identical with the requirements for, e.g., new test methods for effect assessment (Römbke et al., 1995):

a) Theoretical plausibility and relevance
b) Reproducibility and replicability
c) Suitability for standardisation
d) Appropriateness for soil contaminants
e) Status of documentation
f) Practical applicability

In the following we give a preliminary comparative evaluation of the examined approaches on bioavailability in soils with respect to these criteria, based on the approaches and methods raised in chapters 4.3 to 4.5 and exemplified by PAH and cadmium.

<u>Theoretical plausibility and relevance</u>

Suggestions on the assessment of bioavailability by means of mathematical partitioning and uptake models (EPT and extensions) are principally based on a more thorough theoretical concept than the experimental approaches. For the first, a definite exposure scenario is formulated which is - at least partly - supported by empirical evidence: “soil organisms are predominantly exposed via the soil pore-water“. At the same time this means a pronounced simplification of the complex exposure situation in soil. For example, the limitations of the EPT approach were shown by deviations of the calculated bioaccumulation in oligochaetes from experimental values (Belfroid et al., 1996; more hints on this problem can be found in chapter 4.5). Main restrictions of the EPT are especially the neglect of the exposure routes soil matrix, soil air and prey organisms (for predaceous soil organisms), which results in not taking into account, e.g., the uptake of highly lipophilic organic compounds adsorbed to soil organic matter or accumulated in prey organisms. Approaches focussing to soil pore-water are also inappropriate for volatile chemicals, because for their bioavailability primarily the concentration in soil air is relevant. Another principal restriction of the partitioning models is due to the dynamics of the processes involved in bioavailability: the theoretically assumed equilibrium between compartments does often not reflect the real exposure situation. This has to be kept in mind both with respect to the various transport and sorption processes in the heterogeneous soil matrix (e.g., slow diffusion in microprobes ore fast distribution via microprobes) and for a number of biological processes (microbial degradation in the pore-water and processes of uptake, transformation and excretion within the organisms studied; Sijm et al., 2000). Besides these critical points of the models, the second main source of error is the uncertainty with respect to the data base used for their calculation. This holds both for experimental data (soil adsorption coefficients, total concentrations of contaminants, soil organic carbon, pH etc.) and for estimated input data (in particular partitioning coefficients). This has been stressed by the German advisory board for existing substances (BUA) in a compilation of models for calculating soil / pore-water partitioning coefficients for heavy metals and organic compounds (BUA, 2001).

The majority of suggestions on experimental assessment of bioavailability lacks a plausible theoretical conception, for they are exclusively based on the empirical coincidence of results from physical-chemical extractions and measurements (see chapter 4.4) with biological observations (degradation, toxicity and bioaccumulation). Relating these methods to the actual exposure of organisms is especially problematic for the „mild“ extractions with various organic solvents, whereas extractions with water and neutral salt and desorption experiments conceptionally aim at the available and easily mobilisable fractions or the exposure route pore-water, respectively. In contrast to this, the “biomimetic approaches“ aim at mimicking more differentiated exposure scenarios, also here mostly addressing the same fractions that should be covered by the previous methods. The main problem of these methodologically defined bioavailability concepts is their strong dependency upon the respective experimental conditions, which questions the transferability of the results to other substances, soils and organisms, at least it requires considerable effort in method evaluation and standardisation. The general advantage of experimental approaches over the theoretical strategy is their realism ("hard data"). This holds especially true for the badly needed inclusion of the factor time into bioavailability assessments (potential bioavailability, see below), which is probably only realisable by experimental means. The possibilities of an inclusion of this temporal component in theoretical models seems to be questionable.

In summary, the general problematic of both the theoretical and the experimental strategies lies in the necessity to adequately include complex and often still unexplored processes (organism perspective!) in a substance-specific perspective for quantitative assessment. For assessing the fate of contaminants in soil the data basis usually consists of at least some essential abiotic parameters (substance and soil properties) which can be combined with model-theoretical knowledge on substance behaviour - yet detailed knowledge on the biological interdependencies is largely missing. The following two comments cited from literature reviews underline the widespread objections on both present strategies:

„From the foregoing, it is clear that no theoretical approach can adequately estimate the bioavailability of a substance in soil or sediment. Therefore, either organisms need to be used to study bioaccumulation from soil or sediment, or a biomimetic approach needs to be taken." (Sijm et al., 2000).

„It must be stressed that chemical methods to assess bioavailability can never be all-compassing. Since bioavailability (as discussed above) is organism and even species dependent, a single chemical test to determine bioavailability is an impossibility." (Reid et al., 2000a).

Reproducibility and replicability

The criteria reproducibility and replicability should be unproblematic for a wide-spread use of theoretical approaches for bioavailability assessment, as long commonly accepted and recommended models and valid input data are used. This refers only to the method of exposure assessment (calculation of available concentrations). For the experimental approaches examined here, the criteria reproducibility and replicability obviously have only been sufficiently fulfilled for the intensively studied availability of heavy metals for plants (chapter 4.5.2.2), whereas there is no sufficient data base for other exposure scenarios and organisms, especially for organic contaminants, which would allow for evaluating the diverse methods. The main findings (dependency upon experimental conditions, both over- and underestimation of the exposure with single methods; cf. chapter 4.5), reveal great research need.

Suitability for standardisation

A standardisation of theoretical approaches on bioavailability assessment is relatively simple since it requires only conventions on selection and application of models in the respective context (e.g., different models for (non-)ionisable organic compounds and heavy metals), including requirements on the selection, quality and quantity of input data. In contrast, a standardisation of experimental approaches requires high effort and a lot of time (extensive preparatory studies with a wide range of methods and experimental conditions, followed by ring tests). The identification of an appropriate standard set of methods for defined substance classes and exposure scenarios is of central importance (Harmsen et al., 2001).

Appropriateness for soil contaminants

As discussed above, the bioavailability assessment for soil organisms by means of partitioning models (EPT and extensions) is only appropriate for contaminants and organisms that are primarily exposed via the water phase (see also chapter 4.3), but not for highly hydrophobic organic chemicals (BUA, 2001). The advisory board for existing substances (BUA) recommends the EPT method for the (comparable) calculation of effect concentrations of relatively well water soluble organic compounds (log $K_{oc} < 4$ or log $K_{ow} < 5$) in soil. Also equilibrium partitioning models cannot be recommended unrestricted for heavy metal availability in soil, although for this substance group the pore-water is regarded the main exposure route for most soil organisms (chapter 4.5.). Restrictions of this approach must be made because of the complex sorption processes (speciation) and because of difficulties in effect assessment such as, e.g., the regulation of the body concentration of Cu and Zn by oligochaetes. Whereas for the theoretical assessment a range of validity for the contaminants seems to be at least roughly identifiable, for the experimental approaches no such range or common sense (of applications and methods) can be found, except for plant available metals in soils. Extensive studies are required for standardisation (see above), yet due to the large complexity a universal method cannot be expected - rather different procedures for certain contaminant classes and exposure situations will have to be established (Harmsen et al., 2001).

Status of documentation

The documentation of bioavailability assessment of selected contaminants on the base of EPT is relatively scarce and restricted to the primary literature. There are no systematic compilations or data sets, although this should be possible after some transformations (see Hammel & Herrchen, 1999**)**. Except for plant-available heavy metals (see chapter 4.5.2.2), the present status of documentation on experimental approaches is insufficient. Two publications dealing with this are currently being prepared by SETAC (Society of Environmental Toxicology and Chemistry) members (Alexander et al., in press; Lanno et al., in press). Furthermore there are two published position papers commenting experimental methods, one of them in written Dutch (Harmsen et al., 2001; Sijm et al., 2002). Besides these works, the primary literature is hard to oversee. Also we are not aware of a database with extensive documentation of experimental results - which would be necessary for systematically surveying the state of present knowledge.

Practical applicability

In practice, EPT and extensions can presumably be used for assessing bioavailable contaminant concentrations in soils with relatively little effort of time and costs. The only preconditions are well-founded recommendations for calculation defaults on the partitioning of different contaminants in soil and the necessary basic data (soil and substance properties). In contrast, the practical use of experimental approaches requires considerably more effort (equipment, consumables and personnel), especially when it comes to standardisation (comparison with at least one “established” standard such as total soil concentration).

Our study shows that, on the base of present knowledge, the appropriateness of neither theoretical nor experimental approaches on bioavailability of contaminants in soil for risk assessment of chemicals cannot be conclusively judged. Only in recent years (starting around 1995) environmental chemistry and ecotoxicology started to draw more attention to the bioavailability concept, and the experimental design of studies has only inertly started to reflect its integrative character (cf. Fig. 1a). On the one hand this expresses a theoretical deficit (particularly visible from the lack of a commonly-accepted definition, see chapter 2), on the other hand there is a growing diversity of experimental methods without a recognisable trend for standardisation. Also, the theoretical bioavailability assessment by means of partitioning and uptake models cannot generally replace experimental studies. Because no general standards are available, at present it is critical to implement bioavailability into existing strategies for risk assessment of contaminants in soils. To overcome the discussed restrictions, more and interdisciplinary research efforts are necessary (suggestions see chapter 5.3).

5.1 Applicability of bioavailability concepts: evaluation

5.2.1 Bioavailability and risk assessment

Risk assessment of chemicals within the European Union is based on a quotient of the estimated concentration of a substance in an ecosystem [compartment] (PEC = predicted environmental concentration) and the estimated contaminant concentration that causes no toxic effects on the organisms living therein (PNEC = predicted no effect concentration). If the expected exposure (PEC) exceeds the assumed effect concentration (PNEC), i.e. PEC/PNEC > 1, a risk is assumed and measures have to be taken to reduce it. The PEC for (mg / kg dry soil) soil is based on a quantitative exposure analyses of the chemical, which takes into account produced amount, imission routes, substance behaviour, degradation and (if existing) monitoring data. The associated hazard analysis compiles and compares existing toxicity data for the chemical, preferably derived from international (OECD, ISO) standard terrestrial ecotoxicity tests. The PNEC (mg / kg dry soil) is derived from available test data by identifying the lowest NOEC (no observed effect concentration) or ECx (effective concentration, resulting in x % effect) value, respectively, and dividing this by an extrapolation factor that decreases with increasing data quantity and quality. Detailed information on risk evaluation of substances in soil according to the EU Technical Guidance Documents can be found in

TGD (1996), Ahlers & Krätke (1998) and BUA (2001). Soil quality criteria or threshold values, e.g. for the German soil protection law and the associated regulation, are derived by following a comparable scheme (BBodSchG, 1998; BBodSchV, 1999). However, here target values (for measures, tests and precautionary principles) are derived by taking into account the various soil functions, with only the precautionary values aiming at sustaining the multifunctionality of soils (Frische u. Mathes, 1999). Both substance- and soil-related risk evaluation are characterised by only partly or insufficiently considering bioavailability; rather total soil concentrations than any more detailed (relevant) fractions are included in exposure and hazard assessment. Corrections with respect to bioavailability are seldom made, for instance (i) in the TGD for the normalisation of effect data in different soils to a standard soil with a defined organic carbon content, (ii) in precautionary values for the German "Bodenschutzverordnung" (considering different sorption properties with respect to soil type and organic matter content for some heavy metals and organic contaminants) or (iii) when fixing extraction procedures (incl. threshold values) for the availability of heavy metals for plants. Thus, chemical regulation and soil protection reflect the limited knowledge (i.e. no well-founded strategy, insufficient data base) on bioavailability. The BUA summarises in their suggestion for risk assessment: "For determining the exposure of organisms, the bioavailability of contaminants in soils is an essential parameter. Since bioavailability depends both on substance properties and on the respective soil properties and the uptake route of the organisms under study, a sufficiently exact assessment is at present not possible." (BUA, 2001 [translated from German]). Although the BUA was also sceptic towards partitioning models for assessing bioavailable concentrations, these approaches are possibly an important base for future risk assessment discussions. Hammel & Herrchen (1999) and Hammel (1999) exemplarily demonstrate possibilities for applying this theoretical strategy. The authors' concept endeavours a more detailed consideration of the various exposure routes for soil organisms, aiming at calculating bioavailable effect concentrations from toxicity tests with various soils and organisms which then should be normalised for being comparable in a standard scenario. The advantage of this approach is that the already existing pool of (eco)toxicological data could be used for a meta-analysis with respect to bioavailability. Such a scheme would aim e.g., at generating data for a species sensitivity distribution and various contaminants based on normalised data, or at comparing results of present risk evaluations (base: total concentrations) with those derived from normalised "bioavailable" data. However, since existing data are often uncomplete a broad applicability of such an approach seems unrealistic (Hammel & Herrchen, 1999). A comparable suggestion for normalising existing ecotoxicological data for pore-water concentrations of heavy metals was presented by McLaughlin (1999) during the SETAC-Europe conference 1999 in Leipzig. The author emphasised the use of such re-calculations for critically evaluating the relevance of existing data.

Unlike theoretical partitioning models, the different experimental approaches (see chapter 4.4) for bioavailability assessment at present do not allow for integrating them into risk evaluation (exception: plants / heavy metals). A clear (though less critical) application potential is given in remediation research, predicting long- and short-term degradation potential of organic contaminants for microorganisms.

Once more it has to be stated that still much research is needed for replacing the "crutch total concentration" by "bioavailable concentration" for exposure assessment in soils. The conception of future research in this field requires a "turn in ecotoxicological awareness" from the primarily abiotic to the more biotic perspective (cf. Fig. 1b), which can only be achieved by interdisciplinary co-operation between chemists, biologists and soil scientists. This will only be substantially fostered when obligatorily required for project funding.

Considering the manifold objections it has to be critically discussed if the large effort required for integrating bioavailability into risk assessment is justified - in particular when taking into account that the error of the present procedure usually (but not generally - there are exceptions!) overestimates risks of chemicals, thus having a protective character (Alexander, 2000).

5.2.2 Bioavailability and the Critical Loads Concept

The critical loads concept in context with metal bioavailability can almost exclusively be found in the "grey" literature (Curlík et al., 2000; de Vries & Bakker, 1998; Gregor et al., 1998, 1999). Since these articles do not unequivocally use terms and concepts, we will initially put emphasis on definitions. The critical loads concept was developed in the early 70ies in Canada (Hornung et al., 1995) and further refined by the United Nations Economic Commission for Europe (UN/ECE) within the context of water and soil acidification. They defined "critical load" as follows:

„A quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified elements of the environment do not occur according to present knowledge" (Nilsson & Grenfelt, 1988 in Hornung et al., 1995). A further definition, found on the homepage of the enterprise ECOmatters (http://www.granite.mb.ca/~sheppard/projects.html), leads to the "Critical Limits" concept: „The critical load was defined as the flux (deposition of contaminants from the atmosphere), that will increase soil or water concentrations to a level, that approaches but does not exceed concentrations, where ecotoxicological effects may be observed." These concentrations correspond to the critical limits, also called "critical levels". In an UN/ECE "Workshop on critical limits and effect-based approaches for heavy metals and persistent organic pollutants" they were defined as follows (Gregor et al., 1998): "A Critical Level is defined as the concentration of a pollutant in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials, may occur according to present knowledge". Hornung et al. (1995) address further important aspects of the concept: "The critical load concept provides a receptor-based approach to controlling emissions of pollutants, the principle being that the ecological impact of given pollutant emissions depends on where the pollutant is deposited." The "receptor" can be an ecosystem (forest, field, lake...) or an organism.

During the above-mentioned workshop the threshold values for heavy metals in general had not been effect-(receptor-)based and thus not adequate for the protection of ecosystems. The calculation of effect-based critical loads was one of the aims of a series of workshops and methods papers arranged by the UN/ECE (Curlík et al., 2000; de

Vries & Bakker, 1998; Gregor et al., 1998, 1999). A further aim was the calculation of toxicological data on the base of pore-water concentration or free ion activity instead on total concentrations. These phases were regarded the main exposure routes for soil organisms and were selected because the measurement of internal body concentrations is not possible in routine analyses (Gregor et al., 1998). Also pore-water concentrations or ion activity should not be measured directly but calculated on the base of total concentrations, soil properties and transfer functions.

The reason for this is the applied approach of the critical load concept, aiming at quickly achieving results for practical recommendations. At the present state of knowledge this implies simplifications that are prone to falsify results for a number of situations and organisms (e.g. pore-water is assumed as main exposure route, which does not hold for Collembola - see discussion in chapter 4.5.2.3). Besides this, an increase of threshold values based on the assumption that only a part of the total concentration in soil is bioavailable, cannot be justified according to present knowledge: too many unknown factors affect critical limits (Curlík et al., 2000). These factors comprise, e.g., interactions with other metals, ions or complexing substances, receptors more (or less) sensitive than the standard test organisms, chronical effects, food chain effects, global change (warming, acidification, land use...). In summary, the calculation of transfer functions (which is quite advanced in some countries) can be useful for calculating pore-water concentrations in the context of bioavailability studies. On the other hand, further basic research on bioavailability will help in refining and improving the critical load concept.

5.2 Research needs

This chapter summarises essential research need on bioavailability of contaminants in soils as revealed by our study.

Biodiversity and exposure

Until know, bioavailability has been investigated only for a very limited set of soil organisms (chapter 4.1.2), dominated by organisms recommended for standardised terrestrial (eco)toxicity tests - with all their well-known limitations. On the other hand there are no or hardly any information for many ecologically relevant soil organisms, e.g. for fungi and mites. There is a general need for expanding the present knowledge on bioavailability at least to all relevant organism groups (irrespective of the differences within one group, which can be enormous). For principal reasons it is however not possible to experimentally study all species. An extrapolation of species-specific results to functional groups is crucial for integrating bioavailability to risk assessment of chemicals in soils (cf. Mathes, 1997; Frische and Mathes, 1999). Because bioavailability is thought to be species-specific (Reid et al., 2000a), and due to the huge biodiversity in soil, the question has to be asked: “Is it possible to identify main exposure routes for whole groups of organisms, and to what extent can standard test organisms adequately represent them?“. Hammel (1999**,** see Tab. 2) provided a first base of discussion. A further examination and improvement of this list (sound evidence for the species mentioned, addition of other species) is crucial for a solid evaluation of extrapolation possibilities - implying extensive research need. Empirical studies ideally should follow a systematic design with respect to organism groups, exposure routes and chemicals. The evaluation of studies on bioavailability of PAH and cadmium for plants, oligochaeta, Collembola and soil microorganisms (chapter 4.5) mostly coincided with the grouping in Tab. 2. However, in two cases contradictions became obvious: The relative significance of the soil pore-water - regarded as main exposure route - for plant availability of PAH remained unclear. Ditto unclear was the main exposure route for Collembola: studies on PAH hint at pore-water, but for cadmium (or heavy metals in general) this has to be questioned.

In this context it has to be examined to what extent single species can be used for predicting bioavailability to other organism *groups* (with different routes of exposure). The availability of anthracene was higher for bacteria as compared to a lumbricid species (*Eisenia*) and to wheat plants (Tang & Alexander, 1999). Whether bacteria in general are appropriate for predicting the bioavailability of contaminants in soil also for higher organisms can only be answered by additional studies.

Spatial and temporal scale of the exposure

Bioavailability has to be considered as small-scale process taking place at phase boundaries of a contaminated soil and the exposed organism (chapter 2), resulting in a spatial-temporal singularity of each exposure situation. Ultimately, it is not possible to adequately operationalise the concept „bioavailability“ - it can only be approximately estimated by means of theoretical or experimental approaches (chapters 4.3 - 4.4). To what extent spatial and temporal dimensions of the exposure can be taken into account will have great impact on assessment quality, as we summarise in the following.

Due to short- and long-term dynamics of distribution, sorption and degradation pro??cesses of both contaminants in soil and of the organisms (growth, development stage, behaviour, population development etc.) bioavailable contaminant concentrations are subject to permanent change. This was extensively discussed during our workshop (see attachment), ending up in differentiating “actual bioavailability“ and “potential bioavailability“. The actual bioavailability represents the actual exposure concentration for an organism (point measurement) whereas the potential bioavailability integrates over a longer period of time. This includes not only the actual pore water concentration but also processes of desorption and remobilisation, i.e. the accumulated amounts of the contaminant that are available over this period. The actual bioavailability of both organic contaminants and heavy metals can be assessed by all approaches related to soil pore water, mainly the EPT (cf. chapter 4.3) and various experimental approaches (e.g., „biomimetic approaches“ or the measurement of free metal ions). Again, the results will strongly depend on the chosen experimental conditions (cf. chapter 4.4). For comparatively assessing the predictive power and applicability of these approaches, more experimental studies are needed. During the workshop, the actual bioavailability was thought to be decisive for the accumulation of soil contaminants and for the toxicity of a contaminated soil. Again, we have to stress that this approach is only valid for organisms (mainly) exposed via the pore water.

In contrast to this, the potential bioavailability was regarded as a relevant indicator for the biological remediation potential of contaminated soils and for the long-term risk of contaminants. The integration of the temporal dimension in this case is not (yet) possible by theoretical approaches but still requires extensive experimental studies. For determining potentially bioavailable contaminants, diverse methods were suggested, in particular methods based on „mild“ extractions with, e.g., organic solvents (for organic compounds) or neutral salt solutions (for heavy metals) (cf. chapters 4.4 and 4.5.1.4). Physical-chemical characteristics of the contaminant under study and aim of the investigation (biological degradation or ecotoxicological risk) determine the selection of the respective method. To make them applicable, a restriction to very few relevant and standardised methods is necessary. This can only be achieved by further systematic experimental studies.

In summary it has to be stressed (once more) that the differentiation between actual and potential bioavailability is arbitrarily defined and also requires experimental methods on a continuous scale between point measurements of actual exposure concentrations and total soil concentrations. The factor time is particularly relevant for the bioavailability of aged contamination. The consequences of these ageing processes for bioavailability have been repeatedly described in particular for organic contaminants (cf. chapter 4.5.1). It has to be taken into consideration that (i) most of these studies were conducted with spiked soils followed by artificial ageing (usually for max. 100-200 days), (ii) the relevant processes up to now are only partly understood (Marschner, 1999) and (iii) the knowledge on bioavailability of historically contaminated field soils is very scarce (which holds especially for so-called “bound / non-extractable residues“).

Another problematic aspect of bioavailability assessment is the spatial heterogeneity of contaminated soils. This holds both for contaminated field soils and the transferability of experimental laboratory results (which usually investigate homogenised, spiked soils) to the field situation. Small-scale differences both in vertical and horizontal contaminant distribution and in soil properties which influence the bioavailability are typical of historically contaminated soils. This means, e.g., that the evaluation of contaminant bioavailability in such soils depends on the sampling design (single or pooled samples, spatial and temporal resolution) and on the preparation of the samples (mixing, sieving, elution etc.). A balance between effort for sampling and analyses (costs!) and representativeness has to be found for every individual case. Because of the singulary character of each soil sample furthermore a detailed description of the relevant soil properties is crucial. This demand is another result of our literature evaluation, which showed that soil properties were insufficiently described in many studies (chapter 4.1.3). The minimal set of parameters should include main soil type, texture, bulk density, soil water content, pH, organic carbon content, whenever possible also cation exchange capacity or sesquioxide contents (Hammel & Herrchen, 1999). For perspectively predicting bioavailability within the context of prospective evaluation of chemicals, a (still to be identified) set of representative soils for routine use in ecotoxicity soil testing is recommended. These should cover a broad spectrum of soil properties (representing the geographical range they should cover, e.g. Europe) and could be tested in parallel to the widespread OECD soil substrates (1984a, b). Such a scheme aims at better taking into account realistic exposure scenarios in toxicity tests and perspective risk assessment.

Bioaccumulation, toxicity and risk

Our study revealed that bioavailability was less frequently studied in the context of toxic effects than in the context of bioaccumulation (cf. Tables 4.2.4a and b; the special case of biodegradation is excluded here). This bias is critical with respect to the comparable ecotoxicological relevance of bioaccumulation and toxicity. The usual concept of bioaccumulation experiments covers a range of (spiked) concentrations that are below the (at least acute) NOEC level, or the uptake process itself is in focus. In contrast to this, the risk evaluation of existing and newly notified chemicals according to EU legislation (TGD, 1996) is based on effect data derived from (eco)toxicological tests. Further investigations are necessary to better understand the interdependencies between bioavailability and toxicity, with special respect to (estimated or measured) bioavailable contaminant concentrations as related to internal effect concentrations, since toxicity (in most cases) does not occur without uptake into the organism. For that purpose combined experiments on bioaccumulation and toxicity are recommended. The potential use of the concept „internal effect concentrations“ for ecotoxicological risk evaluation has been extensively discussed by Sijm et al. (2000). Using an example with a number of organic chemicals of comparable mode of toxic action, the authors showed that internal effect concentrations were distinctly less variable than the respective external effect concentrations. However, uncertainties exist concerning the variation of internal effect concentrations both within populations of one species and between species, and concerning the dependency of this parameter upon time and concentration. Restrictions of the concept are also discussed for metals: here, the regulation of (in traces) essential metals like copper and zinc (Peijnenburg et al. 1999b) and possible adaptations to contaminants in exposed organisms (resulting in higher internal effect concentrations) are of interest (cf. workshop conclusions, attachment). The applicability of this concept is also restricted due to the methodological effort for chemical analysis of internal effect concentrations - especially with decreasing body size of the organisms. Also calculations using partitioning and uptake models (EPT) or „biomimetic approaches“ (cf. chapter 4.3 and 4.4) can be used for the assessment of internal effect concentrations. For the reasons above, at present the implementation of internal effect concentrations into risk evaluation of chemicals in soil is not possible.

Bioavailability and soil biology

As already mentioned in the context of defining bioavailability (chapter 2), the present studies often consider the organism perspective insufficiently. Besides the problems in extrapolating standardised laboratory tests to real conditions (with respect to exposure), there is ample research need concerning the following soil biological questions:

- Can avoidance behaviour of organisms (e.g., in colonisation experiments, Filser et al., 2000) be used as an early-warning indicator for the bioavailability of contaminants in soil?
- How can the exposure route „food“ be adequately addressed in soil animals, in particular with respect to the widespread omnivory in this group (Filser, 2001)?
- How can the dynamics of the biological processes (life cycles etc.) be adequately addressed?
- Which influence has the organism density (abundance) on bioavailability in contaminated soils?

These questions aim at an increasing understanding of bioavailability in the ecosystem context. For better evaluating ecotoxicological risks or hazards, respectively, of bioavailable contaminants in soils, definitely more investigations on higher levels of biological integration (than the test population of one species in a biotest) at more realistic exposure conditions are required. Soil biological monitoring along contaminant gradients or recolonisation experiments in the field or under controlled (semi-field) conditions could be promising approaches.

Soil contaminants

As shown in chapter 4.1.1, in relation to the large number of potentially environmentally (soil) relevant organic chemicals (note: the European list of existing chemicals EINECS contains 100,000 substances) only few studies especially deal with bioavailability in soil. This reflects the general research deficit concerning environmental risks of organic compounds. To identify priorities for future research needs on bioavailability in soil, the relevant imission routes (e.g., sewage sludge for detergents or slurry for animal medicals) have to be investigated in combination with the intrinsic toxic potential of the chemicals. The example of pesticides shows that this is connected to a considerable conflict potential for the risk discussion: in this case bioavailability and resulting biological effects are *desired,* although - wherever possible - specifically for pest organisms. For an (at least orientating) theoretical bioavailability assessment in soil - regarding the high number of organic chemicals - quantitative structure activity relationships (QSAR) could be an appropriate approach: the potential bioavailability could possibly be estimated for defined exposure scenarios (e.g., different soils) on the basis of molecule structure and physical-chemical substance characteristics. Conceptionally, the expected partitioning of the chemicals over the various exposure routes - with special respect to interactions with the inorganic and the organic soil matrix - should be associated with a model on bioaccumulation. It has to be analysed in detail if the statistical data base is sufficient for calculating quantitative models. A principally comparable approach is used for assessing biological degradation of contaminants in EU risk assessment (TGD, 1996), if no experimental data are available. Aside from the principal restrictions of theoretical approaches, the scientific examination of such an instrument could stimulate the discussion on risks of contaminants in soil.

However, there is research need not only concerning the quantity of investigations on bioavailability in soil. Several environmentally relevant questions thus far have not or only seldom been addressed:

- Which influence has multiple contamination of soils with various chemicals (mixture contamination: combinations of several organic compounds, metals, or both) on the bioavailability of single substances?
- Which influence has repeated (sequential) contaminations of a substance on bioavailability, which is typical of many field situations? This questions is particularly related to the long-term sorption capacity of soils.
- Which role play metabolites of a contaminant with respect to bioavailability in soil? Can they be integrated in one assessment?
- Which influence has the physical state (dissolved, crystalline etc.) of the contaminants on the bioavailability in historical contaminations?

In the end of this study, one has to ask if a combined (and thus relatively complex) risk indicator like „bioavailability in soil“ renders advantages in comparison with “classical” indicators that are based on substance characteristics alone (distribution coefficients, bioaccumulation potential, toxicity). We also dare to question if the bioavailability concept in general is more appropriate for assessing risks of chemicals in soils than the conventional method based on total concentrations: the latter appears sufficient from a precautionary point of view since it rather overestimates the risk of chemicals. At least the necessity of a (more detailed and thus more expensive) bioavailability approach must be clearly identified. For instance, bioavailability is important for the cultivation of food plants on (more or less) contaminated land, which is the case for many arable soils, e.g. due to heavy metal accumulation resulting from Cd-contaminated phosphate fertilisers or in permanent cultures after extensive use of copper fungicides).

6. Summary

This literature study, financed by the Federal Environmental Agency (Umweltbundesamt, UBA) of Germany, examines recent concepts on the bioavailability of contaminants in soil. It is focused on theoretical concepts and methods appropriate for identifying bioavailable fractions of contaminants.

The bioavailability concept integrates soil science, chemistry and biology. Our study is based on the following definition: “Bioavailability describes the complex processes of mass transfer and uptake of contaminants into soil-living organisms which are determined by substance properties, soil properties, the biology of organisms and climatic influences. The bioavailable contaminant fraction in soil represents the relevant exposure concentration for soil organisms.” Bioavailability assessments are principally based on four measurements: 1. total soil concentration, 2. analytically measurable concentration, 3. bioavailable concentration, 4. biologically effective concentration. No. 1 remains unknown and No. 2 is directly measured by e.g. exhaustive extractions (neglecting bound residues that cannot be extracted by conventional procedures). No. 3 is either derived from theoretical models or from measurements following so-called “mild extractions” (e.g. salt solutions) and comparable non-exhaustive procedures. No. 4 includes the whole complex of biological effect assessments (mainly biodegradation, toxicity and bioaccumulation). Whereas 2 and 3 depend on soil properties (in particular texture, organic carbon content, pH, water content and cation exchange capacity) and chemical extraction efficiency or on the quality of the model, 4 is determined by the whole set of soil properties and organism characteristics (size, morphology, physiology, behaviour, life cycle, nutritional state etc.).

Abstracts of over 300 references simultaneously addressing bioavailability and soil were reviewed. A systematic survey revealed a bias towards the physical and chemical perspective of bioavailability whereas the influence of the organisms itself was clearly underrepresented. Ageing of substances was more often investigated for organic chemicals whereas studies on the influence of soil properties on bioavailability were dominated by heavy metals. Biodegradation of contaminants was the main focus in studies addressing contaminant availability for microorganisms, in contrast to toxicity and bioaccumulation in studies involving plants and animals

Based on the abstracts, a selection of articles for more detailed analysis was made, using the criteria (i) theoretical or methodological approach and (ii) quantity of comparable studies. This selection resulted in focussing on two contaminant classes, polycyclic hydrocarbons and heavy metals, for which the problems and possibilities in assessing their bioavailability in soils was examined in detail. Due to the fundamental chemical difference (mainly in lipophilicity), bioavailability of organic compounds and of heavy metals is not comparable.

Exposure routes of (non-volatile) contaminants for soil organisms are (presumably predominantly) the soil pore water, followed by organic and inorganic matrix, soil air and living organisms (predacious organisms only). Therefore, most approaches on bioavailability in soil address soil pore water whereas the other routes of exposure are generally underrepresented, but often very relevant - which became obvious from the few studies examining these.

Theoretical assessments of bioavailability are based on substance characteristics and soil properties, assuming an equilibrium partitioning of the contaminant between soil matrix and pore water (e.g. the equilibrium partitioning theory, EPT). Experimental approaches use various extraction procedures with organic solvents or salt solutions, but also promising new methods that aim at mimicking either sorption processes (ion exchange resins) or uptake into biological tissues (biomimetic approaches).

Most experiments are conducted with artificially contaminated (spiked) soils under standardised laboratory conditions; historically contaminated soils have rather seldom been studied with respect to bioavailability. The biological component is investigated by biodegradation of chemicals, tissue concentrations in organisms (bioaccumulation) or in ecotoxicological standard tests with very few organism species typically used in such tests. Plants, microorganisms and lumbricids (which mostly represent the soil water exposure route) prevail, and some ecologically relevant groups of soil organisms (fungi, mites and predacious organisms) have not been studied at all.

A general problem of most bioavailability approaches is that the temporal and spatial components of the complex processes (e.g. transport, diffusion, organism biology) are not considered at all. This became obvious whenever ageing of the contaminant was studied, or when results from model calculations or standardised laboratory experiments were compared to the real field situation: at the present state of knowledge, transferability is usually not given.

The general problem of both the theoretical and the experimental strategies lies in the necessity to adequately include complex and often still unexplored processes (organism perspective!) in a substance-specific perspective for quantitative assessment. At present neither the theoretical concepts nor the experimental data are sufficient for deriving a widely-applicable concept on the bioavailability of contaminants in soils. Therefore - and because general standards do not exist - this concept cannot be included into risk assessment of chemicals in soils without extensive further research efforts. The present risk assessment scheme - which is based on total contaminant concentrations and worst case scenarios - seems to be sufficient from a precautionary point of view since it rather overestimates the risk of a chemical for the soil ecosystem and its organisms.

Still, bioavailability is a concept worth to follow more detailed, for bioavailable fractions of contaminants in soils can be relevant for human health, e.g. when growing food plants on (moderately) contaminated land. The - thus far - best known problems are related to heavy metal accumulations. The availability of heavy metals for plants is the best-investigated phenomenon within the whole context of bioavailability of contaminants in soils, yet even here some research questions are unsolved. The question of bioavailability is also relevant for soil protection (protection goal: organism biodiversity). Many soil organisms are sensitive to contaminants, and their bioavailability can be crucial for survival or extinction.

Important open research questions with respect to bioavailability of contaminants in soil are

1. the role of mixture contamination for the bioavailability of single substances
2. the significance of sequential contamination
3. the role of metabolites
4. the significance of the physical state (dissolved, adsorbed, crystalline) of the substance
5. the potential use of behavioural reactions of organisms (avoidance) as early-warning indicator
6. the exposure route “food” for soil organisms
7. the influence of the temporal and spatial dynamics within the soil compartment
8. the effect of organism density (abundance) on bioavailability.

Only in recent years environmental chemistry and ecotoxicology started to draw more attention to the bioavailability concept, and the experimental design of studies has only inertly started to reflect its integrative character. On the one hand this expresses a theoretical deficit, on the other hand there is a growing diversity of experimental methods without a recognisable trend for standardisation. Also, the theoretical bioavailability assessment by means of partitioning and uptake models cannot generally replace experimental studies. To overcome the discussed restrictions, more and interdisciplinary research efforts (soil science, biology, chemistry) are necessary.

Within the context of this study, an international workshop “Bioavailability of soil contaminants: problems and perspectives” was held in July 2002 at the University of Bremen, Germany. The main conclusions resulting from the lively and inspired discussions during this workshop are attached to the document.

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Attachment

Workshop “Bioavailability of soil contaminants: problems and perspectives”

UFT Centre for Environmental Research and Technology, University of Bremen

July 18-19, 2002

organised by

Tobias Frische, Klaus-Holger Mebes and Juliane Filser

Within the context of this literature study, an international workshop was held in July 2002 at the University of Bremen, Germany. Our idea had been to foster the interdisciplinary discussion within the context of bioavailability of contaminants in soils. Therefore, chemists, soil scientists and soil biologists were invited to contribute to one of the three sessions:

1. Biodegradation of organic chemicals - biological and chemical availability
   (Keynote: Brian Reid, University of East Anglia, Norwich, UK)
2. Bioavailability - the metal case
   (Keynote: Kees van Gestel & Willie Peijnenburg; Free University of Amsterdam and RIVM National Institute for Public Health and the Environment, Bilthoven, NL)
3. Bioavailability of organic chemicals to soil fauna ant plants
   (Keynote: Martin Krauß, Technical University of Berlin, DE)

About 40 participants from Germany, Denmark, The Netherlands, Great Britain and the USA accepted the invitation. In each session, a plenary lecture was followed by 3-5 oral contributions and a summarising discussion. The workshop ended with a final discussion “Bioavailability - are there unifying conceptual perspectives” following an introductory statement by Joop Harmsen, Alterra, Wageningen (NL) and summarising statements by the keynote speakers and by Juliane Filser, University of Bremen.

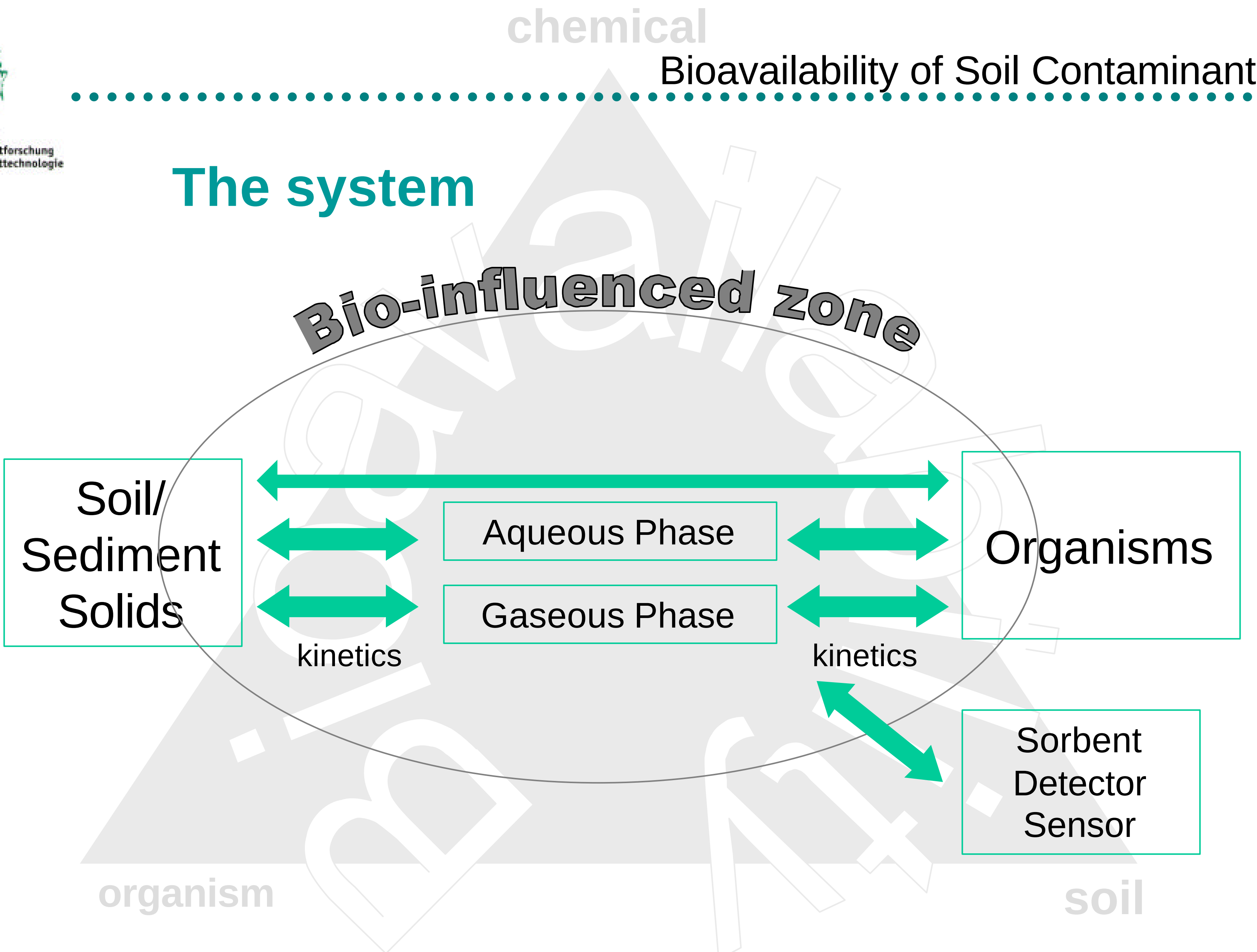
The main conclusions resulting from the lively and inspired discussions during this workshop are summarised on the following pages.

Bioavailability of Soil Contaminants: Problems and Perspectives

- Conclusions -

Juliane Filser, Brian Reid, Kees van Gestel, Martin Krauß, Tobias Frische, Holger Mebes
and all other workshop participants

The system


chemical
Bio-influenced zone
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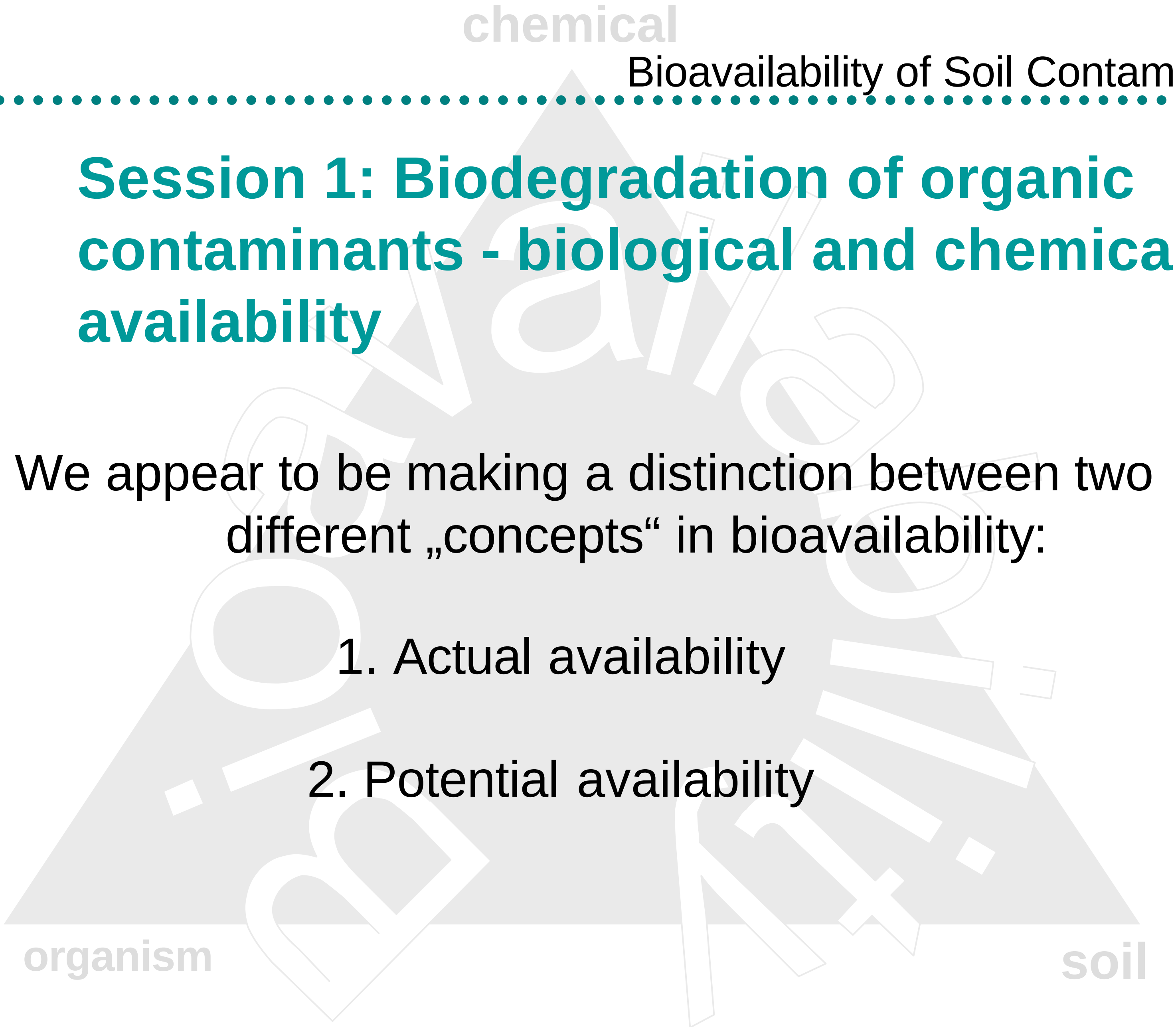
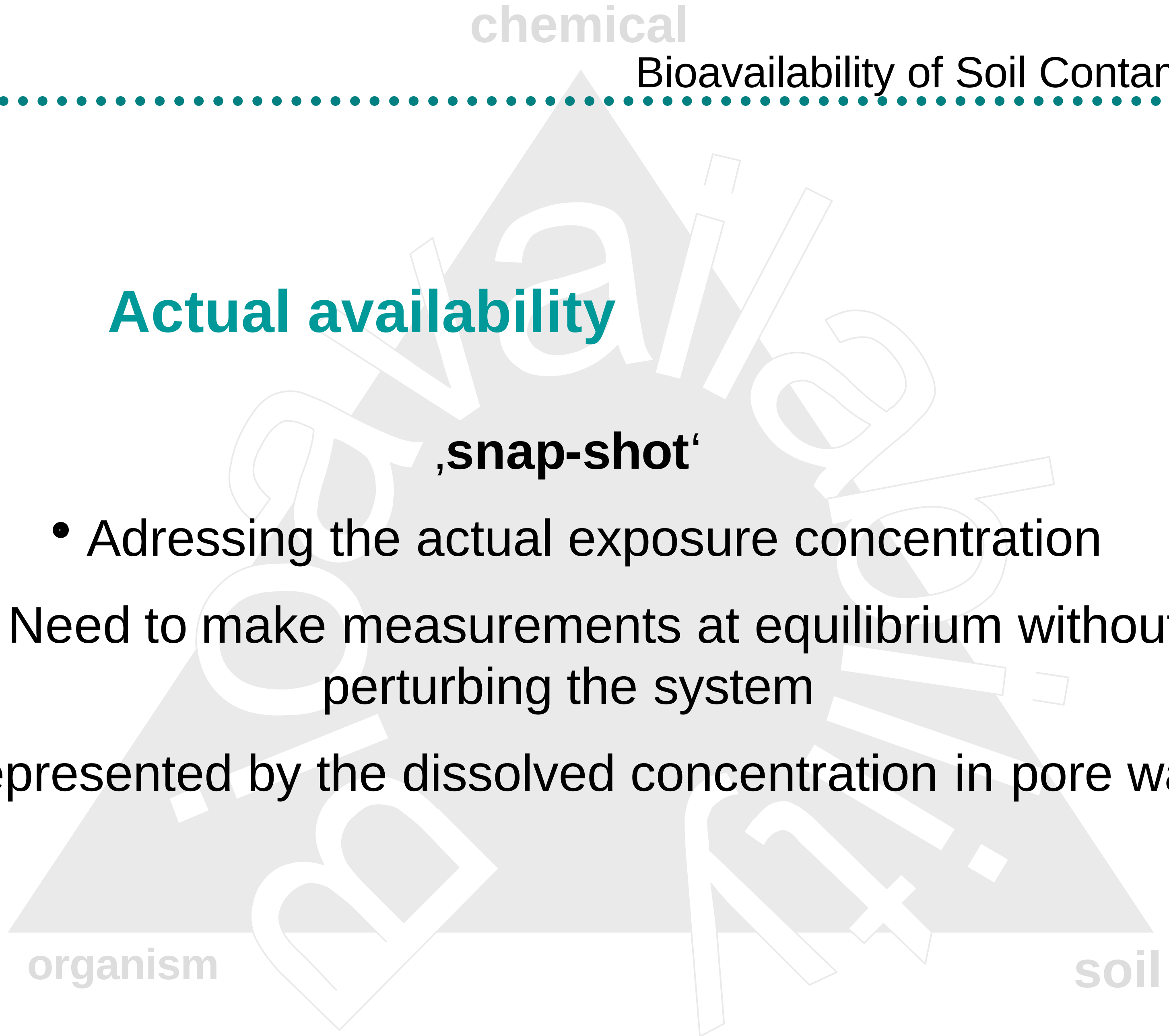


Session 1: Biodegradation of organic contaminants - biological and chemical availability

- We appear to be making a distinction between two different „concepts“ in bioavailability:

1. Actual availability

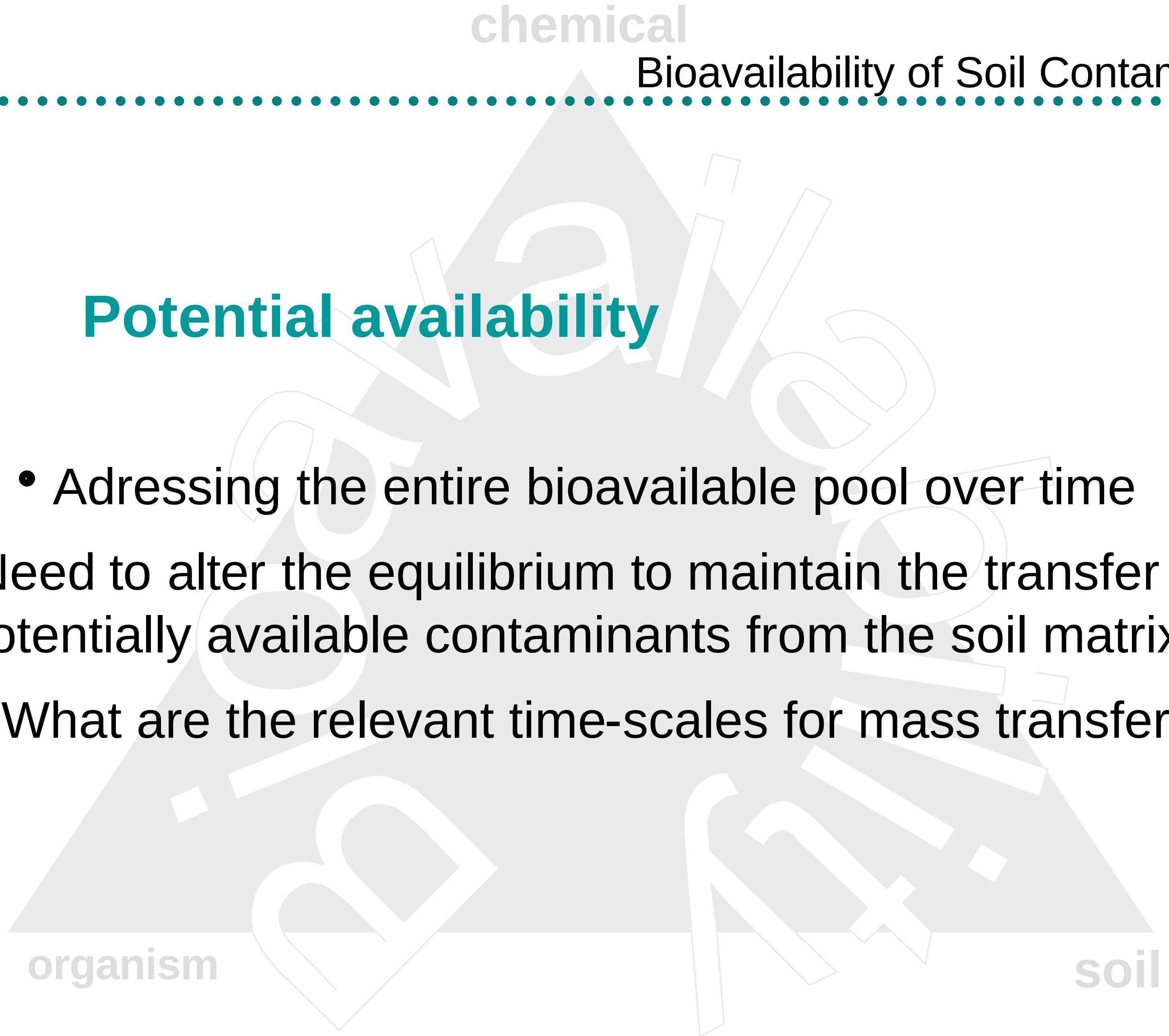
2. Potential availability



Actual availability

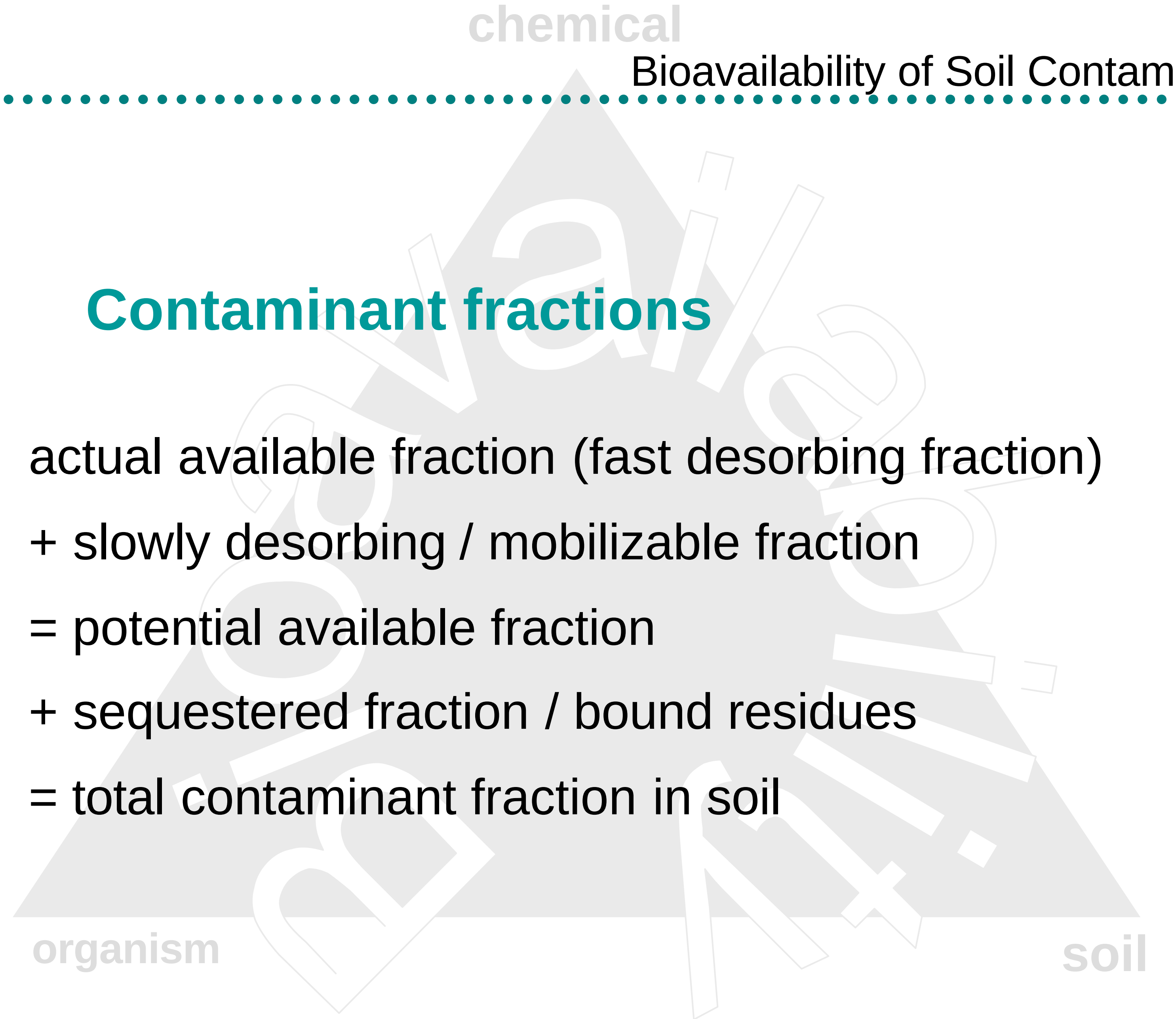
‚snap-shot‘

- Adressing the actual exposure concentration
- Need to make measurements at equilibrium without perturbing the system
- Is represented by the dissolved concentration in pore water?



Potential availability

- Adressing the entire bioavailable pool over time
- Need to alter the equilibrium to maintain the transfer of potentially available contaminants from the soil matrix
- What are the relevant time-scales for mass transfer?



Contaminant fractions

actual available fraction (fast desorbing fraction)

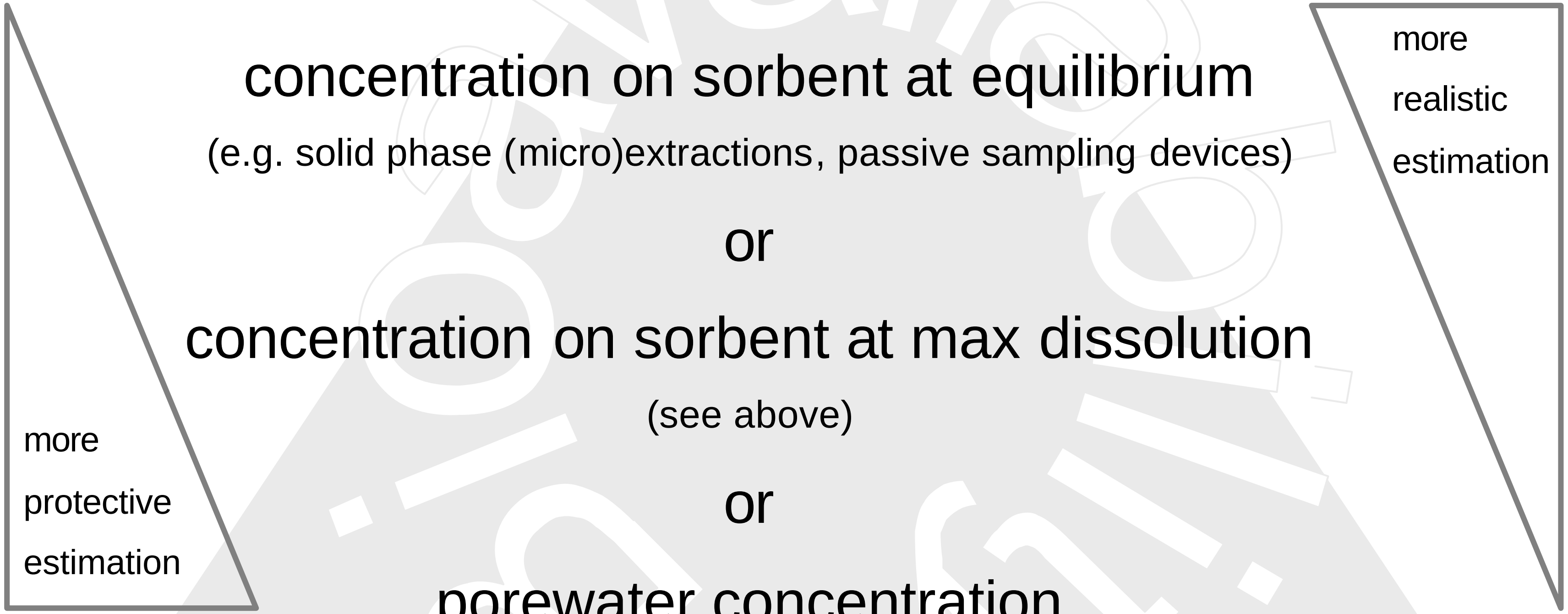
+ slowly desorbing / mobilizable fraction

= potential available fraction

+ sequestered fraction / bound residues

= total contaminant fraction in soil

Actual availability: measurement options



concentration on sorbent at equilibrium

(e.g. solid phase (micro)extractions, passive sampling devices)

or

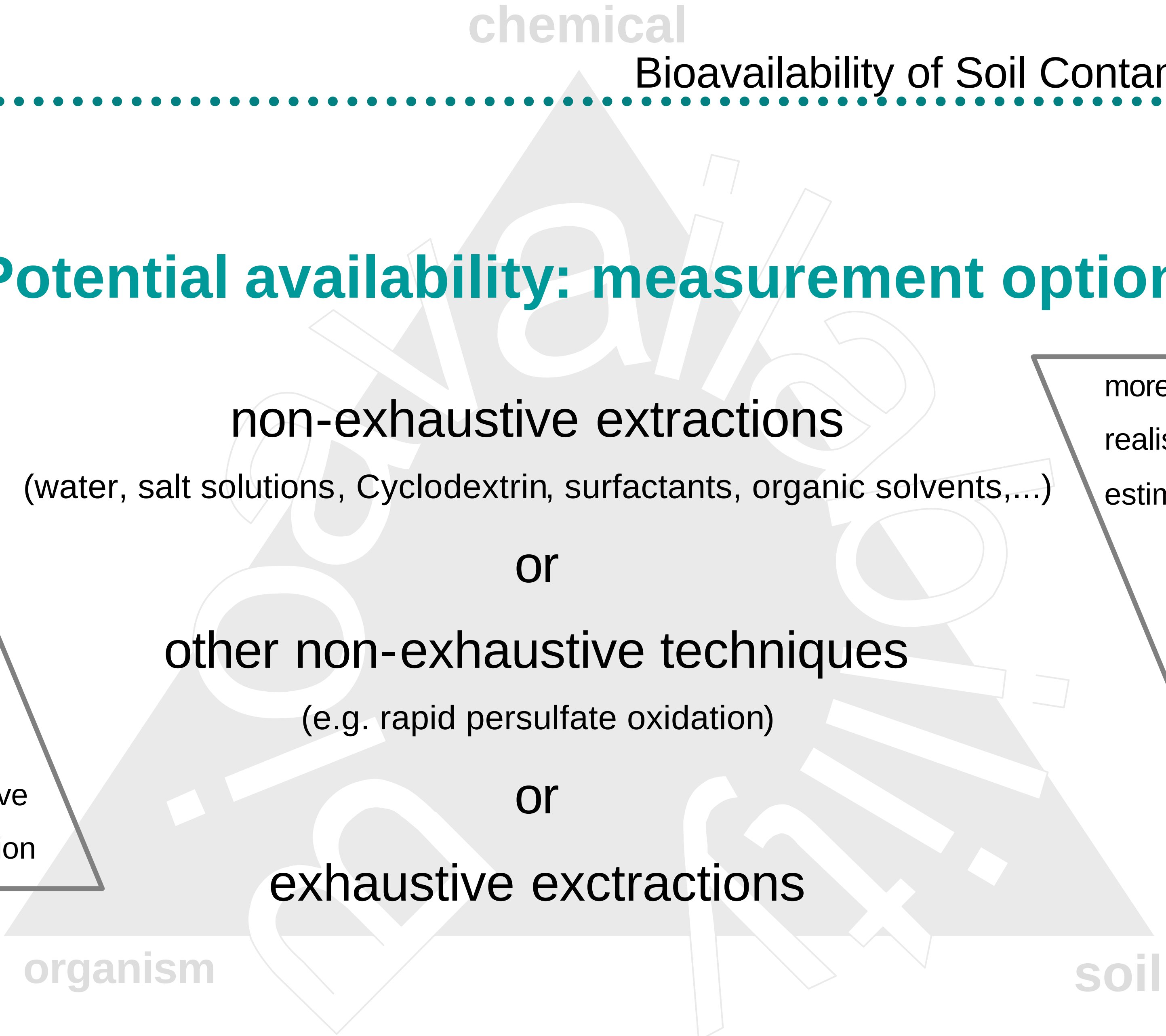
concentration on sorbent at max dissolution

(see above)

or

porewater concentration

Potential availability: measurement options



more
protective
estimation

non-exhaustive extractions

(water, salt solutions, Cyclodextrin, surfactants, organic solvents,...)

or

other non-exhaustive techniques

(e.g. rapid persulfate oxidation)

or

exhaustive exctractions

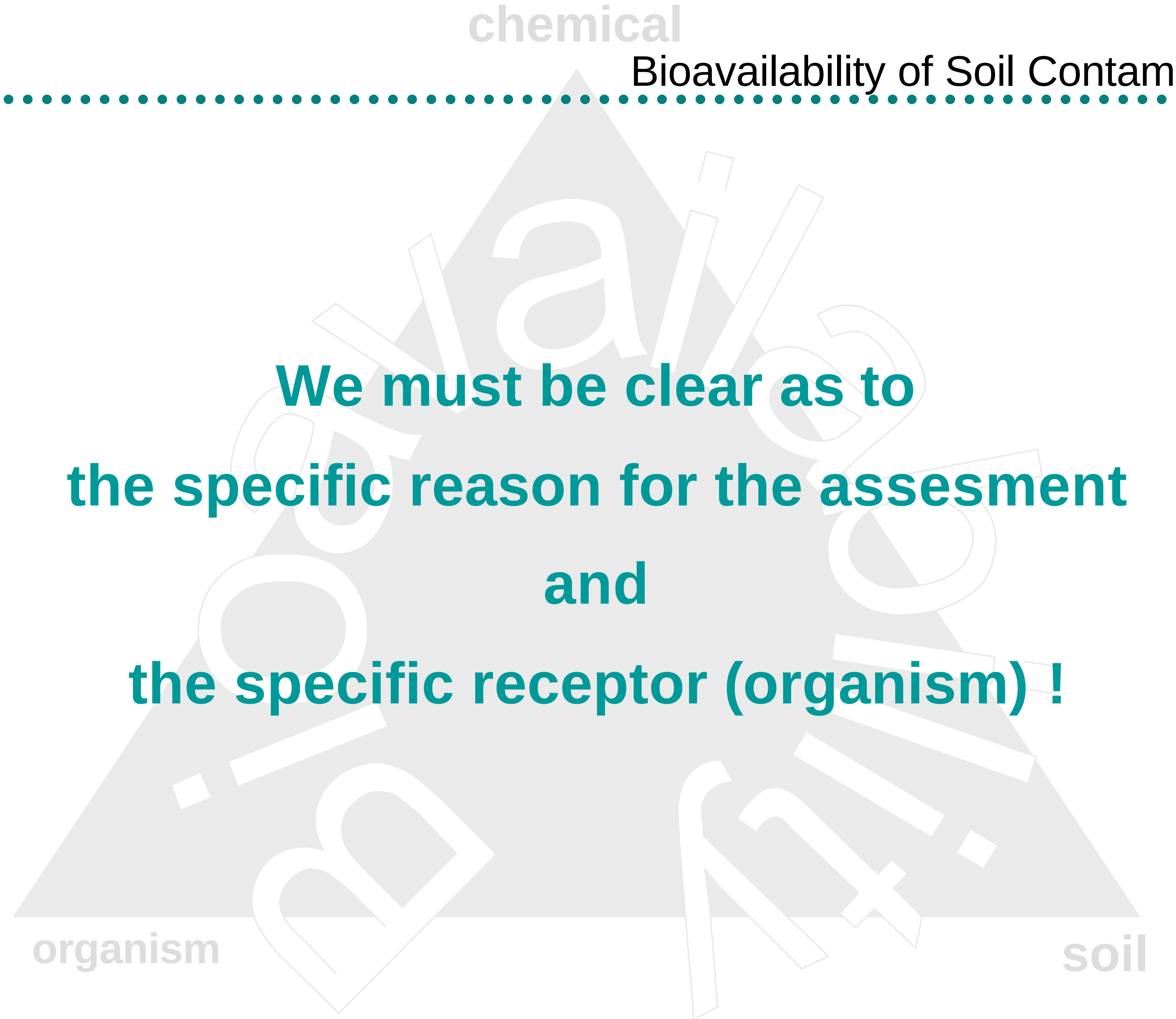
more
realistic
estimation

Applications

Actual BA => Predict organism tissue concentrations

Potential BA => Predict bioremediation potential

Either/both => To evaluate short and long-term risk

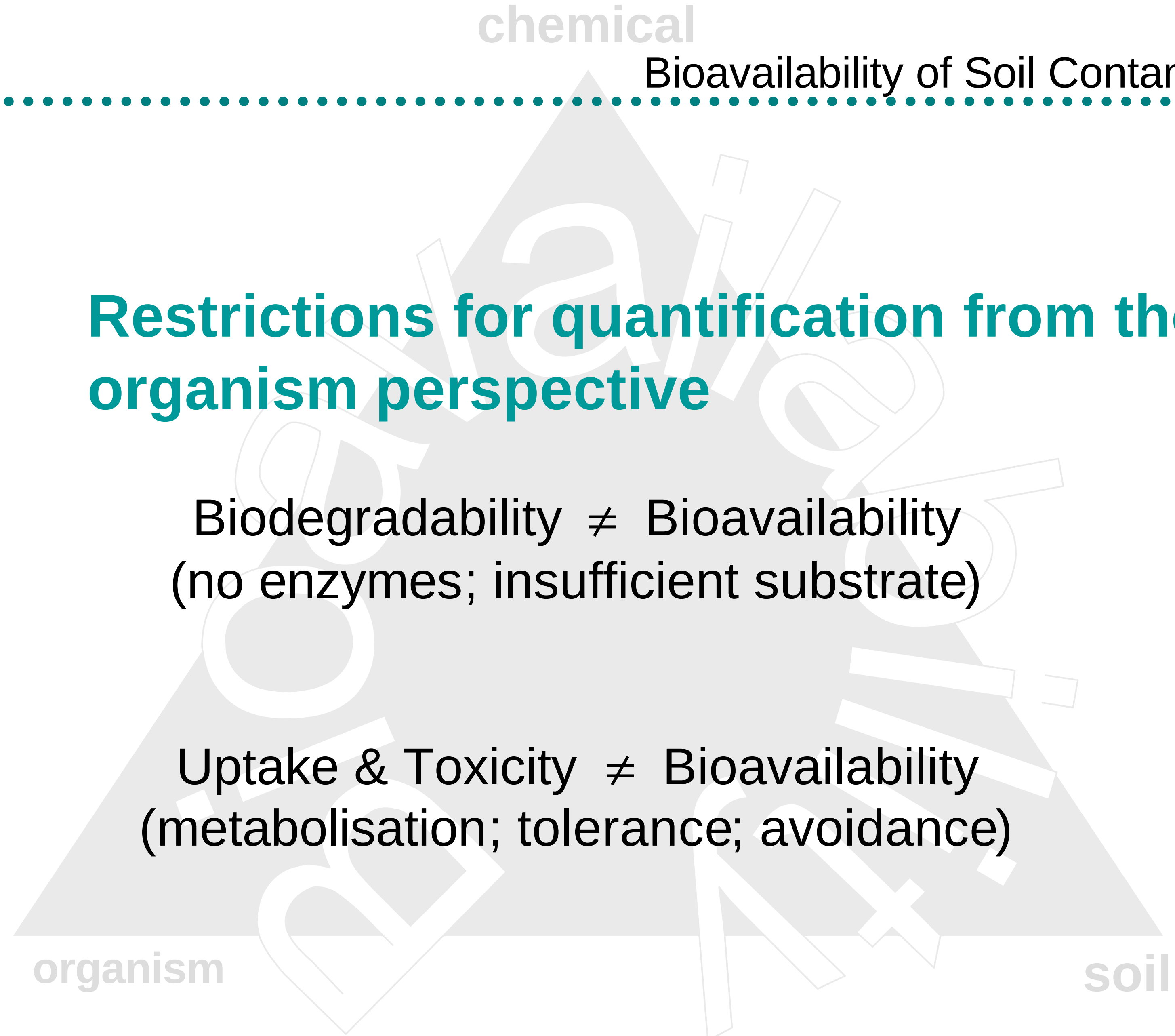


We must be clear as to

the specific reason for the assesment

and

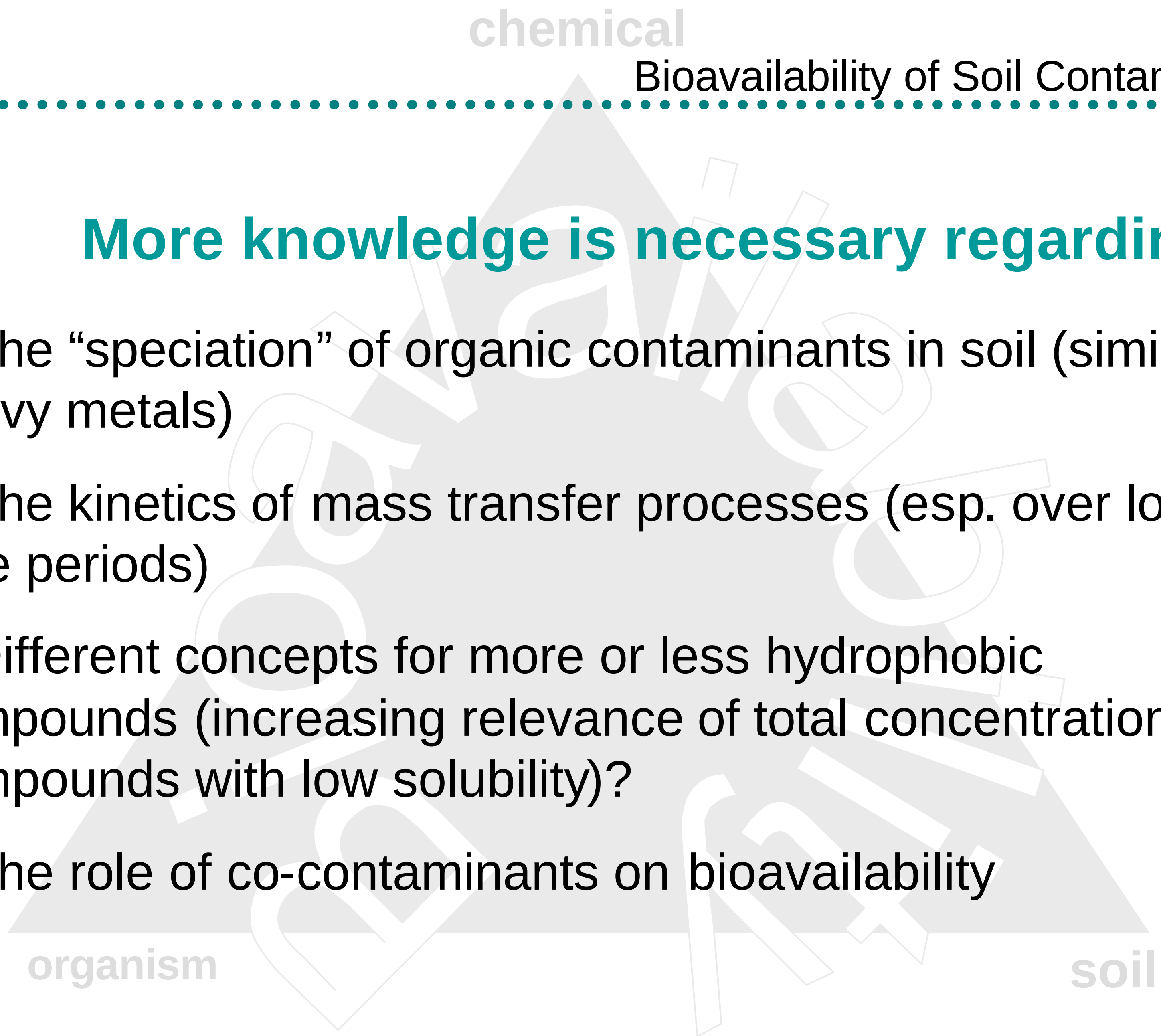
the specific receptor (organism) !



Restrictions for quantification from the organism perspective

Biodegradability ≠ Bioavailability
(no enzymes; insufficient substrate)

Uptake & Toxicity ≠ Bioavailability
(metabolisation; tolerance; avoidance)



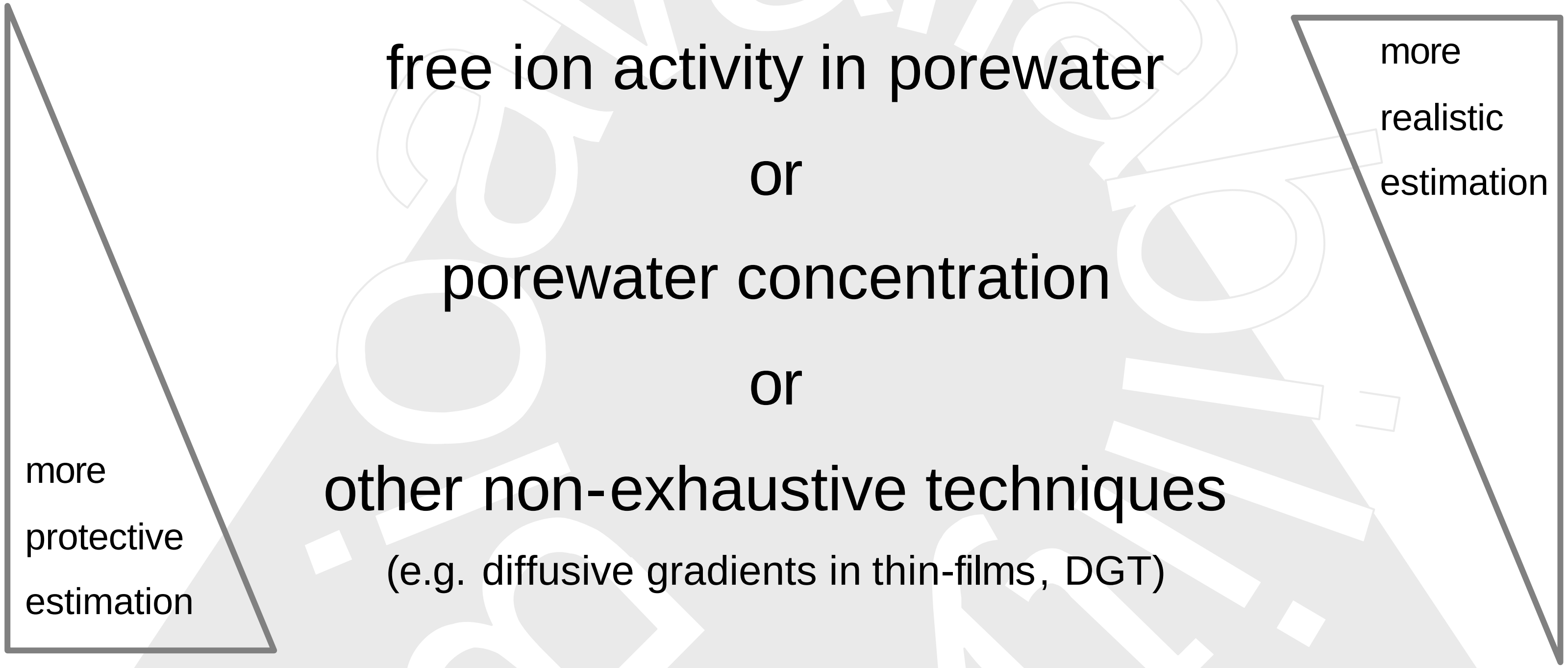
More knowledge is necessary regarding

- The “speciation” of organic contaminants in soil (similar to heavy metals)
- The kinetics of mass transfer processes (esp. over longer time periods)
- Different concepts for more or less hydrophobic compounds (increasing relevance of total concentrations for compounds with low solubility)?
- The role of co-contaminants on bioavailability

Session 2: Bioavailability - the metal case

- The „chemical approach“ seems to work better for heavy metals: important predictors of bioavailability are pH, Ca, SOM, clay
- Metal uptake and toxicity is specific with respect to metals (essential/non essential), speciation, organisms (e.g. soft-bodied, hard-bodied animals, fungi) and soil-properties
- Microbial assays will also be protective for higher organisms
- There is clear evidence for adaptation to occur under field conditions: the critical body burden concept needs modification (see abstract W. Peijnenburg)

Actual availability: measurement options



free ion activity in porewater

or

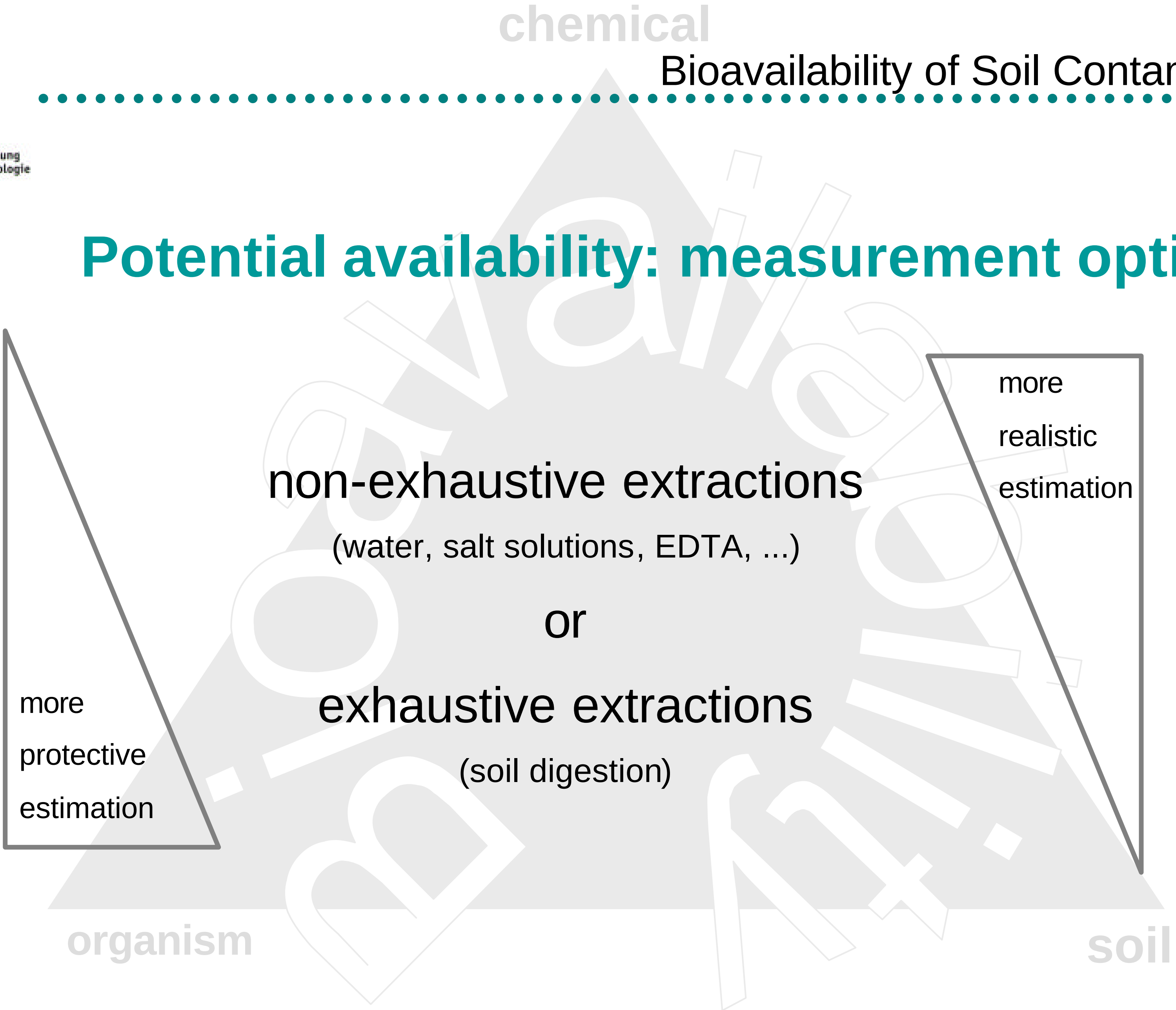
porewater concentration

or

other non-exhaustive techniques

(e.g. diffusive gradients in thin-films, DGT)

Potential availability: measurement options



more
protective
estimation

non-exhaustive extractions

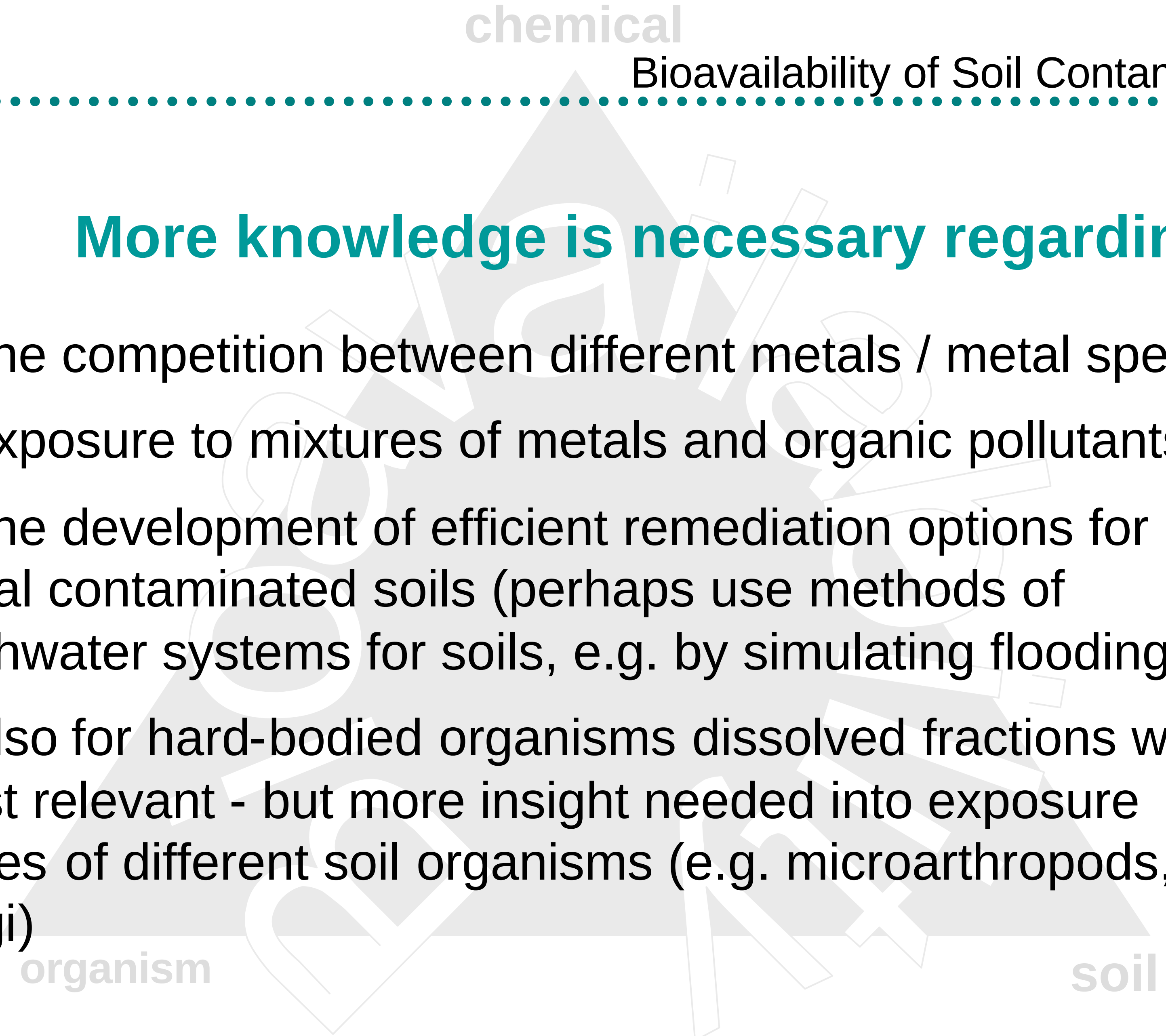
(water, salt solutions, EDTA, ...)

or

exhaustive extractions

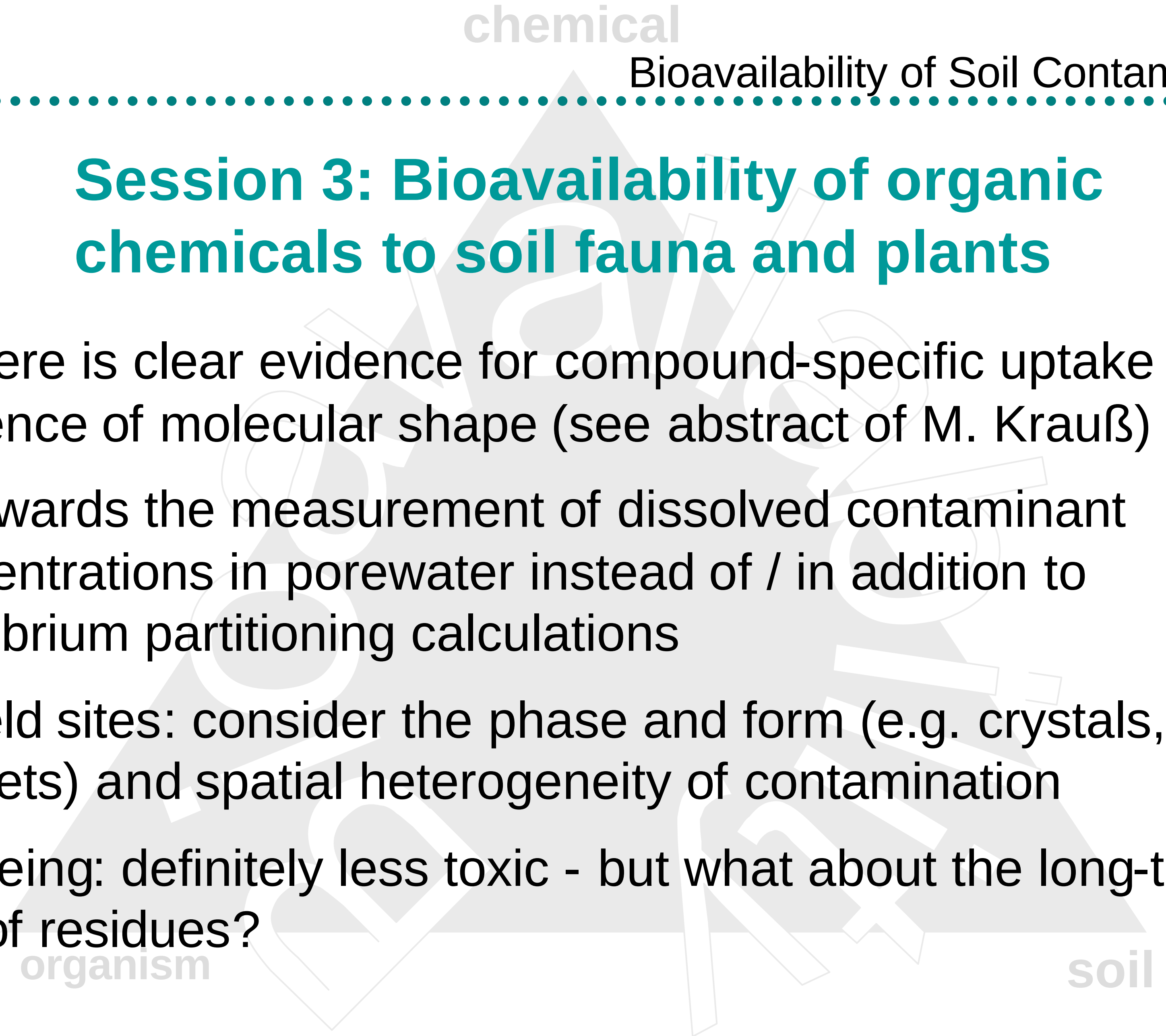
(soil digestion)

more
realistic
estimation



More knowledge is necessary regarding

- The competition between different metals / metal species
- Exposure to mixtures of metals and organic pollutants
- The development of efficient remediation options for metal contaminated soils (perhaps use methods of freshwater systems for soils, e.g. by simulating flooding)
- Also for hard-bodied organisms dissolved fractions will be most relevant - but more insight needed into exposure routes of different soil organisms (e.g. microarthropods, fungi)



Session 3: Bioavailability of organic chemicals to soil fauna and plants

- There is clear evidence for compound-specific uptake / the influence of molecular shape (see abstract of M. Krauß)
- Towards the measurement of dissolved contaminant concentrations in porewater instead of / in addition to equilibrium partitioning calculations
- Field sites: consider the phase and form (e.g. crystals, droplets) and spatial heterogeneity of contamination
- Ageing: definitely less toxic - but what about the long-term fate of residues?

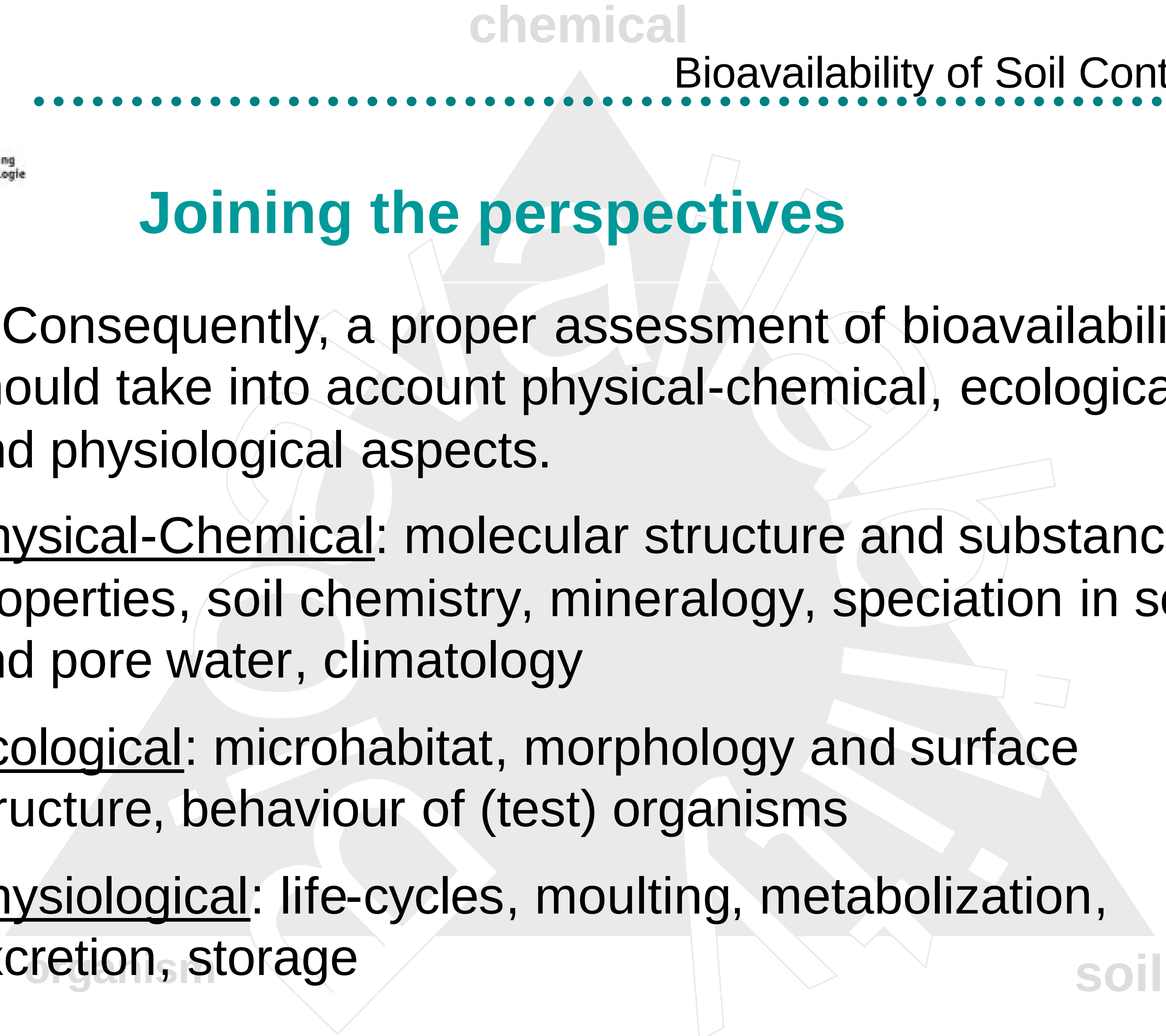
More knowledge is necessary regarding

- How far we can generalise bioavailability models for other compound classes and organisms
- Exposure routes for different soil organisms with special respect to test endpoints (e.g. avoidance)
- Equilibrium thermodynamics vs. soils as non-equilibrium systems?
- How SPE (solid phase extraction) techniques work under non-saturated conditions in the field
- The mechanisms of ageing of chemicals in soil

General conclusions

- Due to the complexity of the processes, a rather open, general definition of bioavailability is preferred for practical reasons (example given below). But, more detailled ones may be provided for special purposes.

Bioavailability describes the complex mass transfer and uptake processes of contaminants into soil organisms conditioned by substance properties, soil characteristics and the biology of organisms under varying environmental conditions. The bioavailable fraction represents the relevant exposure concentration to soil organisms.



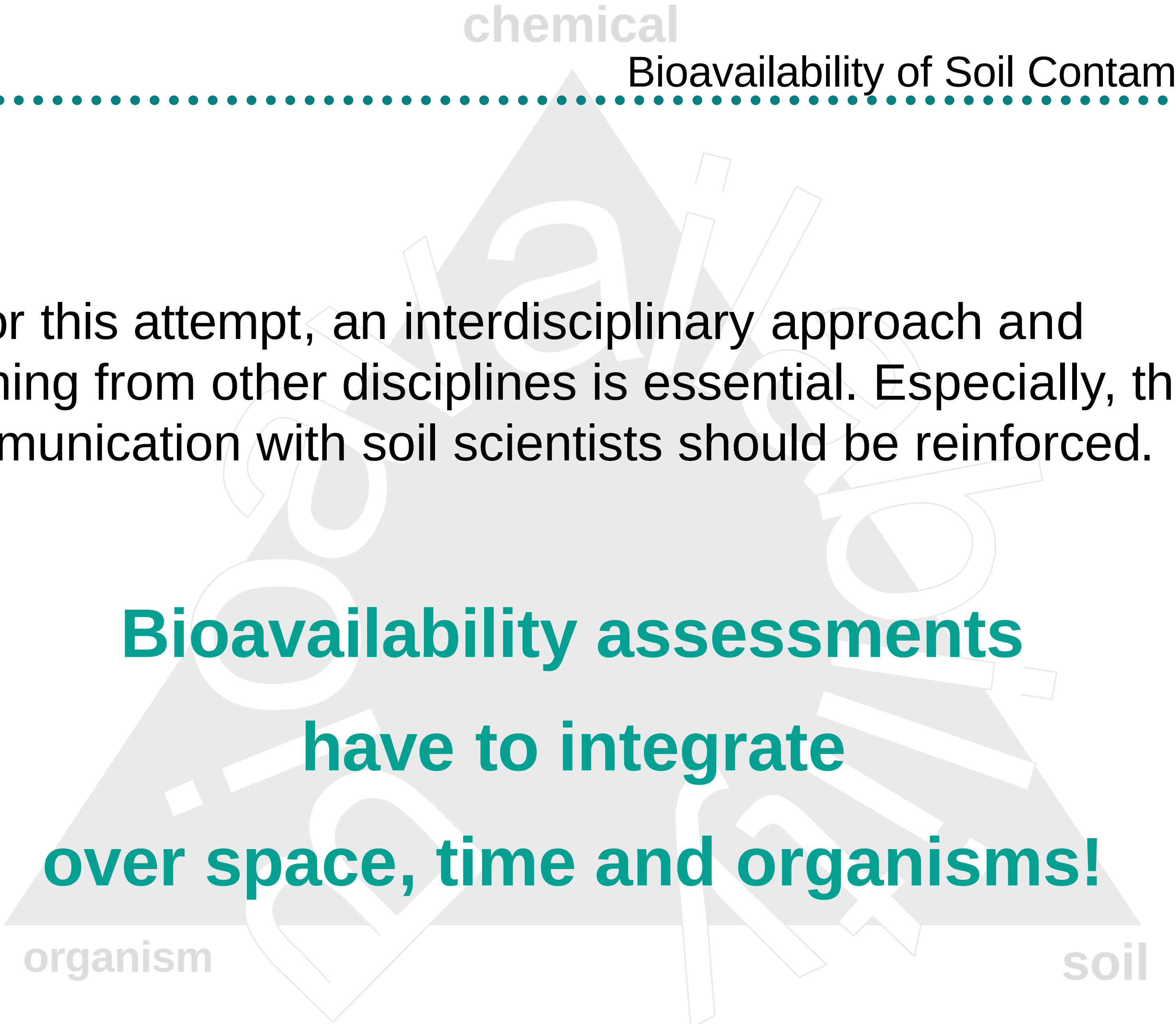
Joining the perspectives

- Consequently, a proper assessment of bioavailability should take into account physical-chemical, ecological and physiological aspects.

Physical-Chemical: molecular structure and substance properties, soil chemistry, mineralogy, speciation in soil and pore water, climatology

Ecological: microhabitat, morphology and surface structure, behaviour of (test) organisms

Physiological: life-cycles, moulting, metabolization, excretion, storage

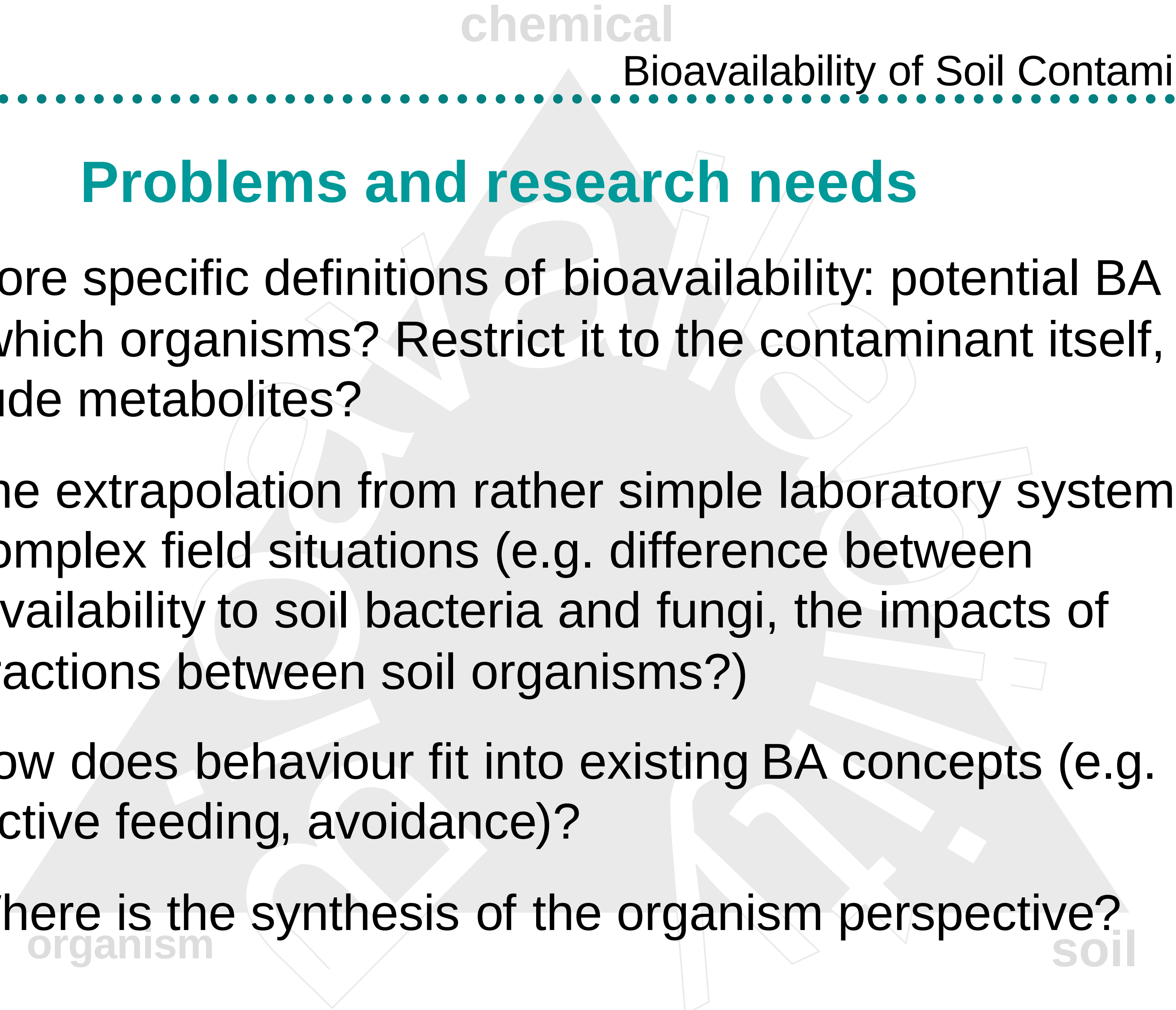


- For this attempt, an interdisciplinary approach and learning from other disciplines is essential. Especially, the communication with soil scientists should be reinforced.

Bioavailability assessments

have to integrate

over space, time and organisms!



Problems and research needs

- More specific definitions of bioavailability: potential BA for which organisms? Restrict it to the contaminant itself, or include metabolites?

- The extrapolation from rather simple laboratory systems to complex field situations (e.g. difference between bioavailability to soil bacteria and fungi, the impacts of interactions between soil organisms?)

- How does behaviour fit into existing BA concepts (e.g. selective feeding, avoidance)?

- Where is the synthesis of the organism perspective?

The regulatory perspective

- Bioavailability in view of the precautionary principle and scientific uncertainty (e.g. release of sequestered contaminants under changing environmetal conditions/ increasing or reducing risk by remediation techniques that enhance bioavailability)?
- For the integration of bioavailability into regulation, further method development / standardisation and especially communication with regulators („What do they want to protect?“) are the major tasks (see talk of Joop Harmsen) !
- What about the real costs of BA assessments („fancy“ devices, analytics,...)?

Once again

We would like to thank all workshop participants for their contributions and the productive discussion!

Bremen, 14. August 2002

Juliane Filser, Tobias Frische, Holger Mebes