National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Advice on implementing bioavailability in the Dutch soil policy framework

User protocols for organic contaminants

RIVM report 711701102/2012

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Colophon

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This investigation has been performed by order and for the account of the Ministry of Infrastructure and the Environment (I&M), Directorate General of the Environment (DGM), Directorate of Sustainable production (DP), within the framework of project 711701, Risk in relation to Soil Quality.

Abstract

Advice on implementing bioavailability in the Dutch soil policy framework
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A more realistic assessment of ecological risks
RIVM proposes a procedure with which the effects of contamination on organisms in soil and sediment can be predicted in a more realistic way. The starting point of this procedure is the concentration of the contaminant in soil with which organisms come into contact (the bioavailable concentration). In the field, part of the contaminant binds to soil particles and no longer has an effect on soil organisms. It is expected that by using this more realistic procedure, the amount of soils that currently are classified as harmful based on the total content will decrease. In this way, less remediation of contaminated sites will be necessary.

Current soil policy based on total concentrations
In ecological risk evaluations of contaminated sites, soil quality is determined on the basis of total contaminant concentration. These measured total concentrations are compared with Soil Quality Standards of the relevant contaminants. If the soil concentration exceeds the Soil Quality Standards, a supplementary risk evaluation can be performed to determine whether a site needs remediation.

Proposal for implementation in soil policy
This report presents five methods and their corresponding user protocols to measure bioavailable concentrations in soil for organic compounds. Furthermore, it describes two procedures on how the results of these measurements can be implemented in the existing soil framework for decision-making in remediation of contaminated soils or sustainable land management.

Keywords:
Bioavailability, organic compounds, soil policy, risk assessment, soil contamination

Rapport in het kort

Advies voor de implementatie van biobeschikbaarheid in het Nederlandse bodembeleid
Gebruiksprotocol voor organische verontreinigingen

Realistischer beeld van ecologische risico's
Het RIVM stelt een werkwijze voor waarmee de effecten van vervuilingen op organismen in bodem en sediment realistischer kunnen worden weergegeven. Uitgangspunt van deze werkwijze is de concentratie van de vervuilende stof waarmee organismen in de bodem daadwerkelijk in contact komen (de biobeschikbare concentratie). In de praktijk blijkt namelijk dat een deel van vervuilingen aan de bodemdeeltjes gebonden blijft waardoor ze geen effect hebben op organismen. De verwachting is dat door deze realistischere werkwijze het aantal bodems dat momenteel als risicovol wordt aangemerkt, kleiner wordt. Hierdoor kunnen er mogelijk minder gevallen voor sanering in aanmerking komen.

Huidige bodembeleid gaat uit van totaalconcentraties
In de bestaande ecologische risicobeoordeling van bodemverontreiniging wordt de bodemkwaliteit beoordeeld op basis van totale concentraties verontreinigde stoffen in de bodem. Deze totaalconcentraties worden vervolgens vergeleken met de interventiewaarden voor de desbetreffende stoffen. Als de gemeten concentraties hoger zijn dan deze norm, kan aanvullend onderzoek plaatsvinden om te bepalen of de bodem moet worden gesaneerd.

Voorstellen voor implementatie in bodembeleid
Dit rapport beschrijft vijf methoden en de bijbehorende gebruiksprotocollen om biobeschikbare concentraties in de bodem te meten voor organische verontreinigingen. Daarnaast worden twee voorstellen gedaan om de uitkomsten van deze methoden te implementeren in het bestaande bodembeleid voor besluitvorming binnen het saneringsbeleid van verontreinigde gronden en voor duurzaam bodemgebruik.

Trefwoorden:
Biobeschikbaarheid, organische stoffen, bodembeleid, risicobeoordeling, bodemverontreiniging

Acknowledgements

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Contents

Summary

In ecological risk evaluations of contaminated sites in the Netherlands, soil quality is determined on the basis of total contaminant concentration. These measured total concentrations are compared with soil quality standards. If the contaminant concentration in the soil exceeds the soil quality standards, a higher tier risk evaluation can be performed.

From practice the perception rises that performing risk evaluation by measuring total concentrations leads to an inaccurate prediction of the risks, as a portion of the contaminants in the soil may not be available for soil organisms. The idea exists that there is often an indication of risks, while the ecosystem is not affected. The assumption that only the bioavailable fraction is available to exert adverse effects on the soil ecosystem can help to improve the risk assessment. It is suggested that if bioavailability is taken into account during a soil risk evaluation, it can reduce the amount of false positives.

In 2008 several analytical methods were selected that can be used to measure bioavailable concentrations of organic compounds in soils. These methods are:

Measuring actually bioavailable concentrations:

- passive sampling with either Solid Phase Micro Extraction (SPME), Polyoxymethylene Solid Phase Extraction (POM-SPE) or silicone rubber;

Measuring potentially bioavailable concentrations:

- by extracting with the strong adsorbent Tenax or the complexing agent cyclodextrin.

In this report these methods will be described in more detail. To date there is no international guideline on how to perform these experiments and therefore, user protocols are described in this report. This was done in analogy with international developments such as the ISO working group on bioavailability and was necessary because the analytical methods are intended for use on a national scale and similarity in execution is desired. This enables a comparison of the results obtained with different methods. Only then can the outcome of the bioavailability assessment of an examined area be compared with data from another contaminated area.

Furthermore, it was necessary to relate the outcome of the measurements to soil quality standards. This should be a higher tier assessment in line with the current way of risk assessment of contaminated soils. We present two options, being:

- Relating the actually bioavailable concentrations to aquatic toxicity standards;
- Relating the potentially bioavailable concentrations to soil quality standards.

This report is written with the aim of describing a series of practical recommendations for a possible implementation of bioavailability in the Dutch regulatory framework as a higher tier risk assessment for decision-making in the remediation of contaminated soils or sustainable land management. In this way the report serves as a foundation for policy making for contaminated soils.

1 Introduction

1.1 Why implementation of bioavailability in a regulatory framework?

In current risk evaluation of contaminated sites or decision-making in sustainable land management, total contaminant concentrations are used to predict possible impacts. Practical experiences have given rise to the perception that performing risk evaluation based on (measured) total concentrations may lead to an inaccurate assessment of the risks. Often risks are predicted, while effects on the ecosystem are not observed and vice versa. A false indication of risk could lead to the application of remediation measures and associated expenses that do not result in an improved ecosystem. On the other hand, in some cases there might be an underestimation of the actual effects, resulting in insufficient protection of the ecosystem. Accounting for bioavailability of contaminants in a higher tier risk assessment might reduce the number of false positive risk evaluations. Furthermore, the assessment is more effect based or in other words, realistic. This allows for more effective expenditure of the scarce economic means available for soil remediation. Accounting for bioavailability in decision-making in sustainable soil management can offer a more effect based risk assessment of the soil to be reused within an area.

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. Only after real exposure of biological receptors there is by definition a certain risk by contaminants, which might be acceptable or unacceptable considering the target quality of our environment. Occurring effects are directly related to the binding capacity of soil and the speciation of the contaminant in pore water[1], as affected by physical-chemical properties of soil and contaminant, and specific properties of biota that are exposed to contaminants.

In 2007 we started a project with the goal to come to a selection and further (quantitative) description of methods to account for the differences in bioavailability in a higher tier risk assessment. In 2008 the Dutch Soil Protection Technical Committee[2] (TCB) published 'Advice on ecological underpinning of soil quality standards'. In this advice it was stated that implementation of bioavailability as part of a site-specific risk assessment is an important issue and that the introduction of standardised analytical methods for in particular organic contaminants, could be realised within a short timeframe. The TCB also addressed the need to develop a corresponding regulatory framework to relate bioavailable concentrations to. The TCB advised developing guidance on how bioavailability could be used within the risk assessment of contaminated soils and sustainable soil management (Stolker-Nanninga, 2008).

Until now, no systematic application or implementation of bioavailability in soil risk assessment has been performed, although a number of pragmatic approaches have been implemented in some countries. An ISO (International Organisation for Standardisation) working group on bioavailability provided guidance (ISO 17402) 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 the implementation of measured bioavailability in soil quality assessment and proposes a generic user

[1] In soils, water that occurs naturally within the pores can also be called interstitial water instead of pore water. In this report we consider both to be equal.
[2] The TCB is an independent scientific committee based on the Dutch Soil Protection Act. The committee provides recommendations on technical and scientific aspects of soil policy in the Netherlands.

protocol on passive sampling (for Solid Phase Micro Extraction (SPME), Polyoxymethylene Solid Phase Extraction (POM-SPE) and on extractions with a strong adsorbent (Tenax) or complexing agent (cyclodextrine). The current project took place in close consultation with this ISO group to develop this protocol and obtain the support needed for implementation. At the time this report was written, the details on the ISO protocol are not yet presentable.

In 2012 a new NTA working group (Dutch Technical Agreement) will start with the development of a protocol (NTA 6751) to measure organic micro-pollutants with Tenax. The NTA 6751 will be developed on the initiative of the Directorate-General of Public Works and water management of the ministry of Infrastructure and the Environment and is also based on the current status of the ISO discussion paper. The Dutch normalisation institute (NEN) will support this working group. The goal is that the new protocol will be published at the end of 2012.

1.2 Aim and boundaries of the research

The goal of this report is to offer the Ministry of Infrastructure and the Environment options for the implementation of bioavailability in the current regulatory framework for decision-making in the remediation of contaminated soils or sustainable land management. This advice is mainly focused on the technical implementation of bioavailability in the regulatory framework. It does not address the policy decisions that still require attention. These decisions are however described in chapter 5 of this report, to give further guidance to the follow-up of this report. This report serves as a foundation for policy making for contaminated soils and focuses on:

- bioavailability of organic contaminants;
- for ecosystems;
- in soils and sediments;
- in higher tier risk assessment;
- using existing information only.

In this way, the first tier assessment by means of total concentrations remains the same. Furthermore, this activity and hence this report will not address aspects of human health. This report is on the bioavailability of immobile apolar organic contaminants only. Highly mobile contaminants and metals are not addressed in this report. A report on heavy metals is scheduled to follow.

The primary compartment of interest 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 when defining the methods. It is realised that many 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 soils and sediments. It is noted that the behaviour and the effects of contaminants on organisms in soil are more complex than in water.

This study is based on currently existing information only and is intended to give advice for implementation in the Dutch soil policy framework. This information will be generated by literature studies. No additional research in the form of laboratory or field experiments will be performed.

This report is a follow-up of the reports of Peijnenburg and Brand (2007) and Brand et al. (2009). We will only consider the methods that have been selected by Dutch experts in 2008 (as described in the report of Brand et al. 2009 and shortly summarised in section 1.4.1 of this report).

Finally, we operationalise several analytical methods that can be used to determine the bioavailability of organic contaminants. A short description of the

technical design of each method will be presented before we advise on a user protocol.

1.3 Chemical vs Biological measurements

According to Harmsen (2007), bioavailability is a dynamic process that can be described by basically three steps (see also Figure 1.1): 1) availability of the contaminant in the soil (i.e., environmental availability), 2) uptake of the contaminant by the organism (i.e., biological bioavailability), and 3) within the organisms, accumulation and/or effect of the contaminant (i.e., toxicological bioavailability).

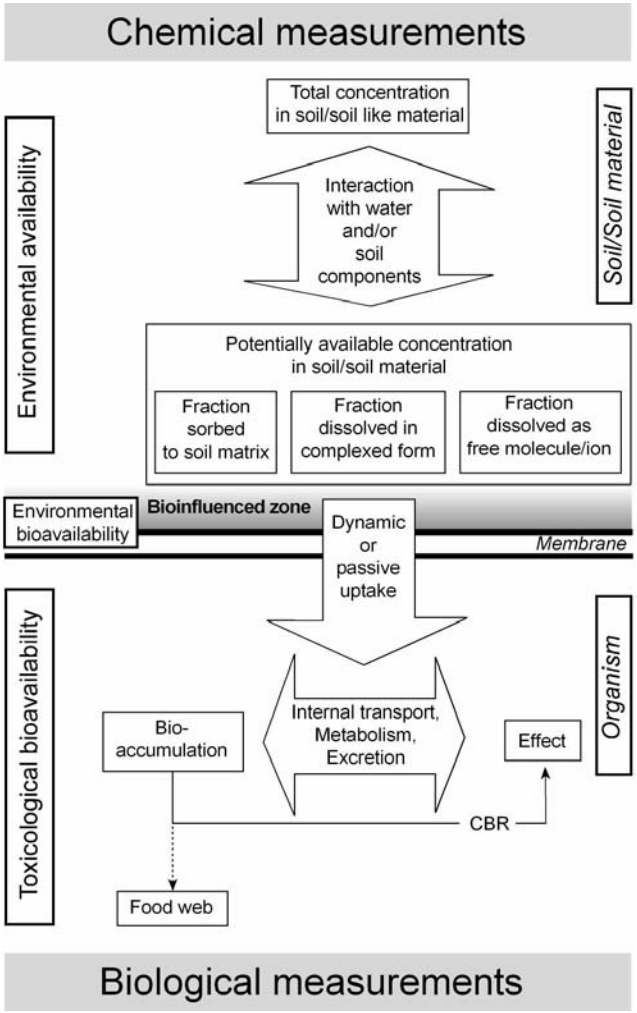

Figure 1.1: There are two complementary ways to assess bioavailability, chemical measurements and biological measurements (Harmsen 2007).

This point of view leads to two complementary ways to assess bioavailability, biological measurements and chemical measurements.

In biological measurements (bioassays) organisms are exposed to soil or soil eluates to monitor effects. If accumulation and/or effects (e.g., mortality and growth inhibition) are present, it is most likely that bioavailable contaminants are present. To close the gap between chemical measurements and biological effects, more knowledge of processes controlling bioavailability is needed (Harmsen 2007).

An alternative to biological measurements is the Critical Body Residue (or CBR) approach. Critical body residue is a concept that examines the relationship between the accumulation of a toxicant in an organism and its effects on an organism. It is based on the premise that a toxicant must reach a critical concentration within an organism before adverse effects are observed (Foulkes 1990). These effects are not necessarily lethal but can range from the lowest observed adverse effect to death. The internal threshold concentration (ITC) is the critical or lethal body residue or burden (this depends on the chosen endpoint). If the critical body burden is exceeded, effects on organisms are imminent.

This approach has been shown to be effective for hydrophobic contaminants that induce toxicity via the mechanism of non-polar narcosis (a nonspecific mode of toxic action) but it is not applicable for contaminants that have a different mode of action.

Aside from biological testing, chemical testing can also be used to address the bioavailability of contaminants. "*Chemical testing can be used if the bioavailability for an organism in soil can be mimicked by a chemical process. However, a function between the bioavailability and the chemical test that estimates the bioavailability is required*" (ISO 17402). Chemical measurements like Tenax and cyclodextrine extraction can be used in the soil matrix. They determine the fraction of contaminants assumed to be available for specific biological receptors. These chemical methods are mainly developed to predict the amount of contaminants taken up by organisms. If a correlation between the measured chemical values and biological effects or accumulation can be demonstrated, these chemical measurements may replace biological testing in a routine assessment of soil quality (Harmsen 2007).

This report will focus on chemical testing only, either by use of passive samplers or by use of a strong adsorbent or complexing agent. The following section will explain more about the selection process of the analytical methods.

1.4 Previous projects

This section shortly summarises activities preceding the current report. For detailed information we refer you to the literature described in the text.

1.4.1 Projects of 2007 and 2008

In 2007 RIVM was asked to investigate the possibilities to implement bioavailability in the Dutch policy framework for the assessment of contaminated soils. A first inventory was made of analytical methods that can measure bioavailability. This inventory could be considered as a basis for the follow-up of the project. This first inventory was mainly based on the report of Sijm et al. (2002) who performed similar research on analytical methods that can measure bioavailability.

In 2008 RIVM was asked to elaborate on the document of 2007 and we started an intensive study on methods that measure bioavailability. Building upon the outcomes of Sijm et al. (2002), the list of methods was completed with a literature study. The methods and their functioning were also briefly described (Brand et al. 2009). The methods and models that we selected in 2008 for further evaluation were as follows:

Organic contaminants

- Passive sampling methods including
  - Solid Phase Micro Extraction (SPME);
  - Semi Permeable Membrane Devices (SPMD);
  - Polyoxymethylene Solid Phase Extraction (POM-SPE);
  - Empore disks;
  - Silicone rubber;
- Tenax extraction;
- Cyclodextrine extraction;
- Supercritical fluid extraction.

Heavy metals

- Acid extractions;
- Extractions with chelating agents;
- Weak extractions;
- Donnan membrane technique;
- Diffusive gradient in thin films;
- Sequential extractions;
- Two-step 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.

1.4.2 Results workshop 2008

In the autumn of 2008 we organised a workshop for which national experts on bioavailability and the Dutch policy framework on soil contamination were invited, to comment on the list of methods.
The following questions were discussed during this workshop:

- which method(s) are considered to be most promising for the implementation of bioavailability?
- how can selected methods be implemented into policy or in which framework can they be used?
- which pre-conditions apply for these methods to be implemented?
- what knowledge is still missing and therefore what further research should be performed?

At the end of the workshop the participants scored the methods based on the following selection criteria:

1) wide ranging applicability, meaning
   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 (e.g., leaching).

Based on this scoring we made a final selection for the methods that were deemed fit for implementation in the Dutch regulatory framework. We made a distinction between measuring potentially bioavailable concentrations and measuring actually bioavailable concentrations. With potentially bioavailable concentrations we mean concentrations in pore water and the rapidly desorbing fraction of concentrations from the soil particles. With actually bioavailable concentrations we mean only the freely dissolved pore water concentration, which can be assumed to be the bioavailable concentration (see also section 3.3).

Taking the difference between actual and potential into account, the following methods were considered to be the most promising:

Organic contaminants
Measuring actually bioavailable concentrations:
- Passive sampling with materials in equilibrium with the pore water phase like SPME, POM-SPE or silicone rubber;

Measuring potentially bioavailable concentrations:
- Using a strong adsorbant or complexing agent depleting the pore water phase like Tenax extraction or cyclodextrin extraction.

Heavy metals
Measuring potentially bioavailable concentrations:
- Acid extraction with 0.43 M HNO_3;

Measuring actually bioavailable concentrations:
- Weak extractions with 0.01 M $CaCl_2$.[3]

In the this report we will work out the methods for organic contaminants that we selected in 2008, in order to give advice to the Ministry of Infrastructure and the Environment on how these methods could be implemented in the current regulatory framework. The bioavailability of heavy metals will be part of a following project.

1.5 Reader's guide

This report has been written for two audiences. On the one hand it is written for policymakers who have to decide whether or not it is desirous and possible to implement bioavailability into the regulatory framework and if so, how this can be done. On the other hand, the report is written to give guidance on how to perform measurements on bioavailability and in this respect the report can be used by laboratories and consultancies. With these two audiences in mind, every chapter starts with a section about the information that is given and at which audience it is aimed.

In brief, the report is divided as follows:
- Chapter 2 describes the basics of the different analytical methods that can be used to determine bioavailability. In this chapter the results of a literature review on how the analytical methods are used in experiments is described. This chapter does not yet advise on a generic user protocol for the selected methods;
- Chapter 3 describes different approaches to relate bioavailability to the current legislative framework. With help of a semi-quantitative case study, a step-by-step implementation of bioavailability will be demonstrated;

[3] In the international ISO standard 0.001 M $CaCl_2$ is recommended because 0.01 M $CaCl_2$ may suppress dissolution of DOC (Dissolved organic carbon) and associated contaminants. In the following project on heavy metals a final choice will be made on this point.

- Chapter 4 will present generic user protocols for the use of the analytical methods. This chapter will also pay some attention to quality assurance, as part of the method development;
- Finally, Chapter 5 gives some concluding remarks and an outlook considering the continuation of this project.

2 Methods

2.1 Introduction

In the projects of 2007 and 2008 we made a first selection for several methods that can determine the bioavailability of contaminants in soils. This selection was necessary due to our aim to give advice on how to implement the measuring of bioavailability in the policy framework and due to the large variety of methods that exist.

For the layman reader this chapter gives a general introduction to the analytical methods that are the subject of this report. *This information is particularly intended to familiarise you with the extraction methods and the basic principle of application of the methods.* This chapter briefly summarises the results of a literature review on each analytical method and does not have the intention to give advice on user protocols or how these analytical methods can be used. Chapter 4 will describe generic user protocols for each of the selected methods.

2.2 Passive sampling

Passive sampling techniques in soils or sediments are all based on the principle that the passive sampler is exposed to a soil or sediment sample until equilibrium is reached between the soil and pore water phase. Such a passive sampler can be any material that absorbs contaminants in a reproducible way and allows them to be recovered by extraction or other desorption systems.

Generally, these materials are polymers that allow compounds to diffuse inside. Polymers used are low density polyethylene (LDPE), polyoxymethylene (POM), polydimethylsiloxane (PDMS) and ethylenevinylacetate copolymers (EVA). In the passive sampling of surface waters, solvent or lipid filled LDPE membranes are also applied but these are rarely used for soils/sediment because they are probably not rigid enough to survive shaking with a soil slurry or sediment.

Due to the selection made in 2008, this report will be limited to describing passive sampling by use of PDMS-coated solid phase microextraction (SPME) fibres, POM and silicone rubber. The research of Ter Laak et al. 2006a; Ter Laak et al. 2006b; Mayer et al. 2000b; Jonker et al. 2007 and Van der Wal et al. 2004 all indicated that these passive sampling methods might be applied in toxicity setups to monitor freely dissolved concentrations in soils and sediments.

When using passive sampling to mimic bioavailability, it is explicitly assumed that exposure of the organism via pore water is the most important exposure pathway. However, it cannot be ruled out that some organisms are also capable of taking up contaminants from the soil phase directly via ingestion. However, most of the exposure will take place via the pore water concentration. By simulating these pore water concentrations of a contaminant, passive sampling techniques should give an indication of possible risks.

2.2.1 Solid phase micro extraction

Solid phase micro extraction (SPME) was introduced in 1990 by Arthur and Pawliszyn and has since then frequently been used in different types of analytical research. It was particularly used for determining aqueous analyte concentrations. These applications were based on the extraction of a constant analyte fraction from an aqueous sample by using internal and external calibration for quantification (Mayer et al. 2000a). However, SPME is nowadays

also more often used for sediments and soils. Mayer et al. (2000b) and Van der Wal et al. (2004) for example, both determined concentrations of hydrophobic organic contaminants by using SPME. Mayer et al. used SPME to determine the bioavailability of PCBs in sediment pore water, whilst Van der Wal et al. determined organic micro pollutants in terrestrial soils. This shows that SPME in addition to traditional extraction of water is also applicable to soils and sediments (Leslie et al. 2002; Jonker et al. 2007; Mayer et al. 2000b).

SPME uses a thin silica fibre, which is coated with a micro layer of organic polymer (usually polydimethylsiloxane or PDMS) (Conder et al. 2003) (see also Figure 2.1). To make the fibres more suitable for extraction of different types of contaminants it is possible to adjust parameters such as the thickness and type of the polymer coating and the agitation method. This makes it possible to measure less hydrophobic contaminants, such as surfactants, as well and it can also have a positive influence on the time needed to perform a measurement (Brand et al. 2009).

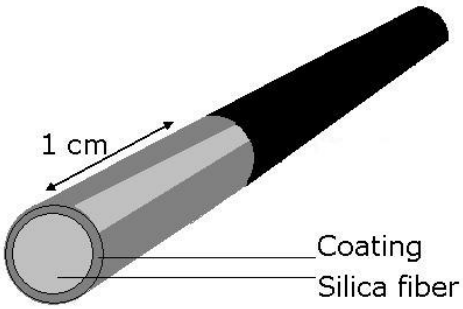

Figure 2.1: SPME fibre (Wikipedia).

Organic contaminants absorb into the SPME fibre coating and after measuring these contaminant concentrations in the fibre with HPLC[4] or GC[5], the pore water concentrations can be calculated with the SPME-water partition coefficient. This means that the measured concentrations of dissolved hydrophobic contaminants can be compared with the soil and water quality standards by means of these sampler-water coefficients.

SPME is used as a method that mimics concentrations that are actually bioavailable to organisms. SPME only extracts the freely dissolved contaminant concentrations in the pore water (Conder et al. 2003). This extraction does not influence the equilibrium between the contaminants bound to Dissolved Organic Carbon (DOC) and freely dissolved chemicals (Ter Laak et al. 2006b; Van der Wal et al. 2004).

SPME can be used for a variety of different contaminants. Successful research has been performed on PAHs, PCBs, chlorobenzenes and pesticides like DDT, DDE and lindane (Van der Heijden and Jonker 2009; Jonker et al. 2007; Mayer et al. 2000b; Van der Wal et al. 2004).

2.2.2 Literature on: Solid Phase Micro Extraction

SPME can be used in two different ways. Firstly, it can be used in the laboratory where the fibres are added to a soil/liquid slurry after which equilibration takes place. Secondly, the fibres can be placed directly on site as an in situ measurement technique. Although the second method presents reality best, this is also a laborious procedure. First of all, SPME fibres are very delicate and can easily break when placed in the field. Secondly, by assuming that pore water concentrations are the primary manner of exposure, this automatically implies

[4] HPLC = High Performance Liquid Chromatography
[5] GC = gas chromatography

that to perform reliable research, the presence of pore water on location is necessary. Although for sediments this is not a problem, for soils this can result in some problems during dry periods like summer.

When performing a laboratory experiment with SPME, firstly a soil/liquid slurry of the soil under investigation is created. This liquid frequently consists of a 0.01 M $CaCl_2$ solution but also Moderately Hard Water (MHW) is used (Trimble et al. 2008). To prevent bacterial growth that could degrade the compounds of interest, a predetermined amount of a biocide is added. Examples are sodium azide, mercury(II)chloride and copper powder (Jonker et al. 2007; Trimble et al. 2008). Which solution is finally used depends on the quantity of soil used. Van der Heijden and Jonker (2009) used a ratio of 2-4 g of sediment with 6.5-7 ml Millipore water containing 0.01 M $CaCl_2$ and 25 $mg.l^{-1}$ sodium azide. In 2007, Jonker et al. used 1-2 g of soil and 5 ml Millipore water containing 0.01 M $CaCl_2$ and 25 mg/l sodium azide. Van der Wal et al. (2004) used 5 g of soil (dry weight) at maximum water holding capacity. They added also 5 ml of 650 $mg.l^{-1}$ sodium azide. However in the ISO standard ISO/TS 21268-2, a $CaCl_2$ concentration of 0.001 M is recommended because a concentration of 0.01 M $CaCl_2$ may suppress dissolution of DOC and the associated contaminant.

Equilibrium times are also an important factor when extracting with passive samplers. There are two types of equilibrium time that need to be accounted for. The first one is the time that is taken to create equilibrium after the (dry) soil sample is mixed with the $CaCl_2$ solution. The second one is the time needed to reach equilibrium between the slurry and the SPME fibre. In the research of Van der Heijden and Jonker (2009), Jonker et al. (2007), Ter Laak et al. (2006a) and Van der Wal et al. (2004) we found no evidence of reaching equilibrium before starting the SPME extraction. All studies directly added one or several SPME fibres and then waited until equilibrium in the total system was reached. Finally, Van der Wal et al. (2004) selected an equilibration period of 20 days whilst Jonker et al. (2007) and Trimble et al. (2008) selected 28 days for measuring PCBs and PAHs. A homogeneous slurry is necessary before and during the experiment. Therefore, manual shaking before adding the fibres and mechanicaly shaking during the entire experiment is required. This provides an optimal contact between the fibres and the pore water concentrations. Furthermore, to prevent any degradation of the contaminant, amber-coloured vials are often used.

Finally, the type and length of SPME fibres added to the slurry are important. The fibre length added depends on the expected concentrations in relation to the limits of quantification. Jonker et al. (2007) and Van der Heijden and Jonker (2009) both used 1 or 2 fibres with a length of 5 cm. Ter laak et al. (2006a) used fibres of 3 to 5 cm. Van der Wal et al. (2004) used 3 fibres with a length of 10 cm. Prior to use, the fibres should be sufficiently cleaned. This can be done by either thermal cleaning at 275 °C for 16 hours under a constant helium flow of 30-35 $ml.min^{-1}$ (Ter Laak et al. (2006a); Jonker et al. (2007); Van der Heijden and Jonker. 2009) or by rinsing the fibres several times with methanol and Millipore water respectively, Jonker and Van der Heijden (2007); Mayer et al. (2000b).

Furthermore, a PDMS coating of about 30 µm is frequently used for PCB and PAH-like compounds (Van de Heijden and Jonker (2009); Jonker et al. (2007); Van der Wal et al. (2004); Jonker and Van der Heijden (2007); Ter laak et al. 2006a). For contaminants that have a higher or lower hydrophobicity than PAH or PCBs, coating thickness can be adapted to meet the circumstances.

The outcome of this extraction is a concentration of contaminants in the SPME fibre ($mg.l^{-1}$ PDMS coating), which is finally converted into a pore water concentration (See also Chapter 4).

2.2.3 *Polyoxymethylene solid phase extraction*

POM-SPE stands for 'polyoxymethylene solid phase extraction' and is a relative new area of research. Still, POM-SPE is nowadays used in the Netherlands, Norway, Finland, Sweden, China, USA, UK, Austria and Germany.

It was originally developed by Jonker and Koelmans in 2001 when they were searching for a method that could measure distribution coefficients for soot-adsorbed PAHs. Other methods to determine the coefficients proved not to be useful because the desorbed concentrations in the water phase were expected to be extremely low.

Polyoxymethylene is a hard and smooth plastic and can be bought in different shapes (see Figure 2.2). However, a simple sheet to cut strips from is mostly used.

Hydrophobic contaminants show reproducible and strong partitioning to the plastic. Because of its smooth surface, soot can easily be wiped off with a moist tissue without having an effect on the distribution coefficient (Jonker and Koelmans 2001). Due to its resistance to organic solvents, it is also possible to use these solvents to extract the contaminants from the plastic for analysis. The trade name of polyoxymethylene is (poly)acetal (formula CH_2O).

Figure 2.2: POM spheres but it is also available in sheets (www.megchems.com).

Conceptually, the POM-SPE method resembles the SPME method, as an additional artificial partitioning phase is added to a slurry to absorb organic contaminants from the aqueous phase. Thereafter, the freely dissolved aqueous concentrations can be calculated with compound-specific passive sample-water partition coefficients (Barthe et al. 2008). However, contrary to SPME, POM-SPE is capable of extracting larger fractions, thereby lowering detection limits (Jonker and Koelmans 2001). This is due to the large capacity (volume) of POM. To measure a negligible fraction of the contaminant a maximum of 5% of the total sorbed contaminant mass may be extracted. By adjusting the used POM mass accordingly, this criterion can be met (Jonker and Koelmans 2001).

The operational time of POM-SPE equals other sampling methods because several weeks are needed to reach equilibrium. There is however a new setup under development at the Institute for Risk Assessment Sciences (IRAS) that only takes 2 days.

The contaminants for which POM-SPE can be used are different types of hydrophobic organic pollutants (HOC), as presented in Jonker and Koelmans (2001), Hong and Luthy (2008) and Cornelissen et al. (2008).

2.2.4 *Literature on: polyoxymethylene solid phase extraction*

Most of the literature found was basically a variation on how Jonker and Koelmans performed the first experiments in 2001 and 2002.

Jonker and Koelmans added 0.6 g of sediment to 300 ml brown-coloured bottles. The bottles were filled with nanopure water containing 25 mg/l sodium azide and 0.01 M $CaCl_2$, leaving a headspace of 10 ml. Finally, 1 g of POM was added. This system was horizontally shaken and allowed to equilibrate for 28 days at 20 °C, which proved to be sufficient (Jonker and Koelmans 2001 and 2002). Cornelissen et al. (2008) used 3-4 g of sediment and 50 ml of distilled

water with 50-200 mg of POM strips. However, Cornelissen et al. (2008) did not achieve equilibrium within the 28 days reported by Jonker and Koelmans (2001) and stated that this is attributed to the POM/sediment ratios, which are much lower for Cornelissen et al. (2008). Hong and Luthy (2008) used 100 mg soil, 0.5 g of POM and 35 ml aqueous solution existing of milli-Q water, 100 mg/l sodium azide and 0.01 M $CaCl_2$. All bottles were tumbled end-to-end in a dark room at 25 °C for 40 days, which proved to be sufficient to reach equilibrium.

Currently there are also experiments in which equilibrium takes two days at IRAS. The results of this setup have still to be published.

Before use, the POM strips need to be cleaned. This can be done by washing with hexane (30 min) and methanol (3 × 30 min) followed by drying for 1.5 hours. Furthermore, the experiments performed by Jonker and Koelmans (2001 and 2002), Cornelissen et al. (2008) and Hong and Luthy (2008) made use of POM strips with a thickness of 0.50 mm and a frequently used total mass of POM strips lies between 0.1 and 2 g.

Except for Cornelissen et al. (2008), all studies used Soxhlet extraction to extract the POM strips. Jonker and Koelmans (2001 and 2002) extracted for 3 hours with 70 ml of methanol to recover the contaminants from the POM strips. Whilst Hong and Luthy (2008) extracted for 16 h with a 120 ml acetone/hexane mixture (1:1). Both experiments proved to be sufficient.

Furthermore, before Soxhlet extraction begins, the POM strips should be wiped with a tissue to remove any particles sticking to the strip which can cause interference when measuring concentrations. 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 the POM strips should therefore have no influence on the measured concentrations.

Jonker and Koelmans (2001 and 2002) also applied a cleanup step after Soxhlex extraction by concentrating the extracts to 1 ml and exchanging them to hexane. The hexane was then cleaned up over Al_2O_3 (for PAHs) or Al_2O_3/silicagel (for PCBs) columns. The eluates were reduced to 1 ml and exchanged to acetonitrile (for PAHs) or isooctane (for PCBs) and reduced to 1 or 0.5 ml. This clean-up step serves to remove interfering chemicals and is especially necessary in soils rich in soot or other organic matter, due to the complexity and interfering nature of these materials. The outcome of this extraction is a concentration of contaminants in the POM strip ($mg.g^{-1}$ POM), which can be translated into a concentration in the pore water with the help of chemical-specific POM-water partition coefficients (See also Chapter 4).

2.2.5 Silicone Rubber extraction

Silicone rubber (also PDMS sheets) (see Figure 2.3) is basically the same material as the coating on SPME fibres. The quantities generally used lie between 0.5 to 3 g and this results in the absorption of high concentrations of contaminant when exposed to soil suspensions. Consequently, detection limits can be up to 50-100 times lower than SPME. Due to its high sorption capacity there is however also a risk for depleting the mixture at low soil or sediment densities.

Extraction with silicone rubber strips is not as frequently applied as SPME or POM-SPE. It is currently being used in the Netherlands and Norway. During an extraction with silicone rubber a small piece of rubber is placed in a soil slurry, after which the mixture is shaken and the rubber can absorb organic contaminants. Instead of rubber strips it is also possible to use bottles of which the inside is lined with a silicone rubber layer. The absorbed fraction of contaminant in the rubber is proportional to the pore water concentration (WGMS 2007).

The surface of silicone rubber (and SPME for that matter) is less smooth than, for example, POM-SPE, therefore soil and soot particles or oil droplets may stick to the surface.

Diffusion through silicone rubber is expected to be faster than any other sampling materials. This means that the uptake rate is not limited by diffusion in the sampler itself (Cornelissen and Kamerling 2003).

Rusina et al. (2007) investigated polymers for passive sampling and compared critical properties like swelling in solvents, diffusion coefficients and partition coefficients. Amongst others, silicone rubber was tested and showed positive results in relation to the other passive samplers.

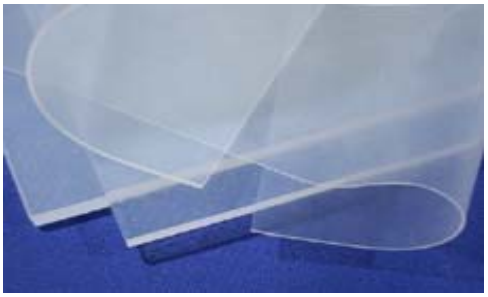

Figure 2.3: Sheets of silicone rubber (www.altecproducts.com).

2.2.6 Literature on: silicone rubber

Passive sampling with silicone rubber in soil or sediments is described by the Working Group on Marine Sediments (WGMS) of the International Council for the Exploration of the Sea (ICES) (WGMS, 2007) and was applied in the 'ICES-OSPAR Passive Sampling Trial Survey' (Smedes et al. 2007).

According to WGMS, either rubber sheets or bottles coated with a thin layer of silicone rubber can be used. Usually 0.3-0.8 g of rubber (either sheet or coating) is needed for 0.5 kg dry weight of soil. Before use, the rubber sheets need to be pre-cleaned by soxhlet extraction for 100 hours with ethylacetate. It is also possible to clean the sheets by shaking them for one week with 20 ml ethylacetate per sheet. The ethylacetate needs to be refreshed twice during this week. Bottles coated on the inside with silicone rubber are shaken three times for 24 hours with 50 ml ethylacetate. This pre-extraction is required to remove unpolymerised material that would disturb analyses. After this pre-extraction both sheets and bottles are dried in a fume hood in a flow of nitrogen (WGMS 2007).

For the bottles it is also necessary to keep track of the weight of the coating used for equilibration. Therefore, it is better not to use labels but engraving a number on the bottle can be an alternative. Furthermore the bottles should be weighed without the cap and all caps used should be air and liquid tight by using an aluminium-lined insert in the cap (WGMS 2007).

WGMS uses between 0.5-0.7 kg of homogenised wet sediment or soil sample. If necessary, some water is added to liquefy the sample. Generally about 80% water content for clay and 25-30% for sandy soils is required. The rubber sheets are added to the sediment or soil suspension and in case of coated bottles, this suspension is transferred to the bottle. Before equilibration oxygen is removed from the bottles by purging, using a nitrogen flow. Subsequently the bottles are placed on an orbital shaker at 125 rpm with an amplitude of 3 cm. This is continued during 3 weeks at 20 °C in the dark. Thereafter the bottles are emptied and quickly rinsed with 50 ml of water to remove any soil that remains. The sheets can be washed, wiped dry and stored in a glass vial with an aluminium foil-lined cap.

Extraction of the rubber-lined bottles is done by shaking twice for 2 hours with 40 ml methanol. Sheets can be extracted by shaking twice with 20 ml methanol per 1 gram of sheet during 24 hours shaking (WGMS 2007).

2.3 Tenax extraction

Tenax extractions are widely used for the assessment of sediment samples. Several laboratories both in the EU (Netherlands, France, Spain, Norway, Sweden and Germany) and elsewhere such as the United States have reported positive results when using Tenax as an adsorbent. The frequent use of this polymer means that several calibration studies have been performed. The method is also representative of 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 (Brand et al. 2009).

Tenax is a porous polymer based on 2,6-diphenyl-*p*-phenylene oxide and is often used as an infinite sink for the desorption of organic pollutants from soils and sediments (see Figure 2.4). Tenax extractions are therefore often exhaustive and represent a worst case with regard to available amounts of contaminants in the soils or sediment. Tenax can have different mesh sizes ranging from very fine powder of 177-250 µm in diameter (60-80 mesh) to beads of 1 mm in diameter (20-35 mesh).

The quantification of Tenax extractable fractions has been used by researchers to measure the potentially available concentration of organic contaminants in soil or sediment. Examples of studies that used Tenax as a sorbent are Trimble et al. (2008); Cornelissen et al. (1997), Harmsen (2004) and Ten Hulscher et al. (2003). They used Tenax to determine the bioavailability of PCBs and PAHs from field collected sediments and soils. Furthermore, Tenax extractions are used by the Dutch 'Directorate-General of Public Works and Water Management (Rijkswaterstaat)' to determine concentrations of organic contaminants in sediments (see also section 3.2).

Extraction of organic contaminants for 6-24 hours by Tenax is thought to capture the fast desorbing pollutant fraction. This fast fraction is assumed to be (the most) available for affecting organisms in soils and sediments. When extractions with Tenax are longer than 24 hours, the slowly and very slowly available fractions can also be adsorbed. These fractions represent potentially bioavailable fractions in the long term.

Tenax can be used in different types of soil but the presence of black carbon, oil, tar or peat can cause problems. These soot-like materials have a tendency to bind to the Tenax beads, which makes separation and cleaning of the Tenax beads before extraction difficult. The soot particles can interfere with the analysis of the extractant and therefore cause higher concentrations than available in reality. This causes an overestimation of the risks.

Tenax can be used for several organic contaminants, including "old" pesticides such as DDT and lindane. Tenax is probably less suited for more polar contaminants (Brand et al. 2009).

Figure 2.4: Tenax powder (anonymous).

2.3.1 *Literature on: Tenax extraction*

Before starting a Tenax extraction, the Tenax beads need to be cleaned. This can be successfully done by rinsing the beads firstly with 10 $ml.g^{-1}$ water. Thereafter, the beads are in succession manually shaken with hexane and acetone or methanol 3 times each. The Tenax is then left to dry overnight at 75°C (Van Noort et al. 2003; Van der Heijden and Jonker 2009; Cornelissen et al. 1997).

Tenax extraction can be used for external or internal adsorption. With external adsorption the Tenax is placed outside the soil-water solution and only part of the liquid is guided over a Tenax bed. With internal adsorption the Tenax is placed inside a separation funnel together with the soil-water solution. The Tenax beads therefore have direct contact with the soil particles.

There are basically two ways of Tenax extraction, multipoint extractions and single point extractions. Multipoint extractions use different batches of Tenax over longer periods of time. At regular time intervals the Tenax is replaced by a fresh batch. These experiments can be continued for longer periods of time because the changing of the batches ensures that the Tenax will not be saturated. With single Tenax extractions only one batch of Tenax is used during the whole experiment. Usually, a single point extraction lasts for 6 or 24 hours or somewhere in between. The aim of a single point extraction is different from a multipoint extraction. Single point extractions are performed to determine the rapidly desorbing fraction in a soil-water solution, whilst multipoint extractions are usually used to determine rapidly and slowly desorbing fractions to establish desorption curves. De Weert et al. (2008) performed experiments with nonylphenol to determine at which point the rapidly desorbing fraction is depleted. The experiments showed that within 5 hours, 75% of the nonylphenol had desorbed from the sediment. After 24 hours this fraction increased to 86%. This indicates that most of the rapidly desorbing fraction can be determined within 6 hours after starting the experiment. The research of Ten Hulscher et al. (2003) and Cornelissen et al. (2001) confirms these findings. However Van Noort (2009a) evaluated the Tenax extraction method and concluded that a 24-hour extraction is preferred because the compound-specific extrapolation factor (E_f), which is used to convert Tenax extracted concentrations into bioavailable concentrations, showed less variance at 24-hours than at a 6-hour extraction. Previously, a generic factor of 2 was used as extrapolation factor for various compounds but this sometimes overestimated the concentrations available to biota.

Furthermore, Tenax-to-soil ratios are important because saturation of the Tenax before the end of the experiment can cause misleading results, as the Tenax needs to function like an infinite sink in this type of experimental setup. Although Tenax-to-soil ratios can vary greatly, between 0.33 to 1.5 for multipoint and single point extractions, Kraaij et al. (2002), Ten Hulscher et al. (2003) and Cornelissen et al. (2001) all had a Tenax-to-soil ratio of 1 or 1.5.

The total solution can best be shaken in a separation funnel of 100 ml. The water frequently used is 70 ml of Milli-Q water and $HgCl_2$ or NaN_3 can be added to prevent microbial activity. Minimal amounts should be used to prevent interference with the measurements. During the whole extraction the funnels need adequate shaking to assure that the soil and Tenax remain in suspension (Ten Hulscher et al. 2003 and Cornelissen et al. 2001).

After the extraction, the Tenax and soil can be relatively easy separated. The Tenax sticks to the wall of the funnel while the soil-water slurry is removed. The Tenax needs to be cleaned of any remaining soil particles before extraction; this can be done by rinsing with Milli-Q water.

Thereafter, the Tenax can be extracted with hexane. Kraaij et al. (2002) and Ten Hulscher et al. (2003) used a single extraction of respectively 20 ml and 30 ml. Others use a combination of acetone and hexane in varying concentrations (Trimble et al. 2008; Oen et al. 2006). Instead of acetone, ethanol can also be used (Moermond et al. (2004); Van der Heijden and Jonker 2009). Finally, the hexane sample can be analysed.

2.4 Cyclodextrin extraction

Hydroxypropyl-β-cyclodextrin (hereafter called HPCD or cyclodextrin) has a hydrophilic cavity but also contains a hydrophobic organic cavity within its molecular structure (see Figure 2.5). These molecules have high aqueous solubilities because of an array of hydroxyl functional groups on the exterior shell. This allows for the formation of a 1:1 water soluble inclusion complex between the cyclodextrin molecule and a low polarity organic compound (Swindell and Reid 2006). But it has also been shown that molecules that are too large to form 1:1 complexes with cyclodextrin, can form 1:2 inclusion complexes. In this case, two cyclodextrin molecules and one guest molecule form a complex (Reid et al. 2000). But larger cyclodextrin molecules can also be used to accommodate larger guest molecules.

Aqueous solutions of cyclodextrins have been used to dissolve a range of contaminants of low aqueous solubility. Examples are PAHs, DDT, chlorobenzenes, 2-methylphenantrene, 1-methylfluorene, 1,2-benzofluorene and β-bromonaphthalene (Reid et al. 2000). Due to its ability to selectively encapsulate freely dissolved organic molecules, which is similar to the membrane permeation limit of 9.5 Å (pore size), HPCD is suitable for sampling in environments with high concentrations of dissolved organic carbons, like peat areas. Cylodextrins also form complexes with polar organic compounds such as carboxylic acids and dissociated organic molecules. This increases the application range of cyclodextrins (Hartnik et al. 2008).

The type of soil has an influence on the amount of extracted contaminant. The highest extraction yields for cyclodextrin are seen with sandy soils (up to 89% for freshly spiked soils). Clay soils show lower but still good extraction amounts of 75% (Reid et al. 2000). Extraction amounts further decrease with aging. The remaining fractions after extraction are not considered bioavailable.

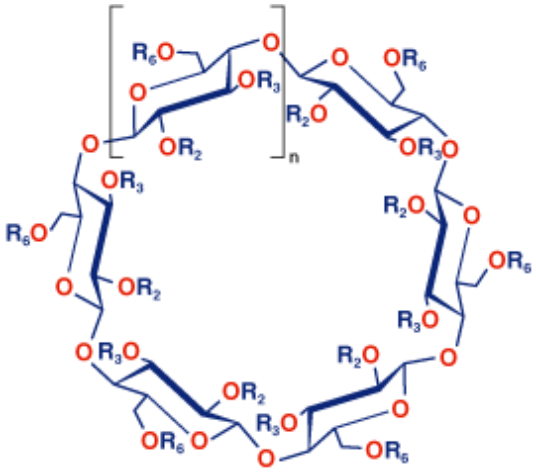

Figure 2.5: Cyclodextrin molecule (www.macherey-nagel.com).

2.4.1 Literature on: cyclodextrin extraction

Reid et al. (2000) performed some experiments in which the optimal experimental setup for cyclodextrin extractions was determined. They used 25 ml (put in Teflon centrifuge tubes) of different solutions of cyclodextrin ranging from 10 to 60 mM for these purposes. Similar experiments were performed to determine the optimal extraction time by testing at 3, 6, 12, 18 and 24 hours. Finally, they determined that at concentrations higher than 40 mM and an extraction time of 6 hours, the amount of extracted contaminant no longer increases. To ensure that the extraction of contaminants is complete, Reid et al. (2000) used an extraction time of 20 hours and a cyclodextrin concentration of 50 mM to extract 1.25 mg of soil during the remainder of the experiments. The tubes with the soil-cyclodextrin solutions were shaken on an orbital shaker at 150 revenants per minute. Afterwards, the tubes were centrifuged at 27,000g. The supernatant (6 ml) was sampled and added to Ultima gold XR Scintillation fluid (14 ml) and analysed (Reid et al. 2000).

Van der Heijden and Jonker (2009) used a slightly modified version of Reid et al. (2000). They used 0.75 g of sediment in 20 ml vials and added 15 ml of 50 mM of HPCD and 25 $mg.l^{-1}$ sodium azide in Millipore water. The vials were then horizontally shaken at 150 rpm for 24 h and centrifuged at 3000 rpm for 20 min. Thereafter, 10 ml of supernatant was extracted with 2 × 3 ml hexane for analyses.

Swindell and Reid (2006) adopted the same method as Reid et al. (2000) but changed the amount of soil to 1.5 mg and the concentration of cyclodextrin to 60 mM per 25 ml. Allan et al. (2006) and Hofman et al. (2008) also used the method of Reid et al. (2000).

Hartnik et al. (2008) also performed some testing to determine optimal experimental conditions for hydrophobic pesticides. Their optimal setting used 24 ml glass vials with PFTE lined caps and 0.6 g soil and a HPCD-to-soil ration of 3.5 (by weight). Then a 15 ml aliquot of 10 mM of sodium azide solution was added to prevent biological activity. Subsequently, the suspensions were shaken at 150 rpm for 48 hours, the vials were centrifuged at 2000 rpm for 20 min and 2 ml of the supernatant was mixed with scintillation cocktail Ultima Gold for scintillation counting. This setting uses a HPCD-to-soil ratio that is twice as high and an extraction time that is twice as long as Reid et al. (2000). This is however not considered a problem as both Reid et al. (2000) and Hartnik et al. (2008) showed that above a certain extraction time and a specific HPCD concentration, the concentration of extracted compounds does not increase. In this way, a large HPCD-to-soil ratio does not have a negative effect on the extractions. However, it should be stressed that too low concentrations of cyclodextrins can have a negative effect on the extraction.

2.5 Field of application

To summarise what is described in the previous sections and in the report of 2009 (Brand et al. 2009), Table 2.1 presents a short overview of the analytical methods and their field of application. It is summarised if the methods can be used in soil and/or sediments, for which contaminants the methods can be used, for which soil type the methods can be used and for which purpose the methods are less suitable. It is worth mentioning that this table is only based on the reviewed literature within this project and that no extensive literature review has been done to derive this table.

Table 2.1: Field of application of 5 analytical methods that can be used to measure bioavailability (based on reviewed literature).

Method	**SPME**	**POM-SPE**	**Silicone rubber**	**Tenax**	**Cyclodextrin**
Use in soil or sediment	- Soil - Sediment	- Soil - Sediment	- Soil - Sediment	- Soil - Sediment	- Soil - Sediment
Suitable for the following contaminants	- PAH - Pesticides (DTT, DDE, DDT, Drins) - PCB's - Chlorobenzenes - Surfactants	- PAH - Pesticides (DTT, DDE, DDT, Drins) - PCB's	- PAH - Pesticides (DTT, DDE, DDT, Drins) - PCB's	- PAH - Pesticides (DTT, DDE, DDT, Drins, lindane) - PCB's - Nonyl-phenol	- PAH - Pesticides (DTT, DDE, DDT, Drins, Lindane) - Mineral oil - PCB - chlorobenzenes, - 2-methylphenantrene - 1-methylfluorene - 1,2-benzofluorene - β-bromonaphthalene
Soil type	Variety of soils except peat soils	Variety of soils including peat soils	Variety of soils except peat soils	Variety of soils except peat soils	Variety of soils including peat soils
Less suitable for	- Polar contaminants - Assessment of plants (because of difference in uptake from other organisms) - Soils with high fractions of black carbon, oil, tar or peat due to separation or fouling problems	- Polar contaminants - Assessment of plants (because of difference in uptake from other organisms)	- Polar contaminants - Assessment of plants (because of difference in uptake from other organisms) - Soils with high fractions of black carbon, oil, tar or peat due to separation or fouling problems	- Polar contaminants - Assessment of plants (because of difference in uptake from other organisms) - Soils with high fractions of black carbon, oil, tar or peat due to separation problems	- Freshly added polar contaminants - Assessment of plants (because of difference in uptake from other organisms)

3 Implementation of bioavailability into the regulatory framework

3.1 Introduction

Various processes can affect bioavailability in the field. For example, strong sorption of contaminants to soot or other organic materials will strongly limit their availability for uptake by plants and animals (and hence, samplers). These processes are time- and space-dependent, creating a complex system which makes it challenging to assess the toxic effects of contaminants. This is why many different methods have been developed over the years to measure bioavailability, making it complex to implement bioavailability into the legislative framework.

The development of methods to quantify the bioavailable concentrations was usually done without the development of a corresponding reference system based on ecotoxicity test data. We therefore need a framework to relate measured bioavailable concentrations with ecosystem protection goals. Such a framework is currently non-existing. The following sections describe a proposal to relate measured bioavailable concentrations to risk values. For a better understanding, the position of bioavailability in the risk assessment of the water compartment will also be explained. *This chapter is particularly written for policymakers to decide on whether or not and if so, how bioavailability can be implemented into the regulatory framework.*

3.2 Bioavailability in water risk assessment

Many of the developments in research on bioavailability are made in the water compartment and it is expected that the general principles of bioavailability in water are not beforehand different from the principles in soil.

The possibility to assess the bioavailability of contaminants in the water compartment was already included in the European Priority Substances Directive (2008/105/EU) as, *"If … hardness, pH or other water quality parameters affect the bioavailability of metals, Member States may take this into account when assessing the monitoring results against the EQS".*

In the Netherlands, the concept of bioavailability has been implemented as a second tier risk assessment of the water compartment. This assessment is restricted to the metals copper, nickel and zinc. For each of these metals Biotic Ligand Models (BLMs) have been derived. BLMs combine chemical equilibrium modelling for the assessment of metal speciation with a toxicity model that relates metal accumulation at the biotic ligand to a toxic effect.

If, in the generic assessment of the water quality (tier 1), the contaminant concentrations in water exceed the ecological targets for surface water or environmental quality criteria for individual pollutants, it is possible to correct the measured concentration in tier 2 for site specific pH and DOC. Through trend monitoring it is determined if the contaminants do not accumulate in sediment, cause problems downstream and if the ecosystem is protected and improved. Every 6 years it needs to be determined if the correction for bioavailability still applies because of changing circumstances (pH and DOC).

The Dutch Directorate-General of Public Works and Water Management also uses bioavailability measurements to determine whether a sediment needs to be remediated/dredged. If the contaminant concentrations in water exceed the chemical or ecological quality targets as defined in the European Water framework Directive (2000/60/EC), it is possible to determine the contribution

from the sediment to this concentration in water by measuring the bioavailable concentration in the sediment. As a first approach, this concentration can be determined with equilibrium partitioning. If higher reliability is desired, bioavailability can be measured with a Tenax extraction.

3.3 Difference between measuring potential and actual concentrations

Until now we have made no distinction in this report between actually bioavailable concentrations and potentially bioavailable concentrations. The basic difference lies in the applied method for the determination of bioavailable concentrations.

Passive samplers, like SPME, POM-SPE and silicone rubber, measure only freely dissolved pore water concentrations, which can be assumed to be the actually bioavailable concentration for most soil organisms. Hence, soil biota are directly aware of these concentrations via contact with soil pore water. Due to the low solubility of most organic contaminants and strong adsorption to the solid matrix, the free concentration of contaminants in the pore water can be very low.

The potentially bioavailable concentration is the concentration which, in time, can become freely available for soil biota. Tenax and cyclodextrin extractions serve as an infinite sink and thereby also extract part of the rapidly desorbing fraction of concentrations from the soil particles. Depletion of the pore water would occur if the extracted fraction is not replaced by contaminants sorbed in the soil matrix. However, the rapidly desorbing part of the sorbed contaminants will replace the depleted freely dissolved contaminants and are considered the potentially bioavailable concentration. The actual exposure of soil biota depends on the site-specific situation, which determines binding of contaminants, e.g., pH or organic matter content. Some organisms are not only exposed to the contaminants via direct contact with pore water, but can also be affected by the ingestion of contaminants bound to soil particles. For these organisms a risk assessment based on actual concentrations (pore water concentrations) could underestimate the risks. One could therefore argue that the potentially bioavailable fraction also covers those organisms that feed directly from the soil matrix. Still, it is generally accepted that pore water concentrations are a reliable measure for determining effects on ecosystems for a wide range of biota.

The slowly and very slowly desorbing fractions will in time also become available, but are not included in the potentially bioavailable concentration which is measured by either Tenax or cyclodextrin extractions of 6-20 hours. As in the name, this process is slow and the amount coming available per unit of time is low and it is assumed that this will not cause risks. Figure 3.1 presents the principle of potentially and actually available concentrations in relation to the selected analytical methods described in this report.

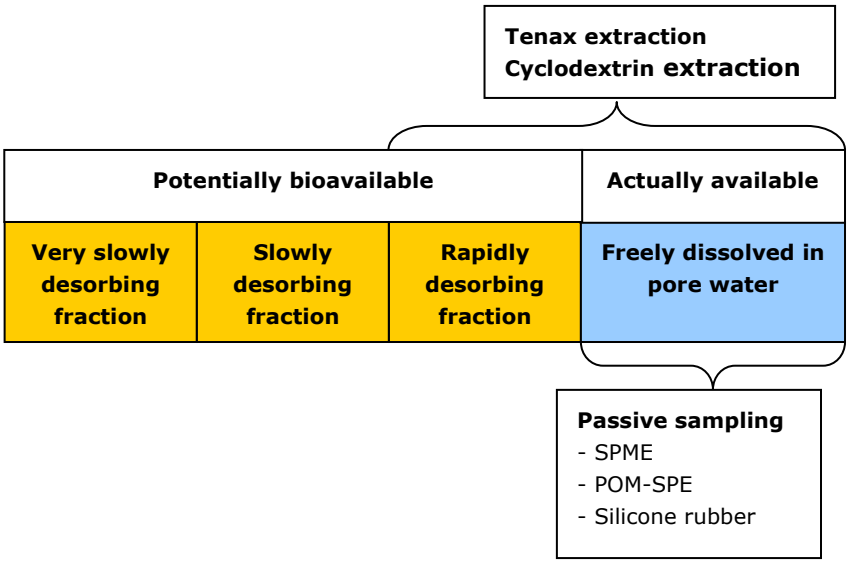

Figure 3.1: Principle of potentially and actually bioavailable concentrations in relation to the selected analytical methods.

Note: the size of the squares does not resemble the realistic ratio between fractions.

Due to the difference between actually available and potentially available concentrations it can be discussed whether actual concentrations or potential concentrations should be used for ecological risk assessment.

In the policy for sustainable land use this could be a key factor. For a soil to be sustainably fit for use means that soil can still be used for the function it is serving in the long term. From a policy viewpoint it can be suggested that potentially available concentrations are a more protective (and more conservative) assessment of risks. After all, rapidly desorbing fractions could become available (under changing conditions) and are included in the assessment when determining potentially bioavailable concentrations. On the other hand, it can be argued that this approach overestimates the risks because at the time organisms are not exposed to these concentrations. Depending on site-specific properties it can be questioned whether these potential bioavailable concentrations will indeed become available in the future. The contamination to which we refer already exists for many years and a change of conditions at the site is not expected. Therefore, accounting only for potentially available concentrations could lead to unnecessarily strict precautionary measures. From this point of view, measuring actually available concentrations is a more realistic approach for decision-making on current soil quality.

This discussion explains why during the workshop of 2008 different experts advised using a combination of the extraction methods when assessing a contaminated site.[6] The current report will not advise on the selection of either potentially or actually bioavailable concentrations because this is a policy decision and has to be discussed within another phase of the project (see also section 5.2.3 of this report).

[6] For more detailed information about the results of the 2008 workshop please refer to Chapter 4 of Brand et al. 2009.

3.4 The current regulatory framework for soil risk assessment and sustainable land management

3.4.1 Determining soil remediation urgency

The current regulatory framework on contaminated soils exists of 3 tiers:
1. Determination of a case of serious soil contamination;
2. Generic risk assessment;
3. Site specific risk assessment.

Tier 1 and 2 are obligatory. Tier 3 can be carried out if deemed necessary by the initiator or competent authority pursuant to the Soil Protection Act. Figure 3.2 shows the steps of risk assessment, remediation and management. The three tiers will briefly be described. For a more extensive explanation see annex 2 of the Soil Remediation Circular 2009 (VROM 2009).

Tier 1
To determine if there is a case of serious soil contamination, the total concentration of the contaminant in the soil is determined. If this concentration exceeds the Dutch Intervention Value for soil, there is a case of serious contamination and a generic assessment has to be carried out to determine the remediation urgency.

Tier 2
The generic assessment is done with help of the web application Sanscrit. By use of default scenarios and exposure pathways, the risks for human health, ecology and contaminant migration in the groundwater are determined. If there is a risk for humans, ecology or contaminant migration it is possible to carry out a site specific risk assessment if deemed necessary. Otherwise a remediation plan needs to be written.

Tier 3
In tier 3 it is possible to use site specific information as input for the web application Sanscrit. This information can range from a measured concentration in vegetables for determining risks for human health to performing a Triad assessment to determine risks for ecology. The following section will elaborate on the triad approach and its relation with measuring bioavailable concentrations.

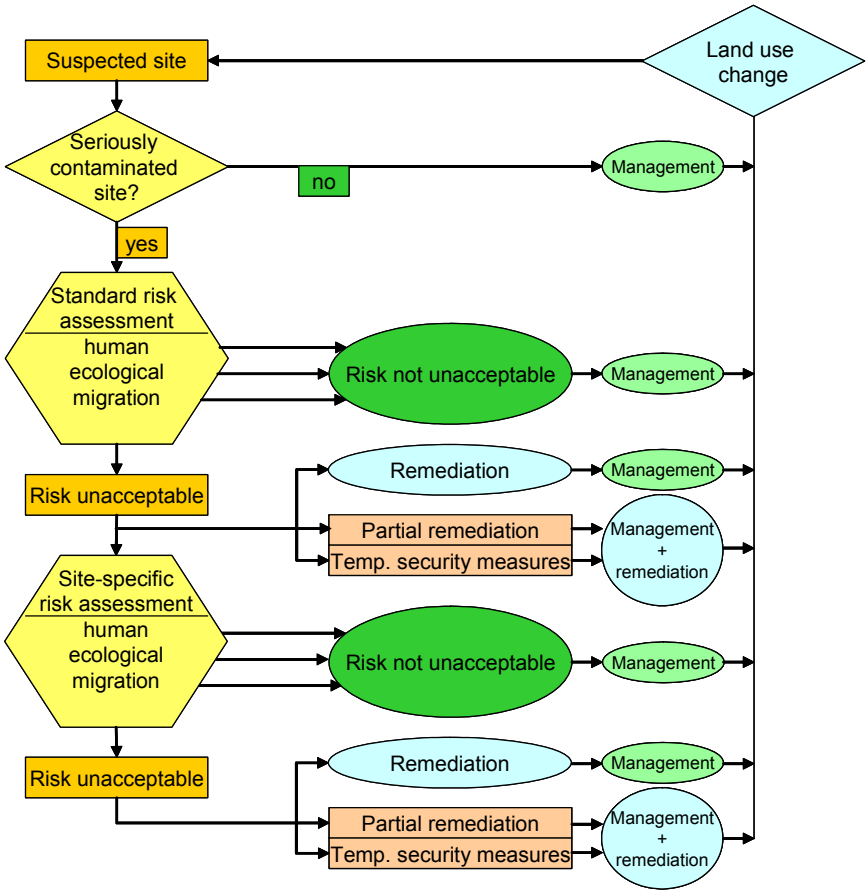

Figure 3.2: Tiers within the current regulatory framework for soil contamination (VROM 2009).

3.4.2 *Bioavailability in the triad approach*

At present, measuring bioavailability of contaminants can already be part of the chemical assessment within the Triad approach. Experiences with measuring the bioavailability of metals have been gained in this way. For organic contaminants less experience is gained this way.

The triad approach (Figure 3.3) combines three disciplines within ecotoxicological research:

- Chemistry, being the concentration of contaminants in the environment measured as total or bioavailable concentration, accumulated in biota or modelled via food-chains and is used for calculation of risks on the basis of toxicity data from the literature;
- Toxicity, contains bioassays with species across genera, in order to measure the toxicity present in environmental samples from the site;
- Ecology, consists of field ecological observations at the contaminated site which are compared to the reference site (Jensen et al. 2006).

There are 2 points of attention with respect to the triad approach. Firstly, until now there is no formalised approach to how bioavailable concentrations can be related to soil quality standards or toxicity data. Secondly, the triad approach gets its strength by combining chemistry, toxicity and ecology assessments. But to do so it can become a time-consuming and costly operation, especially if there are expectations that the majority of the contaminant concentration is not bioavailable.

Efficiency can be improved by creating an additional step within the site specific assessment, prior to the triad approach by measuring bioavailability. Moreover, the bioavailability measurements can be reused as part of the triad approach and in this way can be considered to be a 'no regrets approach'. For more information on the Triad we refer to NEN 5737, Mesman et al. (2011) or Jensen et al. (2006) and Rutgers and Jensen (2011).

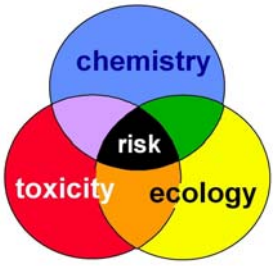

Figure 3.3: The triad principle is a combination of possible instruments for the assessment of ecological risks, based on chemistry, toxicity and ecology.

3.4.3 Sustainable land management

The principle behind sustainable land use is that a soil is fit for current and future use. Therefore, the reuse of soil in different areas is restricted.

The regulatory framework for sustainable land management provides generic standards (background values and Maximum Values (Residential and Industrial)). If the concentrations in the soil are below the background value, it is possible to reuse this soil everywhere. If the concentration is above the remediation criterion, the soil cannot be reused.

In between the background value and the remediation criterion the maximum values 'Residential' and 'Industrial' apply. Excavated soil can only be reused on soils that have the same class or higher. Figure 3.4 presents a schematic overview of the classification of the generic standards.

Local authorities are allowed to derive area specific standards as long as these have a proper underpinning. This allows local authorities to derive more stringent or less stringent standards that fit the local situation as long as the 'standstill principle' applies. The standstill principle means that a (slightly) contaminated site within a certain area may become more polluted as long as another location within this same area gets cleaner. Furthermore, the site specific standards are not allowed to be higher than the remediation criterion. If there are no site specific standards derived by the local authorities, then the generic automatically standards apply. Figure 3.5 presents a schematic overview of the classification of the site specific standards. For more detailed information on this framework please refer to the reports of Senternovem, Bodem+ (2008 in Dutch) and Senternovem, Bodem+ (2007).

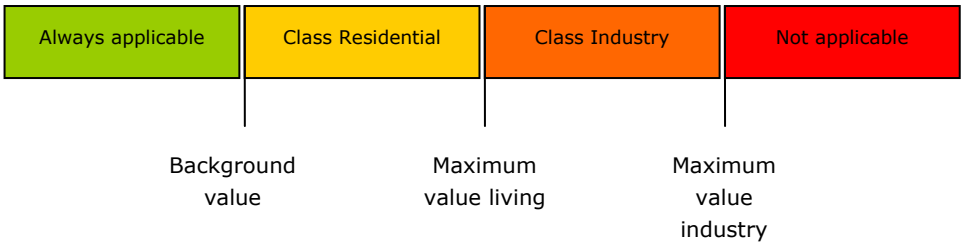

Figure 3.4: Generic standards for the sustainable land use of soil and sediment.

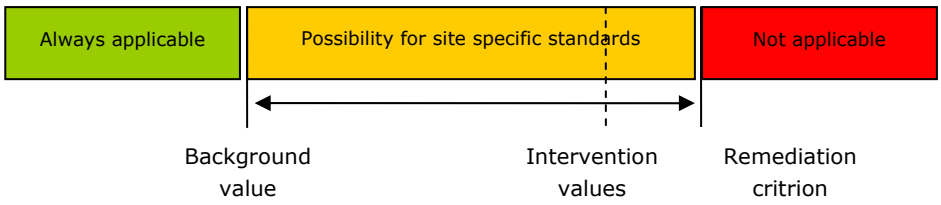

Figure 3.5: Site specific standards for the sustainable land use of soil and sediment.

3.5 Possibilities for implementation

The best way of implementing bioavailability in the current framework is to relate measured bioavailable concentrations in the field to toxicity data for soil biota based on bioavailable contaminant concentrations. These toxicity data are preferably measured with the same analytical method as the field data.

If we look at the Dutch reference system for soil we can observe that:

1. Soil quality standards are based on soil toxicity data for which total concentrations are determined.
2. Several soil quality standards are also derived from aquatic toxicity data, which have been converted into soil concentrations using a distribution coefficient.
3. No soil quality standards are based on aged bioavailable concentrations in soil.
4. Most soil toxicity data are derived from experiments were contaminants have been spiked. Spiked contaminants can be considered to be totally bioavailable because they did not have the time to bind to sites that are not accessible to organisms (also called aging of soils).

If there is a desire to implement bioavailability by relating the measured bioavailable concentrations to toxicity data based on bioavailable contaminant concentrations determined with the same analytical methods, new soil toxicity data and hence, soil quality standards, need to be derived. Because of the restriction that bioavailability should be implemented in the existing regulatory framework, deriving new bioavailability based quality standards is no option here. Therefore, other options to implement bioavailability without the need to derive new soil quality standards were investigated. Sections 3.5.1 and 3.5.2 will describe two options, corresponding to the selected analytical methods for measuring bioavailability.

3.5.1 Relating actually bioavailable concentrations to the toxicity of water organisms

In the workshop of 2008 numerous experts stated that it is possible to compare the extracted soil concentrations directly with the aquatic toxicity data. This is an option because pore water resembles the situation in aquatic toxicity testing in some aspects. This approach is based on two widely accepted assumptions:

1. Bioavailability, bioaccumulation and toxicity are closely related to pore water concentrations, and
2. Sensitivities of aquatic organisms are comparable with sensitivities of terrestrial organisms living in the soil (Verbruggen et al. 2001).

Examples of aquatic toxicity data to relate the measured concentrations to could be the HC5 level, the HC50 or any other HCx level for surface water. The HC5 and HC50 for most relevant contaminants can be found in the report of Verbruggen et al. (2001). A final decision on which risk limit should be chosen as a standard still has to be made and is a policy decision (see also Chapter 5 of this report).

This approach is a quick, transparent, reproducible and relatively representative way of implementing bioavailability for organic contaminants in a higher tier risk assessment. It essentially eliminates the need for toxicity testing when extracted bioavailable concentrations do not exceed the water standards.

As aquatic toxicity data are expressed as $\mu g.l^{-1}$, measured bioavailable pore water concentrations, should preferably also be expressed in $\mu g.l^{-1}$. The passive samplers SPME, POM-SPE and silicone rubber measure the concentration of the contaminant that is freely dissolved as $\mu g.l^{-1}$ pore water. Therefore, measured concentrations can directly be compared to aquatic toxicity data and no conversion is needed. The bioavailable concentration either exceeds the toxicity data or not. If the toxicity data are exceeded, effects on the ecosystem are imminent. This outcome can then be used for short-term decision-making like determining the remediation urgency of contaminated sites.

Technically, it is also possible to translate potentially bioavailable concentrations (given in $mg.kg^{-1}$) as measured with Tenax, cyclodextrin into a concentration in $\mu g.l^{-1}$ by using the distribution coefficient (K_{oc}) between soil and water. However, this calculation introduces an extra uncertainty due the uncertainty in the K_{oc} that is chosen. It is therefore preferable to relate potentially available concentrations directly to soil toxicity data, which are also given in $mg.kg^{-1}$ (see section 3.5.2).

3.5.2 Relating potentially bioavailable concentrations to toxicity of soil organisms

A second approach is to relate the measured bioavailable concentrations to toxicity data for soil organisms. Examples of these toxicity data are the HC5 or HC50 values for soil as found in Verbruggen et al. (2001). As with the actually bioavailable concentrations, a final decision on which risk limit should be chosen still has to be made. This decision has to be made by the policymakers (see also Chapter 5). This approach resembles the current way of risk assessment because the measured concentrations are compared with toxicity of soil organisms.

There is however a point of attention. Some of the Dutch soil quality standards have been derived from aquatic toxicity data. This causes some uncertainty because bioavailable soil concentrations are then compared to aquatic toxicity data that were converted into soil concentrations. For some of the compounds (mostly revised in 2001) the relevant toxicity data can be retrieved to determine whether terrestrial or aquatic toxicity data were used. This is however not the case for all the compounds. A study to recover this information is not always possible because the necessary data are no longer available or it may take a lot of time to retrieve it. Still, this uncertainty does not pose a major problem within current risk assessment or the implementation of bioavailability in the regulatory framework.

The results of the Tenax and cyclodextrin extractions are reported as concentrations in soil in $mg.kg^{-1}$. Therefore, no conversion of the results is required and the outcome of these methods can be directly related to terrestrial toxicity data.

The outcome of this assessment can be used for short-term decision-making like determining the remediation urgency. It is also possible to use the outcome for long-term decision-making like sustainable soil management. This is due to the fact that Tenax and cyclodextrin extractions also measure the rapidly desorbing fraction (potentially bioavailable concentration).

3.6 Step-by-step procedure

To demonstrate how the above procedures for implementing bioavailability can be used for decision-making concerning contaminated soils, a semi-quantitative case study is presented.

Figure 3.6 presents an overview of this step-by-step approach. Table 3.1 shows the required parameters for an assessment of this case study for endrin. The ecological risk limits are presented as HCx, which represents a still to be determined value for soil or water. Here we have used the value of the HC50 for ecosystems in soil or water; however, this could be also another value.

Table 3.1: Required parameters to assess remediation urgency.

Compound	**Measured total conc. in soil ($mg.kg^{-1}$)**	**HCx_{eco_soil} ($mg.kg^{-1}$)**	**HCx_{eco_water} ($\mu g.l^{-1}$)**	**Intervention value soil ($mg.kg^{-1}$)**
Endrin	1	0.095*	0.92*	0.1**

* *Verbruggen et al. (2001).*

** *Soil remediation Circular (VROM 2009).*

3.6.1 *Generic risk assessment (tiers 1 and 2)*

Note: to prevent complications we have simplified tier 1 and 2 (generic risk assessment with Sanscrit[7]). For a full description of these tiers see also annex 2 of the Soil Remediation Circular 2009 (VROM 2009).

We start with an imaginary total concentration of 1 $mg.kg^{-1}$ endrin in soil in more than 25 m^3 soil. This concentration is above the Dutch intervention value of 0.1 $mg.kg^{-1}$ for soil. This means that there is a serious soil contamination and a generic risk assessment is required (tier 2).

Based on the total contaminant concentrations and the Sanscrit model, the risks for human health, ecology and migration of the contaminant in the groundwater are determined.

If tier 2 indicates that there is risk, it can be decided to perform a site specific risk assessment by measuring bioavailable concentrations (tier 3) or a remediation plan is written if no further research is desired.

3.6.2 *Site specific risk assessment including bioavailability (tier 3)*

When it is decided to perform a site specific risk assessment by measuring the bioavailability of endrin, one of the analytical methods (e.g., passive sampling, extraction with Tenax or extraction with cyclodextrin) can be chosen depending on the soil properties, contaminant properties or nature of the assessment (e.g., determining remediation urgency or sustainable land management).

[7] Sanscrit is a decision support system to determine the urgency of remediation of contaminated soils. For more information on Sanscrit see also Otte and Wintersen (2007).

The experimental setup of the measurements is described for each method in Chapter 4.

Using passive samplers provides a bioavailable concentration of A $\mu g.l^{-1}$ endrin. If an extraction with Tenax or cyclodextrin is chosen, the outcome will be in B $mg.kg^{-1}$ endrin.

These values have to be compared with respectively the HCx_{eco_water} of 0.92 $\mu g.l^{-1}$ or the HCx_{eco_soil} of 0.095 $mg.kg^{-1}$. If the bioavailable concentration is smaller than the appointed quality standard, there is no remediation urgency. If the concentration is higher than the appointed quality standard, further research can be done (e.g., in the form of a Triad approach) or a remediation plan is written.

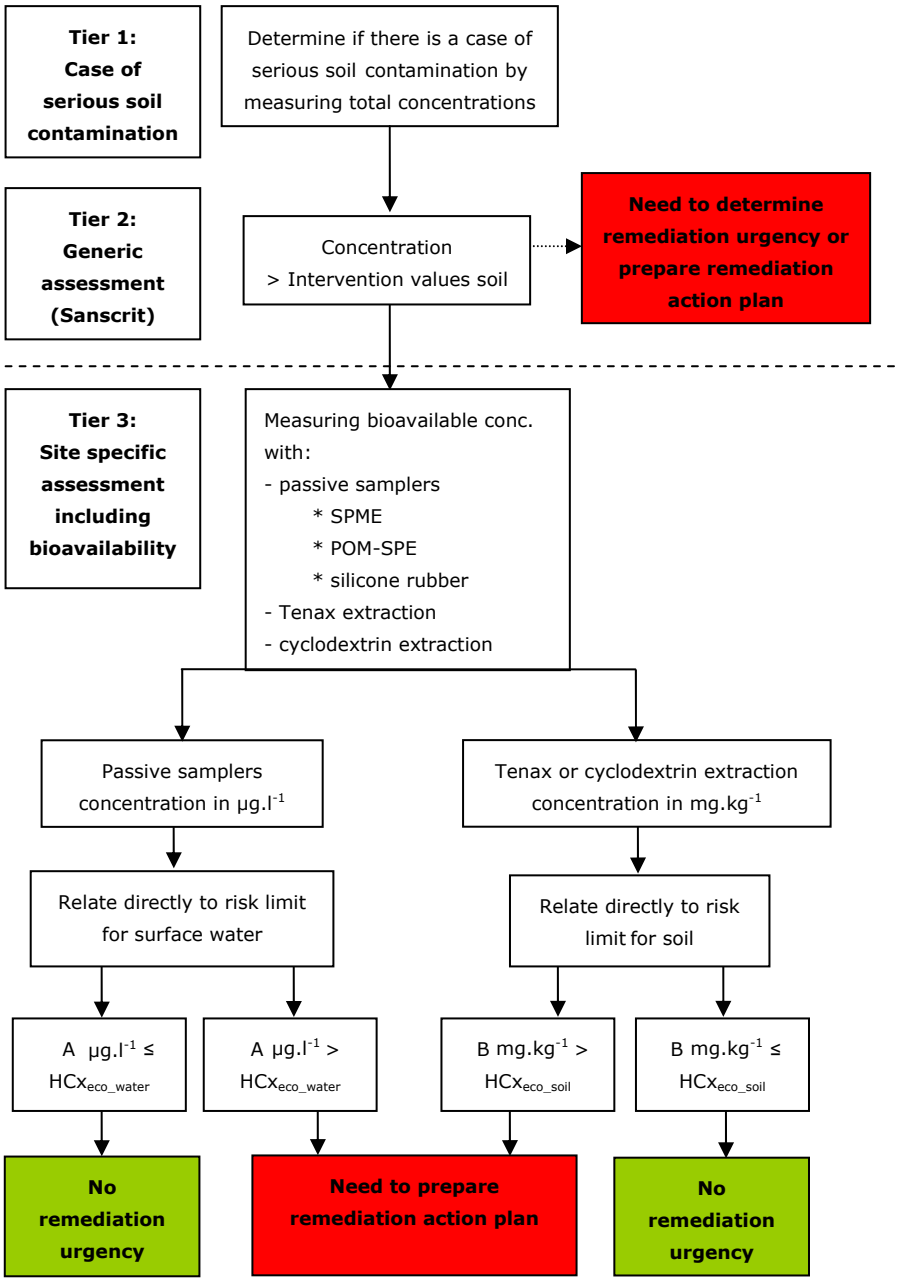

Figure 3.6: Step-by-step approach to implement bioavailability in the present framework.

4 Technical design and user protocol

4.1 Introduction

In the previous chapter we discussed the theoretical principle of the implementation of the methods. In this chapter we will present the practical implementation by describing the user protocols of the methods. Some parts of this chapter are still in the phase of method development (e.g., the exposure times needed, quality assurance like determining depletion factors, spiking, et cetera). Once methods have been used more frequently and laboratories have gained more experience in performing this type of research, these quality control measures can require less attention.

Due to the technical nature of this chapter it is particularly useful for laboratories and consultancies.

The selected methods (Chapter 2) are intended for use on a national scale and some similarity in their execution is desired. This is considered useful as it enables a quick performance of the experiments and allows for a comparison of the results obtained with different methods. Also, only then can the outcome of the bioavailability assessment of an examined area be compared with data from another contaminated area.

It is desirable to use existing international guidelines on how to determine a bioavailable soil concentration to stimulate uniformity and (scientific) acceptance. However, to date there is no international guideline on how to do this. In standard ISO/DIS 17402 of the International Organisation for Standardisation (ISO), guidance is given for the selection and application of methods for the assessment of bioavailability in soil and soil materials.

Currently, there is also progress within the ISO-workgroup Bioavailability on the development of standards on protocols to perform measurements with passive sampling (SPME and POM-SPE), Tenax and cyclodextrin extractions. At the time of writing this report the final outcome was still unclear. Therefore, the situation at the end of 2010 is used to write this report. In analogy with the developments within ISO, we advise using the following approaches. When (inter)national agreement has been reached this advice can be changed if necessary.

4.2 In general

4.2.1 Depletion

A logical criterion for the passive sampling material would be that the rate of diffusion inside the sampler is not limiting the uptake and allows the sampler to be seen as a homogeneous medium.

The partition theory says that in equilibrium the concentration of a compound in the sampler is directly proportional to the freely dissolved concentration of sampled compounds in pore water (by the sampler - water partitioning coefficient, K_{pw}). Because this freely dissolved concentration is considered to be the driving force for transport or the uptake by organisms, it is also considered as a measure for bioavailability. Consequently, it is also the driving force for uptake of passive samplers.

If the proper polymer is selected for the passive sampling of soil or sediment, two factors are crucial for an accurate estimation of the concentration in the pore water:

1. The amount extracted by the passive sampler should be so small that changes in the concentrations in pore water and soil or sediment are negligible;
2. Concentrations in sediment and sampler should be in equilibrium.

The percentage remaining in the soil or sediment is not a proper criterion to represent non-depletion, as limited availability implies that the concentration in the soil or sediment has no relation to the freely dissolved concentration in pore water. An unaffected freely dissolved concentration of contaminant in pore water can be determined when the sampler's sorption capacity is kept well below that of the soil or sediment. In Appendix 1 formula 2 a rule of thumb is given on depletion of the system.

For extractions with strong sorbents the above does not apply because these methods function as an infinite sink and focus on the fast desorbing fraction, which can then be correlated with biological effects. The amount of sorbant (either Tenax or cyclodextrin) should be relatively high, compared to the amount of soil. In other words, the absorption capacity of the sorbent needs to be higher than the total amount of pollutant in the system. As a rule of thumb, a Tenax/cyclodextrin-to-soil ratio of 1 can be used.

4.2.2 Pre treatments

Almost all used materials require pre treatment to remove residues of unpolymerised material and contaminants that may be absorbed during production or storage.

1. SPME fibres can be heated or extracted with methanol to remove impurities;
2. POM sheets can be pre-extracted with hexane[8]/methanol;
3. Silicone rubber samplers require extensive pre-extraction (100 hours soxhlet extraction with ethylacetate) to remove unpolymerised oligomers that would disturb the analyses. Rubber coatings on the inside of glass bottles are extracted 3 times for 24 hours with ethylacetate (this is a one-time action and the samplers can be reused);
4. Tenax can be cleaned by rinsing in turn with acetone and hexane[8];
5. Cyclodextrin does not require pre-treatment.

4.2.3 Spiking with PRCs

Quality assurance can be achieved by monitoring whether a non-depletive situation is really obtained by the addition of Performance Reference Compounds (PRC) to the sampler or sorbent. Adding PRCs is still in the phase of method development. Until enough experience has been gained with the measuring of bioavailability, spiking with PRCs is considered as a necessary step to perform some quality assurance. In the long term it can be considered to reduce the amount of quality assurance once enough experience has been gained.

[8] Recently, the increased toxicity of hexane has become the subject of discussion. Studies are now being performed to find an alternative for hexane as an extractant. Petroleum ether could become the designated alternative. Furthermore, it is determined if the amount of hexane that is used for analyses can be lowered. At the moment of writing this report, the exact outcome of these studies is unclear.

PRCs are compounds not found in the environment but with equal properties as the target compounds e.g., deuterated PAHs and/or PCBs that do not occur in technical mixtures. After equilibration the remaining fraction of the PRCs on the sampler is an indication of the depletion. Compounds over a wide hydrophobicity range should be used. Preferably about five compounds or more covering the whole K_{ow} range of the target compounds.

Amounts spiked should be in the same range as what is absorbed from the soil or higher. The rather high concentrations are required to allow determination of the much smaller residue on the sampler after exposure to the soils or sediments.

Spiking of rubber sheet passive samplers can be performed from a mixture of 20% water in methanol containing the selected PRCs (Booij et al. 2002). Samplers are equilibrated by gently shaking overnight and will all absorb an amount of PRCs in accordance with their weight. This method will also apply to SPME. Spiked samplers not exposed to sediment are analysed to determine the spiked concentrations.

Bottles coated with a thin film of silicone rubber are spiked by dripping a spike solution in hexane[8] at different places on the coating. The solution will be immediately sorbed by the rubber.

Spiked samplers can be stored in diffusion tight jars (e.g., glass with lids lined with aluminium foil) and preferably in the freezer (at least six months).

Presently, no quality assurance is applied to monitor the extraction process for depletive methods like Tenax or cyclodextrin. This is however possible. Adding Performance Reference Compounds to the soil or sediment mixture before the extraction process creates a pool of contaminants that are supposed to be rapidly releasable. Provided the time between adding the PRCs and the start of the extraction does not exceed the prescribed extraction period, it is expected that the PRCs should be fully recovered. This could however be a point of research.

In section 4.6, quality assurance is discussed in more detail.

4.2.4 Exposure

Equilibrium times for a certain compound vary depending on K_{pw} and sampler thickness. Times required to obtain equilibrium increase with hydrophobicity. A very rough rule for the required equilibrium time is given by formula 1 in Appendix 1.

When using strong sorbents like tenax of cyclodextrin, equilibrium between the sorbent and pore water concentrations is not required. But the extraction time needs to be chosen in such a way as to assure that the rapidly desorbing fraction is extracted completely but the slowly desorbing fraction is not.

For routine application it is important to have consensus on the exposure time. Therefore, in user protocols of this report a fixed time is used. After exposure, samplers and sorbents are recovered and cleaned if necessary in the shortest possible time. Preferably, the samples are analysed as soon as possible.

4.2.5 Extraction

SPME samplers are generally desorbed by heating but if extracted, the methods will not differ from other passive samplers.

Extraction of passive samplers or strong sorbents can be done through soaking in solvent, soxhlet extraction or accelerated solvent extraction (ASE). Prior to extraction, recovery standards should be added as quality assurance for the analytical procedure. *Note that during extraction compounds need to diffuse out of the sample/sorbent. This process is faster or slower, depending on the*

polymer type. Consequently, the extraction time should be in accordance with the internal diffusion process. This implies that the extraction time for a recovery check by applying an additional extraction should be tenfold the time period used in the first analytical extraction or perhaps even equal to the exposure period.

Solvents used for extraction vary from methanol to hexane.[8] For silicone rubber, methanol or acetonitril is recommended, as these solvents are strong enough to extract most compounds and they do not dissolve residual oligomers that possibly may be present in the polymer.

4.2.6 Clean-up

Extracts from passive samplers generally contain less matrix then soil or sediment extracts. Cleanup is required if the background of co-eluted compounds disturbs the instrumental analyses. SPME and POM are generally not subjected to cleanup. In extracts of silicone rubber, the removal of oligomers is necessary.

Cleanup methods similar to those applied for sediment or water extracts for the compounds of interest can also be applied to extracts of passive samplers. These methods also remove other highly hydrophobic materials like lipids or grease. This includes the removal of sulphur that is also partly extracted from sediments by passive samplings.

Cleanup if Tenax and cyclodextrin is not required.

4.2.7 Data calculation and quality checks

This section does not apply to Tenax and cyclodextrin extractions.

Depletion factors (DF) can be used to determine if an extraction was complete or not. The depletion factors indicated by the PRCs are calculated from the distribution of the PRCs between sampler and sediment. Formula 3 of Appendix 1 presents the required formula.

The values for DF should be less than 0.1 to give maximum assurance that no depletion has occurred for the compound groups represented by the PRCs.

For all cases where DF<0.1, the freely dissolved concentration in pore water, (C_w) can be calculated formula 4 of Appendix 1.

If DF is above 0.1 the extraction has depleted the system, which can be due to:

- Too high ratio between masses of sampler and sediment;
- Equilibrium was not attained.

If the K_{oc} of the organic carbon is much lower than the capacity assumed there is an overestimation. The sampler size should be decreased or the sampler should be exposed to more sediment. Insufficient equilibrium is indicated by the DF increasing with hydrophobicity. Low hydrophobic compounds exchange faster and DF can be well below 0.1 in a short exposure time. Of course, it is also possible that no equilibrium is attained as well as a too high sampler sediment ratio has been selected. How quality assurance can be implemented in the measuring of bioavailable concentrations will be described in more detail in section 4.3.

In the following sections, protocols based on the draft of ISO standards are described. Although methods are already used in practice there is still a scientific debate on applicability. The next section gives the conditions for applications of the procedures especially on equilibration time and prevention of depletion by using the proper amount of sampler and sample.

4.3 User protocol for passive sampling

Passive sampling is used as a method to measure concentrations that are actually bioavailable to organisms. Passive sampling only extracts a negligible fraction from the rapidly dissociable fraction of contaminants from the soil matter.

Water is added to the soil sample with particle size < 2 mm. A passive sampler is added to this system and equilibrated during 28 days. After the equilibration period, the amount absorbed into the passive sampler is measured. Using the partition coefficient between the sampler and water, the freely dissolved concentration can be calculated.

4.3.1 Reagents

Reagents used shall be of analytical or higher grade. The blank value of the reagents (including water) shall be negligible (<10%) compared to the lowest concentration of organic contaminants to be determined. The following reagents are required:

- Millipore or Nanopure Water;
- Solvent for extracting the passive sampler (acetonitril, cyclohexane, iso-octane, methanol – depending on sampler and analytical technique);
- Sodium azide;
- Calcium chloride.

4.3.2 Apparatus

The following equipment shall be used. All materials that come into contact with the sample (soil or reagents) should not cause contamination by compounds to be determined or adsorb the components of interest.

- Sieves, having a mesh size of 2 mm (for air-dried samples and of 5 mm to 8 mm for freshly collected samples);
- Balance, accuracy 0.01 g;
- Equilibration systems: either glass bottles of about 50 (or more) ml;
- Shaking machine, horizontal (1 or 2-dimensional) movement shaker, capable of 100-180 movements/min^{-1} (for POM-SPE and silicone rubber); or Rock and Roll device (for SPME use).

4.3.3 Procedure

Sample preparation

Fresh samples should be used. Depending on their cohesion, freshly collected samples should be forced by hand through a 2 mm sieve using a large spatula. For samples of mineral soils, particles exceeding about 2 mm in diameter can be picked out by hand. If homogenisation is inadequate, larger sample masses may be extracted but the ratio of freshly collected soil to extraction solution should be kept constant in order to obtain reproducible results.

Note: the use of too large mechanical forces during pre treatment and time-consuming pre treatment procedures may increase bioavailability or stimulate biodegradation.

If samples have to be stored, store them in such a way that processes that have an effect on the bioavailability (biodegradation, change in organic matter composition) are prevented. Cooling is often appropriate.

Determining water content

Determine the water content as specified in ISO 11465

Extraction

SPME

The soil sample with particle size < 2 mm is mixed with water and the system is extracted with the passive sampler. In case of SPME, weigh 10 g of soil material and place this in a glass bottle of about 50 ml or 100 ml. Add 25 ml of Millipore water (filling the vial but leaving some headspace needed for turbulence), containing 0.01 M $CaCl_2$ and 25 mg NaN_3 to prevent biodegradation of the target compounds. Then add pre-cleaned (washed 3x with solvent and air-dried or in case of a polar solvent also 3x with water to remove solvent) SPME fibres (length depending on a priori estimated concentrations or by using the rule of thumb as specified in Appendix 1 formula 4). Place the vials on a Rock and Roll device for 28 days in the dark, at a temperature of 20 ± 2 °C. After 28 days, remove the SPME fibres, clean them with a wet (Millipore water) tissue, cut them, and place in an autosampler vial containing the solvent of choice (volume depending on estimated concentrations). Vortex the vials for 30 sec.

POM-SPE

In case of POM-SPE, weigh about 10 g of soil material and place this in a glass bottle of 50 or 100 ml. Add Millipore water, containing 0.01 M $CaCl_2$ and 25 mg NaN_3 but leave a couple of ml of headspace to ensure sufficient turbulence during shaking. Then add a solvent-extracted and pre-weighed POM strip (mass depending on a priori estimated concentrations or by using the rule of thumb as specified in Appendix 1). Place the bottles on a horizontal shaker operating at 150 rpm for 28 days in the dark, at a temperature of 20 ± 2 °C. After 28 days, remove the POM strips, clean them with a wet (Millipore) tissue, cut them and place them either (depending on the mass and type of POM) in an autosampler vial containing the polar solvent of choice (volume depending on estimated concentrations) or in a Soxhlet extractor. In the latter case, extract with the solvent of choice (methanol or hexane[8]/acetone – depending on the chemicals of interest) during at least 3 hours. Then concentrate the extract, clean-up if necessary and transfer the extract to autosampler vials.

Silicone Rubber

In case of silicone rubber, weigh about 10 g of soil material and place this in a glass bottle of 50 or 100 ml. Add Millipore water, containing 0.01 M $CaCl_2$ and 25 mg NaN_3 but leave a couple of ml of headspace to ensure sufficient turbulence during shaking. Then add a solvent-extracted and pre-weighed strip of silicone rubber (mass depending on a priori estimated concentrations or by using the rule of thumb as specified in Appendix 1). Place the bottles on a orbital shaker operating at speeds of 100-150 rpm with an amplitude of 2-3 cm for 28 days in the dark, at a temperature of 20 ± 2 °C. After 28 days, remove the rubber strips, clean them with a wet (Millipore) tissue. For extraction of silicone rubber methanol or acetonitril is recommended as these solvents are strong enough for most contaminants and they do not dissolve residual oligomers that possibly may be present in the polymer. Then concentrate and clean-up the extract similarly to those methods that are applied for sediment or water extracts and transfer the extract to autosampler vials.

4.3.4 Measurement

Measure the content of organic contaminants in the obtained extract according to the appropriate international standard.

4.3.5 *Blank test*

Perform a blank determination following sections for the applied procedure using the same amount of reagents that are used in a normal procedure. Analyse the blank immediately prior to analysis of the samples to demonstrate sufficient freedom from contamination (50% of the lowest reported value).

4.3.6 *Calculation*

After measuring the concentrations of contaminants in the passive samplers we need to convert these concentrations into freely dissolved pore water concentrations before further assessment can take place. The conversion of concentrations in the sampler to concentrations in the pore water can be done with help of formula 1:

$$C_{Dissolved\_pore\_water} = C_{sampler} / K_{sampler} \qquad \text{Formula [1]}$$

in which:

$C_{Dissolved\_pore\_water}$ = Freely dissolved concentration of the contaminant in the pore water ($mg.l^{-1}$);

$C_{sampler}$ = Concentration of the contaminant in the sampler (either SPME, POM-SPE and Silicone rubber) at equilibrium (in either $mg.l^{-1}$ PDMS coating; or $mg.kg^{-1}$ POM);

$K_{Sampler}$ = Sampler-to-water partition coefficient ($l.kg^{-1}$).

With:

$C_{sampler}$ being calculated by dividing the mass of compound in the passive sampler (= concentration in extract ($\mu g.l^{-1}$) x volume of extract (l)) by the mass (POM or silicone rubber; kg) or volume (SPME; l) of the sampler.

The partition coefficient ($K_{sampler}$) can either be found in the literature or experimentally determined. Every type of sampler (including different samplers of the same material) has a different partition coefficient. Because partition coefficients are sampler and compound-dependent, laboratories have to experimentally determine partition coefficients themselves for each new batch of sampler.

The $K_{sampler}$ can be determined in several ways; however only the most frequently-used method will briefly be described in this section.

An aqueous solution containing sodium azide (as biocide; e.g., at 50 $mg.l^{-1}$) and e.g., calcium chloride (at e.g., 0.01 M; to represent a natural ionic strength) in Millipore or nanopure water is brought into a glass bottle (e.g., 100 or 250 ml). Pre-cleaned SPME fibres, POM- or silicone rubber strips are added to the water and the system is spiked with the contaminants of interest, dissolved in a water-miscible solvent (e.g., acetone or methanol). Preferably, spiking should occur at different concentration levels of contaminants. The system is closed (preferably with a glass stopper or aluminium-coated cap) and shaken for several weeks (depending on the hydrophobicity of the compounds). Upon equilibration, the sampler is removed and either directly transferred to an autosampler vial containing a suitable solvent (in case of SPME fibres or small POM strips) or extracted in a Soxhlet apparatus (or similar), after which the extract is concentrated and transferred to an autosampler vial (in case of large POM or rubber strips). The aqueous concentrations are determined by extracting a water sample three times with, e.g., hexane[8]. After reducing the extract to a smaller volume and possibly solvent-exchange, it can be analysed. The partition coefficient can then be determined according to formula 2:

$$K_{Sampler} = C_{sampler} / C_{aq}$$ Formula [2]

in which:

$K_{sampler}$ = Sampler-to-water partition coefficient ($l.kg^{-1}$);
$C_{sampler}$ = Freely dissolved concentration of the spiking contaminant in the sampler ($mg.l^{-1}$ or $mg.kg^{-1}$);
C_{aq} = The concentration of the spiking contaminant determined in the aqueous solution ($mg.l^{-1}$).

4.4 User protocol Tenax extractions

The soil sample with particle size < 2 mm is extracted with water, containing Tenax beads that act as a 'receiver phase' for organic contaminants. Following extraction, the contaminants adsorbed to Tenax are determined by appropriate analytical methods.

4.4.1 Reagents

Reagents used shall be of analytical or higher grade. The blank value of the reagents (including water) shall be negligible (<10%) compared to the lowest concentration of organic contaminants to be determined. The following reagents are required:

- Millipore or Nanopure Water;
- Tenax TA, 60-80 mesh;
- n-hexane[8];
- Sodium azide or mercury chloride;
- Calcium chloride;
- (Ethanol).

4.4.2 Apparatus

The following equipment shall be used. All materials that come in contact with the sample (soil or reagents) should not cause contamination by compounds to be determined or adsorb the components of interest.

- Sieves, having a mesh size of 2 mm (for air-dried samples and of 5 mm to 8 mm for freshly collected samples);
- Balance, accuracy 0.01 g;
- Separation funnels, 1000 ml;
- Shaking machine, horizontal (1 or 2-dimensional) movement shaker, capable of 100-180 movements/min;
- Kuderna-Danish evaporation equipment or rotavapor.

4.4.3 Procedure

<u>Sample preparation</u>

Fresh samples should be used. Depending on their cohesion, freshly collected samples should be forced by hand through a 2 mm sieve using a large spatula. For samples of mineral soils, particles exceeding about 2 mm in diameter can be picked out by hand. If homogenisation is inadequate, larger sample masses may be extracted but the ratio of freshly collected soil to extraction solution should be kept constant in order to obtain reproducible results.

Note: the use of too large mechanical forces during pre treatment and time-consuming pre treatment procedures may increase bioavailability or stimulate biodegradation.

Note 2: Particle size should not exceed the diameter of the hole in the tap of the separation funnel to be used. If it does, the soil suspension cannot be separated from the Tenax later on.

If samples have to be stored, store them in such a way that processes that have an effect on bioavailability (biodegradation, change in organic matter composition) are prevented. Cooling is often appropriate.

Determining water content

Determine the water content as specified in ISO 11465

Extraction

The soil sample with particle size < 2 mm is extracted with water including Tenax. Shake for 20 hours at a temperature of 20 ± 2 °C in the dark. Mercury chloride or sodium azide should be added to prevent biological degradation of the target compounds. Appropriate glassware and plastic-ware is used in order to make phase separation possible.

Weigh 10 g (dry weight) of soil material and place in a 1000 ml separation funnel. Add 450 ml of Millipore or Nanopure water containing 0.01 M calcium chloride and 50 $mg.l^{-1}$ $HgCl_2$ or NaN_3 to prevent biodegradation of the target compounds. Add 10 g Tenax and close the funnel. Place the separation funnel on a horizontal shaker at 180 rpm for 20 h in the dark.

Phase separation

Remove the separation funnel from the shaker after 20 hours of shaking. Before phase separation, shake the funnel by hand, then immediately remove the stopper and open the tap after a few seconds, while holding the funnel over a beaker. Tenax beads are hydrophobic and will float on water. They will be attached to the wall during phase separation. The soil suspension will leave the funnel but the Tenax will thus remain inside. After separation, add about 100 ml of Millipore water, shake the funnel (with stopper) and drain the water. Repeat this procedure one more time. In this way, the system is cleaned as much as possible from remaining soil particles sticking to the Tenax or glass wall of the funnel.

Extraction from the strong adsorbent

Extract the Tenax in the separation funnel using 100 ml of hexane[8]. Shake during at least one minute and remove the hexane from the funnel. Concentrate with nitrogen evaporation and transfer to autosampler vial.

Alternatively, the Tenax can be extracted with ethanol first (50 ml) and then with hexane[8] (100 ml). This mixture should be concentrated with a Kuderna-Danish or rotavapor apparatus, followed by any solvent-exchange and transfer to an autosampler vial.

4.4.4 Measurement

Measure the content of organic contaminants in the obtained extract according to the appropriate international standard.

4.4.5 Blank test

Perform a blank determination following sections for the applied procedure (extraction, phase separation and extraction of the strong adsorbent), using the same amount of reagents that are used for the normal procedure. Analyse the blank immediately prior to analysis of the samples, to demonstrate sufficient freedom from contamination (50% of the lowest reported value).

4.4.6 Calculation

The percentage of the total, solvent-extractable concentration of contaminants in the soil that is potentially bioavailable can be calculated according to:

$((E_f Q_{ex}/M_s) / C_{s\text{-}tot}) * 100\%$ Formula [3]

in which:

E_f = extrapolation factor of contaminant (-);

Q_{ex} = mass of compound measured in the Tenax extract, calculated by: concentration measured in Tenax extract (C_{ex}; $\mu g.l^{-1}$) × volume of the extract (V_{ex}; l);

M_s = mass of soil extracted in the separation funnel (g);

$C_{s\text{-}tot}$ = total, solvent-extractable concentration of contaminants in the soil ($mg.kg^{-1}$).

In Appendix 2 some generic extrapolation factors can be found for a 20-hour Tenax extraction.

In case no total concentration is available but the residue after Tenax extraction has been determined, this concentration can be obtained by summing the masses of compound extracted from the residue and the Tenax and dividing the summed mass by the intake weight of the soil sample.

Note: to be able to express the results on a dry weight basis, obviously the water content of the soil should be taken into account.

4.5 User protocol cyclodextrin extractions

The soil sample with particle size < 2 mm is extracted with water, containing cyclodextrin that acts as a 'receiver phase' for organic contaminants. Following extraction, the contaminants captured by the cyclodextrin are determined by appropriate analytical methods.

4.5.1 Reagents

Reagents used shall be of analytical or higher grade. The blank value of the reagents (including water) shall be negligible (<10%) compared to the lowest concentration of organic contaminants to be determined. The following reagents are required:

- Millipore or Nanopure Water;
- Hydroxypropyl-β-cyclodextrin;
- n-Hexane[8];
- Sodium azide or mercury chloride;
- Calcium chloride.

4.5.2 Apparatus

All materials that come in contact with the sample (soil or reagents) should not cause contamination by compounds to be determined or adsorb the components of interest. The following equipment shall be used.

- Sieves, having a mesh size of 2 mm (for air-dried samples and of 5 mm to 8 mm for freshly collected samples);
- Balance, accuracy 0.01 g;
- Centrifugation tubes (100 ml) made of glass;
- Centrifuge, capable of centrifuging the centrifuge tubes/vials;
- Shaking machine, horizontal (1 or 2-dimensional) movement shaker, capable of 100-180 movements/min.

4.5.3 Procedure

Sample preparation

Fresh samples should be used. Depending on their cohesion, freshly collected samples should be forced by hand through a 2 mm sieve using a large spatula. For samples of mineral soils, particles exceeding about 2 mm in diameter can be picked out by hand. If homogenisation is inadequate, larger sample masses may be extracted but the ratio of freshly collected soil to extraction solution should be kept constant in order to obtain reproducible results.

Note: Use of too large mechanical forces during pre treatment and time-consuming pre treatment procedures may increase bioavailability or stimulate biodegradation.

If samples have to be stored, store them in such a way that processes that have an effect on the bioavailability (biodegradation, change in organic matter composition) are prevented. Cooling is often appropriate.

Determining water content

Determine the water content as specified in ISO 11465.

Extraction

The soil sample with particle size < 2 mm is extracted with water containing cyclodextrin. Shake for 20 hours at a temperature of 20 ± 2 °C in the dark. Mercury chloride or sodium azide should be added to prevent biological degradation of the target compounds. Appropriate glassware is used in order to make phase separation possible.

Weight 10 g of soil material and place this in a centrifuge tube or vial. Add exactly 100 ml of Millipore or Nanopure water containing 0.01 M calcium chloride, 50 $mg.l^{-1}$ $HgCl_2$ or NaN_3 (to prevent biodegradation of the target compounds), and 100 mM of hydroxypropyl-β-cyclodextrin. Place the tubes or vials on a horizontal shaker at 150 rpm for 20 h in the dark.

Phase separation

Remove the tubes/vials from the shaker after 24 h of shaking. Centrifuge the tubes/vials to obtain phase separation (clear supernatant) during 15 minutes at least 3000 rpm. Pipette off exactly 10 ml of the supernatant solution for further analysis in a clean 20 ml vial. In case the residue needs analysis, discard the rest of the water phase by pipetting off and rinsing three times with Millipore water (shake and centrifuge in between). Store the residual extraction pellet in a freezer.

Note: depending on the soil it may be necessary to use a centrifuge with high g-value and longer time.

Extraction from the strong adsorbent

Extract the 10 ml aliquot of the aqueous cyclodextrin phase twice with 3-5 ml of hexane[8] by shaking on a shaker for 30 min and pipetting off the extracts in a clean tube. Concentrate the combined hexane extract to the desired end volume under a gentle stream of nitrogen and change to another solvent if needed.

4.5.4 Measurement

Measure the content of organic contaminants in the obtained extract according to the appropriate international standard.

4.5.5 Blank test

Perform a blank determination following sections for the applied procedure (extraction, phase separation and extraction of the strong adsorbent), using the same amount of reagents that are used for normal procedures. Analyse the blank immediately prior to analysis of the samples to demonstrate sufficient freedom from contamination (50% of the lowest reported value).

4.5.6 Calculation

The percentage of the total, solvent-extractable concentration of contaminants in the soil that is potentially bioavailable can be calculated according to:

$(1.5*(Q_{ex}/M_s) / C_{s\text{-}tot}) * 100\%$ Formula [4]

in which:

Q_{ex} = Mass of compound measured in the cyclodextrin extract, calculated by: concentration measured in cyclodextrin extract (C_{ex}; $\mu g.l^{-1}$) × volume of the extract (V_{ex}; l);

M_s = mass of soil extracted in the centrifugation tube/vial (g);

$C_{s\text{-}tot}$ = total, solvent-extractable concentration of contaminants in the soil ($mg.kg^{-1}$).

In case no total concentration is available but the residue after cyclodextrin extraction has been determined, this concentration can be obtained by summing the masses of compound extracted from the residue and the cyclodextrin solution (the latter multiplied by 1.5) and dividing the summed mass by the intake weight of the soil sample.

Note: to be able to express the results on a dry weight basis, obviously the water content of the soil should be taken into account.

4.6 Quality Assurance

In analytical procedures analysts include different quality assurance measures to verify the correctness of procedures and possible effects of matrix on the analytical process. In passive sampling of surface waters the effect of different flow regimes is monitored and corrected from the measured release of compounds dosed prior to exposure. Similar procedures can be applied in passive sampling of soils and sediments that aim for determination of concentrations of compounds in pore water. For depletive extractions to determine the fast releasable portion such quality assurance procedures can be included.

4.6.1 Quality assurance for equilibrium passive sampling

Pre-assessment of depletion

When equilibrating a passive sampler with soil compounds that will be extracted from the soil, it will consequently reduce the concentration in the soil. Therefore the estimation of the true freely dissolved concentration (C_w) of contaminants requires that the amount depleted from the soil to obtain equilibrium with the passive sampler should be minimal. This is because every reduction of the concentration in the soil (C_s) will also result in a decrease in the concentration in the aqueous phase. It should be noted that C_w is not necessarily proportional to the total concentration in the soil. A 10% decrease in total sediment concentration could result in a 90% decrease in C_w, depending on the proportion of the target compound that is available for exchange with the water phase.

Consequently a non-depletive situation should be verified by evidence that the concentration in the pore water is little affected instead of the concentration in soil or sediment.

The capacity of the soil for a certain compound can be expressed in the water volume it represents. For soil that is the mass of soil (m_s) multiplied by the soil-water distribution coefficient (K_d) and for a passive sampler this is the mass of the sampler (m_p) multiplied by the sampler-water partition coefficient (K_{pw}). The concentration in pore water is mainly related to the contaminant portion bound to amorphous organic matter (AOC). For this AOC, compounds have partition coefficients (K_{AOC}) related to K_{ow} (Noort 2009a and 2009b). Assuming that a greater portion of organic carbon is AOC, a first rule of thumb for a phase ratio can be given by:

$$capacity\,ratio = \frac{m_p K_{pw}}{m_s f_{aoc} K_{aoc}} \sim \frac{m_p K_{pw}}{m_s f_{oc} K_{ow}} \qquad \text{Formula [5]}$$

in which:
m_p = the amount of sampler (kg);
K_{pw} = sampler-water partition coefficient (l. kg^{-1});
m_s = the amount of soil (kg);
f_{aoc} = the fraction amorphous organic matter in soil (-);
K_{aoc} = amorphous organic carbon – water partition coefficient (l. kg^{-1});
f_{oc} = the fraction organic carbon in soil (-);
K_{ow} = octanol-water partition coefficient (-).

To prevent depleting the soil during equilibration, the numerator should be insignificant compared to the denominator. When the sampler-water partition coefficient is in the range of the $K_{ow,}$ depletion will be negligible if:

$$m_s f_{oc} >> m_p \qquad \text{Formula [6]}$$

in which:
m_s = the amount of soil (kg);
F_{oc} = the fraction organic carbon in soil (-);
m_p = the amount of sampler (kg).

Which essentially means that the mass of the passive sampler should be small compared to the amount of organic carbon in the soil sample intake. This means that allowing a depletion of maximally 10%, the sampler size should not exceed 10 mg in an equilibration of 3 g soil intake and an OC content of 3%.

The above estimation is based on rough assumptions that $K_{pw} \sim K_{ow} \sim K_{oc,}$ which in practice may deviate up to an order of magnitude and deviate differently for various compounds.

Monitoring depletion by passive samplers

As for passive sampling in the water compartment (Smedes 2007) Performance Reference Compounds (PRCs) can be applied in passive sampling of soils (Booij et al. 2003). PRCs are compounds not present in the environment, like deuterated PAHs or PCBs that do not occur in technical mixtures.[9] These PRCs are spiked to passive samplers prior to equilibration with sediment and during

[9] Candidates for PRCs among PCBs are: PCB 4, PCB 10, PCB 14, PCB 21, PCB 26, PCB 30, PCB 50, PCB 55, PCB 78, PCB 104, PCB 112, PCB 145, PCB 155 and PCB 204.

equilibration the PRCs desorb from the sampler and sorb to the soil following a process similar to the uptake of target compounds. Provided that equilibrium is obtained, the distribution of PRCs between the reference phase and the soil phase indicates the capacity ratio of those two phases following Formula [5]. As mentioned in section 4.2.2, depletion can be expressed by the depletion factor (DF) (see also formula 2 of Appendix 1). That is the ratio of the amount of PRCs that remains on the passive sampler and that sorbed by the soil. When DF is 0.1 or less, the true freely dissolved concentration will not be substantially affected by depletion. Situations can occur in which this criterion for DF is met for one compound but not for another as a result of difference in the K_{pw}-K_{oc} ratio. E.g., for PAHs the K_{oc} is often higher than the K_{ow} and using silicone rubber the sampler-water partition coefficient (K_{pw}) is lower than the K_{ow} while for PCBs the K_{pw} is equal to or higher than the K_{ow}. High depletion factor values mean that there is little sorption capacity in the soil compared to the sampler and probably indicates that the sampler material has depleted the soil. The calculated value for the freely dissolved concentration (C_w) will then be an underestimation.

When a set of PRCs covering a larger hydrophobicity range is added to the sampler the depletion factors of those compounds can also give a clear indication of whether equilibrium has been attained because in equilibrium PRCs having large differences in hydrophobicity, similar values for DF are expected. In other words, the depletion factor for PRCs such as PCB10 and PCB 204 should not be greatly different. However, hydrophobic compounds require longer equilibrium times and DF values increasing with hydrophobicity indicate incomplete equilibrium and consequently, the calculated freely dissolved concentration (C_w) for the target compounds will be underestimated.

Spiking of passive samplers

Spiking of passive samples like SPME, POM-SPE and silicone rubber can be performed by exposing multiple samplers to the same solution, as suggested by Booij et al. 2002. Equilibrium time is proportional to the sampler-water partition coefficient. When equilibration takes place in appropriate methanol-water mixtures, the sampler-water partition coefficient of the analytes is sufficiently low such that equilibrium is attained in a short time.

The equilibrium time is however also related to the internal diffusion in the polymer. For silicone rubber it has been demonstrated that internal diffusion is sufficiently fast (Rusina et al. 2007 and Rusina et al. 2010) to obtain equilibrium overnight or even in several hours (Booij et al. 2002). Methods used for silicone rubber can also be applied to PDMS coated SPME. To apply the process quality assurance to POM equilibrations, an adequate procedure for spiking should be developed.

4.6.2 Quality assurance for depletive extraction methods

Depletive extraction methods using Tenax or cyclodextrin estimate the rapidly desorbing fraction of contaminants bound to organic matter in the soil.

Presently, no quality assurance is applied to monitor the extraction process for such depletive methods, which is however quite possible. Adding Performance Reference Compounds to the soil or sediment mixture before the extraction process creates a pool of contaminants that are supposed to be rapidly releasable. Provided the time between adding the PRCs and the start of the extraction does not exceed the prescribed extraction period, it is expected that the PRCs should be fully recovered. Of course, it can be argued that the addition should be aged more or less for the recovery to be a realistic measure of the efficiency of the extraction. Nevertheless, compared to the present

absence of quality assurance, measuring the recovery of freshly added compounds as a minimum requirement is already a step forward.

For the 6-hour extraction using Tenax, extraction of the releasable fraction was considered not complete and a correction factor of 2 was used to calculate the total releasable or fast fraction (Cornelissen et al. 2001).

Van Noort (2009c) listed correction factors that were increasing with hydrophobicity. If the added PRCs reflect the fast fraction, the recovered PRCs may be in accordance with this factor, i.e., the reciprocal fraction recovered should be related to the correction factor. The correction factor lower than 1 implies that part of the slower fractions are also extracted. Therefore, a criterion of 100% recovery for PRCs can be set for a 24-hour Tenax extraction, similarly to the cyclodextrin extraction for which no correction factors are used.

Several PRCs of different natures comprising a large hydrophobic range should be used, since rates of extraction may be related to compound properties.

5 Conclusion and outlook

5.1 Introduction

This chapter presents generic conclusions on the implementation of bioavailability in the regulatory framework. Furthermore, it presents a short outlook on future developments for bioavailability of both organic contaminants and metals.

5.2 Organic contaminants

5.2.1 Implementation of bioavailability in a regulatory framework

This report aims to describe practical recommendations for a possible implementation of bioavailability in the higher tier of the regulatory framework. It not only presents methods that can be used to measure bioavailability, but also shows a way to relate measured concentrations to the existing regulatory framework for contaminated soils and existing quality standards for soil and water. Two different proposals are distinguished, corresponding to the selected analytical methods that can be used to measure bioavailability.

The pore water concentrations measured by passive samplers can be related to aquatic toxicity standards to determine possible effects. The concentrations as measured by extractions with Tenax and cyclodextrin can be related to soil toxicity standards. By presenting both methods the report acts as scientific underpinning of quality standards for contaminated soils.

The bioavailable concentrations can be used in decision-making on remediation urgency of a contaminated site or for decision-making on sustainable land management. The aim of this report is not to use bioavailability in the precautionary framework to prevent soil contamination.

Furthermore, aspects like combination toxicology are not addressed with bioavailability and still require a separate approach similar to the current practice (assessing the risk based on adding up individual risk indices of compounds).

5.2.2 Policy decisions

This report presents a technical approach on how bioavailability can be implemented into the regulatory framework. It does not address some policy questions that require further attention to be able to decide on implementation of bioavailability. The following questions still require attention:

- What is the aim of implementation of bioavailability in a regulatory framework?
    - Implementation within determining the remediation urgency?
    - Local use in sustainable land management?
- Is the implementation of bioavailability as presented in this report possible or are there (non-scientific) issues that hamper its use?
- What are the consequences of the implementation of bioavailability?
    - For future risk assessments and the amount of urgent sites?
    - For past risk assessments of contaminated sites?
- Is additional information needed to come to a decision on the implementation of bioavailability in the current framework?

5.2.3 Follow up

The Ministry of Infrastructure and Environment has to decide about a follow-up on the points mentioned in the previous section. This requires some scientific underpinning. Therefore, the following actions will have to be carried out:

1. Look at the consequence of the required ecological protection limit. Distinction has to be made between the framework of soil remediation and sustainable land management;
2. Determine whether to use potentially bioavailable concentrations or actually bioavailable concentrations (or a combination of both) in risk assessment. This could be different between the framework of soil remediation and sustainable land management.

1. Choosing a risk limit

A crucial point within risk assessment is the selected risk limit. It can be re-evaluated for each policy context at which level an ecosystem is sufficiently protected, without having too strict limitations on soil (re)use?

In chapter 3 it was already stated that existing toxicity data can be used to assess the measured bioavailable concentrations. To support a policy decision on this, a consequence analysis is needed to determine if the current risk limits fits the protection level of the current regulatory framework. It is a policy decision what level of effect is accepted. When choosing a risk limit it is important to consider the differences between the soil remediation framework and policy decisions on sustainable land management.

2. Using potentially or actually bioavailable concentrations

In section 3.3 the policy discussion on whether to use potentially bioavailable or actually bioavailable concentrations is mentioned. As a follow up of this report, it will be determined what generic choice can be made. Therefore, more has to be known about the relation between effects on the ecosystem and the actually or potentially bioavailable concentrations. Actual concentrations represent current risks, whilst potential concentration also addresses potential risks in the future. Considering the differences between the framework of soil remediation and sustainable land management, a proposal is needed.

5.2.4 Future developments with respect to analytical methods

With this report, the implementation of bioavailability into the current legislative framework of contaminated soils is examined. Further improvement of the analytical methods can optimise measuring and implementation of bioavailability. We therefore like to emphasise that the search for an optimal risk assessment based on bioavailable concentrations does not end with this advice. We encourage further work on this matter.

This implies translation of the present knowledge to an internationally accepted (ISO) standard and proper guidelines to use the results of the measurements. In particular, we ask attention for the development of a corresponding regulatory framework along with new methods.

In this respect, international developments on the implementation of bioavailability for soil contaminants can be of value. It can provide unity and a general acceptance of evaluating the risk of contaminated soils. Particularly the progress of the ISO working group on bioavailability should be monitored regularly.

5.3 Metals

This report has focused on organic contaminants. It is desired to do the same for metal contaminants. The report of Brand et al. (2009) provides a good starting point in this respect.

The bioavailability of metals in soil is more complex than for organic chemicals, because measured bioavailable concentrations are not directly related with toxicity data for (water) standards. Models for quantifying the extent of toxic effects still needs to be developed. The possibilities to implement bioavailability for metal contaminants need to be examined. However, for unity in implementation, the outcome of heavy metal assessments needs to be a similar principle as that used for organic contaminants. This research will be part of a follow-up project.

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Appendix 1 Formula

Exposure

$$t_{eq} = 10^4 K_{pw} d$$ Formula [1]

in which:
t_{eq} = required equilibrium time (h);
10^4 = empirical constant (l. kg^{-1} $m.h^{-1}$);
K_{pw} = passive sampler-water partition coefficient (l. kg^{-1});
d = film thickness of the sampler (mm).
Note 1: that if the sampler is exposed from both sides d equals half the thickness.
Note 2: this rule of thumb only applies if the uptake by the sampler is not limited by the diffusion inside the sampler.

Depletion

As rule of thumb, depletion is lower than 10% when the sampler-sediment phase ratios meet:

$$\frac{m_p}{m_s f_{oc}} < 0.1 \frac{K_{ow}}{K_{pw}}$$ Formula [2]

Where m_p and m_s are masses of the sampler and the amount of sediment, f_{oc}. the fraction organic carbon in the sediment and K_{ow} and K_{pw} respectively, the octanol-water and the passive sampler-water partition coefficient. Instead of the K_{ow} the generic K_{oc} can also be used. In case $K_{ow} \approx K_{oc} \approx K_{pw}$ the formula above shows that for a soil or sediment with 2% organic carbon about 500 g or more sediment or soil is required per 1 g sampler to prevent depletion. It is worth noting that in such phase ratios the mass transfer is likely not limited by desorption from sediment and is determined by the sampler uptake process.

The depleting factor can be determined by:

$$DF = \frac{N_p}{N_0 - N_p}$$ Formula [3]

in which:
DF = depletion factors (-);
N_p = amount of PRC recovered from the sampler after exposure (ng);
N_0 = amount of spike analysed in the reference samplers (ng).

The values for DF should be less than 0.1 to give maximum assurance that no depletion has occurred for the compound groups represented by the PRCs.

For all cases where DF<0.1 the freely dissolved concentration in pore water (C_w) can be calculated by:

$$C_w = \frac{N_p}{m_p K_{pw}}$$ Formula [4]

in which:
C_w = freely dissolved concentration in pore water (ng. l^{-1});
N_p = amount of PRC recovered from the sampler after exposure (ng);
m_p = masses of the sampler (kg);
K_{pw} = passive sampler-water partition coefficient (l. kg^{-1}).

Appendix 2 Extrapolation factors for 24-hour Tenax extraction

Table: Extrapolation factors for 24-hour Tenax extraction for different organic compounds taken with permission from Van Noort (2009a).

Compound	Extrapolation factor	Compound	Extrapolation factor
1,1,1-trichloroethane	0.72	DDE	0.73
1,1,2-trichloroethane	0.72	DDT	0.74
1,2,3,4-tetrachlorobenzene	0.72	delta-HCH	0.72
1,2,3,5-tetrachlorobenzene	0.72	deltamethrin	0.73
1,2,3-trichlorobenzene	0.72	demeton	0.72
1,2,4,5-tetrachlorobenzene	0.72	desmetryn	0.72
1,2,4-trichlorobenzene	0.72	diazinon	0.72
1,2-dichlorobenzene	0.72	dibenzo[a,h]anthracene	0.76
1,2-dichloroethane	0.72	dichlofluanid	0.72
1,3,5-trichlorobenzene	0.72	dichloromethane	0.72
1,3-dichlorobenzene	0.72	dichloroprop	0.72
1,3-dichloropropene	0.72	dichlorovos	0.72
1,4-dichlorobenzene	0.72	dieldrin	0.72
1-chloronaphthalene	0.72	diethyl phtalate	0.72
2,3,4,5-tetrachloroaniline	0.72	diethylhexyl phtalate	0.82
2,3,4,5-tetrachlorophenol	0.72	dihexyl phtalate	0.74
2,3,4,6-tetrachlorophenol	0.72	di-isobutyl phthalate	0.72
2,3,4-trichloroaniline	0.72	dimethoate	0.72
2,3,4-trichlorophenol	0.72	dimethyl phthalate	0.72
2,3,5,6-tetrachloroaniline	0.72	di-n-butyl phthalate	0.72
2,3,5,6-tetrachlorophenol	0.72	dinoseb	0.72
2,3,5-trichlorophenol	0.72	dinoterb	0.72
2,3,6-trichlorophenol	0.72	disulfoton	0.72
2,3-dichlorophenol	0.72	diuron	0.72
2,3-dimethylaniline	0.72	DNOC	0.72
2,4,5-T	0.72	endosulfan	0.72
2,4,5-trichloroaniline	0.72	endrin	0.72
2,4,5-trichlorophenol	0.72	ethoprophos	0.72
2,4,6-trichloroaniline	0.72	ethylbenzene	0.72
2,4,6-trichlorophenol	0.72	ETU	0.72
2,4-D	0.72	phenanthrene	0.72
2,4-dichloro-6-nitroaniline	0.72	fenitrothion	0.72
2,4-dichloroaniline	0.72	phenol	0.72
2,4-dichlorofenol	0.72	fenthion	0.72
2,4-dimethylaniline	0.72	Fluoranthene	0.72
2,4-dinitroaniline	0.72	fluorene	0.72
2,5-dichloroaniline	0.72	phoxim	0.72

2,5-dichlorophenol	0.72	heptachlor	0.72
2,6-dichloroaniline	0.72	heptachloroepoxide	0.72
2,6-dichlorophenol	0.72	heptenophos	0.72
2-chloro-4-methylaniline	0.72	hexachloro-1,3-butadiene	0.72
2-chloro-4-nitroaniline	0.72	Hexachlorobenzene	0.72
2-chloroaniline	0.72	Hexachloroethane	0.72
2-chlorophenol	0.72	hydroquinone	0.72
2- chloronaphthalene	0.72	indeno[1,2,3-c,d]pyrene	0.76
2-methylaniline	0.72	irgarol 1051	0.72
2-nitroaniline	0.72	isodrin	0.74
2-nitro-N-phenylaniline	0.72	isoproturon	0.72
2-xylene	0.72	lindane	0.72
3,4,5-trichlorophenol	0.72	linuron	0.72
3,4-dichloroaniline	0.72	malathion	0.72
3,4-dichlorophenol	0.72	m-cresol	0.72
3,4-dimethylaniline	0.72	mecoprop	0.72
3,5-dichloroaniline	0.72	metabenzthiazuron	0.72
3,5-dichlophenol	0.72	metamitron	0.72
3-chloro-4-methylaniline	0.72	metazachlor	0.72
3-chloroaniline	0.72	methomyl	0.72
3-chlorophenol	0.72	metobromuron	0.72
3-methylaniline	0.72	metolachlor	0.72
3-nitroaniline	0.72	mevinphos	0.72
3-xylene	0.72	monochlorobenzene	0.72
4-chloro-2-methylphenol	0.72	N,N,3-trimethylaniline	0.72
4-chloro-2-nitroaniline	0.72	N,N-dimethylaniline	0.72
4-chloro-3-methylphenol	0.72	naphthalene	0.72
4-chloroaniline	0.72	N-methylaniline	0.72
4-chlorophenol	0.72	nonylphenol	0.72
4-methylaniline	0.72	o-cresol	0.72
4-nitroaniline	0.72	octylphenol	0.72
4-nitro-N-phenylaniline	0.72	oxamyl	0.72
4-xylene	0.72	oxydemeton-methyl	0.72
acenaphthene	0.72	ethyl parathion	0.72
acenaphthylene	0.72	parathion-methyl	0.72
alachlor	0.72	PCB101	0.73
aldicarb	0.72	PCB118	0.73
aldrin	0.73	PCB138	0.74
Endosulfan sulphate	0.72	PCB153	0.74
alpha-HCH	0.72	PCB28	0.72
anilazine	0.72	PCB52	0.72
anthracene	0.72	p-cresol	0.72
atrazine	0.72	Pentabromodiphenyl ether	0.74
azinphos-ethyl	0.72	pentachloroaniline	0.72
azinphos-methyl	0.72	Pentachlorobenzene	0.72
benomyl	0.72	pentachloroethane	0.72
bentazon	0.72	pentachlorophenol	0.72

benzene	0.72	permethrin	0.73
benzo(a)anthracene	0.72	perylene	0.73
benzo(a)pyrene	0.73	pirimicarb	0.72
benzo(b)fluoranthene	0.73	propachlor	0.72
benzo(ghi)perylene	0.76	propoxur	0.72
benzo(k)fluoranthene	0.73	pyrazophos	0.72
benzo[b]fluorene	0.73	pyreen	0.72
beta-HCH	0.72	pyridine	0.72
bifenthrin	0.78	quintozene	0.72
butyl benzyl phthalate	0.72	resorcinol	0.72
captafol	0.72	simazine	0.72
captan	0.72	styrene	0.72
carbaryl	0.72	TCMTB	0.72
carbazole	0.72	telodrin	0.72
carbendazim	0.72	tetrachlorethene	0.72
carbofuran	0.72	tetrachlormethane	0.72
catechol	0.72	tetrahydrofuran	0.72
chlordane	0.72	tetrahydrothiophene	0.72
chlorfenvinphos	0.72	thiram	0.72
chlorpyriphos	0.72	tolclofos-methyl	0.72
chlorthalonil	0.72	toluene	0.72
chloridazon	0.72	triallate	0.72
chrysene	0.72	triazophos	0.72
coumaphos	0.72	Trichloroethene	0.72
cyanazin	0.72	trichlorophon	0.72
cyclohexanone	0.72	trichloromethane	0.72
cypermethrin	0.72	trifluralin	0.72
DDD	0.73	vinyl chloride	0.72

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