rivm
National Institute
for Public Health
and the Environment

Report 711701084/2009

E. Brand et al.

Towards implementation of bioavailability measurements in the Dutch regulatory framework

rivm

RIVM Report 711701084/2009

Towards implementation of bioavailability measurements in the Dutch regulatory framework

E. Brand, RIVM
W. Peijnenburg, RIVM
B. Goenenberg, Alterra
J. Vink, Deltares
J. Lijzen, RIVM
D. Ten Hulscher, RWS
C. Jonker, IRAS
P. Romkens, Alterra
E. Roex, Deltares

Contact:
Willie Peijnenburg
Laboratory for Ecological Risk Assessment
willie.peijnenburg@rivm.nl

ALTERRA
WAGENINGEN UR

Deltares
Enabling Delta Life

Rijkswaterstaat

Institute for Risk Assessment Sciences

This investigation has been performed by order and for the account of The Ministry of Housing, Spatial Planning and the Environment, Directorate General for the Environment (DGM), Directorate of Sustainable production, within the framework of project 711701, Risk in relation to Soil Quality.

RIVM, P.O. Box 1, 3720 BA Bilthoven, the Netherlands Tel +31 30 274 91 11 www.rivm.nl

Abstract

Towards implementation of bioavailability measurements in the Dutch regulatory framework

A research group composed of RIVM, Alterra, Deltares, IRAS and RWS has selected several methods that can improve the accuracy when used to perform risk assessments of contaminated soils. These methods can be employed to determine which fraction of the contaminants present in the soil is actually biologically available and forms a risk to plants and animals living in the soil. Scientific studies have proven that only the bioavailable fraction of the contaminant is able to exert negative effects on the soil ecosystem. Not all of the contaminants, both in terms of quantity and species, are taken up by plants or animals.

The proposed methods can supplement the current approach used to perform risk assessments of soil ecosystems in the Netherlands. This assessment is based on the determination of the total contaminant concentration and the prediction of possible impacts from these data. There is an increasing realization, however, that risk evaluations based on the total contaminant concentrations lead to an incorrect assessment of the actual risk, as they may indicate a potential ecological risk when one is not present. This can result in drastic and often costly soil management measures aimed at improving the soil ecosystem – but which are entirely unnecessary.

The methods were selected on the basis of available scientific information and input from an expert workshop. All methods are in a sufficient state of development to allow for implementation in soil risk evaluation programmes. The bioavailability of chemicals in the environment has been a focus of scientific research for many years. Substantial progress has been made in unraveling the complex interaction between soil biota and the chemical speciation of contaminants in soil environments. At the same time, methods have been developed and tested that reliably extract and analytically determine the (bio)availability of contaminants in the terrestrial environment.

Key words:
bioavailability, heavy metals, organic contaminants, soil quality criteria, regulatory framework

Rapport in het kort

Naar het gebruik van biobeschikbaarheid in het Nederlandse bodembeleid

Een onderzoeksgroep bestaande uit onderzoekers van het RIVM, Alterra, Deltares, Iras en RWS heeft enkele methoden geselecteerd waarmee risicobeoordelingen van verontreinigde bodems nauwkeuriger kunnen worden uitgevoerd. Met deze methoden kan worden bepaald welk gedeelte van stoffen die in de bodem zitten daadwerkelijk vrijkomt en risico's vormt voor planten en dieren in de bodem. Het is wetenschappelijk aangetoond dat alleen deze zogeheten biobeschikbare fractie van de verontreiniging het bodemecosysteem negatief kan beïnvloeden. Niet alle delen van verontreinigende stoffen komen in planten of dieren terecht.

De voorgestelde methoden kunnen de huidige risicobeoordeling aanvullen. De huidige risicobeoordeling van de bodemkwaliteit gebruikt totaalgehalten van verontreinigingen in de bodemecosystemen. Deze manier van risico beoordelen blijkt de risico's van bodemverontreiniging onjuist te kunnen weergeven. De indruk bestaat namelijk dat het meten van totaalgehalten er regelmatig toe leidt dat beleidsnormen worden overschreden, hoewel het ecosysteem niet lijkt aangetast. Vanwege deze overschrijdingen kunnen ingrijpende en vaak kostbare (sanerings-) maatregelen worden opgelegd die echter niet nodig zijn om het ecosysteem te verbeteren.

De selectie van de methoden is gemaakt op basis van beschikbare wetenschappelijke informatie en een workshop met deskundigen op het gebied van biobeschikbaarheid. De biologische beschikbaarheid van stoffen staat al jaren in de wetenschappelijke belangstelling. Daarbij is meer inzicht verkregen in de interactie tussen bodemorganismen en de manier waarop verontreinigingen chemisch over het bodemecosysteem verspreid raken. Gelijktijdig zijn er methoden ontwikkeld en getest die de biobeschikbaarheid van verontreinigingen kunnen meten.

Trefwoorden:
biobeschikbaarheid, zware metalen, organische contaminanten, bodemkwaliteitscriteria, beleid

Acknowledgements

The initiators of the project (W. Peijnenburg and E. Brand) like to acknowledge: B. Groenenberg (Alterra), P. Römkens (Alterra), D. Ten Hulscher (RWS[1]), C. Jonker (IRAS[2]), J. Vink (Deltares), J. Lijzen (RIVM), M. Janssen (RIVM) and E. Roex (Deltares) as members of the research group for their devotion and help during the duration of the project.

We would also like to thank the participants of the workshop for their attendance in such large numbers. We would especially like to thank our colleague D. Sijm for his role as chairman of the workshop. The results of the workshop show the large attention that bioavailability has in the field of risk assessment. The supplements and comments collected during the workshop are an important aspect of this report and the final decision for the selection of the bioavailability methods.

Finally we would like to acknowledge our RIVM-colleagues T. van der Linden and T. de Nijs for their final supplements and comments to the report as part of a reviewing session.

---

[1] RWS stands for: Rijkswaterstaat

[2] IRAS stands for: Institute for Risk Assessment Sciences

Contents

Summary

In current risk evaluations of contaminated soil in the Netherlands, the quality is determined on the basis of total contaminant concentration. These measured total concentrations are compared with soil quality standards (SQS) or maximum values that also represent total concentrations of contaminants in soil and take account of potential risks. If the soil concentration exceeds the Soil Quality Standards a second tier risk evaluation can be performed. This second evaluation can be both chemical and/or biological of nature and is usually directed towards assessing actual risks. Although a risk evaluation can also relate to the protection of human health, this report focused on the protection of the soil ecosystem, only.

From practice the perception rises that performing risk evaluation by measuring total concentrations leads to an incorrect prediction of the actual risks. The idea exists that there is too often an indication for risks, whereas the ecosystem seems not to be affected. The total concentration is a poor indicator of the actual risk because it does not account for the impact of the binding capacity of the soil on the availability (and risks) of contaminants in soil. There are circumstances in which the soil physical-chemical properties can reduce the effective available concentrations of the contaminants in the ecosystem. It is only the bioavailable fraction that can exert adverse effects in the soil ecosystem. It is suggested that if bioavailability is taken into account during a risk evaluation, it can reduce the amount of false positives and false negatives (i.e. soils in which the actual bioavailability due to the soil parameters affecting actual exposure is higher than expected on the basis of the total concentration of the contaminants) in the soil risk evaluations.

Bioavailability of chemicals in the environment has been a topic of scientific research for a large number of years. Great improvements have been made in regard to increasing our understanding of the chemical and ecological mechanisms responsible for making chemicals available for uptake and toxicity. The legislators face the challenge of finding a way to implement this knowledge in present risk evaluations.

In 2007 the National Institute for Public Health and the Environment (RIVM) was commissioned with the task to investigate whether it is possible to implement bioavailability in the current risk evaluation of contaminated soils. This report presents the outcome of a project in the Netherlands that was aimed at selecting methods that measure bioavailability, to include these methods in a second tier risk evaluation for soils. Based on available scientific information an overview of the possible methods that are in a sufficient state of development to allow for implementation in soil risk evaluation and their functioning was written. This overview was presented at a workshop with Dutch specialists on bioavailability and the Dutch legislation to make a definitive selection between the methods.

After careful consideration and with help of predetermined selection criteria the following methods were considered as most promising for implementation into policy:

organic contaminants:
- passive sampling with either SPME, POM-SPE or silicone rubber (actual concentrations);
- tenax extraction (potential concentrations);
- cyclodextrin extraction (potential concentrations).

heavy metals:
- weak extractions with 0.01 M $CaCl_2$ (actual concentrations);
- acid extraction with 0.43 M HNO_3 (potential concentrations).

Although a first step towards implementation is taken within this project, follow up is needed. For the continuation it is advised to determine a political framework to which the measured concentrations can be related. Also the technical design of each method and a user protocol are needed. Future research is, especially for heavy metals, needed on the link between the measured concentration by the method and the actual uptake in and toxicity to the organism and finally it is advised to perform pilot testing. It is expected that on the short term methods for organic compounds are most successful.

Samenvatting

In Nederland wordt in de huidige risicobeoordeling van bodemverontreinigingen de kwaliteit bepaald aan de hand van totale concentraties in de bodem. Gemeten totaalconcentraties worden vergeleken met interventiewaarden en maximale waarden voor bodem welke eveneens op totaalconcentraties gebaseerd zijn. Als de gemeten concentraties de interventiewaarden overschrijden kan een tweedelijnsbeoordeling worden uitgevoerd. Deze tweedelijnsbeoordeling kan zowel chemisch als biologisch van aard zijn en is meestal gericht op het beoordelen van actuele risico's. Hoewel risicobeoordelingen van bodems zowel bescherming van de mens als van ecosystemen kunnen omvatten, richt dit rapport zich alleen op het beschermen van ecosystemen.

Vanuit de praktijk is aangetoond dat het uitvoeren van risicobeoordelingen op basis van totale concentraties tot incorrecte inschatting van risico's kan leiden. Het idee bestaat dat er te vaak een indicatie voor risico's is terwijl het ecosysteem niet is aangetast. Een risicobeoordeling gebaseerd op totale concentraties is derhalve een slechte maat om daadwerkelijke risico's te voorspellen. Hierbij wordt namelijk geen rekening gehouden met de invloed die de bindingcapaciteit van de bodem heeft op de beschikbaarheid (en risico's) van de contaminanten in de bodem. Er zijn omstandigheden waar de fysisch-chemische eigenschappen van de bodem de effectieve beschikbaarheid van concentraties in het ecosysteem kan reduceren. Daarom kan alleen de biobeschikbare fractie het ecosysteem negatief beïnvloeden. Door rekening te houden met biobeschikbaarheid tijdens de risicobeoordeling van bodems kan het aantal fout -positieve en fout -negatieve risicobeoordelingen (bodems waarin de biobeschikbaarheid hoger is dan verwacht op basis van totaalconcentraties in verband met lokale bodemeigenschappen) worden gereduceerd.

Vanuit verschillende hoeken van de wetenschap wordt de laatste jaren al onderzoek gedaan naar de biologische beschikbaarheid van stoffen in het milieu. Daarbij zijn grote verbeteringen geboekt in het begrip van chemische en ecologische mechanismen die verantwoordelijk zijn voor het beschikbaar maken van de stoffen voor opname en toxiciteit. Beleidsmakers staan nu voor de uitdaging om deze kennis te implementeren in het beleid ten aanzien van risicobeoordeling van verontreinigde bodems.

In 2007 is het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) verzocht om te inventariseren of het mogelijk is om biobeschikbaarheid in het huidige beleid van risicobeoordeling van verontreinigde gronden op te nemen. Dit rapport presenteert de resultaten van het project dat is uitgevoerd om methoden te selecteren om biobeschikbaarheid te kunnen meten, met als uiteindelijk doel deze te implementeren in het beleid. Dit kan voor het bepalen van spoed om te saneren maar mogelijk ook ten behoeve van bodembeheer.
Gebaseerd op wetenschappelijke informatie wordt een overzicht gegeven van methoden welke in voldoende mate gereed zijn om in het beleid te implementeren. Daarnaast is de werking van de methoden beschreven. Deze beschrijving is tijdens een workshop gepresenteerd aan experts op het gebied van biobeschikbaarheid en het Nederlandse bodembeleid met als doel te komen tot een selectie van de methoden. Na zorgvuldige afweging is besloten om met behulp van enkele selectiecriteria en de uitkomsten van de workshop de volgende methoden te selecteren:
organische verontreinigingen:
- passive sampling met hetzij SPME, POM-SPE of silicone rubber (actuele concentraties);
- Tenax-extractie (potentiële concentraties);
- cyclodextrine-extractie (potentiële concentraties).

zware metalen:
- zwakke extracties met 0,01 M $CaCl_2$ (actuele concentraties);

- zure extracties met 0,43 M HNO_3 (potentiële concentraties).

Hoewel met dit rapport een eerste stap richting de implementatie van biobeschikbaarheid in het beleid is genomen, zijn er nog vervolgacties nodig. Als vervolg op dit project wordt geadviseerd om een referentiekader op te stellen waarmee de gemeten biobeschikbare concentraties kunnen worden vergeleken. Daarnaast moeten voor enkele methoden nog een technische beschrijving en gebruiksprotocol worden geschreven. Daarnaast is er met name voor de metalen nog onderzoek nodig om de relatie tussen de gemeten concentraties met de desbetreffende methode kwantitatief te relateren aan de toxiciteit van deze concentraties voor biota. Ten slotte wordt er geadviseerd om de methoden na afronding van de bovenstaande vervolgacties te testen door middel van enkele pilotstudies. Naar verwachting kan op de korte termijn het meeste succes worden geboekt met de methodes voor organische contaminanten.

1 Introduction

1.1 Current policy in the Netherlands

In current risk evaluations of contaminated soil in the Netherlands, the quality is determined on the basis of total contaminant concentrations. These measured total concentrations are compared with Soil Quality Standards that also represent total concentrations of contaminants in soil and take account of potential risks. This is called a first tier risk evaluation. If the soil concentration exceeds the Soil Quality Standards a second tier risk evaluation can be performed. This second evaluation can be both chemical and/or biological of nature and is usually directed towards assessing actual risks. Although a risk evaluation can also relate to the protection of human health, this report will focus on the protection of the soil ecosystem only.

1.2 Why implementation of bioavailability?

Practical experiences have given rise to the perception that performing risk evaluation based on (measured) total concentrations may lead to an incorrect assessment of the actual risks. The idea exists that on the one hand there is too often an indication of risks, while the ecosystem is not affected (false positive indication of risk). On the other hand, there are indications that some soil ecosystems are affected by contaminants at contaminant levels well below the Soil Quality Standards (false negative indication of risk). A false positive indication of risk could lead to the application of remediation measures and associated expenses that do not result in an improved ecosystem. A false negative indication induces insufficient protection of the integrity of the ecosystem, which is against the basic principles of Dutch environmental policy of protection of 95 % of the species potentially present in an ecosystem.

The basic problem is that the mere presence of a contaminant does not by definition mean that there is an actual risk or measurable effect on the ecosystem. Actually occurring effects are directly related to the binding capacity of the soil and the speciation in the pore water, as affected by the physical-chemical properties of the soil and the contaminant and specific properties of the biota that are actually exposed to contaminants. Binding of contaminants to the solid soil matrix in combination with the composition of the soil pore water (especially pH and dissolved organic carbon (DOC)), can reduce the concentrations of the contaminants in the ecosystem to which biotic species are actually exposed. In other words, depending on the soil matrix, organisms may actually experience less effect of contaminants than potentially expected from total soil concentrations. Therefore it is only the bioavailable concentration that is able to exert adverse effects in the soil ecosystem. It is suggested that if bioavailability is taken into account during a risk evaluation, the number of both false positives and false negatives (i.e. soils in which the actual bioavailability due to the soil parameters affecting actual exposure is higher than expected on the basis of the total concentration of the contaminants) can be reduced in the soil risk evaluations, thus properly protecting the ecosystem and allowing for effective expenditure of the scarce economic means available for soil management and soil remediation.

Various processes can affect bioavailability in the field. The first type of processes is physico-chemically driven in nature and relates to sorption, precipitation and occlusion in mineral and organic matter particles. Factors influencing this process are substance- and soil specific parameters, such as hydrophobicity, aqueous solubility, dissociation, cation exchange capacity (CEC), pH, clay content and organic matter type and content, thereupon the chemical composition of the pore water is of importance as this determines the speciation of the chemical. These processes determine the chemical availability of contaminants, i.e. the actual exposure of the organism. The second type of processes are physiologically driven biological uptake processes. These biological processes are controlled by species specific parameters like anatomy, surface-volume relationship, feeding strategy and related uptake routes of nutrients and contaminants, and preferences in habitats. This second type of processes determine the biological availability of a chemical. The third type of processes that are of relevance for bioavailability, are internal allocation processes controlled by the organisms. Examples are metabolism, detoxification, storage capacity, excretion and energy sources. This last type of processes determine the toxicological availability (Styrishave et al., 2008). These processes are of importance as they allow organisms to respond to (natural) shifts in exposure of the organisms to contaminants.

All processes have in common that they are time and space dependent, creating a complex system. This is one of the main reasons why legislators have been reluctant to implement bioavailability in risk assessment procedures. However, bioavailability in the environment has been a topic of scientific research for a large number of years. Great improvements have been made in regard to increasing our understanding of the chemical and ecological mechanisms responsible for the availability of chemicals for uptake and toxicity.

Legislators now face the challenge of finding a way to implement this knowledge in present risk evaluations (Peijnenburg et al., 2004). Up till now, no systematic application or implementation of bioavailability in soil risk assessment has been performed, albeit that a number of pragmatic approaches were implemented in some countries. An ISO working group (ISO/DIS 17402) provided guidance for the selection and application of methods for the assessment of bioavailability in soil and soil materials. This guidance provides an overview of methods that are potentially ready for implementation of bioavailability in soil quality assessment. However further action is needed to perform the actual implementation. Nevertheless, there is increased international awareness of the need to include bioavailability in risk assessment. The European Food Safety Authority (EFSA) has for instance initiated a working group which is to prepare a white paper that is to be the basis for the preparation of scientific opinion on 'the usefulness of total concentrations and pore water concentrations as metrics for the assessment of ecotoxicological effects in soil'. The scientific opinion focuses on organic compounds and will be supported by public consultation. In anticipation of the foreseen contents of the scientific opinion (is expected to become available in 2009), fate and effect models for organic chemicals are being prepared to allow for actual implementation of pore water as the metric for risk assessment of plant protection products in Europe.

Another obstacle hindering implementation of methods developed to quantify the bioavailable fraction is probably the large number of methods that are available. The development of these methods was done without proper consideration of the need to develop the corresponding reference system for linking chemical availability to biological and toxicological availability. Thereupon, field validation and extrapolation of testing results typically obtained in a laboratory setting to realistic (varying!) field conditions, has usually been ignored. At a smaller scale, bioavailability considerations are taken into account in for instance site-specific risk assessment. In such cases, often biota specific and chemical specific information on the link between chemical availability and biological/toxicological bioavailability is used to deduce whether (part of) the ecosystem is at risk. Large scale applications of these approaches are virtually non-existing.

1.3 Goals and boundaries of the research

The need to implement bioavailability in tiered risk assessment of contaminants in soil is increasingly recognized. Therefore given the Dutch aim of implementing bioavailability in the risk evaluations of soils, a study was started with the goal to come to a selection and further (quantitative) description of methods to describe the differences in bioavailability in a higher tier (second tier) risk assessment. For practical reasons it was decided not to suggest implementation of bioavailability in a first tier risk assessment.

This aim has been materialized in a stepwise approach for selection of methods that are in a sufficient state of development to allow for actual implementation in the risk evaluation of soils. Amongst others, this requires a clear description of the methods, substantial proof of the added value of the methods, proof of the robustness of the method in dynamic field situations, and the presence of a reference framework in which toxic effect can be quantified on the basis of relatively simple and standardized methodologies.

The stepwise approach includes:
1 – an update of the methods recommended during a Dutch Workshop on bioavailability that was organized in 2002;
2 – selection of methods appropriate for implementation in soil risk assessment;
3 – actual implementation, amongst others by establishing a reference framework.

The research reported here is on the topics 1 and 2 of the stepwise approach. This report is the result of a cascade of activities:
1 – A first inventory of methods potentially suited for inclusion in risk assessment was made.
2 – A draft report was prepared and discussed by a research group composed of: W. Peijnenburg (RIVM), B. Groenenberg (Alterra), P. Römkens (Alterra), D. Ten Hulscher (RWS), C. Jonker (IRAS), J. Vink (Deltares), J. Lijzen (RIVM), M. Janssen (RIVM), E. Roex (Deltares) and E. Brand (RIVM).
3 – This report was used as the starting point for a workshop. This workshop was organized in October 2008 by the research group and invited national experts form different institutes to give their opinion about the draft report. An overview of the participants of the workshop can be found in Appendix 1.
4 – Following the workshop, the report was finalized by selecting the final methods and establishing some recommendations for the necessary steps to actually implement the methods.

The focus of this project is on bioavailability of both heavy metals and organic contaminants and the primary compartment is soil. It is, however, expected that most methods are not limited to the soil compartment but are also applicable to sediments. Therefore this project will also focus on sediments as a secondary compartment.
It is realized that much of the developments in bioavailability research are most advanced in the water compartment. It is assumed in this study that the general principles of bioavailability in water do not at forehand differ from the principles in soil and sediments. However it is noted that the behaviour and effects of contaminants on organisms in soil are more complex than in water.
This study is based on available information to give an advice for implementation in the Dutch soil regulatory framework. Remaining technical details of the methods will be concluded in a later stage, where needed.

This study does not consider bioassays of field locations as a possibility for measuring bioavailability, solely for the reason that the current project focuses on compound specific risk assessment. Bioassays are particularly useful to assess chemical mixtures as found in the field. Risk assessment of chemical mixtures (and the accompanying combination toxicology) is however not part of the current research.

1.4 Readers guide

In chapter 2 of this report a general framework for the implementation of bioavailability into policy is described. Chapter 3 gives a short overview of available research and also gives an overview of methods that can determine bioavailability. Also the functioning of these methods is described. Chapter 4 gives a summary of the workshop that was organized within this project. In chapter 5 the selected methods, will be related individually to selection criteria. Finally chapter 6 presents some follow up actions that should be taken before the selected methods can actually be implemented into policy.

2 Bioavailability in policy framework

2.1 What is bioavailability?

Bioavailability is a comprehensive subject that is dependent on many different chemical, biological and environmental factors, such as pH and organic matter content. Therefore it is not easy to give just one distinct definition of bioavailability.

Semple et al., (2004) proposed the introduction of the term ‘bioaccessibility’ to complement bioavailability. According to Semple et al.„. bioavailable material is ‘freely available to cross an organism’s cellular membrane from the medium to the organism inhabits at a given time’ whilst bioaccessible material is ‘that which is available to cross an organism’s cellular membrane from the environment, if the organisms has access to the chemical’. The key advance offered by Semple’s, definitions is the inclusion of a time element. It should be noted that many classical chemical extraction methods for measuring ‘bioavailability’ actually measure bioaccessibility and that, for risk assessment and remediation it is often bioaccessibility rather than bioavailability that is of concern. Semple et al., also make the points that bioavailability and bioaccessibility will vary between organisms and that the ‘membrane’ in question can vary with the organism. Consequent to Semple’s paper the term ‘bioaccessibility’ is gaining usage in common practice.

The International Standards Organisation (ISO) has established a working group on bioavailability (ISO/TC190 – Soil Quality). Harmsen (2007) summarises their activities. The definition of bioavailability used by this organization is; “*Bioavailability is the degree to which chemicals present in the soil may be absorbed or metabolised by human or ecological receptors or are available for interaction with biological systems*” (ISO, 2005). However, Harmsen (2007) argues that bioavailability is best presented as a concept that is then related to specific situations or measurements on a case by case basis.

To conclude, a variety of definitions for bioavailability have been proposed in the literature. There is a growing consensus that in broad terms it is a measure of uptake of and consequent cellular interaction with a contaminant by an organism. There is also a growing acknowledgement that bioavailability will: 1) vary between organisms, 2) be a function of time of contact between the organism and contaminated media and, 3) that further, more precise definitions will only be applicable on a case by case basis.

In this report we define the bioavailability of a chemical, in line with Peijnenburg and Jager (2003), as:

> *“The fraction of a total amount of a chemical present in a specific environmental compartment that, within a given time span, is either available or can be made available for uptake by (micro)organisms or plants, from either the direct surroundings of the organisms or the plant or by ingestion of food.”*

This definition implies automatically that some of the methods recommended in this report for quantifying bioavailability, will be seen by some of the readers as actually mimicking bioaccessibility. In order not to distract from the core activity, no further distinction will be made.

It should explicitly be noted that although the definition of bioavailability as applied here is valid also for human beings, the focus of this report is on contaminants in terrestrial and benthic ecosystems only.

2.2 Purpose of bioavailability in policy

Considering current knowledge and learning from the experiences gained in implementing bioavailability of metals and organic compounds for the water compartment, bioavailability should be implemented in a policy framework aimed at properly protection of the soil ecofunctioning. This implies that the focus of this report is on improved risk assessment regarding the extent of chemicals on plants and organisms present in soil ecosystems. Although similar approaches are in a number of cases also applicable to issues like leaching of contaminants from soil to groundwater, assessment of adverse effects of soil pollutants on humans (for instance via soil ingestion or by consumption of home-grown vegetables), quality and quantity of agricultural crops, and well-being and functioning of agricultural cattle, these issues are not dealt with in this report. Actual implementation of the recommended methods is foreseen in the 'Risicotoolbox Bodem' (www.risicotoolboxbodem.nl).

In the Netherlands, the risk assessment of soil contamination is performed in a tiered approach in which the complexity and need for data gradually increases. As mentioned in the previous chapter it is, for practical reasons, not desirable to change the first tier of the risk assessment process. This means that in the first tier assessment the use of quantitative standards based on total concentrations of contaminants will remain in use, making sure that the standards are not too conservative or too progressive.

In the higher tiers of risk assessment it will be possible to account for bioavailability; however also in these tiers a few practical preconditions are enforced. The implementation of bioavailability should be based on validated studies which are again not too conservative or too progressive. The methods should also be relatively easily executable in the daily practice. This means that the methods should not be too costly, too time consuming, or too specialized (i.e. there needs to be the possibility to execute the underlying methods by more than one laboratory), whereas the interpretation of the results of tests prescribed should be transparent. An important aspect in this respect, is the development of a reference framework in which results of (relatively easy to perform and standardized) physico-chemical determinations are linked to actually occurring adverse effects. In terms of policy aspects, this will allow for a more accurate risk assessment in which 'false' expressions of risk or no-risk are minimized. Overall, the following requirements apply for suited methods (in decreasing order of importance):

1. scientifically justified (validated studies);
2. accepted by policy makers;
3. easily executable;
4. yielding comparable results;
5. cheap;
6. fast.

3 Measuring bioavailability

It has become more and more clear that the current generic way of risk assessment should be replaced with a site specific alternative, which takes into account all local factors that may affect actual local risks to the environment. Preferably this risk assessment should be based on bioassays, as these tests include all local factors influencing final contaminant concentrations in organisms on site. However bioassays take a long time to perform, are laborious, expensive and ask for a constant supply of test organisms which can be problematic and unethical. In time several analytical methods have been developed that could serve as replacements for bioassays. These methods try to estimate either the actual or the potential concentrations of contaminants available for uptake by organism from the soil matrix (Jonker et al., 2007), and can be divided into models and chemical extractions. Due to the differences in their behaviour, methods vary for inorganic and organic contaminants.
In this chapter an overview is given from the most promising methods currently developed with the final intention to select one or more of the methods as an option for implementation into policy within a short time frame.

3.1 Translation from water to soil

Bioavailability considerations for contaminants present in the water phase are increasingly implemented in aquatic risk assessment, such as the use of Tenax extractions for risk assessments of sediments. This is due to the fact that the understanding of the fate and effects of organic and inorganic contaminants is quickly increasing. This is in part facilitated by the mere observation that water is a more homogeneous compartment than either soil or sediment whereas in general the behavior and exposure pathways of chemicals are relatively simple to deduce in a laboratory setting.

In general, for neutral organic compounds with moderate hydrophobicity (i.e. chemicals for which the log of the octanol-water partitioning coefficient is roughly in between 3 and 6), sorption of the chemical to particulate and dissolved organic matter has been shown to be the main factor affecting the bioavailable fraction of these chemicals. As this sorption is only to a limited extent impacted by additional water properties, generally available models describing the sorption of organic contaminants to natural organic matter are recommended for correction for bioavailability in the aquatic compartment (Zwolsman and Peijnenburg, 2007).

Driven by research that was carried out within establishment of Risk Assessment Reports (RARs), as required within the EU legislation on new and existing chemicals, a new generation of effect models has been developed for the most common metals in the aquatic compartment: Cu/Zn/Ni/Pb. The models are based on the quantification of specific interactions of cations at the biotic receptors for toxicity. In case of fish, the gill has for instance been shown to be the biotic ligand at which toxicity is induced. In this case, adverse effects have been shown to be proportional to the concentration of metal at the fish gill. In turn, the concentration of metal at the fish gill is on the one hand proportional to the activity of the free metal ion in solution, whereas on the other hand the concentration of the metal at the biotic ligand (i.e. the gill in this example) is affected by competition for sorption of the metal with all cations present in the water phase. This implies that for instance in case of zinc, zinc is less toxic in waters in which the concentrations of Ca, Mg, or Na are higher, as higher concentrations of these cations increase the competition for sorption of zinc at the fish gill. Similarly, a lower pH (i.e. more competing H-ions) will induce less toxic effects of free zinc ions present in surface water.

In turn, the activity of free metal ions is affected by the water composition, and especially pH and concentrations of dissolved organic carbon strongly affect metal activities, with metal activities in general declining upon increasing concentrations of dissolved organic carbon.

Figure 3.4 in section 3.4.3.5 provides a schematic overview of the principles that provide the fundamentals of the new class of so-called Biotic Ligand Models that are currently used to quantify bioavailability in the aquatic risk assessment of metals. A central place in this scheme is taken by the free metal ion as this is assumed to be the main toxic metal 'species'. However this does not mean that exposure via the water phase is the only important route of up take. Uptake via food is also considered important.

A priori, especially for soil organisms primarily exposed via the (pore) water phase (like plants, worms and nematodes), there is no reason to assume that the principles of the aquatic biotic ligand model are not applicable for soil organisms too. Therefore a more elaborate explanation of the terrestrial BLM will be presented.

3.2 Former research

In 2002, a first step towards implementation of bioavailability was made by Sijm et al. (Sijm et al., 2002; Peijnenburg et al., 2004). Within the field of risk assessment advice was asked on the future role of biological availability. A workshop was organized to respond to this request. A number of selected methods that can determine bioavailability were discussed during the workshop. The individual strengths and weaknesses of methods identified were written down in the accompanying report 'Bioavailability in standard setting, results of a workshop' (in Dutch) *(*Sijm et al., 2002).
Also points for further research and improvements are described, as are the expected timelines before a method becomes suitable for implementation into policy.

The following methods were under consideration by Sijm et al:

- passive sampling methods such as solid phase micro extraction (SPME) and Semi Permeable Membrane Devices (SPMD) for organic contaminants in water, soil and sediment;
- Tenax extraction for organic contaminants in soil and sediment;
- normalization to organic carbon content for organic contaminants in soil and sediment. The elementary carbon is measured and converted to organic carbon on behalf of normalization of the measured concentrations in soils and sediments;
- bioavailability tests for hard and soft skinned organisms for organic contaminants and heavy metals in soil, based on the route of exposure by the organisms;
- correction for local background concentration for metals in soil;
- transfer functions to relate heavy metal concentrations in pore water and 0.01 M $CaCl_2$ extractions to soil, based on soil characteristics, pH and reactive metal;
- 0.43 M HNO_3 extraction to determine the potential available fraction of heavy metals in soil;
- 0.01 M $CaCl_2$ extractable fraction for heavy metals in soil;
- determining the bioavailable fraction for heavy metals in soil, water and sediment with DGT (Diffusive Gradients in Thin films) based on binding of the metals in a Chelex gel after diffusion through a hydrogel;
- DOC correction for limited availability of copper in water and sediment due to binding at DOC;
- succession or parallel extractions of heavy metals in water and sediment. In this way actual and potential concentrations can be measured at once;
- two tiers evaluation of zinc in anaerobic sediments. If the zinc concentrations exceed the standard, a conversion can be made based on AVS (Acid Volatile Sulphide) and organic carbon.

Based on the workshop Sijm et al. recommended the following actions:

- *"Implement the normalization of the concentrations of organic contaminants on the basis of elementary carbon present in the substrate towards the levels in (Dutch) standard soils and sediments;*
- *Adapt the current standards for local background concentrations, as a short term solution to substitute for the current practices in the Netherlands of normalization on the basis of so-called standard soil or sediment;*
- *Establish research groups that are commissioned to prepare proposals for the implementation of six methods*[3] *for which their implementation can be expected within 1-3 years. This includes an investigation into means of financing the necessary research activities;*
- *Look for a broader (international) support for further research activities and means of implementation within an international framework (EU) for all options with a time frame > 3 years.*
- *Stay alert on new developments within the broad area of bioavailability and continue looking for means of implementing the most promising new insights".*

The report of Sijm et al., (2002) did not give advice on a particular bioavailability method to implement in risk evaluation. However the report presented a good overview of the practical and political assets of several methods to determine bioavailability in the form of fact sheets. Therefore it was decided that the current project should use these fact sheets as a basis for further research. In the next section a short summary of the selected methods by Sijm et al., is presented. Some methods that are not considered by Sijm et al., but are currently of interest will also be shortly described.

3.3 Points of attention

This section will discuss several topics that have an influence on most of the discussed methods in the following sections.

3.3.1 Black carbon

Black carbon can be found more and more in the environment due to the large emissions by traffic and increased biomass burning. Black carbon is a collective term for remnants of incomplete combustion, such as soot. It is thought to serve as an additional sorptive phase, next to natural organic matter (such as dissolved organic matter (DOM)), for organic contaminants. Due to its ubiquitous presence in atmospheric aerosols, sediments and soils it has an influence on the transport and bioavailability of these contaminants.

Although the characteristics of black carbon vary widely, usually it is comprised of a high carbon content and relatively few functional groups making it relatively inert. This causes long residence times in the environment. The size of black carbon particles can span several orders of magnitude. This last property means that the lower range particles can not be separated with the common techniques such as filtration or centrifugation. Depending on the type of black carbon, very small particles can make up a large part of the entire carbon mass (Jonker and Koelmans, 2001).

Black carbon is believed to contribute to enhanced sorption of contaminants and hence extremely high distribution coefficients, nonlinear sorption isotherms for organic contaminants, very slowly desorbing contaminant fractions and reduced bioaccessibility of hydrophobic organic contaminants. Black carbon can be held responsible for 80-97% of PAH sequestration in soils and sediments even though it

---

[3] These methods are: tenax extraction, 0.43 M HNO_3 extractable metal fraction, 0.01 M $CaCl_2$ extractable metal fraction, measuring actual dissolved and total concentrations of pollutants in water and DOC correction for copper in water.

represents only 1 – 20% of the total organic carbon content and less than 1% of the total sediment mass. This means that the presence of black carbon can lead to lower environmental risks of contaminants (Rhodes et al., 2008a).

Soot is known to sorb strongly to surfaces of well-known sorbents such as Tenax, Empore disks et cetera. Separation afterwards proves to be difficult or not possible due to the rough surface of these devices. In addition, some methods are simply not sensitive enough to measure the extremely low contaminant concentrations, resulting from the strong sorption to black carbon.

In short, when measuring bioavailability, sequestration of organic contaminants to black carbon should be taken into consideration.

3.3.2 Relationship between measured concentration and toxic concentration

Most of the methods described in the next sections have a long history of research. Much of this research is focused on validating the relationship between measured freely dissolved concentrations by the methods in relation to the actual concentrations in pore water or biota. Less research has, until now, been performed on the relation between the measured concentrations by the methods described here and the actual toxicity of these concentrations to biota. In general there is a lack of toxicity and bioassay data in which, apart from adverse effects, the underlying bioavailable fraction is quantified. For this reason, the position of a measurement in the total risk assessment and the reference framework to be built upon measuring bioavailable fractions, has special interest in this research.

3.3.3 Passive uptake primary uptake route via pore water

When measuring bioavailability it is generally assumed that passive uptake of persistent organic pollutants via pore water (usually dermal uptake) is the main route of exposure.

However, for organisms living in the soil and sediment this assumption is not sufficiently covering all possible uptake pathways (Sijm et al., 2002). Next to dermal uptake via pore water, the following routes may also contribute to uptake of contaminants:

- ingestion of food or soil particles and/or water;
- topical uptake (mostly limited to spiders);
- inhalation of air present in the soil pores.

In its broadest context, three issues are determining the way biota is exposed to their local environment:

- the morphology of the organism;
- the physiology of the organism;
- the behavior of the organism.

Consequently, soil dwelling organisms are exposed to chemicals by a variety of pathways. Most organisms share the feature that the relative contribution of each pathway varies. On top of ecological impacts, these contributions depend on factors like the hydrophobicity of the chemical and variations in environmental conditions like soil type, climate, et cetera.

The relative importance of each of these uptake routes is determined by morphological (e.g. structure of the epidermis), physiological (e.g. mode of uptake of water [drinking versus uptake via the skin], mode of uptake of oxygen, feeding habits) and behavioural properties. A general sub-division may be made into so-called 'soft-bodied' organisms (like nematodes, earthworms, enchytraeids and some insect larvae) and 'hard-bodied' invertebrates (arthropods like spiders, mites, insects, millipedes, centipedes, harvestman, isopods, and some other terrestrial crustaceans like some crab species). 'Hard-bodied' organisms have evolved special organs for assimilation of oxygen and water, while for 'soft-bodied' biota uptake via the skin is the most important route of uptake of water and oxygen. Contaminants and nutrients may also be taken up via these distinct exposure routes while uptake of contaminants via food is possible for all biota. Some insight in the physiology and ecology of terrestrial organisms is needed to enable evaluation of the importance of the various uptake routes. The structure of the skin, mode of uptake of water, mode of uptake of oxygen and feeding habit are important variables in this respect. Thereupon, the behaviour of the organisms is of importance.

Knowledge on uptake routes of organic contaminants and of metals by soil invertebrates is far from complete. Most information is available for earthworms, springtails and isopods. The equilibrium partitioning theory appears to be valid for earthworms and springtails in laboratory settings, although some specific uncertainties like food type need further investigation. Overall it is clear that uptake of contaminants depends on species, soil type, and the chemical properties. The contribution of oral uptake may vary within a specific taxon but for soil organisms in close contact with the soil solution, pore water mediated uptake is in general the dominant pathway. It is commonly modified by soil specific ageing and speciation and by specific factors of the organisms, like nutrition status. Belfroid et al.,. (1995) modelled the importance of non-dermal uptake routes and found that the contribution of the latter routes in the uptake of hydrophobic organic contaminants is at maximum a factor of 2 as compared to the contribution of passive dermal uptake.

3.3.4 Measuring actual concentrations in relation to future changes

Most methods that measure freely dissolved concentrations of organic contaminants only measure the actual concentrations and do not account for possible changes (positive or negative) in the future. Although several methods (e.g. sequential metal extraction) can measure the potential concentrations that can become available in the future, this is not a given certainty. When deciding, based on bioavailability measurements, that currently there is no risk present for the ecosystem, future monitoring is needed to prevent risks in the future.

3.4 Methods under consideration

3.4.1 Organic contaminants

3.4.1.1 Introduction

There are several forms in which organic contaminants can be present in the soil ecosystem. They can be freely dissolved in pore water or sorbed in the solid fractions of the soils. When measuring bioavailability it is often assumed that organisms are mainly exposed to the contaminant via pore water. Therefore the freely dissolved concentrations of contaminants are considered to be the bioavailable concentration that can have (potentially) negative effects on organisms. By measuring these freely dissolved concentrations insight in potential negative effects can be given.

The freely dissolved concentrations are influenced by the tendency of a contaminant to adsorb to the soil particles. The sorption of organic contaminants to soil particles can be reversible or irreversible. The reversibly sorbed fractions are usually called the rapidly desorbing fraction and can be estimated with the help of the Freundlich-equation. It is assumed that the pore water concentration is in equilibrium with the rapidly desorbing fraction. Furthermore there are also slowly (days/weeks), very slowly (months/years) and non-desorbing fractions to be distinguished. The slow and very slow fractions show, in contrary to the rapid fraction, a Langmuir like sorption (Cornelissen et al., 2000). Which fraction (rapidly, slowly or very slowly) is extracted depends on the extraction time. For the assessment of the bioavailability of pollutants it is usually assumed that the slow and very slow fractions are not available to cause ecological risk.

By measuring the rapidly desorbing fraction, the freely dissolved concentrations can be calculated using the equilibrium partitioning theory.

For the methods selected for measuring bioavailability, a difference is made between measuring the free pore water concentrations and the sorbed concentrations. In this report the free pore water concentrations are considered to be the actual concentrations of contaminants present in the pore water and the sorbed concentrations the potential concentrations of contaminants that can desorb towards the pore water within several hours or days.

The following methods can be used to determine the bioavailability of organic contaminants and will be discussed in the following sections:
methods to determine actual concentrations:
- passive sampling methods;

methods to determine potential concentrations:
- Tenax extraction;
- cyclodextrine extraction;
- supercritical fluid extraction.

3.4.1.2 Passive sampling

Passive sampling comprises many different methods that can be used to determine the bioavailability of organic pollutants. In this section the following methods will be discussed: Solid Phase Micro Extraction (SPME), Semi Permeable Membrane Devices (SPMD), Polyoxymethylene Solid Phase Extraction (POM-SPE), Empore disks and silicone-rubber. All these methods have in common that they can be used to determine the concentration of the contaminant freely dissolved in pore water. Although some of the methods are also able to extract sorbed fractions of contaminants due to stronger sorption to the device than to the soil particles.

When using these methods it is assumed that exposure via pore water is the most important exposure pathway for organisms. Therefore measuring the pore water concentrations of a contaminant with passive sampling techniques should give an indication for possible risk. Although passive sampling techniques are originally designed for liquid phases and much of the scientific development is achieved for the water compartment, more and more research is performed for soil and sediments. The research of Ter Laak et al., 2006a and b ; Mayer et al., 2000b; Jonker et al., 2007 and Van der Wal et al., 2004 all indicated that passive sampling methods might be applied in toxicity setups to monitor freely dissolved concentrations in soil and sediments.

Passive sampling methods work by obtaining equilibrium with the contaminated soils or sediments after which freely dissolved concentrations of contaminants can be calculated if the partition coefficient between the sampler and the aqueous phase is determined (Ter Laak et al., 2006b; Van der Wal et al., 2004).

According to Ter Laak et al., (2006b) accurate measurements with passive sampling techniques require the following criteria:

1. the sampler should be in equilibrium with the contaminated soil-water system;
2. partition coefficients to the fibres or disks should be known;
3. the sampler should not influence the concentrations in the soil pore water (it should only absorb a negligible amount);
4. no substantial fouling on the fibre surface.

Following from these requirements it can be concluded that equilibrium between the sampler and the soil or sediment is crucial.

<u>SPME:</u> SPME finds its origin in the food industry. This sampling technique consists of a device containing a small segment of fused silica fibre with a thin polymer coating (usually polydimethylsiloxane or PDMS) for the sampling of analytes. Organic contaminants bind to this device

and after measuring with HPLC[4] or GC[5] the pore water concentrations can be calculated with the SPME-water partition coefficient. With SPME only a negligible fraction of the contaminant is extracted from the pore water. This fraction does not influence the equilibrium between the bound contaminants and freely dissolved chemical (Ter Laak et al., 2006b; Van der Wal et al., 2004). Next to measuring freely dissolved contaminant concentrations SPME can potentially also be used to sense fugacity. These measurements could be very valuable because fugacity determines the direction and extent of diffusive mass transport within the environment (Mayer et al., 2000b; Van der Wal et al., 2004).

SPMD: The device consists of low-density polyethylene membrane tubes containing a thin layer of neutral glycerol triolein inside. This layer is supposed to mimic organism use of organic pollutants. The free pollutant can pass through the membrane and accumulate in the internal lipid, which is similar to how organisms absorb and accumulate the organic pollutants (Huckins et al., 1990). SPMD is specifically developed for the environmental assessment. Due to its slow uptake kinetics SPMD is typically limited to kinetic sampling (Mayer et al., 2000b).

POM-SPE: POM-SPE stands for 'polyoxymethylene solid phase extraction' and was developed by Jonker et al., (2001) when they were searching for a method that could measure distribution coefficients for soot adsorbed PAHs. Other methods to determine this fraction of contaminants proved not to be useful because the desorbed concentrations in the water phase were expected to be extremely low (Jonker et al., 2001). According to Cornelissen and Kamerling (2003) POM-SPE has a detection limit of ten times lower than SPME, but five to ten times higher than silicone rubbers.
Polyoxymethylene consists of a hard and smooth plastic and hydrophobic contaminants showed reproducible and sufficiently strong partitioning to the plastic. According to Jonker et al., (2001) soot can easily be wiped of with a moist tissue without having an effect on the distribution coefficient. The study of Mayer et al., (2000a) confirms that contaminants are absorbed *into* instead of *onto* the coating of the SPME fibres when using PDMS coatings. Wiping the surface of POM should therefore have no influence on the measured concentrations.
POM is resistant to organic solvents, meaning that it is possible to extract the contaminants from the plastic for analyses. From the research of Jonker et al., (2001) it can be suggested that the operational time equals other sampling methods (several weeks are needed to reach equilibrium) and also the costs of POM-SPE are considered low. Depending on the system design, POM can strongly extract larger amounts from the matrix than for example SPME. Thus unlike SPME, POM can deplete the natural system (up to several percentages) and therefore change the equilibrium in it.

Empore Disks: The principle behind Empore disks is equal to that for SPME and SPMD. After usage Empore disks are extracted with an organic solvent (e.g. acetone, hexane et cetera). There are still some biofouling problems with these disks when being used (fact sheets Deltares). Sometimes ultrasonic frequencies and rinsing can overcome this problem.
In contrast to the other passive sampling techniques the disks are relatively large in size, meaning that equilibrium times can take longer and therefore also the measurements. Still the disks are easy to use and are not expensive. The use of Empore disks gradually declines, due to alternatives which have a higher sensitivity and shorter equilibrium times (Cornelissen and Kamerling, 2003).

Silicone-rubber: A small piece of silicone-rubber is placed in a sediment water mixture after which the mixture is shaken and the rubber can adsorb organic contaminants. The adsorbed fraction is proportional to the pore water concentration (fact sheets Deltares). Silicone rubber is a very sensitive material and can reach detection limits up to 50-100 times lower than SPME, enabling the use of very

---

[4] HPLC = High Performance Liquid Chromatography

[5] GC = Gas chromatography

small strips of rubber. Due to its high sensitivity there is a risk for depleting the sediment mixture at low sediment densities. The surface of the rubber is less smooth than for example POM-SPE, therefore binding of DOM and black carbon can be a risk. However the rubber is cheap and easy to obtain and diffusion through silicone rubber is expected to be a little faster than in other sampling materials. This means that the needed equilibrium times are also a little shorter (Cornelissen and Kamerling, 2003).

Strong points of passive sampling

An advantage of passive sampling is that the measured concentrations of dissolved hydrophobic contaminants can be easily compared with the soil quality standards by use of sampler-water coefficients. Therefore no filtration or conversion is needed. There is also a high sensitivity of these methods in comparison to the conventional analyses with water and there is less organic solvent needed in comparison to classical measurements. These methods are relative cheap and easy to perform and the actual hands on time is according to Mayer et al., (2000b) much shorter than conventional methods.

There is a possibility to adjust parameters such as thickness of the polymer coating, agitation method and detector type. This makes it possible to measure also less hydrophobic contaminants such as surfactants and it can have a positive influence on the time that is needed to perform a measurement.

Weak points of passive sampling

The time for equilibrium after placement of the devices can be long (several weeks), depending on the diffusion rate of the contaminant, turbulent mixing of the soils and desorption of the contaminant from the matrix constituents. Due to this long extraction time it is advised to perform the extractions in parallel rather than in series. This however requires large number of SPME fibres which could in turn be costly (Mayer et al., 2000b). However according Ter Laak et al., (2006a) adjusting the dimensions, properties and agitations of the exposure vessel and the size of the passive sampler it is possible to sample detectable concentrations in a practical time span.

Fouling of the fibres or disks can have an influence on the uptake process by the sampler. Some researches indicate that this influence is of minor significance. Also the use of disposable fibres could reduce this influence (Ter Laak et al., 2006a). Another option is to correct for fouling. This correction factor can be determined by applying a substance on the sampler before the assessment is started. The influence of fouling on the decline in concentration of this substance is equal to the process of uptake by the contaminant (fact sheets Deltares).

Depletion of the soil matrix is possible, which influences the results or make measurements of total content necessary.

Concluding

In conclusion it can be said that a lot of research has been done considering passive sampling and there are several techniques to choose from. Most of these methods have been validated for the measured concentration in the sampler and in the biota (e.g. Styrishave et al., 2008; Jonker et al., 2007; Van der Wal et al., 2004). The most important information that is still lacking is the relationship between the measured concentrations by de sampler and the actual toxic concentrations for the organism. If this relationship is established the method can be applied within three years for the soil compartment according to Sijm et al., (2002). It is anticipated by the experts that aquatic toxicity data can be used for this purpose.

3.4.1.3 Tenax extraction

Much like the passive sampling methods that have been described above, Tenax (a polymer of diphenylphenylene oxide) can be used to adsorb organic pollutants. Unlike passive sampling, that measures the freely dissolved concentrations of contaminant, Tenax works by extracting the contaminant that can desorb from surrounding soil particles within a few hours or the potential concentration of contaminants. It is still assumed that the freely dissolved organic pollutants play a role in the uptake of pollutants by organisms, but the rapidly desorbing fraction is in equilibrium with this freely dissolved concentration. By measuring the rapidly desorbing fraction, the freely dissolved concentrations can be calculated using the equilibrium partitioning theory. Tenax extraction is usually performed by adding Tenax to a sediment – water mixture in a separation funnel. This funnel is then shaken for a specified amount of time (for example 6 or 24 hours) after which the Tenax is separated from the mixture and extracted with acetone and hexane. Multiple extractions on the same sediment are also possible (Cornelissen et al., 1997). There are also other ways of using Tenax, in which the Tenax does not come into direct contact with the soil or sediment. In these setups the soil moisture is guided over a Tenax column as described by Shiaris et al., 1980.

A study of Ten Hulscher et al., (2003) showed that the rapidly desorbing fraction is known to better predict the uptake of pollutants by organisms than total contaminant concentrations. According to Cornelissen et al., (2001) this rapidly desorbing fraction can be easily determined with help of a 6 h Tenax extraction. Which fraction (rapidly, slowly or very slowly) is extracted depends on the extraction time.

Strong points of Tenax

Tenax extraction of organic contaminants in sediment and soil can be used for a site specific risk assessment. The method is easy to use and can, next to contaminated water, also be used for contaminated soils. Analytical quality control proved to be positive but are limited to PCBs and PAHs. The costs of Tenax extractions are acceptable and reuse of cleaned Tenax is, for several compounds, possible. Compared to passive sampling methods Tenax takes far less time (1 or 2 days) to measure bioavailability because equilibrium is not needed. Also a considerable amount of research has been performed on Tenax and there are a fair number of laboratories that are able to perform measurements (Factsheets Deltares).

Weak points of Tenax

A disadvantage of Tenax is that the method is less useful for peaty, oily and sooty soils, because of separation problems between the soil and the Tenax-beads. Also the outcome of Tenax extraction can not directly be related to soil quality criteria. This requires a generic corrected partitioning coefficient which can relate the (measured) sorbed concentrations with the freely dissolved concentrations. But this introduces an uncertainty as the correction is laborious. Tenax can not deal with large quantities of soil and hence sample heterogeneity can have a large influence on the 6/24 h measurement. This can be overcome by using a large number of samples but this increases the analysis costs correspondingly. The time needed for extracting the contaminants (rapidly desorbed fraction) varies with soil and/or contaminant type. This makes the 6 or 24 h Tenax extraction less accurate and requires another correction factor to relate the rapidly desorbing fraction with the measured 6/24 h fraction (Cornelissen and Kamerling, 2003).

Concluding

Concluding it can be said that Tenax extractions have been studied intensively and the measured concentrations can be validated for concentrations in biota. As with passive sampling the information that is still lacking is the relationship between the measured concentrations by de sampler and the actual toxic concentrations for the organism. If this relationship is established the method can be applied within three years for the soil compartment according to Sijm et al., (2002). As for passive sampling, it is anticipated by the experts that aquatic toxicity data can be used as a reference for this purpose, especially for the rapidly desorbing fraction.

3.4.1.4 Cyclodextrine extraction

Recently several studies have been performed that use aqueous cyclodextrine extractions as alternative for the prediction of PAH availability and degradability in soils. Cyclodextrines are cyclic oligosaccharides with a hydrophilic exterior and hydrophobic toroidal-shaped interior. The

cyclodextrines use this distinctive structure to form inclusion complexes with a variety of hydrophobic organic contaminants, among which PAHs.
One of the most frequently used cyclodextrine is Hydropropyl-β-cyclodextrin (HPCD). This six-glucose cyclodextrine has been chemically modified to enhance water solubility without affecting the basic structure and characteristics (Rhodes et al., 2008b; Stokes et al., 2005). As with Tenax extraction this method focuses on the weakly bound fraction (rapidly desorbing fraction) (Ter Laak et al., 2006b).

The cyclodextrine solution is added to a soil sample and is shaken for a predetermined time. After separation (via centrifugation or filtration) the extracted concentrations can be determined.
Reid et al., (2000) performed several tests to determine optimum conditions in relation to cyclodextrine concentrations and shaking time and found that 40 mM of cyclodextrine and 6 hours of shaking were sufficient. However this research was performed on spiked soils. Stokes et al., (2005) and Cuypers et al., (2002) performed some tests with aged field contaminated soils and also found that cyclodextrin extractions can be used to determine the bioavailability of organic contaminants. However they both used different concentrations of cyclodextrine and shaking times. It can therefore be concluded that until now no uniform protocol has been developed for cyclodextrine extractions.

Strong points of cyclodextrine extractions
The time needed to perform a cyclodextrin extraction is roughly the same as for a Tenax extraction (several hours). However the principal advantage of HPCD is considered to be the ease of sample handling. Especially the separation of de HPCD from the soil/sediment particles is less laborious than for example separation of the Tenax from the soil/sediment (Cuypers et al., 2002).

Weak points of cyclodextrine extractions
A disadvantage of this method may be the high amount of cyclodextrine needed. Until now regeneration of this compound is not easily achieved and this could make the technique relatively expensive compared to other methods (Cuypers et al., 2002). Although several studies have been performed on cyclodextrine extraction the knowledge about this method is, in contrast to other methods (Tenax, Passive sampling) rather low.
Jonker and Koelmans (2002), suggested that aqueous based extractants, like cyclodextrines, may have difficulties in penetrating black carbon matrices that contain organic contaminants. The highly hydrophobic nature of black carbon leaves the cyclodextrine useless for the extraction of soils with a high organic carbon content or soot due to its low displacement capacity. Rhodes et al., (2008a) assume that cyclodextrine is no longer useful if the black carbon content of the soil is higher than 2.5 to 5%.

Concluding
Cyclodectrine extraction is a relative new method, developed between 1998 and 2000 by Reid et al., and is found to be a promising method to determine the bioavailability of PAHs in several studies. There are still some validating steps required, but this can take less than 3 years if requested (Jonker oral announcement).

3.4.1.5 Supercritical fluid extraction

Several studies (Björklund et al., 1999; Hawthorne et al., 2002; Hawthorne et al., 2001; Hawthorne and Grabanski.,2000) have demonstrated that supercritical fluid extraction (SFE) can be used as a replacement for classical extraction methods for the extraction of PCBs and PAHs from soil and sediments.
SFE is a process in which components (e.g. contaminants) are extracted from the soil matrix (but also from a water matrix) by use of an extractant under specified pressure and temperature (both above the critical values). By varying this temperature and pressure, the properties of the supercritical fluid and,

with it the extraction, can be made more selective, providing the possibility to extract specific fractions of the contaminant.
As with Tenax extraction this method focuses on the weakly bound fraction of contaminants (Ter Laak, et al., 2006b). Therefore adapting the pressure and or temperature provides the researcher with the possibility to only extract the rapidly desorbing fraction. This corresponds, according to Hawthorne & Grabanski, (2000) and the general assumption, with the bioavailable fraction of the contaminant in soil. Carbon dioxide (CO_2) is usually used as the extractant because its polarity is similar to biological lipids and under the correct temperature and pressure the solubility of low and high molecular weight PAHs in water and the supercritical CO_2 are similar (Hawthorne et al., 2001). Another advantage is that CO_2 is easily removed after the extraction. Sometimes methanol and ethanol are added as modifiers but unless described otherwise this report focuses on unmodified CO_2.

Strong points of supercritical fluid extraction
SFE is considered much cleaner than organic solvent extractions and SFE does not appear to affect the soil and sediment bulk organic content. Also SFE is a very rapid extraction taking, depending on the pressure, temperature and the desired contaminant fraction, only several minutes to hours to perform. Hawthorne and Grabanski (2000), found in their experiments that the rapidly desorbing fraction can be measured in 0-30 minutes after starting the extraction. The moderate, slow and very slow fractions could be measured in respectively 30 – 60 min, 60 – 90 min and 90 -120 minutes after starting the extraction.

Weak points of supercritical fluid extraction
In contrast with the traditional extraction methods SFE is more costly. However the main disadvantage of SFE is that the relationship between contaminant behaviour in SFE and the actual behaviour in the field is mostly conjectural (Hawthorne and Grabanski, 2000). It is not always clear if the extracted fraction by SFE comprises the total available fraction and there is still some research needed concerning optimal pressure and temperature. Finally some expensive and intricate equipment is needed (Cornelissen and Kamerling, 2003).

Concluding
Despite the fact that the studies mentioned before indicate that SFE can be used as a method to measure the bioavailability of organic contaminants in the environment, this method requires more research to optimize. Also the expensive and intricate equipment may cause problems for acceptance. Therefore it is estimated that SFE is not the optimal method to implement bioavailability into policy, also in comparison to other methods.

3.4.2 Organic contaminants and heavy metals

Measuring pore water concentrations directly can be used to determine the bioavailability of both organic contaminants and heavy metals and will be discussed in the following section.

3.4.2.1 Measuring pore water concentrations (directly)

By measuring pore water concentrations of organic contaminants or heavy metals directly, the mobile part of the contaminants can be determined. Some common techniques to sample pore water are: centrifugation of soils, vacuum extraction with a suction cell or rhizon sampler (also called vacuum sampling), undisturbed sediment probing and sampling with a zero-tension sampler (also called passive sampling). The most common treatment is centrifugation.

Centrifugation of the soil: Soils are sampled in the field and brought to the laboratory where with centrifuging at high speed, soil water is separated from the soil. Soils can either be centrifuged at their actual moisture content or the soil can be centrifuged at a standardized moisture content by adjusting the moisture content of the soil to a pre-set condition (for example 80% of the field capacity). The moisture content can be adjusted by adding demi-water or a dilute salt solution (e.g. 0.002 M $CaCl_2$). After adding solution the soil is allowed to equilibrate after which the soil is centrifuged. The collected pore water is then filtered over a 0.45 μm filter to remove colloidal particles.

Vacuum sampling: The most common technique of vacuum sampling is by use of suction cells. The suction cell is a porous cup installed in the field, where it is in contact with the surrounding soil. At the time of sampling a vacuum is created which sucks the pore water into a porous cup. In simple systems, the water is stored in the suction cell and is then sucked or blown into a sample flask placed on the ground surface. Vacuum sampling is also possible with rhizon samplers installed in pots in the laboratory.

Undisturbed sediment probing: Pore water is probed under undisturbed circumstances, using a corer device and pore water sampling probes. The technique is known as 'Sediment or Fauna Incubation Experiment' (SOFIE) and allows for repeated and redox-dependent measurements of metal concentration gradients (Vink, 2002).

Zero-tension sampler (passive sampling): The zero-tension sampler only collects water when the pore water content of the soil exceeds the water holding capacity. It collects pore water seepage in a collection chamber. The pore water is then removed from the chamber by pumping or syphoning it, sucking it by vacuum or by lifting and emptying the collection chamber.

Strong points of measuring pore water concentrations (directly)

One of the most important advantages of measuring pore water concentrations directly is that there are no conversion or translation steps needed to compare the measured concentrations to the legal standards. In other words the measured pore water concentrations can be directly compared to groundwater concentrations as described in the soil quality framework. The methods also provides an accurate account of the actual concentrations on site, however these concentrations vary strongly and are very time an space depended (e.g. rain versus no rain, summer versus winter et cetera).

Weak points of measuring pore water concentrations (directly)

Suction cells and vacuum samplers are time consuming procedures which are only used when repeated sampling is foreseen at several times. Centrifuging is only used in some laboratories and is also a time consuming technique. An alternative for solution sampling with the above described techniques is the use of dilute salt extractions to mimic pore water. This method will be discussed in section 3.4.3.2.

It can also be a problem to obtain enough pore water to perform a measurement (for example due to drought) also the measured concentrations are very time and space dependent.

Finally when measuring solution concentrations in sampled pore water, total concentrations are determined which include contaminants bound to DOM (Dissolved Organic Matter). DOM is known for its high affinity for organic contaminants. It is possible to estimate the free pore water concentrations by correcting the measured concentrations for DOM. This requires information about the DOM concentrations in the aqueous phase and the sorption of the compounds to DOM (Ter Laak et al., 2006a).

Another way to eliminate the binding of compounds to DOM is by flocculation. Aluminium potassium sulphate can be added to a solution, however this requires and adjustment of the pH and this might change the equilibrium between the freely dissolved and bound contaminants. This method can however only be used for (non-dissociated) organic contaminants.

Finally it is also possible to use a dialysis membrane, in which the freely dissolved concentrations pass the membrane and the DOM does not. However this technique is very labour intensive and requires large amounts of water inside the membrane to detect the very hydrophobic contaminants (Ter Laak et al., 2006a).

Concluding

Although this method can and is already used to perform risk assessments and a policy framework is already present, it is concluded that there are too many practical problems with this method to be used on routine basis. Therefore it is not considered fit for implementation. In situ sampling of pore water is however interesting in case of repeated sampling in monitoring studies.

3.4.3 Heavy metals

3.4.3.1 Introduction

Metals in the soil environment are present in the soil solid phase and soil solution phase. Within the solid phase metals can be present within mineral lattices such as clays and oxides, occluded in mineral or organic particles, sorbed to the surface of mineral and organic particles or present in (surface) precipitates (see also Figure 3.1). Only part of the metals in the solid phase is available for exchange with the soil solution within a for biological uptake relevant time scale. This reactive fraction is referred to in literature as the potential available or geochemical active metal. This fraction includes reversibly sorbed metals ions and precipitated minerals. The inert fraction is not readily available and contains metals in minerals and occluded metals. Exchange between the inert and reactive phase takes place via slow processes such as weathering and ageing. Within the solution phase metals exist as free metal ions or as metals bound to inorganic (e.g. HCO_3^-, Cl^-) and organic ligands present in dissolved organic matter (DOM).

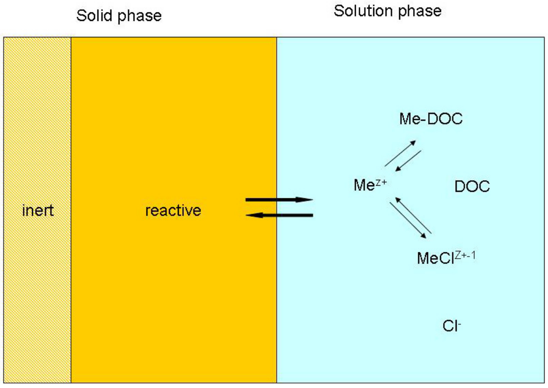

Figure 3.1: Schematic presentation of metal speciation and partitioning between the soil solid phase and soil solution.

Several methods were developed to quantify the chemical- or bioavailability of metals in soil. Methods can be divided into extractions that either determine a measure for the available metal content in the soil solid phase (named potential available content, reactive content, geochemical active content et cetera) or determine a measure for the available metal concentration in the water phase (actual available content). For both, several extractions or analytical techniques were developed which will be discussed in the following sections. Figure 3.2 gives an overview of the different methods and how these are related to each other. Several mechanistic and empirical models are available to calculate the partitioning of metals over the soil solid and water phase. To quantify effects, both empirical and more mechanistic based models are being developed or are under development to relate metal contents to internal concentrations or toxic effects. Furthermore models are available to calculate metal speciation in soil solution. With these models the free metal ion concentration can be calculated from total solution concentrations, DOC and pH.

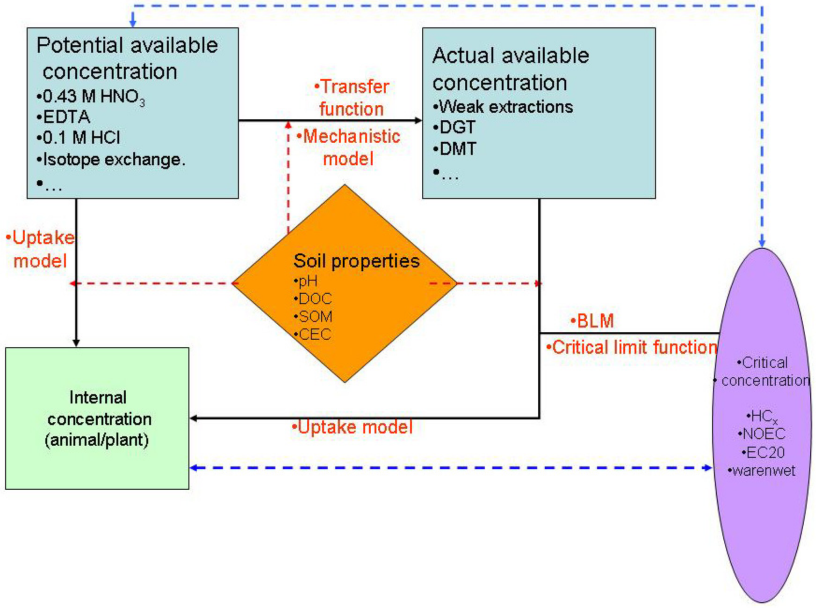

Figure 3.2: Schematic overview of relations between different methods to determine bioavailability of metals and how these are related to each other.

The following methods which can be used in a framework to determine the bioavailability of heavy metals will be discussed in the next sections:
Methods for extraction of the potential available metal content
- acid extractions;
- extractions with chelating agents;

Extractions to extract the actual available content;
- weak extractions;
- Donnan membrane technique;
- diffusive gradient in thin films;
- sequential extractions;
- two steps risk assessment;

Models to calculate metal speciation and/or partitioning in the soil solid phase and soil solution:
- mechanistic assemblage models;
- empirical models such as transfer functions;
- models to calculate internal concentrations and effect levels:
    - terrestrial Biotic Ligand Models (BLM);
    - empirical effect models.

Total metal concentrations or pseudo total concentrations can be determined with X-Ray Fluorescence Spectroscopy (XRF) or Aqua regia extractions. However these methods include metals occluded in mineral and organic soil particles which are not available for exchange with the soil solution within time scales relevant to biota.

Several techniques were developed to extract only ‘available’ metal contents. The difficulty in finding an extractant that measures the availability accurately is that the availability varies amongst different organisms. As such one extraction method to measure the available metal content for all the different metals (e.g. copper, lead et cetera) does probably not exist (Peijnenburg et al., 2007). In this section we distinguish between extractions which measure the potential metal content in the solid phase and extractions which give a measure for the actual available metal concentration in the soil solution phase.

3.4.3.2 Methods for extraction of the potential available metal content

The potential available metal content is a measure for the total amount of metal which is available for desorption/dissolution within a relevant time scale. Single as well as sequential and parallel extraction schemes were developed. Within single extractions a distinction can be made between two groups of extractants i.e. extractions with acids (e.g 0.43 M HNO_3, 0.1 M HCl) which push metals from the solid phase and extractions with chelators (e.g EDTA, DTPA) which pull metals from the solid phase.
When using the potential available metal content it is assumed that uptake by biota takes place via the soil solution phase. Potential available metal contents can be used in models to calculate concentrations in soil solution or can be used in models to calculate internal concentrations or effect levels using empirical or mechanistic models such as BLMs. As such, potential available metals can be compared with existing toxicity data for soils when it is assumed that the added metal content in the toxicity experiment is totally available. This assumption needs to be tested.

Acid extractions: dried soil or sediment is shaken with 0.43 M HNO_3 or 0.1 M HCl for several hours. After centrifuging and filtration, the concentrations of metals in the extract can be measured with for example ICP-AES[6]. Both HNO_3 and HCl extractions measure the potential available fraction of heavy metals from the soils and sediments, but metals bound in the crystalline lattice are not extracted. Still a part of the background concentration is extracted and the extent of this process is hard to predict.

Strong points of acid extractions
The measurements done with this method can be directly related to existing toxicity data, which means that no fundamental changes in the current policy framework are necessary (Sijm et al., 2003). The extraction with 0.43 M HNO_3 is shown to be useful as a measure for the potential chemical availability when predicting solution concentrations of cationic metals (cadmium, copper, nickel, lead and zinc) with mechanistic models (Dijkstra et al., 2004; Bonten et al., 2008) and transfer functions (Römkens et al., 2004; Tipping et al., 2003; Groenenberg et al., 2008). The method is expected to work for other cationic metals as well; however at present no literature is available to confirm this.

Weak points of acid extractions
The method still needs to be tested whether extraction is also suitable for metals present as anions (As, Cr(VI), V et cetera). A part of the background concentration in the soil solid phase is extracted and the extent of this process is hard to predict.
Acid reactions only measure the potential risk of the chemically available fraction of the contaminant. The actual exposure is however determined by local soil parameters (such as organic matter content, clay percentage and pH) and the uptake characteristics of the biota. Therefore this method always requires additional testing for determining the actual availability (Sijm et al., 2003). Also careful interpretation of results obtained by dilute acids in calcareous soils is needed since at high $CaCO_3$ levels, all acid can be buffered by lime instead of releasing the metals from soil. Houba et al., (2003), gives an indication how to correct for $CaCO_3$ by adding 0.2 ml 5M HNO_3 per % of $CaCO_3$.
As with other methods described in this report the relation between measured concentrations and actual toxicity for biota still needs further research.

---

[6] ICP-AES = Inductively coupled plasma atomic emission spectroscopy

Concluding
Much information is available on extracting soils with acids. If the relation between measured concentrations and actual toxicity for biota is validated the method can be implemented into policy. This can be expected within three years (Sijm et al., 2002; fact sheets Deltares).

Extractions with chelating agents: another common extraction of the potential available metal concentration is performed with complexing or chelating agents. The most familiar reagent is EDTA (ethylenediaminetetraacetic acid), but also DTPA (diethylenetriaminepentacetic acid) and NTA (nitrilotriacetate) are used (Papassiopi et al., 1999). The soil and sediment samples are extracted with the reagents and the chelating agents work by forming soluble complexes with heavy metals. The solution is then centrifuged and after separation the supernantant analyzed. Extraction with complexing agents is shown to be useful as a measure for the potential chemical availability when predicting solution concentrations of cadmium, chromium, copper, nickel, lead and zinc (e.g. Quevauviller et al., 1998; Manouchehri et al., 2006). The concentrations of EDTA and DTPA needed are still subject of discussion however there are several studies (e.g. Quevauviller et al., 1998; Fangueiro et al., 2002) that indicate that 0.05 mol/L (pH 7) for EDTA and 0.005 mol/L (pH 7.3) for DTPA are high enough.

Strong points of extractions with chelating agents
For both EDTA and DTPA extraction protocols have been composed by the 'Measurements and Testing Program' (former BCR) of the European Commission (Quevauviller et al., 1998). EDTA and DTPA are widely used, not only for the prediction of the bioavailability of heavy metals but also for supplying micronutrient cations to plants and soil remediation processes (Manouchehri et al., 2006). Several studies have shown the similarity between metal extractions with EDTA and 0.43 M HNO_3 for Cd, Cu, Pb and Zn e.g. Groenenberg et al., (2002), Tipping et al.,(2003) and De Vries et al., (2005).

Weak points of extractions with chelating agents
Little attention has, until now, been paid to the influence of major elements such as calcium. In several studies evidence is found that calcium rich soils use the majority of the added EDTA to form Ca-complexes as the primary process, only thereafter the heavy metals are bound in complexes. Therefore it is necessary to determine the soil major elements content to determine the competition between the metals to be chelated by the reagent (Manouchehri et al., 2006). This is in order to add an excess of chelating agent to calcareous soil and sediment samples. Further research on this subject is needed (Manouchehri et al., 2006; Quevauviller et al., 1998). Furthermore, chelating agents possibly deplete soil samples.

Concluding
As with extractions with acid much information is known on extracting soils with chelating agents. If the relation between measured concentrations and actual toxicity for biota is validated the method can be implemented into policy. This is to be expected within three years (Sijm et al., 2002; fact sheets Deltares).

3.4.3.3 Methods for extraction of the actual available metal content

The background for these methods is that it is assumed that organisms and plants are exposed to the metals via the water phase. Within these methods we can distinguish between 1) methods which are a measure for the total concentration in solution (including metals complexed with organic and inorganic ligands) and 2) methods which give a measure of the free metal ion concentration in solution and 3) methods in between.

Weak extractions: weak extractions such as 0.01 M and 0.0025 M $CaCl_2$ and 0.0025 M $NaNO_3$ extractions are used to extract the actual available metal concentration in solution. In general the extraction is performed by shaking dried soil with the extractant. After a fixed time of equilibration the suspension is centrifuged to separate the solution from the solid phase and the supernatant is used for analysis. Metal concentrations are usually measured with ICP-AES or ICP-MS[7]. In addition also pH and DOM can be determined. Factors like the electrolyte concentration, and the solid to liquid ratio of the extraction, affect the concentration of metals and DOM in the extracted solution (Fest et al., 2008).

There is still some discussion about which concentration of extractant should be used. A modified approach of the weak extraction was given by Schröder et al., (2005). They studied the solid/solution partitioning in a large number of soils, using a weak extraction method (both 0.01 M and 0.0025 M $CaCl_2$) and pore water sampling. Schröder et al., concluded that a 0.0025 M $CaCl_2$ extraction was more robust than 0.01 M $CaCl_2$ extraction, since this electrolyte is more in agreement with the strength and composition of regular pore waters than 0.01 M $CaCl_2$. They recommended this modified extraction for future monitoring programmes.

With the use of speciation models like NICA-Donnan (Kinniburgh et al., 1999) and WHAM/Model VI (Tipping, 1998) the free metal ion concentration in solution can be calculated from the total metal concentration, pH and DOM. Measured values can be interpreted using BLMs as far as they are present at the moment.

Strong points of weak extractions

Weak salt extractions in general correlate, in general, well with soil solution concentrations (e.g Degryse et al., 2003). For several plants and organisms good relations could be derived between the 0.01 M $CaCl_2$ extractable metal concentration and internal concentrations and toxic effects. The method is easy to perform, cheap and reproducible. To which extent the use of 0.0025 M $CaCl_2$ presents more representative results than 0.01 M $CaCl_2$ is a topic of further research.

Weak points of weak extractions

A limitation of the $CaCl_2$ the method is that there are no effect concentrations for soil organisms available against which the results can be compared. Another limitation of $CaCl_2$ and other weak extractions is that it is species dependent. In relation with the previous, the method still needs to be tested for different types of organisms, soils, soil characteristics and contaminant sources. This will bring high costs and will probably take more than three years (Sijm et al., 2002). Finally the method gives the total concentration of metals in soil including metal bound to DOM and inorganic ligands.

Concluding

There is already an amount of data available, however the methods still need more testing for different types of organisms, soils, soil characteristics and contaminant sources. It is expected that this takes more than 3 years (Sijm et al., 2002).

Donnan Membrane Technique (DMT): DMT makes it possible to determine the free metal ion concentration for several cationic metals simultaneously. The method is based on the assumption of (pseudo) equilibrium between a donor (the sample solution) and an acceptor solution in a Donnan cell separated by a cation exchange membrane. The membrane allows fast transport of free cations but hinders the transport of complexed cations and anions. The concentrations of metals in the acceptor cell equals the free metal ion concentrations in the donor cell. The concentration in the acceptor cell is measured with ICP-MS. The method is proven to be successful for the metals cadmium, copper, nickel, lead and zinc (Temminghoff et al., 2000; Weng et al., 2001a; Pampura et al., 2006; Koopmans et al., 2008) but in principle it can be used for all cationic metals.

---

[7] ICP-AES = Inductively coupled plasma atomic emission spectroscopy

ICP-MS = Inductively coupled plasma mass spectrometry

Strong points of DMT

A big advantage of the DMT method compared to other methods to determine free metal ion concentrations, like ion selective electrodes, is the fact that with DMT the free metal ion of several methods can be determined simultaneously.

Weak points of DMT

The method is time consuming and not (yet) suitable for routine analysis. A limitation of the method is the detection limit of the ICP-MS. With ongoing improvements of the ICP-MS technique detection limits are still decreasing. Furthermore methods were developed to overcome this problem by adding humic acid (with well known binding constants) to the acceptor solution to raise the concentration to be analyzed (Kalis et al., 2006). Large volumes of pore water are needed (50 ml).

Concluding

Although several positive results are reported for different metals, this method is not considered an option for implementation into policy, due to the fact that it can not (yet) be implemented at a routine basis.

Diffusive Gradient in thin Films (DGT): With DGT the labile fraction of metals in soil solution is measured. This fraction includes both the free metal ion as well as weak complexes of metals with organic and inorganic ligands. A DGT sensor consists of a layer of hydrogel (0.4-2 mm thick) with an overlying layer of Chelex resin beads. Concentration gradients are established in the gel layer as metal species diffuse through it (planar diffusion) and accumulate in the resin. The lability and diffusion coefficients of penetrating complexes determine the amount of metal collected in the resin (Van Leeuwen et al., 2005). A recent publication suggests that bioavailable copper (for aquatic mosses) comprises more than the free inorganic copper and includes also weak organic complexes as determined with DGT, however more work is needed to confirm this.

Strong points of DGT

Indirectly it accounts for speciation in the water phase and the desorption from the solid phase. The method presents a time integrated view of the availability of the metals depending on the physical-chemical changes. It can also be used on many different types of soils.

Weak points of DGT

A disadvantage of the technique is that the measured concentration is operationally defined. DGT is not sufficiently tested for metal concentrations in sediments and can not be used for dry soil as there is no reference to compare with. Therefore the method seems limited to water and sediments.

One of the primary assumptions of DGT is that it simulates the uptake of metals by an organism and that this organism has no influence on the rate of uptake. This makes DGT less useful for elementary metals because organisms have an influence on the uptake rate of these metals. Also this method only gives an indication for risks to animals that have a high uptake rate for metals. Also a lot of specialistic expertise is needed.

Concluding

DGT is a relative new technique; this implies that still a lot of testing is needed. This will take probably more than 3 years (Sijm et al., 2002; fact sheets Deltares).

Sequential extractions: With the use of sequential extractions one soil sample is extracted with several extractants with increasing strength in sequence. The first extraction is usually a mild salt extraction which could be used as a measure for the actual available metal concentration. Subsequent extractions are used to desorb metals with different bonding strength. Usually these fractions are linked with a specific soil constituent to which the metals are assumed to be bound, however the different fractions are operationally defined. The last fraction is usually the inert fraction metal which is not available. The sum of all extractions, except the last inert fraction, gives a measure for the reactive metal content. Sequential extractions are described by Tessier et al., (1979) and BCR by Rauret et al., 1999. Tessier et al. includes the following fractions:

- exchangeable (MgCl or Na-acetate);
- bound to carbonate (Na-acetate);
- bound to Fe- and Mn-oxides ($Na_2S_2O_4$ or NH_2OH-HCL with acetic acid);
- bound to organic matter (HNO_3 + H_2O_2 followed by NH_4-Acetate);
- other fractions ($HF+HClO_4^-$).

And BCR[8] by Rauret et al. includes the following fractions:

- exchangeable including bound to carbonate (acetic acid);
- bound to Fe- and Mn-oxides (NH_2OH-HCL);
- bound to organic matter (H_2O_2 followed by NH_4-Acetate).

An alternative to sequential extractions is the use of parallel extractions when methods for actual and potential available metals are used in parallel on subsamples from the same sample. The use of parallel extractions is time saving compared to sequential extractions.

Vink et al. (1999) suggested a parallel extraction procedure for metals, based on weak ($CaCl_2$) extraction, immediately followed by a HNO_3 extraction of the residue of the same sample. The large advantage is that information is acquired on both (part of) the solid and the dissolved phase (i.e., potential and actual availability, respectively). The division of the two gives additional information on the distribution coefficients of various metals, which may aid in distinguishing and prioritizing soils or locations based on availability. This procedure is simple to implement in regular laboratory procedures. Vink advises to use the modified weak extraction of 0.0025 M $CaCl_2$ as described by Schröder et al. (2005).

Strong points of sequential extractions

Until now it was assumed that the extractions to measure the actual and potential available fraction were performed one at the time. In other words; a test is performed and finished before starting a next one. However with help of sequential extraction it is possible to determine the actual and potential availability of chemicals directly after each other. So the sample of the first test is also used for the second, third et cetera. Measuring both the freely adsorbed concentration and the bound concentrations has the advantage that for this method it is not only assumed that exposure via pore water is the most important exposure pathway, but also direct contact via soil and sediment. The application of several extractions delivers insight in the actual and potential risks at once and is supplementary to determining total concentrations. The parallel extractions discount the influence of soil and sediment characteristics that modify availability.

Weak points of sequential extractions

As with other extractions the extracted concentration does not always correlate with the accumulated concentrations in the organism. It is, however, unclear how extra information, which results from the different extractions, can be used to interpret the bio-availability. Therefore the link with ecotoxicological effects needs to be confirmed still. Sequential extractions are laborious, costly and more prone to analytical problems compared to single extractions. In addition they give no extra information than a combination of a single extraction for the potential available concentration and a single extraction for the actual available concentration.

---

[8] Bureau Commune de Reference, Commission of the European Union.

Concluding

This method experiences the same difficulties as the weak extractions and acid extraction, because they are based on the same principle. Therefore the possibility of implementation for this method will probably takes between 1-3 years (Sijm et al., 2002; fact sheets Deltares).

<u>Two steps risk assessment for zinc in anaerobic sediments</u>: if zinc concentrations in anaerobic sediments exceed the legal limit it is possible to apply a correction for zinc by use of AVS (Acid Volatile Sulphide) and organic carbon as described by the EU-risk assessment for zinc. This assessment is described in Figure 3.3 below. The method assumes that under anaerobic circumstances zinc is bound to sulphides and in this way reduces the availability of zinc to organisms. The concentration of sulphides is determined with AVS. When determining the concentration of sulphides also the concentrations of bivalent cations are determined (SEM or Simultaneously Extracted Metals). These cations consist mostly out of Zn^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+}. If there is not enough organic matter to bind the metals, these metals will spread over the pore water and other ligands.

Strong points of two steps risk assessment for zinc

Under anaerobic conditions the bioavailability of metals can be limited. It is estimated that with this method zinc in sediments will be less of a problem because this limited availability is accounted for. The method also gives a clear minimum beneath which no effects are expected.

Weak points of two steps risk assessment for zinc

A large disadvantage of this method is that it can only be used under anaerobic conditions and for non-suspended sediment. Therefore use in aerobic terrestrial soils in not possible. The method does not account for different AVS concentrations with different depths and there is only a small dataset available. The relationship with ecotoxicity is weak and should be given a better scientific underpinning. The method has high costs, because AVS is currently not measured on a routine basis.

Concluding

This method can only be used for anaerobic sediments and not for terrestrial soils. Implementation into policy is therefore limited to aquatic systems. Implementation will probably take more than three years (Sijm et al., 2002).

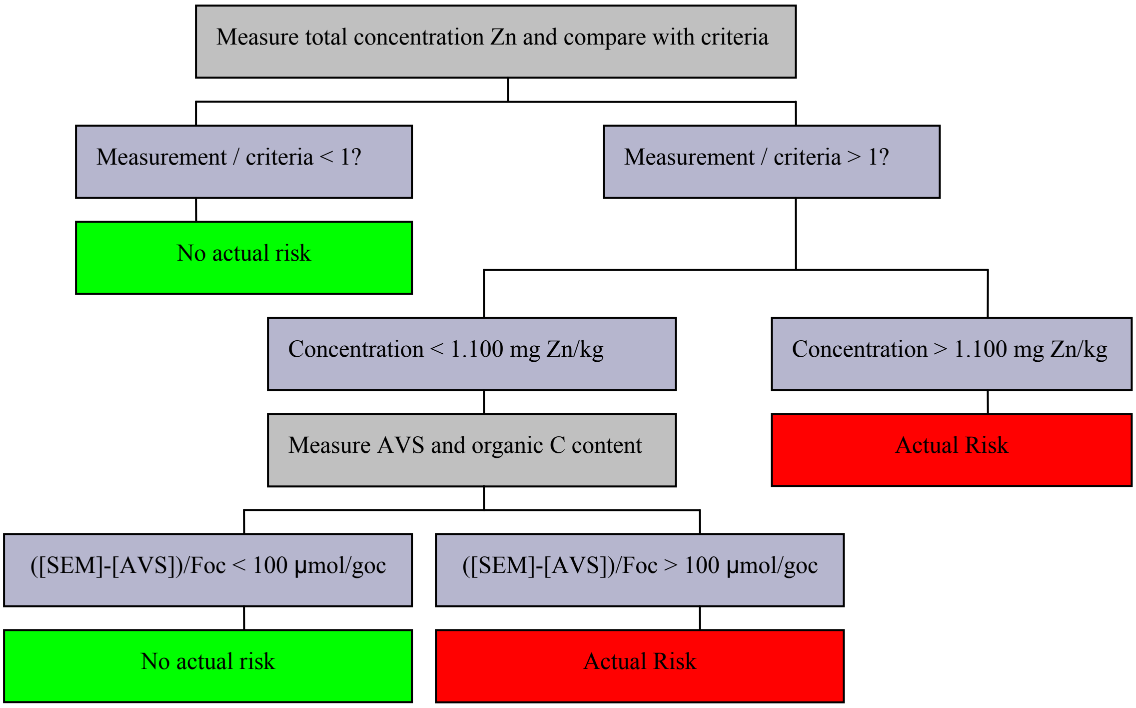

Figure 3.3: A proposal for a two step risk assessment for zinc in anaerobic sediments. If the measured total concentrations exceed the quality criteria it is possible to apply a correction for zinc by use of AVS (Acid Volatile Sulphide) and organic carbon as described by the EU-risk assessment for zinc (Sijm et al., 2002).

3.4.3.4 Models to calculate metal speciation and partitioning in soils

Speciation models for the soil solution can be used to calculate the concentrations of free metals in soil solution, i.e. metals not bound to inorganic (e.g Cl^-, $HCO3^-$) and organic ligands (DOC), based on measured total concentrations. Models to calculate metal ion binding to organic matter (in this case DOC) are a crucial. WHAM/model VI (Tipping 1998) and the NICA-Donnan model (Kinniburgh et al., 1999) are at present the most advanced models to calculate metal speciation including the metal organic interaction. Several studies have shown the applicability of these models (e.g. Weng et al., 2001b; Ge et al., 2005). Pampura et al., (2006) and Groenenberg et al., (2008) show that predictions with these models have mean absolute errors between 0.2-0.6 log units.

Models for metal partitioning between the soil solid phase and the soil solution are the link between the potential available metal content in the solid phase and the actual available concentration in solution. Both mechanistic based as well as empirical models for metal partitioning are available.

Mechanistic assemblage models: Mechanistic assemblage models consist of a set of models which each describe the binding of metals to a different soil constituent and precipitation/dissolution reactions. Usually these models contain sub models for the binding to organic matter in soil and soil solution (NICA-Donnan model (Kinniburgh et al., 1999) and WHAM Model VI (Tipping, 1998)), clay and aluminum and iron oxides (Dzombak and Morel (1990). It is assumed that the binding to the different fractions is additive. Examples are the models by Cancès et al., (2003), Weng et al., (2001b) and Dijkstra et al., (2004). Bonten et al., (2008) showed that concentrations of Cd, Cu and Zn can be predicted well for a large set of soils with a large variety in soil properties. Solution concentrations for Pb were however overestimated. The model can be used both to predict total metal concentrations and free metal ion concentrations in solution. With these models it is possible to account for changes in availability due to changes in the environmental conditions such as pH and organic matter content.

Strong points of mechanistic models
These models have a sound mechanistic basis including competition effects which give them a firm basis for predictions. Changing environmental effects can be taken into account (change in organic matter, pH). These models can be linked to BLMs.

Weak points of mechanistic models
Until now the models are only validated for cadmium, copper, zinc and lead..

Concluding
Models provide an easy, cheap and quick insight in the bioavailability of metals. However due to the limited validation for different types of metals in different soils, they are not (yet) considered ready for implementation.

Transfer functions: for some metals empirical relations for metal partitioning (transfer functions) have been established which can be used to relate metal concentration (free or total) in pore water (and 0.01 M $CaCl_2$) to the reactive metal concentrations (0.43 M HNO_3), soil properties and pH. There are reliable transfer functions for cadmium, nickel, zinc, copper and lead, for other metals functions are still lacking (Groenenberg et al., 2008).

Strong points of transfer functions
An advantage of these functions is the possibility to estimate the actual risks on basis of soil characteristics and the chemical reactive fraction (0.43 M HNO_3). The functions can next to or instead of weak salt extractions account for future changes in soil characteristics (for example changing pH or organic matter content). Also the method delivers insight in chemical factors that influence the bioavailability. Recently transfer functions for free metal ion activity were shown to be widely applicable by validating them on independent data sets (Groenenberg et al., 2008).

Weak points of transfer functions
Due to the lack of reliable functions for other metals than cadmium, nickel, zinc, copper and lead the method is not ready for implementation. Research for these new functions may take three years (Sijm et al., 2002). This however is more or less the same for other models discussed here.

Concluding
Next to or as replacement of weak extractions, transfer functions can be a useful addition in determining bioavailability. Implementation can probably be achieved in 1-3 years (Sijm et al., 2002; fact sheets Deltares).

3.4.3.5 Models to calculate internal concentrations and effect levels

Models can be used to calculate internal body concentrations or effect levels from measured potential or actual available metal concentrations. The simplest models empirically relate internal concentrations to measured concentrations, for example regression relations between metal concentrations in plants and animals and concentrations in soils, accounting for soil properties like pH and organic matter content. The more complex models like the Biotic Ligand Models describe metal uptake or toxicity on a more mechanistic basis and account for speciation of metals and competition effects.

Biotic Ligand Models: BLMs are originally designed to predict metal toxicity to aquatic organisms by integrating important determinants for toxicity. For that purpose they take into account both the metal speciation and interactions of metals at the site of toxic action. In the models it is assumed that there is a competition by cations (including Ca^{2+}, Mg^{2+}, Na^+ and H^+) to negatively charged binding places, such as gill surface sites (Koster et al., 2006). See also Figure 3.4. The bioavailability of metals to soil organisms is also influenced by parameters such as DOC and pH. It is likely that the BLM principle can also be applied to the terrestrial environment due to the assumption that the mechanisms of toxicity in aquatic and terrestrial organisms are the same (Steenbergen et al., 2005).

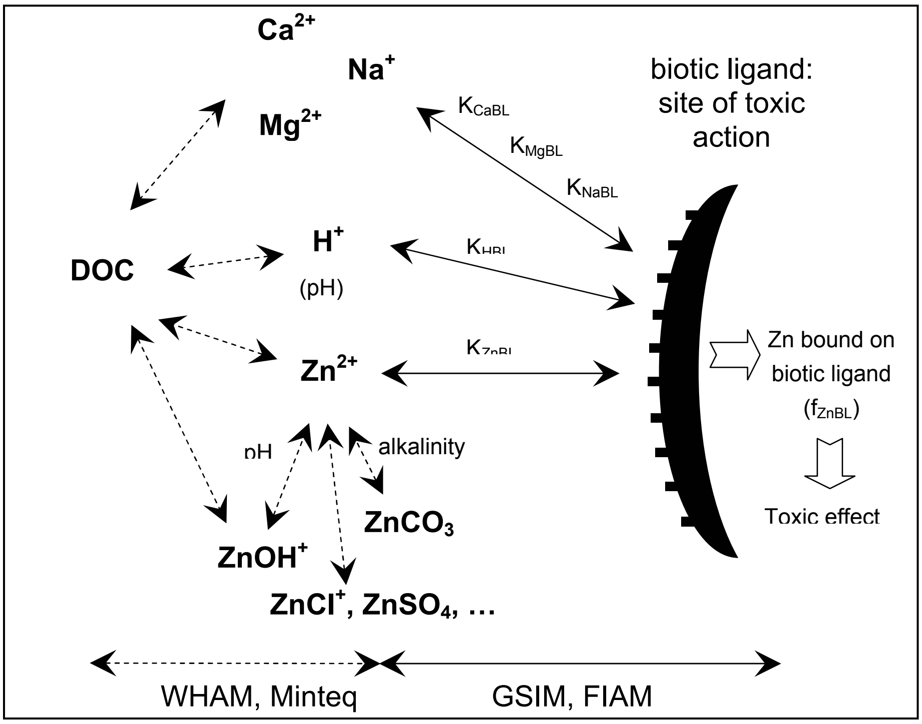

Figure 3.4: Schematically overview of the Biotic Ligand Model for bioavailability and toxicity of zinc (this scheme also applies to other metals). The dotted Lines show the interaction of the zinc ion with the different ligands in surface water (left side of figure), the other lines show the binding of the free metal-ions to the so-called biotic ligands. The extent of binding of the free zinc ion is diminished as the concentration of the competitive ions (Ca^{2+}, Mg^{2+}, Na^+ and H^+) increases. It is assumed that the toxicity is proportional to the amount of bound zinc to the biotic ligand. (By kind permission of Dr. K de Schamphelaere of the University of Gent, Belgium).

Strong points of BLM

The model is flexible and can include several important aspects of the soil chemistry that affect toxicity. These are: temperature, pH, DOC, major cations (calcium, magnesium, sodium and potassium), major anions (sulphate and chloride) and alkalinity. The first developments where obtained from the water compartment, therefore a basic knowledge about how to work with the model and how to derive relations is present. The model is cheap in use once it is established.

Weak points of BLM

A disadvantage of BLMs is that a lot of new experimental research is needed to establish BLMs for different metals and species. It is not possible to use data from the large set of toxicity tests that are already available. Until now the terrestrial BLM concept has only been scarcely researched. This is mainly due to the complexness of the routes of metal uptake in soils in relation to water, since exposure via soil particles and pore water are in principle both of substantial importance. Also it is problematic to manipulate the composition of the soil/water system and to control the metal concentrations in the pore water. This is due to the re-equilibration of the system after modification of the soil properties (Steenbergen et al., 2005). Although promising, the above-mentioned model has not yet been validated in soils, and more research is required before these models can be used for the environmental risk assessment of metals in soils. Kinetic effects, if present, are not accounted for.

Concluding
Due to the fact that for the derivation of BLMs a lot of new research data is needed, this method can not be implemented on the short term.

Empirical effect models: To overcome the problem mentioned for BLMs (lot of new research needed) Lofts et al., (2004) and De Vries et al., (2007) derived empirical critical limit functions using available toxicity data. The main assumption in this model is that organisms are affected primarily by the free metal ion in solution. Toxicity can be counteracted by the competition of other cations in solution, for example calcium. Because protons are important competing cations and concentrations of other cations are often correlated to the pH a lumped pH parameter for competition is used in the model. For most toxicity data information on the pH is available. The free metal ion activity in the toxicity test is in analogy with transfer functions assumed to be related to the organic matter content and the pH. Based on these assumptions relations were developed which can predict a HC_x-concentration in terms of free metal concentrations depending on pH. The method at present is available to predict HC_5 concentrations for cadmium, copper, lead and zinc.

Strong points
As with other models it empirical models are easy to use. Also the model is cheap in use once it is established.

Weak points
Although emperical effect models are promising, they have not yet been validated in soils. Therefore more research is required before these models can be used for the environmental risk assessment of metals in soils. As with BLMs kinetic effects are not accounted for and only proton competition is taken into account.

Concluding
Due to the lack of validation on terrestrial soils empirical effect models are not considerd an option for implementation into policy.

3.5 Selection of methods

3.5.1 Selection criteria

Based on the requirements mentioned in section 1.3 some selection criteria have been determined. These selection criteria are used to score the candidate methods on their applicability for implementation into policy. These selection criteria are carefully chosen in such a way that the selected method(s) can easily be implemented in the current way of risk assessment without the necessity of large changes.

The following selection criteria were used:

1) Wide ranging applicability
Wide ranging applicability of methods comprises different factors. In this report it is decided that the following factors are important:

a) The possibility to perform the technique in a standard laboratory. Meaning, that no excessive costs or training are needed to adapt the laboratory to use this method;
b) The possibility to assess more than one type of organism. Meaning that the risk assessment is not only representative for one type of organism, but represents the assessed environment;
c) The possibility to assess more than one type of soil. Meaning that the method selected can determine bioavailability for more than one soil type, to prevent a jumble of methods;
d) The possibility to assess more than one type of contaminant. Meaning that more organic contaminants or heavy metals can be determined with one method or that one method can be used for both metals and organic contaminants.

2) Practical use
The methods should be practical in their use, meaning that the methods should not be too labour-intensive or time consuming. Examples are the time that is required to perform a sample analysis (this should not take several weeks) or that the methods require so much specific know how that it can not be performed on a routine basis.

3) Added value compared to total content
The methods selected should have an added value in relation to the current way of risk assessment. This added value is specified in costs and a more realistic representation of the availability of the contaminants. This means that the methods selected are not allowed to bring an excessive increase in costs for performing a risk assessment. Also the methods should be demonstrably more realistic than measuring total concentrations.

4) Validity for ecotoxicity
Much research has been performed relating the measured concentration to the actual concentration present in the soil. However still some research is lacking on the relation between the measured concentration by a method and the toxic concentrations to organisms. When selecting a method this relationship between measured concentration and toxic concentrations should be validated or have the possibility to be validated within a short time frame (approximately one year). Also the methods should be widely accepted (in the Netherlands) as a representative of actual bioavailability.

5) Applicability for more than ecotoxicity
Not so much a selection criteria but more an advantage for selection is the possibility of a method to be used for more than the determination of bioavailability of contaminants to ecosystems. If for example the method can also be applied to determine the toxicity of contaminants to humans or chemical availability for leaching to the groundwater this could be a large advantage.

4 Outcome expert workshop

4.1 Goal and set up

As mentioned before in chapter 3, a first workshop about bioavailability was organised by the group of Sijm et al., in 2002. In this workshop, reported in the report 'Bioavailability in standard setting, results of a workshop' (Sijm et al., 2002), methods were evaluated to determine their use in policy. Fact sheets were produced mentioning the strong and weak points of each of the selected methods and giving a concluding remark. Afterwards a general conclusion was drawn with advice about the implementation of some of the methods. The purpose of this workshop was not so much to give an advice about one or more methods that could be implemented into policy, but more to give a generic advice about further needs and research of the methods before they could be implemented.

The goal of the current project is the opposite; to give a specific advice about one or more methods for actual implementation and not so much an inventory of the methods available. Therefore a second workshop was organised. The goals of this workshop were:

- to discuss about which method(s) are considered to be the most promising for the implementation of bioavailability;
- how the selected methods could be implemented into policy or in which framework it can be used;
- under which pre-conditions these methods could be implemented;
- which knowledge is still missing and therefore which further research should be performed.

A group of Dutch experts from different research institutes and universities was composed (see Appendix 1 for a full overview of the participants). The participants were send an overview of the methods reported in chapter 3 and they were asked to score these methods on the selection criteria mentioned in section 3.5.1. The exact scoring is presented in a table in Appendix 2. After scoring, the methods with the most positive scores were selected and discussed in smaller discussion groups. The outcomes of these discussions were thereafter reported to the whole group and shortly discussed. The outcomes of these discussions and some general discussions are summarized in the next sections.

4.2 The use of bioavailability in soil policy

According to the participants bioavailability should only be implemented in the risk assessment and management of contaminated soils and should due to practical reasons not be used to derive generic soil quality standards. It was recognized that policy makers are requesting for fully developed and validated methods to measure bioavailability which are currently not available. But it was also stated that the time it takes to answer all known deficiencies of a particular method in a traditional way (by doing research) will probably take more than three years. It was therefore suggested that the implementation of methods with knowledge on preconditions for implementation, would provide a faster way of eliminating these deficiencies. To do so the preconditions for implementation have to be determined and constitutionalized in the following year.

According to the participants not only the degree of (un)certainty is a determining factor for the selection of a method, but also the improvement of including bioavailability in relation to measuring total concentrations should be considered.
Finally it was suggested that a difference should be made between exotic contaminants and generally occurring contaminants. In other words, selecting a method for measuring bioavailability should not only depend on which methods are available, but should partly be depending on contaminants that are regularly found in the Dutch soils and which are therefore considered as problem contaminants.

4.3 Organic contaminants

The following methods to measure the bioavailability of organic contaminants were discussed during the group discussions:

Methods to measure potential concentrations:
- Tenax extraction;
- cyclodextrine extraction;
- super critical fluid extraction (SFE);

Methods to measure actual concentrations:
- passive sampling
    - SPME;
    - SPMD;
    - POM-SPE;
    - empore disks;
    - silicone rubber.

4.3.1 General advices in relation to organic contaminants

In general it was mentioned that:
1. When introducing a method to measure bioavailability, the performing laboratories should be assisted in executing the measurements. Therefore some form of generalisation about how to use the method is necessary.
2. Methods that can be used to determine the bioavailability of contaminants in sediments can probably also be applied to determine the bioavailability of contaminants in dry soils.
3. Attention should be paid to the presence of very high concentrations of carbon rich particles such as soot and high mineral oil fractions, because these could severely influence the measured bioavailability.
4. Usually a good relationship is present between the measured concentration and the concentration actually found in biota when using methods to simulate bioavailability for organic contaminants. However the relationship is less for plants. Therefore further research should be performed to strengthen this relationship.
5. Although good results can be achieved with one method in one compartment (for example the use of Tenax in sediment) this should not lead to a forced implementation of this one method for other compartments. The selection should be based on which method is considered the best option under the given circumstances.

4.3.2 Tenax extraction

Tenax extractions are widely used for the assessment of sediment samples. For soil this use is less frequent. However based on the assumption that the processes influencing the bioavailability of contaminants in sediments are equal in soil, Tenax can also be used for soils. Tenax is used in several laboratories both in the EU (Netherlands, France Spain, Norway, Sweden and Germany) and elsewhere such as United States. Calibration research has been performed. The method is representative for several organisms inhabiting sediments, however it can also be applied to terrestrial worms, which form an important link in the food chain of terrestrial ecosystems. Still some doubts are pronounced by the participants about if Tenax actually measures the concentrations relevant for biota (does it actually measure what the biota experience) but this applies to more of the selected methods. The method is less applicable for plants, because plants are exposed by active instead of passive uptake of contaminants. However it is again suggested that this limitation applies to more methods. Tenax can be used in different types of soil but the presence of black carbon, oil, tar or peat can cause problems. Tenax can be used for several organic contaminants including old pesticides such as DDT and lindane. For more polar contaminants Tenax can probably not be used. In general Tenax is considered as a possible method for implementation. However it is argued that cyclodextrine extraction (which is based on the same principle as Tenax) is easier in use.

The added value of Tenax to measuring total concentrations is considered large. As with passive sampling the measured concentrations can be compared with water standards. This is not considered a problem, because most of the soil standards are based on water standards. Tenax can also be used to give information on the leaching of freely dissolved contaminants to groundwater.

It is mentioned that the validity of Tenax in relation to ecotoxicity needs further research. However this applies to most of the bioavailability methods. It is suggested that measuring bioavailability should be part of the ecological argumentation in the triad approach[9].

Finally according to one group of participants this method is already implemented for sediments and can be directly implemented for soil. Still some education and standardization for field and laboratory work should be performed.

4.3.3 Cyclodextrine extraction

It was recognised that extraction with cyclodextrine is a very promising method to determine bioavailability. The method is based on the same principles as Tenax extraction and therefore some equal points of discussion apply to it. However in contrast to Tenax, cyclodextrine extractions can be used on soils with high concentrations of mineral oil. The extraction with cyclodextrine is however more expensive and regeneration of the used cyclodextrine is not possible. The method is only used in some laboratories in Europe (UK, Denmark and the Netherlands). In origin this method was developed for bacteria, however in the Netherlands it is also used for aquatic worms and in Denmark also for terrestrial worms. It is suggested that this method can probably be used on more soil types than Tenax because there are no separation problems between the cycodextrine and the soil particles (this is an often heard limitation of Tenax extraction). Although the research until now focused on PAH contamination, it is expected that this method can be used on the same contaminants as Tenax extractions. Cyclodextrine is less suitable for new polar contaminants because these do not bind well with the hydrophobic core of cyclodextrine. The method seems easy and practical in use.

Before the method can be implemented more research needs to be performed on different contaminants and soil types. Also the reproducibility of the measured results should be determined. It is suggested that implementation of this method may take 1-3 years.

4.3.4 Supercritical fluid extraction (SFE)

It was generally suggested that supercritical fluid extraction (SFE) is not useful for implementation in policy. The mentioned reasons for this were that equipment required for SFE is too costly and that very

---

[9] The triad approach is based on the simultaneous and integrated deployment of site specific chemical, toxicological and ecological information in the risk assessment. For a more elaborate description of the Triad approach see Jensen & Mesman (2006).

specialistic knowledge is needed and the method is very labour intensive. Also the method is not broadly used by laboratories and it does not fit with the international way of measuring bioavailability. Application of SFE is not standardized and currently, SFE is a method being applied by trial and error. Therefore in contrast to other methods it is not considered an option.

4.3.5 Passive sampling

It was generally agreed that passive sampling with SPMD and Empore disks are not useful for the implementation in policy. Although passive sampling with SPMD and Empore disks is not expensive and does not require a lot of specialistic knowledge, these methods are currently replaced with more advanced and more useful methods like SPME, silicone rubber and POM-SPE.

SPME and POM-SPE are used in several laboratories inside and also outside the Netherlands. POM-SPE for example is also used in Norway and the United States. SPME is even more broadly used but much of this use is also related to the food industry. Silicone rubber is only used in several laboratories in the Netherlands. SPME, POM-SPE and Silicone rubber are mostly used for sediment sampling although they are also used for soils. All the samplers can be used in a wide variety of soils, however POM-SPE is considered more appropriate for soils with a high oil, soot or peat content, because of separation and fouling problems with SPME and silicone rubber. SPME can be used for both hydrophobic and polar contaminants such as pesticides and surfactants. This is due to the possibility to adapt the coating of the SPME sticks. POM-SPE can be used especially for hydrophobic contaminants. The applicability for polar contaminants is still unknown. Silicone rubber can only be used for (neutral) hydrophobic contaminants.

From the three methods, SPME was considered to be the easiest in use. POM-SPE is the most difficult because it is more labour intensive than the other methods. All three methods intend to measure the free pore water concentration even though a part of the sorbed fraction can also be extracted due to a larger sorption capacity of the device than the soil particles. The measured pore water concentrations can be directly compared with water standards. It is suggested that this is no problem, because most of the soil standards are based on water standards. However it should be determined to which legislative standards passive sampling can be compared with. All measured concentrations can be converted to internal concentrations in biota. All methods are therefore validated for the exposure of biota via pore water. However if other exposure is present (e.g. biomagnification) this is not accounted for. The methods can be used in site specific risk assessment, determining leaching and transport of freely dissolved contaminants. Transport of DOC sorbed contaminant is not accounted for. The biggest disadvantage of passive sampling is considered to be the time it takes for several methods before equilibrium is reached. Finally a question that needs answering is which methods should be selected or should any of these methods be used according to which is best for the given circumstances.

4.3.6 Concluding

It was advised that the selection of a method should be based on which method is considered the best option under the given circumstances. It should not be based on good results that have been achieved with one method in a different compartment (e.g. Tenax in sediment), while other methods are considered more appropriate for soil.

In general it was concluded that passive sampling with SPME, POM-SPE and silicone rubber and Tenax extractions are the most promising methods for implementation. This being said still the policy framework and protocols how to use the methods have to be determined. It was advised by the participants to implement both Tenax and SPME as a combined procedure because this would provide information about the actual bioavailability by measuring freely dissolved pore water concentrations with SPME and potential available concentrations by use of Tenax extractions for measuring the rapidly desorbing fraction.

Also cyclodextrin extraction is a promising method for future use. However it still needs validation studies and studies to determine its reproducibility.

4.4 Heavy metals

The following methods to measure the bioavailability of heavy metals were discussed during the workshop:

Methods for extraction of the potential available metal content
- acid extractions:
    - 0.43 M HNO_3;
    - 0.1 M HCL;
- extractions with chelating agents:
    - EDTA;
    - DTPA;
    - NTA;

Extractions to extract the actual available content:
- weak extractions
    - 0.01 M $CaCl_2$;
    - 0.0025 M $NaNO_3$;
- Donnan membrane technique;
- diffusive gradient in thin films;

Models to calculate internal concentrations and effect levels:
- terrestrial Biotic Ligand Models (BLM).

4.4.1 General advices in relation to heavy metals

In general it was mentioned that:
1. There is some discussion about which end points needed protection. Is it necessary to measure the bioavailability for a number of species or is there satisfaction if only the bioavailability for the most vulnerable species is measured?
2. There is some discussion about which availability should be measured, chemical, biological or toxicological availability and are some complexes (e.g. cadmiumchloride) also considered to be a freely dissolved concentration?
3. Chemical availability should be measured and that these concentrations should be used to relate to the biological and toxicological availability. In the future, determining biological and toxicological availability is preferred by use of models.
4. As with organic contaminants it was suggested that both the actual (freely dissolved) and potential available fraction should be measured during a risk assessment.

4.4.2 Acid extractions

In general an acid extraction with 0.43 HNO_3 was preferred above 0.1 M HCL. For the use of acid extractions standard protocols have already been prepared and the method is used by many different laboratories. If the measured concentrations are directly related to the concentrations in biota, acid extractions are only to a limited extend representative of uptake and effects of metals in different organisms. However if the measured concentrations are used in combination with a model it becomes more appropriate for more biotic species. The method can be used for all types of soil, as long as attention is being paid to the calcium concentrations. If the calcium concentrations are high, it is sometimes possible that extraction of the soil matrix is not complete. This should be accounted for.

Acid extractions can also be used for several metal species, but are limited to cations. For anions it is not clear if acid extractions are representative. According to the participants, the method is easily performed and not expensive. The method is suitable for determining potential concentrations of metals in soils, however it is less suitable for determining actual concentrations in soil. Next to ecotoxicity, acid extractions can also be used (in combination with a model) to determine leaching possibilities of metals. Overall this method is considered to be a useful addition to risk assessment.

4.4.3 Chelating agents

Chelating agents can be used in the same manner as weak extractions and have some similarities with acid extractions, such as the frequent use by several laboratories and for different metals. Still also some differences were mentioned. Chelating agents are strongly influenced by a matrix effect, meaning that it is not always possible for the agents to form adequate metal complexes. Also it is suggested that chelating agents are useful for different types of biota but especially plant species and less for organisms. The method is also relatively practical in use, however the measurement with ICP-AES[10] is more difficult than for acid extraction. There are no indications that chelating agents can be used for other uses (e.g. leaching studies) outside the field of risk assessment of soils.

Although the method is considered to have an added value in comparison to measuring total concentrations, it is suggested that compared to the regular acid extractions this method has no added value.

4.4.4 Weak extractions

In general, extraction with 0.01 M $CaCl_2$ was preferred over 0.0025 M $NaNO_3$. Weak extractions are also considered to be a useful addition to policy as these methods are frequently used by several laboratories and are part of ring testing. A protocol about the standard use is however still needed. It can be used for different types of biota. The method has been validated for plants and soft bodied biota but not for hard bodied biota. With the exception of peaty soils this method is suitable for different soil types. It can also be used for several metals under the condition that DOC is accounted for because DOC can cause an overestimation of the free metal concentrations. Next to risk evaluation this method can also be used to determine leaching of metals.

4.4.5 DMT and DGT

Both the methods DMT and DGT were not considered appropriate for the implementation into policy. Although DMT is important for further development and several scientific issues have been resolved, it is no option yet for routine use. DGT is not considered an option because it does not correspond to current models to translate the measured concentrations to actual concentrations in biota, it also does not relate to speciation of metals.

4.4.6 Terrestrial BLM

There was some discussion in the groups about how the BLMs, that are currently developed for nickel, copper and zinc, should be handled. Some participants argued that these BLMs should be implemented directly but other participants argued that there is still too much uncertainty. Although BLMs are in potency considered suitable for the determination of bioavailability of metals, for now BLMs are not an option for implementation into policy. It was suggested that very specific expertise is required from the user. Therefore until a user friendly version has been developed this method is not practical in use. Another limitation was that for now BLMs are too species and contaminant specific and there is too little information about these species. In the future it may eventually be possible to use BLMs for more species and contaminants. BLMs can not be used outside the world of risk assessment.

---

[10] ICP-AES = Inductively coupled plasma atomic emission spectroscopy

4.4.7 Concluding

It is suggested that a combination of acid extractions and weak extractions has the best potential for implementation. It is believed that 0.43 M HNO_3 and 0.01 M $CaCl_2$ are the best options. 0.43 M HNO_3 is considered the best option due to the possibility to directly use the measured concentration in models to relate the measured concentration to the concentration actually present in the biota.
For $CaCl_2$ extractions it is said that a lot of research has been done and therefore a lot of knowledge is present, but some preconditions for $CaCl_2$ extractions are; 1) it is known to measure also a part of DOC bound metals fractions and can therefore be considered conservative and 2) there is a good relation between the measured concentrations and biota exposed via pore water; however the relation to other biota is still unknown and needs further research.
Both methods are not (yet) suitable for measuring anionic metals and this requires some further research. Also there is not yet a regulatory framework to compare the outcome of the measurements with. It is however suggested that considering these preconditions, the methods should already be implemented for measuring availability for every biota it can be applied to.

4.5 Conclusion

During the progression of the project it was recognized that the selection of one method to measure bioavailability is a challenging task without ending up with questionable results about usefulness and accuracy of the method. Especially during the workshop it was noticed that future users prefer suitable methods under different circumstances. In other words it was advised not to select one method in particular but to have the ability to select a suitable method for different circumstances. This leads to the conclusion that it is, also considering the objective of this project, the best option to select only a few of the most promising methods that together form the advised methods for implementation in policy. This allows the user to choose between the methods suitable for their specific circumstances and allows policy makers to form a framework for implementation of bioavailability.

In doing so it was advised by the participants of the workshop to make a difference between measuring actual concentrations and potential concentrations for both heavy metals and organic contaminants. Following this division the participants selected for organic contaminants a combination of passive sampling with SPME as measure for the actual concentrations of contaminant. For the determination of the potential concentration of organic contaminants Tenax extraction was selected. For heavy metals a combination between HNO_3 extractions to measure the potential concentrations and weak $CaCl_2$ extractions to measure the actual concentrations was selected.
During the workshop there was still some discussion about the molar concentrations that should be used to perform extractions with $CaCl_2$. The final decision of a concentration will be part of the user protocol needed before the methods can be used in the field. Some further research from the project group is needed in this aspect.

5 Selected methods

5.1 Final selection

After careful consideration of the steering committee it was decided to adopt the decisions of the participants of the workshop for the continuation of this project.
As an addition to this selection the discussion group also selected, for the organic contaminants, cyclodextrine extraction for the continuation of this project. The reasons for this decision are that cyclodextrine extraction is a promising method based on the same principle as Tenax extractions. However there are some indications that this method, if further validation takes place, is more appropriate for the use in soil systems because there are studies that indicate that cyclodextrine presents bioavailability more accurate than Tenax extractions (Van der Heijden and Jonker, 2008). However further research in this area must still be conducted.
For passive sampling it was decided to also include POM-SPE and silicone rubber as promising methods for implementation. The reason for this is that POM-SPE can be used in soils with high soot, peat, oil and tar concentrations. The soot can easily be wiped of the plastic with a moist tissue without having an effect on the distribution coefficient. SPME may experience some separation trouble in this matter. Also both POM-SPE and silicone rubber can measure lower concentrations of contaminant than SPME. Silicone rubber as much as 50-100 times lower. Still both the operational procedure of SPME, POM-SPE and Silicone rubber are similar and require therefore a comparable user protocol.

The methods that have finally been selected for the continuation of this project are for organic contaminants:
- passive sampling with either SPME, POM-SPE or silicone rubber;
- Tenax extraction;
- cyclodextrin extraction.

And for heavy metals:
- acid extraction with either 0.43 M HNO_3;
- weak extractions with either 0.01 M $CaCl_2$.

5.2 Individual scoring of the methods

In this chapter the selected methods will individually be compared with the selection criteria as mentioned in chapter 3. This comparison is based on information described in chapter 3 and the results obtained from the workshop described in chapter 4. By comparing the methods with the criteria the advantages and the points of attention of the methods in relation to the possible implementation is described. This gives an indication of the follow up actions. After comparing the methods they will be scored with the selection criteria accordingly in a table.

The selection criteria are as follows:
1) wide ranging applicability
In this report it is decided that the following factors are considered important:
  a) the possibility to perform the technique in a standard laboratory;
  b) the possibility to assess more than one type of organism;
  c) the possibility to assess more than one type of soil;

    d) the possibility to assess more than one type of contaminant;

2) practical use;
3) added value compared to total content;
4) validity for ecotoxicity;
5) applicability for more than ecotoxicity.

5.3 Actual concentrations: Passive sampling

5.3.1 Wide ranging applicability

The possibility to perform the technique in a standard laboratory
According to literature, passive sampling is used by many different laboratories both inside and outside the Netherlands. If a difference is made between SPME, POM-SPE and silicone rubber it can be said that SPME is the most broadly used, whereas silicone rubber is only used in the Netherlands. Although research on silicone rubber is limited to the Netherlands, the method is considered to be suitable for routine use.

The possibility to assess more than one type of organism
SPME, POM-SPE and silicone rubber are all considered suitable to assess the risk for several types of organisms, especially for aquatic and terrestrial worms which are considered to be important constituents of the ecosystem.

The possibility to assess more than one type of soil
Although in principle all passive sampling techniques can be used on soil, mainly SPME has been used for soil analyses. POM-SPE and silicone rubber are more frequently used in the assessment of sediments. All three methods can also be used on different soil types, however SPME and silicone rubber are less suitable for use in soils with high black carbon, oil and tar concentrations due to separation problems. Especially POM-SPE can be used under high soot concentrations.

The possibility to assess more than one type of contaminant
Passive sampling can be used for different types of contaminants. SPME can be used for both hydrophobic and polar contaminants such as pesticides and surfactants. POM-SPE can be used especially for hydrophobic contaminants. The applicability for polar contaminants is not yet known. Silicone rubber can only be used for (neutral) hydrophobic contaminants.

5.3.2 Practical use

Passive sampling is considered to be easy in use and applicable to soil ecosystems. A large disadvantage however is the time it takes to reach equilibrium between the soil and the sampling devices. This may take about a month. However the actual hands-on time is considered normal. Of the three methods selected POM-SPE is considered the most labour intensive. SPME is considered as most practical in use.

5.3.3 Added value compared to total content

Compared to measuring total concentrations, passive sampling is considered to have an added value. By measuring pore water concentrations the actual concentrations affecting the organism are measured more accurately, even though exposure via pore water is not always the most important exposure route. The costs of passive sampling are not substantially larger than measuring total concentrations.

5.3.4 Validity for ecotoxicity

Both SPME and POM-SPE have been validated by relating the measured concentrations with concentrations in biota. It is still to be investigated whether measured concentrations by SPME and POM-SPE can be compared with water toxicity data to determine adverse effects and possible ecotoxicological risks. For silicone rubber no results have been found on these issues.

5.3.5 Applicability for more than ecotoxicity

Passive sampling techniques can be used for more than just risk assessment of soils. It can also be used to monitor the leaching of freely dissolved contaminants and SPME can potentially also be used to sense fugacity (the tendency of a chemical for respectively the solid, liquid or gas phase). Sensing fugacity could be very valuable because it determines the direction and extend of diffusive mass transport within the environment.

5.4 Potential concentrations: Tenax extraction and cyclodextrine extraction

5.4.1 Wide ranging applicability

The possibility to perform the technique in a standard laboratory

Both Tenax extractions and extractions with cyclodextrine can be easily performed in a standard laboratory. Tenax is already widely used both inside and outside of Europe. Cyclodextrine extractions are however not yet used outside Europe.

The possibility to assess more than one type of organism

Both extraction methods can be used for different types of biota. As with passive sampling both methods can be used for the risk assessment for both aquatic and terrestrial worms, which are considered an important link in the ecosystem. Cyclodextrine extractions can also be used for biodegradation assessments (with help of bacteria). Both methods are considered less applicable for the assessment of plants, as these can be exposed to contaminants by active uptake instead of passive uptake.

The possibility to assess more than one type of soil

Tenax can be used on different types of soil. However it is less suitable for peaty soils and soils with high concentrations of black carbon, oil or tar due to phase separation difficulties. Although it is not fully tested it is expected that cyclodextrine extractions can be used on the same soil types as Tenax extractions. In contrast to Tenax, cyclodextrine is in solution and it is therefore assumed that cyclodextrine can also be used on peaty soils because there are no separation problems between the cyclodextrine and the soil particles.

The possibility to assess more than one type of contaminant

Tenax can be used in several organic contaminants and older pesticides (e.g. drins). Analytical quality control proved to be positive, but is up till now limited to PCBs and PAHs. For more polar contaminants Tenax can probably not be used. Until now research with cyclodextrine extractions has focused on PAH contaminations and very recently cylodextrine was applied to assess pesticide (cypermetrin) uptake in earthworms (Hartnik et al., 2008). It is however expected that cyclodextrine can be used for the same types of contaminants as Tenax. Cyclodextrine extraction is less suitable for newer polar contaminants because they do not bind to the hydrophobic core of cyclodextrine.

5.4.2 Practical use

Both Tenax and cyclodextrine extractions are practical in use. Some researchers consider cyclodextrine easier in use than Tenax, especially in view of the ease of sample handling. Depending on the type of Tenax extraction, 6 to 24 hours are needed for an extraction. The extraction time for cyclodextrine lies within the same order of magnitude (24 hours is applied most).

5.4.3 Added value compared to total content

Both methods are considered to have an added value in relation to measuring total concentrations. Another advantage is the measuring of potential contaminant concentrations. Both Tenax extraction and cyclodextrine extractions are more expensive than measuring total concentrations but the extent of this increase is limited. When comparing Tenax and cyclodextrine extractions, the latter is more expensive because cyclodextrine is a relative expensive extractant and re-use is until now not possible. Although Tenax is in purchase rather expensive it holds the possibility of cleaning and re-using the used Tenax, thus lowering the costs.

5.4.4 Validity for ecotoxicity

Although there are researchers that indicate that Tenax is validated for measuring ecotoxicity for both aquatic and terrestrial biota, there is some doubt about Tenax measuring the actual concentrations that biota are exposed to. The same applies for cyclodextrine extractions. It is therefore safe to conclude that the link between measured concentration and measured toxicity to biota needs further evaluation.

5.4.5 Applicability for more than ecotoxicity

Tenax and probably also cyclodextrine extractions are capable of assessing leaching of freely dissolved contaminants to groundwater.

5.5 Actual concentrations: 0.01 M $CaCl_2$ extraction

5.5.1 Wide ranging applicability

The possibility to perform the technique in a standard laboratory

Extractions with 0.01 M $CaCl_2$ are already performed on a routine basis and therefore every standard laboratory can perform this analysis. $CaCl_2$ extractions have also been part of ring-testing. Standard user protocols have however not been written and there is also some discussion about which concentrations of $CaCl_2$ should be used. Some researchers indicate that 0.0025 M is more representative of soil pore water salt concentrations (Schröder et al., 2005).

The possibility to assess more than one type of organism

For several plants and soft bodied organisms good relations could be derived between on the one hand measured concentrations and on the other hand actual internal concentrations and toxicity to biota. These relations are species dependent and the method therefore needs some further investigation for other species and in particular for hard bodied species it is doubtful whether $CaCl_2$ extractions can mimic adverse effects.

The possibility to assess more than one type of soil

With the exception of peaty soils this method can be used on different soil types. However the measured concentrations may be soil type dependent. This means that testing of different soil types and characteristics is import to get a good understanding of the outcome.

The possibility to assess more than one type of contaminant
As with soil type and type of organism the measured concentration by $CaCl_2$ is contaminant specific. Still $CaCl_2$ extractions can be used for several metals under the condition that DOC is accounted for. Part of the metal concentration is bound to this DOC which means that it is less available to exert negative effects on biota. With $CaCl_2$ DOC is (partly) extracted and therewith the contaminants bound to it. The availability of the contaminants is therefore overestimated. This makes the assessment a little conservative.

5.5.2 Practical use

The method is easy in use and can be performed by every standard laboratory on a routine basis.

5.5.3 Added value compared to total content

Compared to measuring total concentrations $CaCl_2$ extractions are considered to have an added value, because the actual available concentration is measured. Weak salt extractions correlate in general well with soil solution concentrations and therefore the measured concentrations are considered more representative than total concentrations. The outcome of the measurement is also reproducible. The costs of this method are low.

5.5.4 Validity for ecotoxicity

The method has been validated for plants and soft bodied organisms, including terrestrial worms which are considered to be an important part of an ecosystem. For hard bodied organisms this validation has not yet been achieved and it is questionable whether there is a link between 0.01 M $CaCl_2$-extractable concentrations and internal concentration in hard bodied organisms and/or toxic effects on hard-bodied biota. Methods to determine potentially available fractions might be more suited for this latter purpose.

5.5.5 Applicability for more than ecotoxicity

Next to risk assessment this method can also be used to determine leaching of metals.

5.6 Potential concentrations: 0.43 M HNO_3 extraction

5.6.1 Wide ranging applicability

The possibility to perform the technique in a standard laboratory
Extractions with 0.43 M HNO_3 are frequently used by different laboratories around the world and standard protocols have already been made.

The possibility to assess more than one type of organism
0.43 HNO_3 extractions are representative for a limited amount of organisms if the measured concentrations are directly related tot the organism. HNO_3 extractions can however become more representative for more types of organisms by using transfer functions. In these functions, HNO_3 extractable concentrations are linked to actual (bio)available concentrations (e.g. $CaCl_2$).

The possibility to assess more than one type of soil
The HNO_3 extractions can also be used on different types of soil. But an import parameter influencing the outcome of the extractions in terms of amount of metal extracted is the concentration of calciumcarbonate in calcareous soils. If this concentration is too high the digestion of the soil is incomplete, influencing the applicability of the method. An essential part of the method therefore is to make sure that acid conditions are retained during extraction.

The possibility to assess more than one type of contaminant
HNO_3 extractions are proven to be representative for the metals cadmium, copper, zinc, nickel and lead. However it is also expected that this method can be used for other cationic metals. The applicability for anionic metals is expected to be limited. This needs further testing.

5.6.2 Practical use
The method is easy in use and can be performed by every standard laboratory on a routine basis. Also the outcomes of this method can be directly related to the current policy framework, therefore no fundamental changes are needed.

5.6.3 Added value compared to total content
Compared to measuring total concentrations HNO_3 extractions are considered to have an added value, because the potential chemical bioavailability is measured. However the actual concentrations that organisms may experience have to be determined with other methods, amongst others using transfer functions. Also a part of the background concentrations is measured and it is hard to determine the extent of this process. The costs of HNO_3 extractions are low.

5.6.4 Validity for ecotoxicity
HNO_3 extraction is considered validated for determining the potentially available concentration of contaminants in pore water. But the link between measured concentration and toxicity to biota still needs some further research. The method is less suited to measure actual concentrations that biota are exposed to.

5.6.5 Applicability for more than ecotoxicity
In combination with models this method can also be used to determine the leaching of metals to groundwater.

5.7 Final scoring of the methods

The above described compassion of the methods with the selection criteria resulted in Table 5.1. In Table 5.1 the individual selected methods are given a positive (+), negative (-) or average (0) score for their correspondence with the selection criteria.

Table 5.1: The correspondence between the selected methods to determine bioavailability of contaminants and the selection criteria. + stands for positive, - stands for negative and 0 stand for average

Method	**Selection criteria**							
	1a	**1b**	**1c**	**1d**	**2**	**3**	**4**	**5**
Organic contaminants								
Actual concentrations								
Passive sampling								
- SPME	+	+	0	+	0	+	+	+
- POM-SPE	+	+	+	+	0	+	+	+
- Silicone rubber	+	+	0	0	0	+	0	+
Potential Concentrations								
Tenax extraction	+	+	0	+	+	+	0	+
Cyclodextrine extraction	+	+	+	0	+	0	0	+
Heavy metals								
Actual concentrations								
Acid extractions								
- 0.43 M HNO_3	+	+	+	+	+	+	-	+
- 0.1 M HCl	+	+	+	+	+	0	-	+
Potential Concentrations								
Weak extractions								
- 0.01 M $CaCl_2$	+	+	+	+	+	+	0	+
- 0.0025 M $NaNO_3$	+	+	+	+	+	+	+	+

6 Recommendations for implementation

In this report a first selection has been made of methods that are considered to have a high potential for implementation in policy (decisions) as analytical methods to measure bioavailable concentrations of contaminants in soils. With the help of a steering committee and a workshop which allowed experts from different institutes to give their opinion, a total of 5 types of methods are advised for further development. However, before these methods are actually ready for implementation some actions are still needed. It is therefore advised to give this project a follow up in 2009. In the following sections the proposed actions for the follow up of this project are mentioned.

6.1 Policy

6.1.1 Policy framework

Measuring bioavailability to perform a risk assessment means also to interpret the outcome of this measurement in relation to soil quality standards that are also based on bioavailable concentrations. In other words there is a need of a framework that underlines the policy aim of protection of ecosystems, to which the measured concentrations can be related to. This can be implemented in the soil management policy and the soil remediation policy. In this project it was for practical reasons decided not to change the first tier of risk assessment. So the first tier remains measuring total concentrations and comparing these with Soil Quality Criteria which are also based on total concentrations. We propose to implement bioavailability in a second tier risk assessment. By using the bioavailable concentration a more effect directed way of measuring the actual concentrations is used and effects can be predicted more accurately. It is however deemed desirable to maintain the current protection level for ecosystems at the 95 and 50 % protection levels.

In the report it is mentioned several times that the outcome of some of the methods can be directly related to the current policy framework. However, the current framework (Tier 1) is based on the principle of measuring total concentrations. Therefore hazardous concentrations that are currently being used (generally: HC5 or HC50) are also based on toxicity tests in which total concentrations are measured. If the bioavailable concentrations are compared to the legislative total concentrations it will result in protection levels that in fact are less protective of the ecosystem as the hazardous concentrations (HC5 or HC50) are based on total instead of bioavailable concentrations. To maintain the current level of protection (protecting 95% and 50% of the species present in the ecosystem) hazardous concentrations based on bioavailable concentrations are required for the second tier risk assessment. It needs to be determined whether this is desired and achievable in common practise, or that the current Soil Quality Criteria should be used. As a follow up of this project, research and consultation with VROM should therefore take place on how measuring bioavailability can be implemented in the current framework of soil management and remediation without changing the protection level.

6.1.2 Contaminants

During the workshop participants mentioned that attention should be paid to the contaminants that are considered to be the main 'problematic' contaminants in the Netherlands. In other words: contaminants that are frequently found and result in remediation of sites. Less attention should therefore be paid (according to the participants) to exotic contaminants that cause less frequently remediation measures (for example flame retardants). Inquiry learned that currently the following contaminants are mostly the reason for remediation (Versluijs et al., 2006)[11].

Organic contaminants:

- mineral oil;
- benzene;
- PAH-total;

Heavy metals:

- copper;
- lead;
- arsenic.

It is a political decision to select whether only problem contaminants should be assessed with help of the selected methods or also the more exotic contaminants. However to implement bioavailability on a short term it is conceivable to agree with this suggestion. On the long term however it is advisable to also take into account the more exotic contaminants.

6.2 Relation between measured concentration and actual toxicity to biota

Bioavailability can only be implemented if the measured concentrations of contaminants can be related to toxicity data for biota. In other words, there needs to be a link between the measured concentrations by the methods described in this report and the toxic effects imposed by these concentrations to biota. This requires some additional testing as these required toxicity data are only available on the basis of total concentrations. Attention should be focused on soil extractions in which the extracted concentrations of contaminants in the soil are related to extracted concentrations in toxicity testing with soil organisms and the corresponding effect levels. This requires some laboratory testing.
For modelling purposes the influence of soil properties on both the extracted concentration and toxicity should be accounted for. This type of research could be performed in cooperation with some Dutch Universities like VU Amsterdam, Wageningen University and Research Centre, and the University of Utrecht (more particular IRAS).

Organic contaminants: For the methods of passive sampling, Tenax extraction and cyclodextrine extractions that have been selected during this project, it is stated by the experts that the extracted concentrations (representing bioavailable concentrations in pore water (mg/l)),can be directly compared with the current toxicity data used for deriving water standards. Conversion of the measured pore water concentrations to soil concentrations is possible by the use of a partitioning coefficient. This process is overall not considered to be a problem because most of the soil standards are already based on water standards and this approach would basically not be different from the current procedure. This enables a quick and representative way for implementing bioavailability for organic contaminants, as toxicity testing is in principle no longer necessary. Thus, by using a combination of methods that measure actual and potential bioavailable concentrations of organic contaminants, a good quantitative expression of the risk to the ecosystem is obtained.

Heavy metals: For metals, dilute salt solutions (0.0025 or 0.01 M $CaCl_2$) in combination with dilute acid extractions are believed to represent the amounts of contaminants that can be taken up by

---

[11] These contaminants are considered problem contaminants when measuring total contents of contaminant. If bioavailability is considered the list of problem contaminants may change due to soil characteristics that influence the availability of a contaminant. However the magnitude of this situation is not known (Versluijs et al, 2006).

organisms or are related to internal levels, or are representative of actually occurring toxic effects. This does not imply that the amounts of metals measured by means of $CaCl_2$-extraction are actually *equal* to the bioavailable fraction but merely that such extracts are able to qualify the link between metals in soil and internal levels of plants and other soil dwelling organisms.
Prerequisites for one or more extracts (or combinations of extracts such as the proposed sequential salt-acid extraction) to be implemented are:

1. A proven quantitative correlation between the measured concentrations in extracts and internal concentrations (plant/organism) or adverse effects;
2. Reproducible measurements on similar samples or at various moments in time;
3. Preferably a broad range of organisms has been tested for which the previously mentioned positive correlation is valid. Present results seem to indicate that for various plants for example, a similar response to increasing $CaCl_2$ extractable metal levels is found although model coefficients describing the relation between $CaCl_2$ extractable metal levels and internal plant levels differ.

Unlike organic contaminants, measured concentrations of heavy metals cannot be easily related to toxicity data for water standards as models for quantifying the extent of toxic effects still need to be developed. This means that toxicity testing in which extractable concentrations are linked to adverse effects as described above, is necessary.
An alternative is the use of a combination of $CaCl_2$ and HNO_3 extraction to get a relative understanding of the bioavailability of contaminants, whilst explicitly taking the impact of soil properties into account. This procedure would amongst others be a useful tool in determining priorities for remediation of contaminated sites.

Additionally, it is advised to measure DOC and pH in the weak extraction eluate. This aids in the interpretation of data, but also prepares the results for input in modelling exercises, among which BLMs. Following additional research, models like BLMs can be used in future to link the measured concentrations to the actual toxicity for biota. However, until now BLMs are not considered applicable for soils. More simple models that empirically relate internal concentrations to measured bioavailable concentrations hold more promise in the short term.

6.3 Technical design of methods

During the course of the project there was some discussion about the technical design of the methods. Some of the questions asked are:

- Is sampling with SPME, POM-SPE and silicone rubber a turbulent extraction or passive? (mixing yes or no)
- What type of Tenax extraction is considered the most appropriate 6 hour extraction or 24 hour extraction? And should it be a one step or multiple step procedure?
- Which molar concentrations of extractant should be used? (0.01 M $CaCl_2$ versus 0.0025 M $CaCl_2$)
- Which preconditions are currently set before a method can be implemented into policy?

Answering of these questions requires some more research in future projects (both literature and laboratory research).

Of course next to the technical design of the methods it is very important that further development of these and other methods is not stopped due to the selection made in this report. Most of the selected methods have some preconditions to take into account. Therefore further development or validation is needed to make them usable for other soil types and/or contaminant types. For example the methods selected in this project have a limited use for anions such as arsenic. It is however not foreseen to perform this, mostly laboratory, research within this project.

6.4 User protocol

During the project and the workshop several discussions were held about the number of methods that should be advised. Should only one or two methods be chosen per contaminant type (metal or organic) or should a few methods be advised from which the executors can choose? For the purpose of this project it was desirable to give a concrete advice about one method. However during the development of the project it was realised that one method per contaminant type was not considered realistic and would result in questionable results/concentrations. It was also mentioned by the participants of the workshop that although good results have been achieved with one method (for example the use of Tenax in sediment) this should not lead to a forced implementation of this one method under different circumstances. In other words: there was a desire to have the possibility to choose between several methods depending on the situation that needs assessing.
Therefore a few promising methods have been selected based on the many aspects mentioned in chapters 3 and 4. These methods are advised as being the most promising. This allows the executor to choose the method which is considered the best option under the given circumstances, while leaving open the possibility for other institutes to gather and develop other methods.

However, for many of these selected methods there is still some discussion about how to use them, like for example the molar concentrations that should be used for $CaCl_2$ extraction. It is considered useful (both by the members of the steering commission and the participants of the workshop) to guide the implementation of bioavailability by the development of a protocol. Such a protocol was considered useful as it assures comparable results between risk assessments of different locations, which enhances the understanding of the method. It also prevents ‘shopping’ of customers between executors that may use more favourable ways to perform risk assessment. Therefore user protocols should be written on how and when to use the methods in practice and relate the outcomes to the policy framework. This is part of the real implementation. It is realised that before user protocols can be written a political framework must be established to relate the outcomes of the measurement to.

6.5 Pilots

Experience of routine measurements with the selected methods within the policy framework is still limited for the soil compartment compared to sediment. By use of pilot testing this experience can be enhanced and it can be determined if the methods are applicable for routine risk assessment. It is proposed to allow one or two consultancies to use the methods in such a way as would be customary in policy. The protocols, the technical design and the interpretation of outcome of the methods can be compared and evaluated with the criteria in chapter 3. This testing may also allow (a part of) the uncertainties (preconditions) of the methods to be resolved and prevents large investments of other laboratories before the methods become common policy.

References

Belfroid, A., Seinen W., van Gestel K., Hermens J., van Leeuwen K. (1995). Modelling the accumulation of hydrophobic organic chemicals in earthworms: Application of the Equilibrium Partitioning Theory. Environmental Science Pollution Research 2 (1) 5-15.

Björklund E., Bøwadt S., Mathiasson L., Hawthorne S.B., (1999). Determining PCB sorption/desorption behaviour on sediments using selective supercritical fluid extraction. 1. Desorption from historically contaminated samples. Environmental Science and Technology 33 (13) 2193-2203.

Bonten, L.T.C., Groenenberg J.E., Weng L., van Riemsdijk W.H.,(2008). Use of speciation and complexation models to estimate heavy metal sorption in soils. Geoderma 146 (1-2), 303-310.

Brand E., (2006). Desorption characteristics of Drin's from sediments at different mixing intensities. Wageningen University & Research Centre, Department of Environmental Technology. Report no. 0620.

Cancès B., Ponthieu M., Castrec-Rouelle M., Aubry E., Benedetti M.F., (2003). Metal ions speciation in a soil and its solution: experimental data and model results. Geoderma 113 (3-4), 341-355.

Cornelissen G., van Noort P.C.M., Govers A.J., (1997). Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons and polychlorinated biphenyls: sediment extraction with Tenax and effects of contact time and solute hydrophobicity. Environmental Toxicolgy and Chemistry 16 (7), 1351-1357.

Cornelissen G., Rigterink H., van Noort P.C.M., Govers H.A.J., (2000). Slowly and very slowly desorbing organic compounds in sediemts exhibit Langmuis-type sorption. Environmental Toxicology and Chemistry 19 (6), 1532-1539.

Cornelissen G., Rigterink H., ten Hulscher T.E.M., Vrind B.A., van Noort P.C.M., (2001). A simple Tenax extraction method to determine the availability of sediment-sorbed organic compounds. Environmental Toxicology and Chemistry 20 (4), 706-711.

Cornelissen G., Kamerling G.E., (2003). Ecotoxicologische risico's en water-bodem-normen. Wat anders ?!. Waterdienst Lelystad, The Netherlands, AKWA reportno. 03.006 (in Dutch).

Cuypers C., Pancras T., Grotenhuis T., Rulkens W., (2002). The estimation of PAH bioavailability in contaminated sediments using hydroxypropyl-β-cyclodextrin and Triton X-100 extraction techniques. Chemosphere 46 (8), 1235-1245.

Degryse F., Broos K., Smolders E., Merkx R., (2003). Soil solution concentration of Cd and Zn can be predicted with a CaCl2 soil extract. European Journal of Soil Science 54 (1), 149-157.

De Vries, W., Čurlík J., Murányi A., Alloway B., Groenenberg B.J., (2005). Assessment of relationships between total and reactive concentrations of cadmium, copper, lead and zinc in Hungarian and Slovakian soils. Ekologia Bratislava 24 (2), 152-169.

Dijkstra J.J., Meeussen J.C.L., Comans R.N.J., (2004). Leaching of Heavy Metals from Contaminated Soils: An Experimental and Modeling Study. Environmental Science and Technology 38 (16), 4390-4395.

Dzombak D.A., Morel F.M.M., (1990). Surface complexation modeling: Hydrous Ferric Oxide. Wiley-Interscience New York.

Fact sheets Deltares, distributed by Erwin Roex on 10-06-2008 during workshop 'Toetsingskader waterbodems'. Deltares Utrecht, the Netherlands

Fangueiro D., Bermond A., Santos E., Carapuça H., Duarte A., (2002). Heavy metal mobility assessment in sediments based on a kinetic approach of the EDTA extractions: search for optimal experimental conditions. Analytica Chimica Acta 459 (2), 245-256.

Fest E.P.M.J., Temminghoff E.J.M., Comans R.N.J., van Riemsdijk W.H., (2008). Partitioning of organic matter and heavy metals in a sandy soil: Effects of extracting solution, solid to liquid ratio and pH. Geoderma 146 (1-2), 66-74

Ge Y., MacDonald D., Sauvé S., Hendershot W., (2005). Modeling of Cd and Pb speciation in soil solutions by WinHumicV and NICA-Donnan model. Environmental Modelling and Software 20 (3), 353-359.

Groenenberg, J.E., Römkens P. F.A.M., Comans R.N.J., Luster J., Pampura T., Shotbolt L., Tipping E. and De Vries W., (2008). Transfer functions for solid solution partitioning of cadmium, copper, nickel, lead and zinc: Derivation of relations for free metal ion activities and validation on independent data. Submitted

Groenenberg J.E., Römkens P.F.A.M., Tipping E., Pampura T., de Vries W., Schutze G., (2002): Transfer functions for the calculation of critical loads for lead, cadmium and mercury. In: Proceedings Expert meeting on critical limits for heavy metals and methods for their application. Berlin 2–4 December 2002, held under the UN/ECE Convention on Long-Range Transboundary Air Pollution. UBA-Texte 47/03. Berlin. Umweltbundesamt.

Harmsen J., (2007). Measuring bioavailability: From a scientific approach to standard methods. Journal of Environmental Quality 36 (5), 1420-1428.

Hartnik T., Jensen J., Hermens J.L.M. (2008). Non-exhaustive β-cyclodextrin extraction as a chemical tool to estimate bioavailability of hydrophobic pesticides for earthworms. Environmental Science and Technology 42 (22), 8419-8425.

Hawthorne S.B., Grabanski C.B., (2000). Correlating selective supercritical fluid extraction with bioremediation behavior of PAHs in a field treatment plot. Environmental Science and Technology 34 (19), 4103-4110.

Hawthorne S.B., Poppendieck D.G., Grabanski C.B., Loehr R.C., (2001). PAH release during water desorption, Supercritical carbon dioxide extraction, and field bioremediation. Environmental science and technology 35 (22), 4577-4583.

Hawthorne S.B., Poppendieck D.G., Grabanski C.B., Loehr R., (2002). Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. Environmental science and technology 36 (22), 4795-4803.

Houba V.J.G., Lee J.J.G. van der, Novozamsky I., (2003). Soil and plant analysis Part 1: Soil analysis procedures. Wageningen University, Wageningen.

Huckins J.N., Tubergen M.W., Manuweera G.K.,(1990). Semi permeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential. Chemosphere 20 (5), 533-552.

ISO. 2005. 11074: Soil quality–Vocabulary. ISO, Geneva, Switzerland.

Jensen J., Mesman, M. (eds.) (2006). Ecological Risk Assessment of Contaminated Land. Decision Support for Site-specific Investigations. RIVM, Bilthoven, the Netherlands. ISBN 90-6960-138-9 and 978-90-6960-138-0.

Jensen J., Montforts M., Liess M., Luttik R., Kula C., Römbke J., Sousa P., Nienstedt K., Peijnenburg W., (in press). The usefulness of total concentrations and pore water concentrations as metrics for the assessment of ecotoxicological effects of Plant Protection Products in soil. European Food Safety Authority.

Jonker M.T.O., Koelmans A.A., (2001). Polyoxymethylene Solid Phase Extraction as a partitioning method for hydrophobic organic chemicals in sediment and soot. Environmental Science and Technology 35 (18), 3742-3748.

Jonker M.T.O., Koelmans A.A., (2002). A extraction of polycyclic aromatic hydrocarbons from soot and sediemtn: solvent evauluation and implications for sorption mechanism. Environmental science and technology 36 (19), 4107-4113.

Jonker M.T.O., van der Heijden S.A., Kreitinger J.P., Hawthorne S.B., (2007). Predicting PAH bioaccumulation and toxicity in earthworms exposed to manufactured gas plant soils with Solid-Phase Microextraction. Environmental science and technology 41 (21), 7472-7478.

Kalis E.J.J., Weng L., Dousma F., Temminghoff E.J.M., van Riemsdijk W.H., (2006). Measuring free metal ion concentrations in situ in natural waters using the Donnan Membrane Technique. Environmental Science and Technology 40 (3), 955-961.

Kinniburgh, D.G. van Riemsdijk W.H., Koopal L.K., Borkovec M., Benedetti M.F., Avena M.J., (1999). Ion binding to natural organic matter: competition, heterogeneity, stoichiometry and thermodynamic consistency. Colloids and Surfaces A: Physicochemical and Engineering Aspects 151 (1-2), 147-166

Koopmans G.F., Schenkeveld W.D.C., Song J., Luo Y., Japenga J., Temminghoff E.J.M. (2008). Influence of EDDS on metal speciation in soil extracts: Measurement and mechanistic multicomponent modelling. Environmental Science and Technology 42 (4), 1123-1130.

Koster M., de Groot A., Vijver M., Peijnenburg W., (2006). Copper in the terrestrial environmt: Verification of a laboratory-derived terrestrial biotic ligand model to predict earthworm mortality with toxicity obesreved in field soils. Soil biology & biochemistry 38 (7), 1788-1796.

Lofts S., Spurgeon D.J., Svendsen C., Tipping E., (2004). Deriving soil critical limits for Cu, Zn, Cd, and Ph: A method based on free ion concentrations. Environmental Science and Technology 38(13), 3623-3631

Manouchehri N., Besancon S., Bermond A., (2006). Major and trace metal extraction from soil by EDTA: Equilibrium and kinetic studies. Analytica Chimica Acta 559 (1), 105-112.

Mayer P., Vaes W.H.J., Hermens J.L.M., (2000a). Absorption of hydrophobic compounds into the Poly(dimethylsiloxane) coating of Solid-Phase Microextraction Fibers: High Partition coefficients and fluorescence microscopy images. Analytical Chemistry 72 (3), 459-464.

Mayer P., Vaes W.H.J., Wijker F., Legierse K. C.H.M., Kraaij R. (H.), Tolls J., Hermens J.L.M., (2000b). Sensing dissolved sediment pore water concentrations of persistent and bioaccumulative pollutants using disposable solid-phase microextraction fibers. Environmental Science and Technology 34 (24), 5177-5183.

Pampura T., Groenenberg J.E., Rietra R.P.J.J., (2006). Comparison of methods for copper free ion activity determination in soil solutions of contaminated and background soils. Forest Snow and Landscape Research 80 (3), 305-322.

Papassiopi N., Tambouris S., Kontopoulos A., (1999). Removal of heavy metals from calcareous contaminated soils by EDTA leaching. Water, Air and Soil pollution 109 (1-4), 1-15.

Peijnenburg W.J.G.M, Jager T., (2003). Monitoring approaches to assess bioaccessibility and bioavailability of metals: Matrix issues. Ecotoxicology and Environmental Safety, 56: 63-77.

Peijnenburg W., Sneller E., Sijm D., Lijzen J., Traas T., Verbruggen E., (2004). Implementation of bioavailability in standard setting and risk assessment. Environmental Sciences, 11 (3), 141-149.

Peijnenburg, W.J.G.M., Zablotskaja, M., Vijver, M.G., (2007). Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. Ecotoxicology and Environmental Safety, 67 (2), 163-179

Quevauviller P.H., Lachica M., Barahona E., Gomez A., Rauret G., Ure A., Muntau H., (1998). Certified reference material for the quality control of EDTA- and DTPA-extractable trace metal contents in calcareous soil (CRM 600). Journal of Analytical Chemistry 360 (5), 505-511.

Rauret G., López-Sánchez J.F., Sahuquillo A., Rubio R., Davidson C., Ure A., Quevauviller P., (1999). Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soils reference materials. Journal of Environmental Monitoring 1 (1), 57-61.

Reid B.J., Stokes J.D., Jones K.C., Semple K.T., (2000). Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability. Environmental science and technology 34 (15), 2000.

Rhodes A.H., Carlin A., Semple K.T., (2008a). Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental science and technology 42 (3), 740-745.

Rhodes A.H., Dew N.M., Semple K.T., (2008b). Relationship between cyclodextrin extraction and biodegradation of phenanthrene in soil. Environmental Toxicology and Chemistry 27 (7), 1488-1495.

Römkens P.F.A.M., Groenenberg J.E., Bonten L.T.C., de Vries W., Bril J., (2004). Derivation of partition relationships to calculated Cd, Cu, Ni, Pb and Zn solubility and activity in soil solutions. Wageningen, the Netherlands. Alterra report no. 305.

Schröder, T.J., Hiemstra, T., Vink, J.P.M., van der Zee, S.E.A.T.M. (2005). Modeling of the solid-solution partitioning of heavy metals and arsenic in embanked flood plain soils of the rivers Rhine and Meuse. Environmental Science & Technology 39 (18), 7176-7184.

Semple K.T., Doick K.J., Jones K.C., Burauel P., Craven A., Harms H., (2004). Defining bioavailability and bioaccessibility of contaminated soil and sediment is complicated. Environmental Science and Technology 38 (12), 228A-231A.

Shiaris M.P., Sherrill T.W., SAYLER G.S. (1980). Tenax-GC Extraction Technique for Residual Polychlorinated Biphenyl and Polyaromatic Hydrocarbon Analysis in Biodegradation Assays. Applied and Environmental Microbiology 39 (1), 165-171.

Steenbergen N.T.T.M., Iaccino F., de Winkel M., Reijnders L., Peijnenburg W.J.G.M., (2005). Development of a biotic ligand model and a regression model predicting acute copper toxicity to earthworm Aporrectodea caliginosa. Environmental Science and Technology 39 (15), 5694-5702.

Sijm D., Lijzen J., Peijnenburg W., Sneller E., Traas T., Verbruggen E., (2002). Bioavailability in standard setting, results of a workshop. RIVM Bilthoven The Netherlands. RIVM reportno. 607220006 / RIZA report no. 2002.003 (in Dutch).

Stokes J.D., Wilkinson A., Reid B.J., Jones K.C., Semple K.T., (2005). Prediction of polycyclic aromatic hydrocarbon biodegradation in contaminated soils using an aqueous hydroxypropyl-β-cyclodextrin extraction technique. Environmental Toxicology and Chemistry 24 (6), 1325-1330.

Styrishave B., Smortensen M., Henning Krogh P., Andersen O., Jensen J., (2008). Solid-Phase Microextraction (SPME) as a Tool to Predict the bioavailability and toxicity of pyrene
to the springtail, Folsomia candida, under various soil conditions. Environmental Science and Technology 42 (4), 1332-1336.

Temminghoff E.J.M., Plette A.C.C., van Eck R., van Riemsdijk W.H., (2000). Determination of the chemical speciation of trace metals in aqueous systems by the Wageningen Donnan Membrane Technique. Analytica Chimica Acta 417 (2), 149-157.

Ten Hulscher T.E.M., Postma J., den Besten P.J., Stroomberg G.J., Belfroid A., Wegeneer J.W., Faber J.H., van der poll J.J.C., Hendriks A.J., van Noort P.C.M., (2003). Tenax extraction mimics benthic and terrestrial bioavailability of organic compounds. Environmental Toxicology and Chemistry 22 (10), 2258-2265.

Ter Laak T.L., Agbo S.O., Barendregt A., Hermens J.L.M., (2006a). Freely dissolved concentrations of PAHs in soil pore water: Measurements via Solid Phase Extraction and consequences for soil tests. Environmental Science and Technology 40 (4), 1307-1313.

Ter Laak T.L., Barendregt A., Hermens J.L.M., (2006b). Freely dissolved pore water concentrations and sorption coefficients of PAHs in spiked, aged and field–contaminated soils. Environmental science and technology 40 (7), 2184-2190.

Tessier, A., Campbell, P.G.C., Blsson, M., (1979). Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51 (7), 844-851.

Tipping, E., (1998). Humic Ion-Binding Model VI: An Improved Description of the Interactions of Protons and Metal Ions with Humic Substances. Aquatic Geochemistry 4 (1), 3–48.

Tipping E., Rieuwerts J., Pan G., Ashmore M.R., Lofts S., Hill M.T.R., Farago M.E., Thornton I., (2003). The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales. Environmental Pollution 125 (2), 213-225.

Van der Heijden S.A., M.T.O. Jonker. PAH bioavailability in field sediments: Comparing different methods for predicting in situ bioaccumulation. 2008, submitted.

Van der Wal L., Jager T., Fleuren R.H.L.J., Barendregt A., Sinnige T.L., Van gestel C.A.M., Hermens J.L.M., (2004). Solid Phase Mircoextraction to predict bioavailabilty and accumulation of organic micropollutants in terrestrial organisms after exposure to a field contaminated soil. Environmental science and technology 38 (18), 4842-4848.

Van Leeuwen H.P., Town R.M., Buffle J., Cleven R.F.M.J., Davison W., Puy J., Van Riemsdijk W.H., Sigg L, (2005). Dynamic Speciation Analysis and Bioavailability of Metals in Aquatic Systems. Environmental science and technology 39 (22), 8545-8556.

Versluijs C.E., Mulder H.F.H.M., Wijnen van H.J., Van de Broek H.H., Bogte J.J., (2006). Survey of compounds and concentrations of MKBA database. Extract for work document process and data-analyses for MKBA soil remediation version 17-11-2006. RIVM Bilthoven.

Vink, J.P.M., van de Guchte, C., Zwolsman, J.J.G., van der Heijdt, L.M., van Steenwijk, J.M., Tuinstra, J. (1999). Naar een nieuwe beoordeling van zware metalen in sediment. Analyse van biologisch beschikbare fracties en toetsing aan risicogrenzen. RIZA/AKWA rapport 99.007, Lelystad (In Dutch)

Vink, J.P.M. (2002). Measurement of heavy metal speciation over redox gradients in natural water-sediment interfaces and implications for uptake by benthic organisms. Environmental Science & Technology 36 (23), 5130-5138.

Vijver, M.G., Spijker, J., Vink, J.P.M., Posthuma, L. (2008). Determining metal origins and availability in fluvial deposits by analysis of geochemical baselines and solid-solution partitioning measurements and modelling. Envrionmental Pollution (in press).

Weng L., Temminghoff E.J.M., van Riemsdijk W.H., (2001a). Determination of the free ion concentration of trace metals in soil solution using a soil column Donnan membrane technique. European Journal of Soil Science 52 (4), 629-637.

Weng L., Temminghoff E.J.M., van Riemsdijk W.H., 2001b. Contribution of individual sorbents to the control of heavy metal activity in sandy soil. Environmental Science and Technology 35 (22) 4436-4443.

Zwolsman J.J.G., Peijnenburg W.J.G.M., (2006). 2^e lijnsbeoordeling ecolopgische risico's in oppervlakte water – wat te meten in 2007. KIWA water research, Nieuwegein, the Netherlands. Report no. KWR 06.112 (in Dutch).

Appendix 1 List of participants workshop

Chairman of the day: D. Sijm (RIVM)
Minutes secretary of the day: E. Brand (RIVM)

Discussion group	**Organic contaminants (1)**	**Organic contaminants (2)**	**Metals (3)**	**Metals (4)**
	Chairman: W. Peijnenburg (RIVM) Minutes secretary: M. Janssen (RIVM) Participants P. van Noort (Deltares) E. Verbruggen (RIVM) J. Harmsen (WUR) J. Tuinstra (TCB)	Chairman: J. Lijzen (RIVM) Minutes secretary: D. Ten Hulscher (RWS) Participants A. Roeloffzen (DCMR) H. Maas (RWS) C. Jonker (IRAS) J. Wezenbeek (Grontmij)	Chairman: B.J. Groenenberg (Alterra) Minutes secretary: M. Vijver (University Leiden) Participants J. Appelman (VROM) W. van Tilborg (VTBC) R. Comans (ECN) B. Koelmans (WUR)	Chairman: J. Vink (Deltares) Minutes secretary: P. Römkens (Alterra) Participants E. Temminghoff (WUR) M. Rutgers (RIVM) G. Kamerling (-) S. Jansen (Deltares)

Appendix 2 Scoring table as filled in during workshop

For a more elaborate explanation of the criteria see section 3.5.1. The following scoring criteria are used:

1) Wide ranging applicability

Wide ranging applicability of methods comprises different factors. In this report it is decided that the following factors are considered important:

a) The possibility to perform the technique in a standard laboratory.
b) The possibility to assess more than one type of organism.
c) The possibility to assess more than on type of soil.
d) The possibility to assess more than one type of contaminant.

2) Practical use
3) Added value compared to total content
4) Validity for ecotoxicity
5) Applicability for more than ecotoxicity

The correspondence between the selected methods to determine bioavailability of contaminants and the selection criteria as filled in by the participants of the workshop. + stands for positive, - stands for negative and 0 stand for average. The number of +/-/0 within a cell stands for the number of participants that gave this method a score (e.g. ++ = two participants gave a positive score).

Method	**Selection criteria**							
	1a	**1b**	**1c**	**1d**	**2**	**3**	**4**	**5**
Organic contaminants								
Passive sampling	+	+	+	+ 0	+ -	++	- 0	+
- SPME	++++ -	++++ -	++++ 0	++++ 0	++ 0	+++	+++ 00	+ -
- SPMD	+++ -	++ - 0	+++ 0	+++ 0	+ 0	+++	+ 00	+ -
- POM-SPE	+++ -	+++ -	++++	+++ 0	+ 0	+++	++	+ -
- Empore disks	++ -	++ -	+++	++ 0	+	++	+	+
- Silicone rubber	+++ - 0	+++++	+++++	++++ 0	+++	++++	++ -	++ -
Tenax extraction	++++++	++++++	+++++ 0	++++++ 0	+ +++ 0	++++++	+++++ +	++++
Cyclodextrine extraction	+++ 0	+++	+++	+++	++	+ 0 -	+ 0	++
Supercritical fluid extraction	----	+ 0 -	++-	+++ -	+ --	+ 0 -	0 -	+ -
Organic contaminants and heavy metals								
Measuring pore water concentrations	++ --	++++	+++	++++	+ ----	+++	+ 0	++
Distinction between hard and soft bodied		+ -	+	+				

species								
Heavy metals								
Acid extractions	+	+	+	+	++		-	-
- 0.43 M HNO_3	+++++ +	+++++ -	++++++	+++++ 0	++++ + 0	++++ -	+ 0 --	+++++ -
- 1 M HCL	++++ -	+++ --	++++ -	+++ - 0	++ - 0	+ -	---	++ --
Extractions with chelating agents								
- EDTA	++ - 0	++ - 0	+++ -	++ -- 0	+ - 0	+ -	- 0	--
- DTPA	++ - 0	++ - 0	++ - 0	++ - 0	+ - 0	+ -	- 0	--
- NTA	++ - 0	+ - 0	++ -	+ - 0	- 0	+ -	-0	--
Weak extractions								
- 0.01 M $CaCl_2$	+++++ ++++	++++++ ++ 0 -	++++++ ++ 0	++++++ ++ 0	++++ + 0 -	++++++ +	++++ + 0	++++++ +
- 0.0025 M $NaNo_3$	+++++ +	+++++ -	+++++ 0	++++ 0 -	+ 0	++	++	+++0
Donnan Membrane technique (DMT)	-				--		++++	+++
Diffusive gradient thin film (DGT)	-				-			
Sequential extraction	0 --	---	+ --	+ --	0 --	+ --	---	+ --
Two step zinc	+	+	+	+	+	+	+	+
Mechanistic assemblage models	+	+	+	+	-	+	+	+
Transfer functions	+ -	+ 0	+++	+ 0	++ 0	+++	+ --	++
Terrestrial Biotic Ligand model (BLM)	0 --	++ -	++++	++++ -	++ 0 - -	++++ 0	++++ +	+ -

RIVM

National Institute
for Public Health
and the Environment

P.O. Box 1
3720 BA Bilthoven
The Netherlands
www.rivm.com