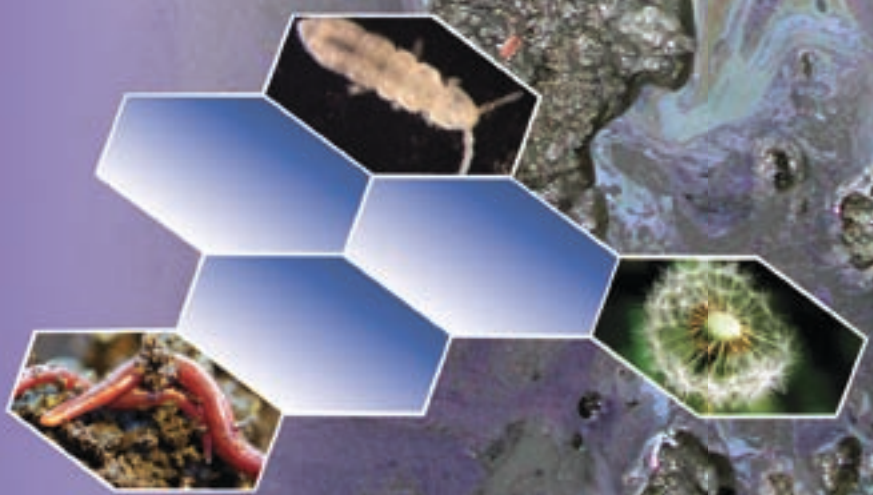
Ecological Risk Assessment of Contaminated Land

LIBERATION

Decision support for site specific investigations



John Jensen and Miranda Mesman

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Edited by
John Jensen and Miranda Mesman

ISBN 90-6960-138-9
978-90-6960-138-0
RIVM report number 711701047

Contents

Foreword

Ever since we entered the field of ecotoxicology and environmental chemistry (dating back to the last millennium) the topic of this book has been one of the hottest issues. It has been the subject of numerous research projects and always promotes lively dialogue amongst soil scientists, ecotoxicologists, regulators and local authorities. It seems on the one hand very obvious and simple, but at the same time difficult to simplify and predict in detail. Consequently it has therefore often been neglected and circumvented with possible economic and social consequences as a result. Although it has often been addressed under the generic term bioavailability it encompasses a wide range of physical, chemical and biological processes as this book hopefully shows.

We had the fortune to receive EU funding for a four-year research project in 2002. It was from the beginning of the project our intention not only to conduct research, which could elucidate the mechanisms and problems associated with ageing and reduced bioavailability, but also to use this information in practise to develop the field of ecological risk assessment of contaminated land. This was reflected in the title of our project "Development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution of organic pollutants" or in short "LIBERATION".

It is to our knowledge one of the first times measures of bioavailability have been incorporated systematically into a framework of ecological risk assessment for contaminated soil. Since most techniques for assessing bioavailability are relatively novel and hence not yet fully validated we expect a number of experts and regulators to challenge our proposals. We highly welcome this. Together with a large number of co-authors we have deliberately suggested pragmatic solutions when needed in order to make the decision support system operational and, hopefully, more widely used by contaminated land managers. It is our hope and intention that by doing so more practical experiences in site specific assessments will be gained throughout Europe. Far more practical experience is, together with more research of the mechanisms controlling bioavailability and techniques to predict it, what we need in order to convince authorities, the research community and other stakeholders to move away from using total concentrations in the assessment of ecological risk of contaminated land.

We hereby hope that this book will be used widely by risk assessors as intended i.e. as a useful tool supporting decision making in ecological risk assessment of contaminated land.

Silkeborg and Bilthoven, March 2006
John Jensen and Miranda Mesman

List of authors

John Jensen
Department of Terrestrial Ecology
National Environmental Research Institute
PO Box 314, Vejlsøvej 25
DK-8600 Silkeborg, Denmark
Email: john.jensen@dmu.dk
Phone: +45 89201565

Miranda Mesman
Laboratory of Ecological Risk Assessment
National Institute of Public Health and the Environment
P.O. Box 1, A. van Leeuwenhoeklaan 9
3720 BA Bilthoven, The Netherlands
Email: miranda.mesman@rivm.nl
Phone: +31 30 2743138

Michiel Rutgers
Laboratory of Ecological Risk Assessment
National Institute of Public Health and the Environment
P.O. Box 1, A. van Leeuwenhoeklaan 9
3720 BA Bilthoven, The Netherlands

Liesbet Dirven-van Breemen
Laboratory of Ecological Risk Assessment
National Institute of Public Health and the Environment
P.O. Box 1, A. van Leeuwenhoeklaan 9
3720 BA Bilthoven, The Netherlands

Neal Sorokin
National Centre for Environmental Toxicology (NCET)
WRc plc
Frankland Road, Blagrove
Swindon, SN5 8YF, United Kingdom

Thomas L. ter Laak
Institute for Risk Assessment Sciences (IRAS)
Utrecht University
Yalelaan 2, PO box 80176
3508 TD Utrecht, The Netherlands

Johan Bierkens
Centre of Expertise Integrated Environmental Solutions
Flemish Institute for Technological Research (Vito)
Boeretang 200
B-2400 Mol, Belgium

Andreas Paul Loibner
University of Natural Resources and Applied Life Sciences, Vienna
Department for Agrobiotechnology, IFA-Tulln
Institute for Environmental Biotechnology
Konrad-Lorenz-Str. 20
A-3430 Tulln, Austria

Elisabeth Erlacher
University of Natural Resources and Applied Life Sciences, Vienna
Department for Agrobiotechnology, IFA-Tulln
Institute for Environmental Biotechnology
Konrad-Lorenz-Str. 20
A-3430 Tulln, Austria

George Archibald Clark Ehlers
University of Natural Resources and Applied Life Sciences, Vienna
Department for Agrobiotechnology, IFA-Tulln
Institute for Environmental Biotechnology
Konrad-Lorenz-Str. 20
A-3430 Tulln, Austria

Björn Tobias Bogolte
University of Natural Resources and Applied Life Sciences, Vienna
Department for Agrobiotechnology, IFA-Tulln
Institute for Environmental Biotechnology
Konrad-Lorenz-Str. 20
A-3430 Tulln, Austria

Rafael Celis
Institute for Natural Resources and Agrobiology of Seville (CSIC)
Avda Reina Mercedes 10, Apdo 1052
41080 Sevilla, Spain

Thomas Hartnik
Norwegian Institute for Agricultural and Environmental Research
Soil and Environment Division
Frederik A. Dahls Vei 20
N-1432 Ås, Norway

Preface

This book brings together the work from a four-year (2002-2005) European research project Liberation (Development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution of organic pollutants) [EVK1-CT-2001-00105].

Contaminated sites are a national problem in most European countries. How to assess and handle risk in an effective and responsible way is therefore a cross-national challenge. One of the main obstacles in assessing the actual risk of contaminants is the observed reduction in toxicity and mobility of hazardous substances in soil as a result of ageing. Often inconsistency is found between toxicity, mobility and degradation rates in freshly spiked soil and observations made in the field. This is typically explained by ageing and by reduction in bioavailability over time.

A major innovative achievement of Liberation was the possibility to link chemical and biological measures of bioavailability with observed ecotoxicological and genotoxic effects in soil and pore waters, i.e. potential surface water, and at the same time to study the underlying physico-chemical processes that may explain these observations. However, the challenge to fully understand the processes controlling bioavailability in order to be able to predict the uptake and toxicity of aged pollutants is still immense. Hence, it has only been possible to include measures of bioavailability that are not yet fully validated in the present decision support system. It is nevertheless the hope that this book will generate a platform for many discussions and continuation of the work in the description, understanding and prediction of bioavailability.

The decision support system described in this book is based on a tiered approach for assessing ecological risk of contaminated soil that originates from years back and is based on the so-called Triad approach described by among others Chapman (1986) and Rutgers et al. (2000). This book is an attempt to continue and expand on this work e.g. by suggesting methodologies to include measures of bioavailability. The Decision Support System (DSS) is separated in three different stages, i.e.

- Stage I. Site characterisation and description of land-use.
- Stage II. Determination of ecological aspects.
- Stage III. Site-specific and tiered assessment (The Triad):
  - Tier 1. Simple screening
  - Tier 2. Refined screening
  - Tier 3. Detailed assessment
  - Tier 4. Final assessment.

Each of these tiers is based on a weight of evidence (WoE) approach combining three lines of evidence (chemistry, toxicology and ecology).

The authors would like to thank all participants in the Liberation project, the following project officers in the European Commission: Jürgen Büsing, Giuseppe Borsalino and Costanza Calzolari, and Lene Birksø NERI and Simon Blake from WRc for critical comments.

Finally, acknowledgement should be given to colleagues (with whom we had many lively discussions) in two other European research projects dealing with bioavailability and decision support systems. These were the EU-funded project ABACUS (Evaluation of availability to biota for organic compounds ubiquitous in soils and sediments) and the Italian ERAMANIA project funded by the Italian Agency for Environmental Protection and Technical Services (APAT). The feedback from the conference *"Decision Support Systems for the Integration of Bioavailability in ERA. Results from the three European Projects Liberation, Abacus and Era-Mania"*, held on the 28th-29th of November 2005 in Venice, Italy in association with APAT, is also highly valued. More information about this workshop, possibilities for downloading part of this book and much more can be obtained from the following homepage: www.sofar.dk.

CHAPTER 1 PRINCIPLES AND CONCEPTS IN ECOLOGICAL RISK ASSESSMENT

Jensen J., Mesman M., Bierkens J. and Rutgers M.

1.1 Scope and content

Numerous decisions have to be made before, during and after conducting an ecological risk assessment. This book is an attempt to guide risk assessors and stakeholders in the decision making process. Its main objectives are to:

1. Present and describe the basic structure of a decision support system (DSS) for assessing site specific risk from contaminated land to ecosystems.
2. List, review and provide further references for useful tools for site specific risk assessment with special attention to bioavailability.
3. Present a basic flow chart for assisting in the selection of risk assessment tools.
4. Present and recommend ways to weight, scale and use the results of the tests in a DSS.

This book represents the final outcome of the EU project Liberation (Development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution of organic pollutants, EVK1-CT-2001-00105). Although special attention was given to organic pollutants, most of the conclusions, the choice of bioassays, scaling of results etc. can also be used in the case of heavy metal contaminated sites provided adjusted tools for estimating bioavailability of heavy metals are applied. Useful and more detailed information about heavy metals and possibilities to assess their bioavailability can be found in e.g. Peijnenburg et al. (1997), Plette et al. (1999), Zhang et al. (2001), Ehlers and Luthy (2003) and Van Straalen et al. (2005).

This DSS is not a comprehensive “all-you-need-to-have” document for managing risk of contaminated land, as it focuses primarily on supporting decisions made when assessing risk to the terrestrial environment. It addresses only indirectly the risk to ground water and associated (connected) fresh water systems. Nevertheless information about e.g. reduced bioavailability may be useful when assessing potential risk for leaching of contaminants to ground water or fresh water. Recognising these limitations is important in the decision making process. Therefore, risk assessors focusing on the ecological impact of a contaminated site need to collaborate closely with risk assessors concerned about the risk to humans and ground water. Moreover, all risk assessors need to have close contact with the relevant stakeholders and other parties involved in the management and the current and future use of the site.

This book is organised as follows: after a brief introduction to the overall principles and concepts in ecological risk assessment and decision support systems in Chapter

One, Chapter Two presents the challenges and solutions for including bioavailability of organic pollutants in an ecological risk assessment framework. In Chapter Three there is a more detailed presentation of decision support systems for evaluating environmental risk of contaminated soil including a description of the different stages of the Ecological Risk Assessment (ERA) process. Chapter Four gives a more detailed description of the principles in the Triad approach, which we recommend as a powerful tool in the final stage of the ERA. Chapter Five provides guidance for a tiered assessment of ecological risk of contaminated soil including decision charts and selecting of site-specific assays in the various tiers. Chapter Six contains a presentation of the most common and useful tools for assessing fate and effect of pollutants found at contaminated sites. The tools are organised in various toolboxes each allocated to a specific tier of the ERA process. Finally, Chapter Seven is a short review of the outcome of using the Triad approach in a case-study with a contaminated site in Denmark.

1.2 Ecological risk assessment in brief

Ecological risk assessment (ERA) is a process of collecting, organising and analysing environmental data to estimate the risk of contamination for ecosystems. Assessing the ecological risk of contaminated soil is, however, a complicated task with many obstacles associated with the process. Terrestrial ecological risk assessment was developed later than aquatic risk assessment, and is furthermore complicated by the fact that soil systems are very heterogeneous. As a consequence of this soil pollution is often less uniform and more difficult to define than fresh water ecosystems. Moreover, land and hence soil is typically private property and traded as real estate. Professional and economically divergence between the interests of land-owners, scientists, national authorities, engineers, managers, lawyers, NGOs and regulators is therefore not unusual.

There are typically two major types of ERA. The first one is predictive and often associated with the authorisation and handling of hazardous substances like pesticides or new and existing chemicals in the European Union. This type of ERA is ideally undertaken prior to environmental release of the substance. The second type of ERA is a description or estimation of changes in populations or ecosystems at specific sites or areas already polluted and should hence be conveyed as impact assessment rather than risk assessment. The principles of ecological risk assessment are described in numerous review papers and books, e.g. Ferguson et al. (1998), US-EPA (1998), Suter et al. (2000), Lanno (2003), Weeks et al. (2004) and Thompson et al. (2005).

Often ERA is performed in phases or tiers, which may include predictive as well as descriptive methods. The successive tiers require, as a rule of thumb, more time, effort and money. The paradigm for ERA may vary considerable but is typically based on an initial problem formulation based on a preliminary site characterisation, a screening assessment and a risk characterisation followed by risk management.

Prior to actually performing an ecological risk assessment it has to be decided whether (cheaper) alternatives are available. The size and the location of the contaminated area has to be evaluated in order to assess whether, for example, a simple dig-and-dump exercise would solve the problem at a much lower cost. This may be the case of very small plots within a larger area. On the other hand, remediation could be out of the question due to logistical problems, e.g. physical deterioration of a larger area in order to clean up a smaller area or disturbance of protected species.

In most European countries the first stage of the ERA of contaminated soils consists of rather simplified approaches including comparison of soil concentrations with soil screening levels (SSL, also known as quality objectives, quality criteria, benchmarks, guideline values etc.). Methodologies for deriving SSLs are described elsewhere, e.g. Wagner and Løkke (1991) and Posthuma et al. (2002). One of the keystones in deriving environmental quality criteria is the use of standardised test procedures. A collection of terrestrial soil tests can be found in for example Sheppard et al. (1992), Keddy et al. (1994), Van Gestel and Van Straalen (1994), Tarradellas et al. (1997), Løkke and Van Gestel (1998). Furthermore, a list of guidelines can be found at www.iso.org and www.oecd.org.

Soil screening levels (SSL) are common and very useful screening tools for assessing ecological risk. However, large discrepancies have been observed between effect levels derived from spiked experiments conducted in the laboratory, i.e. SSL, and the effect levels found when organisms are exposed to soil collected at contaminated sites or when monitored in the field. Many reasons are given for the contradiction between observed toxicity, mobility and degradation rates in freshly spiked soil and the observation made in the field. One explanation frequently given is a reduction in bioavailability over time as pollutants become sequestered into the soil matrix. This phenomenon is particularly important for a sound evaluation of risk and remediation options for contaminated sites. Chapter Two gives a short introduction to the problems and solutions associated with ageing of soil contamination.

Although single species laboratory tests with spiked materials have their obvious benefits, e.g. they measure direct toxicity of chemicals and interpretation is therefore simple, other supplementary tools are often needed to assess risk. Bioassays, performed with contaminated soil *ex situ*, are one of the more frequently used higher tier alternatives. Bioassays have the advantage, compared to the use of spiked soil samples, that the toxicity of a specific soil may be assessed directly. Bioassay testing has therefore the ability to account inherently for the complete mixture of contaminants, including degradation products and metabolites, in the sample. This is important, as generic calculations of the combined effect of mixed contamination are prone to large uncertainties. Furthermore, the *in situ* bioavailability of that specific soil is (at least almost) maintained in the laboratory during the exposure period. Consequently, bioassays are often considered more realistic tools for risk assessment than generic soil screening levels based on spiked laboratory soils.

A number of uncertainties or limitations may, however, be associated with the use of bioassays and the interpretation of the results obtained in these. The test species are exposed to the polluted soil for a short period as compared to the permanent exposure condition found at contaminated sites. Furthermore, beside the presence of contaminants, test species are exposed in an (almost) optimal environment as stress factors like predators, inter- and intra-species competition, drought, frost and food depletion are eliminated during the controlled exposure in the laboratory. Finally, a limited number of species are available for testing.

Contaminated soil may be assessed using multi-species tests, lysometers or terrestrial model ecosystems (TME). In these, species interaction may be evaluated by introducing several species to the systems or monitoring the endogenous populations. Natural climatic conditions may be included if the test systems is kept out-doors.

A crucial issue when analysing the result of bioassays, TME and field studies in all tiers of ERA is the presence or absence of a proper reference site or soil. This is true for chemical information (i.e. background levels in that region), toxicological data from bioassays (i.e. site relevant reference soil and control soil in order to verify the test performance) and ecological field surveys. The reference soil should in principle resemble the contaminated soil in all relevant parameters, e.g. texture, pH, organic matter, water-holding capacity, nutrient content. Since matched reference soils are often lacking this is a practical problem that often is difficult to solve and hence should be considered and discussed in detail before initiating the ERA. The lack of reference sites in field surveys may, however, in some cases be solved by the use of multivariate techniques (e.g. Kedwards et al., 1999ab), which relate the species composition and abundance to gradients of pollutants. Increased computer power and the presence of new easy-to-use soft-ware tools have increased the possibility to move away from more conventional uni-variate statistics like ANOVA to more powerful multivariate statistics, which use all collected data to evaluate effects at a higher level of organisation. Statistical methods like the power analysis may also be very useful in planning and designing large-scale ecotoxicity studies like mesocosms, TME or field surveys (Kennedy et al., 1999).

1.3 Concepts of ecological risk assessment

Schemes, paradigms or programs for conducting ecological risk assessment of contaminated sites have been developed in a number of countries or regions. It is beyond the scope of this book to present all of these in detail. The outline of ERA paradigms is presented below for a few selected countries, i.e. USA, the Netherlands, United Kingdom and Canada.

The US Superfund program for assessing ecological risk is one of the first and also most developed initiatives on ERA of contaminated sites. The Superfund program aims at quantifying potential effects on human health and ecological risks at inac-

tive hazardous waste sites. The Superfund risk assessments determine how threatening a hazardous waste site is to human health and the environment. Risk assessors should seek to determine a safe level for each potentially dangerous contaminant present. For humans, this is a level at which adverse health effects are unlikely and the probability of cancer is very small. For ecological receptors, determining the level of risk is more complicated, as it is a function of the receptors of concern, the nature of the adverse effects caused by the contaminants, and the desired condition of the ecological resources.

The US-EPA has published an Ecological Risk Assessment Guidance, which should be followed when assessing risks at Superfund sites. As all sites are considered unique this should always be done in a site-specific manner.

The ERA process suggested by the US-EPA for Superfund sites follows an eight step process, which can be broken down into four categories, i.e. 1) planning and scoping, 2) problem formulation, 3) stressor response and exposure analysis and 4) risk characterisation. An overview of the eight steps is presented in Figure 1.1 (more details can be found on the web pages of the US-EPA). Essential for all steps are a negotiation and agreement of the need for further action between the risk assessor, the risk manager and other stakeholders, the so-called scientific-management decision points (SMDP).

SMDP made at the end of the screening-level assessment will not set an initial cleanup goal. Instead, hazard quotients, derived in this step, are used to help determine potential risk. Thus, requiring a cleanup based solely on those values would not be very likely, although it is technically feasible. There are three possible decisions at the SMDP:

1. There is enough information to conclude that ecological risks are very low or non-existent, and therefore there is no need to clean up the site on the basis of ecological risk.
2. The information is not adequate to make a decision at this point, and the ecological risk assessment process will proceed.
3. The information indicates a potential for adverse ecological effects, and a more thorough study is necessary.

In the Netherlands contaminated sites are first determined using a set of soil screening levels called target and intervention values, which take both human and ecological risks into account (Swartjes, 1999; VROM, 2000; Rutgers and Den Besten, 2005). At seriously contaminated sites remediation or other soil management decisions are required if the risks cannot be neglected based on a site-specific ecological and human risk assessment, and the chance for dispersion of the contaminants. Until now, the ecological risk assessment has been based on chemical analysis, including a Decision Table harbouring critical dimensions of the impacted area (Table 3.3). Recently, an up-date of the soil protection act is foreseen (VROM, 2003), clearing the way for a site-specific risk assessment based on the Triad (Rutgers and Den Besten, 2005).

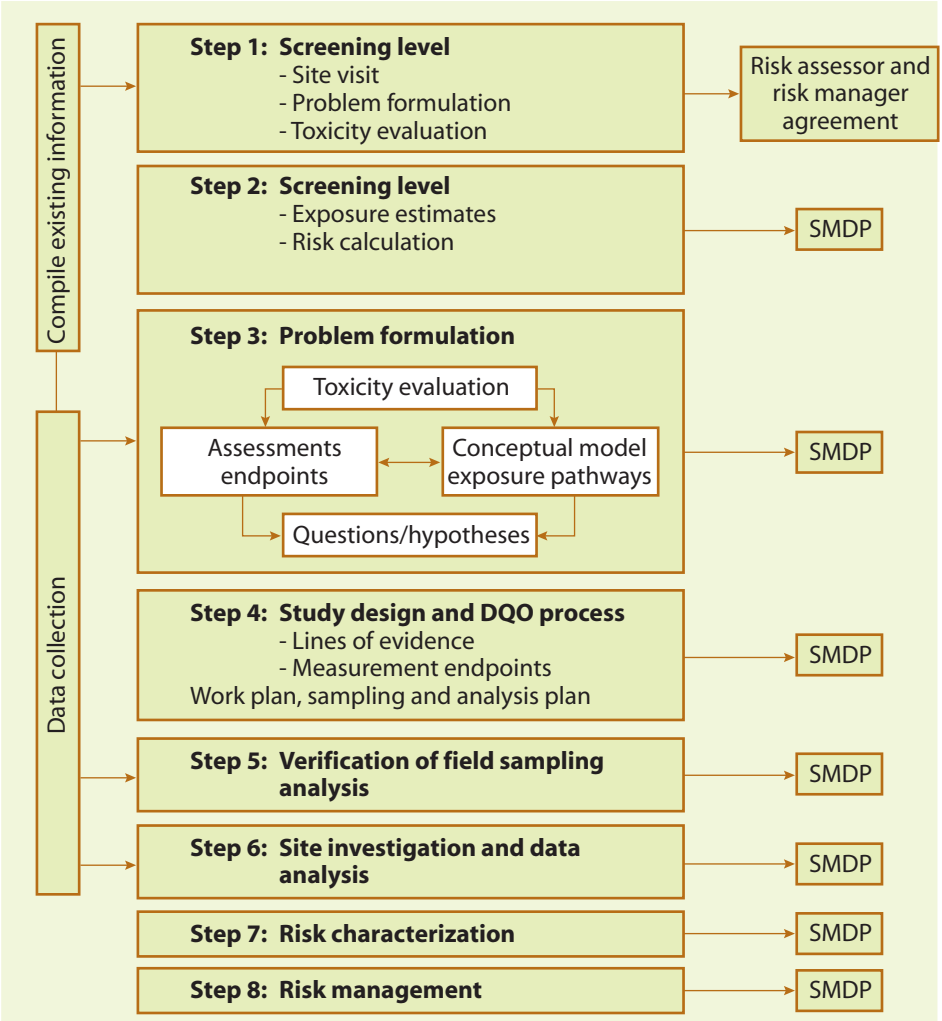

Figure 1.1 The eight steps in the US-EPA framework for risk assessment of contaminated Superfund sites. DQO = data quality objectives

The United Kingdom and Canada have also developed framework for ecological risk assessment of contamination land (e.g. CCME, 1997a; Weeks et al., 2004). A cornerstone in the UK framework of ERA is the connection to the statutory regime for identification and control of land potentially affected by contamination, also known as Part IIA of the Environmental Protection Act of 1990. This act defines land as contaminated if:

- A contaminant source and a pathway along which the contaminant can move is present and the contaminant (potentially) can affect a specified receptor.
- There is a significant possibility of significant harm.
- Pollution of controlled waters is occurring or is likely to occur.

Currently only risk to controlled waters and certain protected habitats (defined in Part IIA) are covered. The Framework does, however, also address how to perform ERA in areas not currently covered by Part IIA. The UK framework is based on schemes found in e.g. USA, Canada and the Netherlands. Like these it is a based on a tiered approach where the initial Tier 0 aims to determine whether a site falls under the Part IIA of the legislation. It involves the development of a Conceptual Site Model (CSM), which described what is already (historically) known about the site, e.g. whether there is a likely source-pathway-receptor linkage.

The conceptual site model is followed by an initial screening phase (Tier 1) and an actual site-specific characterisation (Tier 2). Tier 1 is a simple deterministic comparison of chemical residue data and the soil quality guideline values supplemented with simple soil-specific toxicity testing. The final step (Tier 3) involves more detailed *in-situ* studies and for example ecological modelling based on a more advanced ecological theory. Tier 3 is not likely to be conducted at many sites. More details on for example the selection of tests in the various Tiers can be found in Weeks et al. (2004) and on the web page of the Environment agency in UK (www.environment-agency.gov.uk).

General and technical information about the framework for ERA of contaminated land in Canada can be obtained from the homepage of the Canadian Council of Ministers of the Environment (CCME) (www.ccme.ca) or in two reports from CCME published in 1996 and 1997 (CCME, 1996; 1997a). In the Canadian approach soil quality guidelines are derived for four different land-uses, i.e. agricultural, residential/parkland, commercial and industrial. More information about the paradigms for deriving the Canadian soil quality guidelines can be obtained in CCME (1997b).

1.4 Principles and concepts for decision support systems

Chapter 3 to 5 in this book describes a newly developed system to support the decision-making process when assessing the risk of contaminated land to the environment. The target of the risk assessment is to provide the assessor with an objective and scientific evaluation of the likelihood of unacceptable impacts of the contaminants to the environment. It is, however, recognised that guidance on how to assess environmental risk is only part of the whole picture when stakeholders face the challenge of handling and managing a potentially contaminated site.

Decisions on how to manage the risk at a particular site depends on a number of parallel considerations. The outcome of the risk assessment (may or may not) form the foundation for taking a decision regarding appropriate risk management options. Identifying suitable technical solutions to soil contamination for example is not solely based on the outcome of the risk assessment. Risk management options needs therefore to be addressed in a holistic manner. Key factors include aspects such as the driving forces for remediation, e.g. environmental or human risk, recrea-

tional value or urban development plans, and availability, feasibility, suitability and cost of technical solutions to remediation.

Generally the decision-making process for contaminated land management includes several phases (Bardos et al., 2003), e.g.

- An identification phase in which the (magnitude of) problem is identified.
- A development phase in which likely solutions are identified and (further) developed.
- A selection phase in which the solution is chosen and implemented.
- A monitoring phase in which the effectiveness of the above decisions are examined and evaluated.

The DSS on environmental risk assessment in this book is clearly meant to support the identification phase as outlined above. However, the outcome of the process, e.g. which organisms are at risk and where and when they are at risk, may be very useful when, at a later stage, suitable options for remediation have to be identified and selected.

For a more comprehensive discussion about DSS the reader is referred to a review performed by CLARINET (Contaminated Land Rehabilitation Network for Environmental Technologies) under the 5th Framework Programme of EU and published by the Austrian Federal Environment Agency in 2003 (Bardos et al., 2003).

CHAPTER 2 SORPTION AND AGEING OF SOIL CONTAMINATION

Loibner A., Jensen J., Ter Laak T., Celis R. and Hartnik T.

In the field it is a common phenomenon to observe a relatively rapid initial decrease of organic contaminants followed by a subsequent slow disappearance of the residual fraction. Pollutant retention is a phenomenon that usually occurs in soil and sediment. It increases over time (ageing), which might be explained by ongoing sorption and reduced bioavailability.

Major processes involved are diffusion into nano-pores and sorption (adsorption and absorption) to soil organic matter. Strong sorption and slow release processes are responsible for the sequestration of hydrophobic pollutants and are a major obstacle for the successful application of bioremediation techniques. Moreover, they urge the reconsideration of concepts for deriving soil screening levels and understanding and estimating risks (Luthy et al., 1997; Semple et al., 2003).

Bioavailability as related to organisms living in contaminated soil comprises several phase transition and mass transfer processes. Therefore it has to be recognised that bioavailability is governed by dynamic processes comprising several distinctive phases (e.g. Lanno, 2003). The first processes are physico-chemically driven (chemical availability) like adsorption, desorption and diffusion controlled by substance and soil specific parameters like hydrophobicity, aqueous solubility, pK_a, Cation Exchange Capacity (CEC), pH, clay- and organic matter content. The second are physiological driven uptake processes (biological availability) controlled by species-specific parameters like anatomy, surface-volume relationship, feeding strategy and preferences in habitats. Thirdly, there are internal allocation processes (toxicological availability) controlled by organism specific parameters like metabolism, detoxification, storage capacity, excretion and energy resources. This is illustrated in Figure 2.1. Chemical availability is very site specific, whereas biological and toxicological availability general is more species- or process specific than site specific although physiological and genetically adaptation to contaminants, as well as life stage and seasonal differences in behaviour may alter uptake and allocation processes.

2.1 Sequestration of hydrophobic organic contaminants

Sequestration of hydrophobic organic contaminants (HOCs) refers to a limited availability of contaminants in soil and is a result of physical and chemical mechanisms such as adsorption, absorption and retarded diffusion. Differences in structure and chemical characteristics of soil constituents as well as the spatial arrangement of these constituents are responsible for differences in sorption and desorption of HOCs. Interactions between contaminants and natural soil organic matter (flexible and condensed), combustion residues (“black carbon”) and inorganic micro-pores are responsible for a retarded release of pollutants.

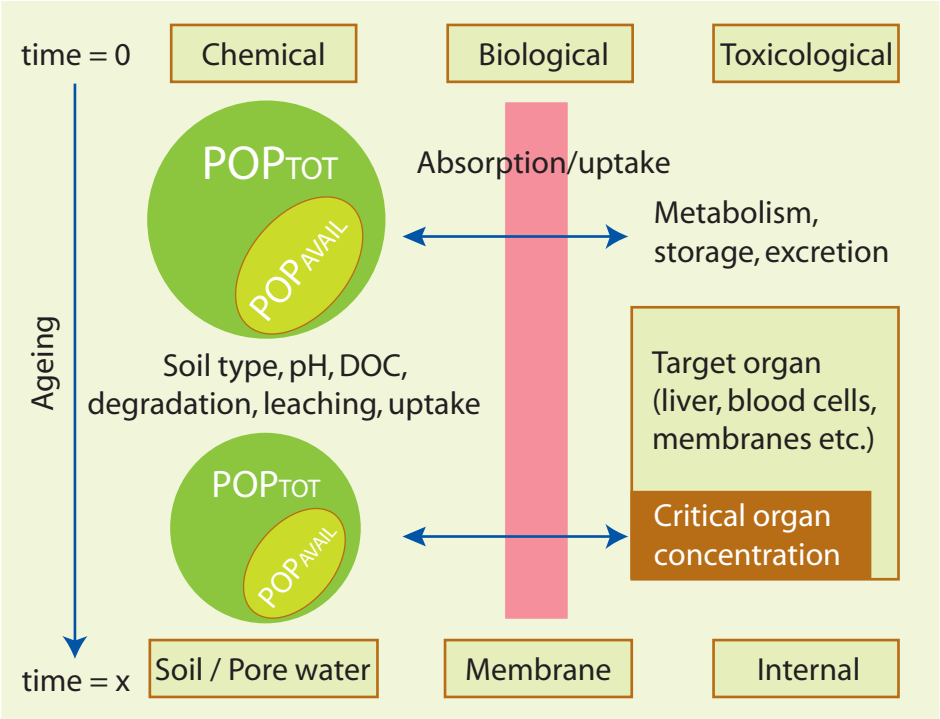

Figure 2.1 Three different types of processes govern the concept of bioavailability and ageing: (i) physico-chemically driven processes (chemical availability) controlled by substance and soil specific parameters; (ii) physiological driven uptake processes (biological availability) controlled by species-specific parameters; (iii) internal allocation processes (toxicological availability) controlled by organism specific parameters (details are given in text). POP = persistant organic pollutant.

Mechanisms involved in pollutant sequestration are (see also Figure 2.2):

1. Adsorption onto mineral surfaces.
2. Absorption into flexible/soft natural organic matter.
3. Adsorption on condensed/hard natural organic matter.
4. Diffusion in micro-porous media.
5. Encapsulation.

The first two mechanisms are usually fast and reversible whereas the latter three mechanisms exhibit slow desorption rates, pronounced sorption-desorption hysteresis, and largely irreversible retention of contaminants. Moreover, solvent extractability is reduced for the last three mechanisms, and alteration of sorption and diffusion kinetics by changes in temperature can be expected at least for mechanism three and four (Luthy et al., 1997). Therefore, extraction procedures using varying extraction conditions (e.g. changing temperature, pressure or solvent type) such as sequential supercritical fluid extraction may reveal information on the prevailing retention mechanisms.

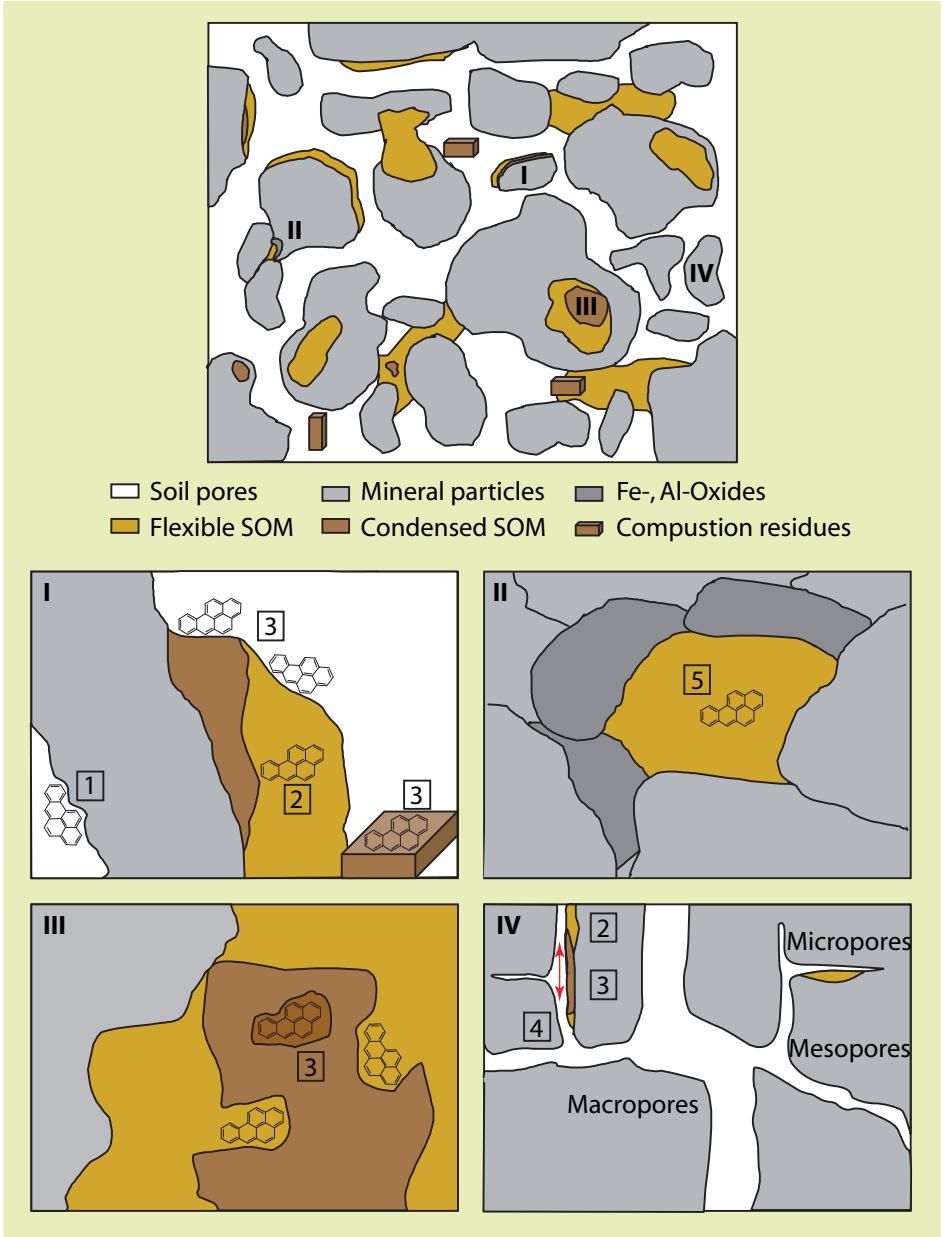

Figure 2.2 Top: schematic representation of potential sites for pollutant sequestration. Bottom: zoomed sections (varying scales): I – adsorption onto mineral surfaces and partitioning between water and organic phases; II – encapsulation of pollutants partitioned to soil organic matter; III – adsorption onto hard organic matter and capture of pollutants in hydrophobic cavities; IV – (retarded) diffusion of HOC in meso- and micro-pores (arrow). Numbers in rectangles refer to the sequestration mechanisms given in the text. Schemes are based on Luthy et al. (1997) and Szolar et al. (2002). SOM= soil organic matter.

Adsorption of organic contaminants onto particle surfaces generally happens rapidly and occurs at organic and mineral surfaces. However, adsorption of high-molecular PAHs may preferably take place at unpolar, condensed organic domains due to the increased hydrophobicity of these compounds.
Absorption into flexible organic matter comprises fast equilibration and almost linear isotherms whereas condensed organic matter may show linear as well as non-linear behaviour.

Diffusion kinetics into micro-voids of different pore size (actually the case for most soils) are non-linear. Tortuosity and interaction with pore walls limit diffusion, particular in pores with diameters in the range of the contaminant's molecule size. Moreover, the occurrence of humic material on the surface of larger pores may retard diffusion processes significantly as it may favour sorption mechanisms 2 and 3 (Grathwohl and Reinhard, 1993; Grathwohl, 1998). Encapsulation of soil organic matter by coatings of Fe and Al oxides, clay minerals or reactive $CaCO_3$ is known as one of the reasons for the stabilisation of soil organic matter (Baldock and Skjemstad, 2000). Encapsulation can occur at various scales ranging from nanometres to centimetres. Particularly at the smallest scales, impermeable mineral coatings may be formed. Isolation of organic matter with adhered contaminants may result in reduced accessibility of the toxicant to the exterior.

In addition to the five pollutant retention processes discussed above, the presence of liquid organic phases may have an influence on the sequestration of HOCs. Usually, free phase liquids are contaminants on their own that occur at very high concentration levels (above saturation) and that may harbour further toxicants. For PAH pollution, such an organic phase (e.g. tar, oil or its fractions) comprises a large variety of individual compounds with PAHs being enriched in this non-aqueous phase liquid. Amongst others, mass transfer is dependent on conditions at the contact area between the organic and aqueous phase. Time dependent alterations at the interface may result in the formation of viscous films that influence interphase mass transfer (Nelson et al., 1996).

2.2 Organic matter and chemical availability

Pollutant sequestration is a result of the presence of some or all above described retention mechanisms. The extent to which individual mechanisms contribute is determined by the composition of the soil. Addressing pollutant sequestration to the quantity of soil organic matter by using linear partitioning model derived K_{OC} values may be insufficient to describe the phenomenon of reduced chemical availability. Linear partitioning models for sorption equilibria (Karickhoff et al., 1979) consider organic matter as a relatively homogenous and amorphous gel-like matrix with pollutants distributed between this organic and the aqueous phase. However, observations such as very slow sorption-desorption rates, varying non-linearity of sorption isotherms, and sorption-desorption hysteresis are inconsistent with simple partition-

ing models. Supplementary to inter- and intra-aggregate diffusion, heterogenity of soil organic matter (SOM) is responsible for the phenomena of variation in pollutant retention observed in many studies (e.g. Karapanagioti et al., 2001). Sorption processes of organic chemicals in soil are strongly influenced by SOM characteristics and a significant correlation of sorption with the quantity of organic carbon is not always possible (Haberhauer and Gerzabek, 2002; Allen-King et al., 2002). In a long-term experiment comprising various agricultural land management practises it has been demonstrated that sorption of herbicides was dependent on the origin of organic matter introduced into soil (Haberhauer et al., 2001).

Beside amorphous humic matter (such as flexible or soft SOM) that exhibits almost linear partitioning, soils also accommodate different kinds of black carbon and kerogen. Sizing from a few micro metres up to 100 µm these reveal low H/C and O/C atomic ratios when compared to humic acids. In soils of industrialised regions, kerogen and black carbon may account for up to 80% of the total organic carbon (Song et al., 2002). The latter two have molecular structures different from humic acids and are considered to be part of the condensed or hard SOM.

Kerogen and black carbon are constituents of the non-extractable humin fraction besides lignin or polysaccharide derived polymers, mineral adhered lipids, and humic acid materials (Song et al., 2002; Huang et al., 2003). Matured kerogen comprises the main fraction of natural organic matter in sedimentary rocks. At the surface, it may undergo alterations and become a major constituent of SOM. Kerogen consists of cross-linked stacks of aromatic sheets (up to 24) with gaps between the individual layers. These micro-voids, with an approximate size of 30-40 nm, may capture planar organic pollutants like PAHs, chlorobenzenes and chlorophenols (Durand, 1980). Black carbon is a product of incomplete oxidation of fossil fuels (frequently referred to as soot) or biomass (usually referred to as char). It is considered to be a strong sorbent for HOCs (Jonker and Koelmans, 2002; Burgess and Lohmann, 2004; Cornelissen and Gustafsson, 2005). The structure and properties of black carbon are governed by combustion conditions and the origin of the incinerated organic material. Due to drastic formation conditions and increased rigidity, black carbon is assumed to show predominantly surface adsorption whereas diagenetically matured kerogen is expected to exhibit dual mode sorption comprising partitioning and adsorption. As a consequence, the sorption behaviour of a particular soil depends on the relative amount of humic matter, black carbon and kerogen. The sorption capacity of HOCs correlates with the O/C atomic ratio of SOM: the lower the O/C ratio is, the higher is the sorption potential. This relationship indicates that hydrophobic interactions may play a significant role and, therefore, that the presence and properties of kerogen and black carbon may directly be related to reduced bioavailability of HOCs in soil (Song et al., 2002; Jonker and Koelmans, 2002; Cornelissen and Gustafsson, 2005). This hypothesis is supported by findings of Haeseler et al. (1999) who characterised SOM using a method initially developed to assess the maturity of kerogen present in rocks. They observed an association of PAHs with heavy non-extractable organic matter in gasworks soils and suggested this was an obstacle for biodegradation.

2.3 Integrating bioavailability in ecological risk assessment

The current practice for assessing risk posed by contaminated soil or landfills does not sufficiently cover the problems associated with the reduction in bioavailability of pollutants often seen in historically contaminated and aged soils. As a result, the actual risk is often over-estimated and improved tools for measuring the bioavailable fraction of contaminants are needed.

As described above, generic modelling of sorption is not sufficient to estimate the risks of hydrophobic contaminants like PAHs in field soils. In field-contaminated soils, pore water concentrations (and hence exposure) can be much lower and soil sorption coefficients can be much higher than equilibrium partitioning models predict. Not only the quantity but also the quality of the organic carbon seems to play an important role in controlling bioavailability and hence risk.

Bioassays – performed with contaminated soil *ex situ* - are one of the more frequently used higher tier alternatives. Bioassays respond only to the biologically active fraction of toxicants. Hence, they may be used as bioavailability estimators for an individual contaminant. Furthermore bioassays have the advantage, compared to the use of spiked soil samples, that the site-specific effect of mixtures of contaminants and their metabolites can be assessed. Besides potential interference from e.g. environmental fluctuations or varying growth conditions, the application of bioassays may be time consuming and laborious. Therefore, a number of physical-chemical techniques have been developed to gain knowledge about the extent of pollutant retention within shorter periods and lower budgets and to get more precise information on mechanisms and soil constituents being responsible for the sequestration of HOCs in soil. The list of techniques includes:

- Desorption experiments (Pignatello, 1991; Cornelissen et al., 1997; 1998).
- Gas purging (Lüers and Ten Hulscher, 1996).
- Solid phase micro extraction (Ramos et al., 1998; Sijm et al., 2000; Verbruggen et al., 2000; Van der Wal et al., 2004; Ter Laak et al., 2005, 2006).
- Rapid persulfate oxidation (Cuypers et al., 2000).
- Surfactant extraction (Volkering et al., 1998).
- Cyclodextrine extraction (Reid et al., 2000; Cuypers et al., 2002; Stokes et al., 2005).
- Tenax extraction (Pignatello, 1991; Cornelissen et al., 1997; Cornelissen et al., 2001; Ten Hulscher et al., 2003).
- XAD-2 Extraction (Northcott and Jones, 2001).
- Semipermeable membrane devices (Södergren, 1987; Huckins et al., 1990; Booij et al., 1998; Macrae and Hall, 1998).
- Electron paramagnetic resonance spectroscopy (Dumestre et al., 2000).
- Solvent extraction techniques (Chung and Alexander, 1998, 1999; Nam et al., 1998; Tao et al., 2004).

- Supercritical fluid extraction techniques (Weber, Jr. and Young 1997; Loibner et al., 1997, 1998; Pörschmann et al., 1998; Hawthorne et al., 1999).

Whereas desorption experiments may take several days to weeks, the application of the extraction techniques presented above allow a more rapid prediction of pollutant sequestration. This chapter does not provide details for the listed methodologies. Instead more information is available from the publications cited above or in the toolboxes in Chapter Six. General information regarding bioavailability can be obtained from e.g. Sijm et al. (2000), Mayer et al. (2003), Escher and Hermens (2004), Dean and Scott (2004) and Reichenberg and Mayer (2006).

The short review on bioavailability presented in this chapter clearly indicates that bioavailability and ageing is dependent on many parameters and that no easy to use concept will enable the incorporation of all its implications in an ERA. The methodologies presented in this book instead aim to provide relatively simple and quick methods to screen for potential risk of contaminants in a more realistic way than using total concentrations. On the basis of this the following two approaches are chosen in this DSS:

1. In the refined screening phase (Tier 2) to use simple non-exhaustive organic solvent extractions and to compare the extracted concentration with soil screening levels.
2. In the detailed assessment (Tier 3) to use passive sampling devices to estimate soil pore water concentrations and to compare these with water quality objectives.

The scientific reasoning and background behind these approaches is given below.

Tier 2. Non-exhaustive solvent extraction

The overall principle in this refinement of ecological risk assessment is to extract a fraction of the contamination, rather than the total concentration, as this reflects more accurately the toxicity of historically contaminated soil when compared to freshly spiked laboratory soil. The extractable concentration (mg kg^{-1} dry soil) from the contaminated soil samples is compared directly to the SSL and the result is used in the Triad after scaling (see Text Box 4, Chapter 4).

It is a prerequisite of this comparison that the selected method is able to extract (almost) 100% of the spiked concentration used in the tests for deriving SSL. If this is the case, then it can be anticipated that the reduced extractability typically found in contaminated soil samples correspond to reduced toxicity when compared to freshly spiked soil samples (see an example in Table 2.1).

In most short-term (< four weeks) laboratory toxicity tests it is reasonable to assume that little “true” ageing or strong sequestering occurs and hence a majority of the spiked chemicals adsorbing to the soil matrix are still extractable by methods using organic solvents like the ones presented below. However, for most methods this is

Table 2.1 An example on how data from solvent extraction methods can be used to adjust the initial risk assessment of historically contaminated sites.

Total soil concentration (mg kg^{-1})	150	Typically determined by exhaustive extraction methods like Soxhlet.
Extractability (% of total)	30	The extractability of the soil samples is determined by mild organic solvents (see text).
Recalculated concentration (mg kg^{-1}) (PEC)	45	A pragmatic approach, as it is acknowledged that "chemical availability" is only part of the total bioavailability concept (see Figure 2.1).
SSL (PNEC)	80	If extractability by the selected solvent is documented to be less than 100% in spiked soils, then the SSL can be adjusted according to this (see text).
Risk (PEC/PNEC) – Total concentration	1.9	Normal risk assessment procedure.
Risk (PEC/PNEC) – Extractable concentration	0.6	Text Box 4, Chapter 4, shows an example of how to scale the results so it can be used in the Triad.

only partly validated. Kelsey et al. (1997) used various acetonitrile-, ethanol-, methanol-water mixtures with/without agitation during extraction of the pesticide atrazine. Methanol-water and methanol alone removed more than 99% after 11 days of ageing. Approximately 70% (with agitation) of atrazine was removed by methanol-water (1:1) mixture and methanol after a 54 days ageing period. Methanol-water (45:55) at 40°C extracted 84.4% of phenanthrene (50 days of ageing) and acetonitrile-water (1:1) extracted 71% (120 days of ageing). Up to 83% of spiked phenanthrene was recovered by different solvent-water mixtures. Tang and Alexander (1999) used butanol, ethyl acetate and propanol to extract PAH. For butanol, 98, 71.3 and 82.9% was extracted from freshly spiked anthracene, fluoranthene and pyrene samples. With ageing, this declined to 63.2% (anthracene, 207 days of ageing), 53.4% (fluoranthene, 140 days) and 52% (pyrene, 133 days). With ethyl acetate they extracted 100, 74.1 and 61.8% from freshly spiked anthracene, fluoranthene and pyrene samples (day 0). This declined to 82.5, 60 and 27.6%, respectively, with increasing ageing times. With propanol 76.6 and 74.4% was extracted at day 0 for fluoranthene and pyrene, respectively. This was reduced to 53.9 and 51.0% after 133 days of ageing. Chung and Alexander (1998) extracted between 53 and 92% of freshly added phenanthrene from soils using 71% ethanol in water.

Based on the information from the examples above and other studies, it is reasonable to anticipate that between 70 and 100% of organic chemicals like PAH are extractable from most freshly spiked soils using mild organic solvents such as methanol, ethanol, butanol or propanol. It is recognised that the fraction of pollutant extracted by these methods does not necessarily correspond fully to the fraction of substances available for uptake in biota. However, the same would be true for aged soils. It should rather be conceived as a pragmatic way to incorporate ageing in the chemical LoE of the ERA. Furthermore, the inaccuracy of comparing the extractable

concentration from historically contaminated soil samples with the SSL (based on toxicity data from tests using freshly spiked soils) is considered to be limited compared to the other uncertainties in the ERA process. If more precise information is available about the efficiency of a specific solvent to extract a pollutant from freshly spiked soils, the SSL can be adjusted according to this knowledge, i.e.

$SSL_{adjusted}$ = SSL * Extraction efficiency (ranging from 0-1)

Tier 3. Passive sampling devices used in detailed assessment of ecological risk
Generic modelling of sorbed and freely dissolved contaminants is not sufficient to estimate the risks of hydrophobic contaminants like PAHs in field soils. In field-contaminated soils, pore water concentrations can be much lower and soil sorption coefficients can be much higher than equilibrium-partitioning models predict. Therefore, the risk should be assessed in a site-specific manner.

If accepting that soil dwelling species are likely to take up the majority of contaminants through the pore water, then tools to estimate the freely dissolved pore water concentration becomes important. The importance of pore water exposure has been demonstrated (e.g. Belfroid et al., 1994ab, 1996; Jager, 1998; Jager et al., 2003). However, particularly for low water soluble contaminants transfer dynamics, i.e. replenishing contaminants in pore water that have been taken up by target organism, can be an important factor as it changes the amount of toxicant reaching the target organism/organ/biomolecule over time.

The overall principle in this refinement of the ecological risk assessment is to extract the fraction of the contamination that is freely dissolved in the pore water. The pore water concentration (µg L^{-1}) extracted by passive samplers from the contaminated soil samples is compared directly to existing water quality objectives and the result used in the Triad.

Numerous studies have for example used polydimethylsiloxane (PDMS) coated fibres (Solid Phase Micro Extraction or SPME) to measure free aqueous pore-water concentrations of PAHs in spiked and/or field-contaminated soils (Heringa and Hermens, 2003; Mayer et al., 2003; Van der Wal et al., 2004; and Ter Laak et al., 2005). The requirements for accurate measurements of freely dissolved pore water concentrations and soil sorption coefficients via a passive sampler such as the SPME fibre are:

- The sampler is in equilibrium with the soil-water system.
- Partition coefficients to the fibres are known.
- The sampler should not affect the concentration in soil.

Even when these requirements are fulfilled, the estimated pore water concentration might, however, still be different from the field situation, since free concentrations in the field can also be affected by biological (and chemical) degradation and losses due to evaporation or leaching (Mulder et al., 2001; Volkering and Breure, 2003;

Artola-Garicano et al., 2003; Hwang and Cutright, 2003; Huesemann et al., 2004). If these processes are faster than desorption from the soil, free pore water concentrations will continuously be at a steady state level below the chemical equilibrium as determined under sterile, controlled conditions in the laboratory. Soils with very slow desorption kinetics are especially prone to be affected by these processes. *In vivo* measurements of pore water concentrations using passive samplers should therefore only be regarded as estimates of potential pore water concentrations in the field.

The SPME method described above is considered a non-depleting technique as it has only limited influence on the micro surrounding. Other passive sampling devices using a depleting technique may also be useful for estimating the uptake and hence risk of organic pollutant. Extraction with synthetic polymers like XAD2-resin, Tenax and cyclodextrin have the advantage compared to mild extractants like butanol or methanol that they are based on the principle that chemicals released from soil into the pore water are removed from the solution by the synthetic polymer (e.g. Reid, 2000). This is comparable to the situation for biodegradation or uptake in organisms as well. It is therefore likely that only freely dissolved or easily desorbing molecules are encapsulated with organic polymers like cyclodextrins or Tenax.

When pore water concentrations have been estimated they are compared to water quality standards in order to evaluate risk. The precondition for doing this comparison is that it is anticipated that the sensitivity of aquatic and terrestrial species is similar and that the major uptake route of organic pollutants is through the water phase. There is no strong evidence for or against that terrestrial species, as a general rule, are either less or more sensitive than aquatic species.

A comparison between calculated HC_5 concentrations for the soil and the water environment showed poor correlation between the species sensitivity of the two compartments for 10 organic and 8 heavy metals (Van Beelen et al., 2003) (Figure 2.3a). This was also confirmed in a collection of acute toxicity data for earthworms and *Daphnia* used in the evaluation of pesticides in Denmark (Data not published) (Figure 2.3b). Van Beelen et al. (2003) concluded that the use of transfer functions (equilibrium partitioning) between the two compartments sometimes overestimated other times underestimated the risk in the terrestrial compartment when based on aquatic data. Nevertheless, they advocated that aquatic data could be used for assessing terrestrial risk when a limited set of soil data was available.

In comparison to other uncertainties associated with the risk assessment procedure of contaminated land, it would therefore be reasonable to assume that a comparison between water quality objectives and pore water concentrations are a sensible tool also to predict site-specific risk or hazard of contaminated soil to terrestrial species.

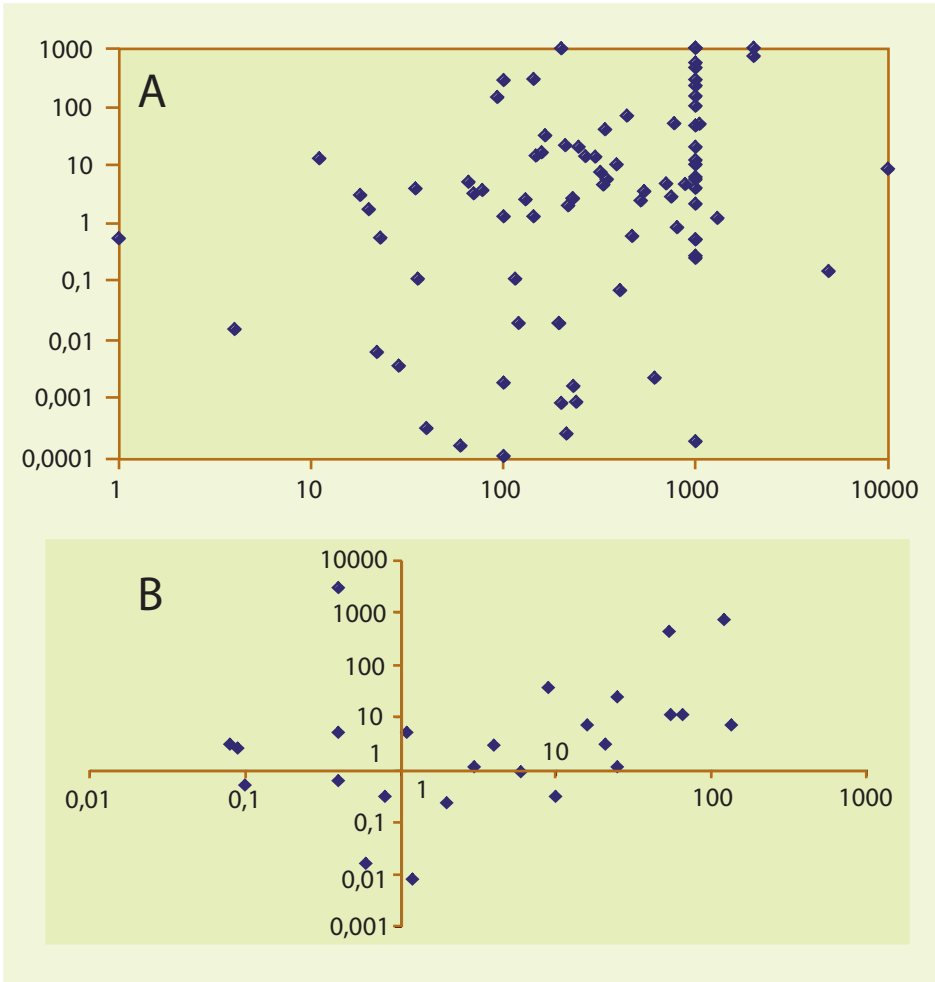

Fig. 2.3 A) Correlation between the species sensitivity (logHC_5) of terrestrial species (x-axis) and aquatic species (y-axis). Number of chemicals was 18 and the total number of tests going into the SSD calculations was 429 for the terrestrial and 967 for the aquatic environment. Data from van Beelen et al. (2003). B) The correlation of acute LC_{50} values for earthworms (x-axis) and Daphnia *(y-axis) for various pesticides. For 85 pesticides data were available for both the aquatic and soil invertebrate species.*

2.4 Conclusions

The Decision Support System presented in this book is a pragmatic example on how to incorporate measures of bioavailability into a conceptual framework for ecological risk assessment and risk management. To validate the suggested approach fully and to gain more insight into the complex processes behind ageing, more work is still needed in this research area. Nevertheless, although there is still a large degree of uncertainty it is believed that including measures of bioavailability in ERA will improve the realism of site-specific ecological risk assessment in comparison with the current use of total concentration.

CHAPTER 3 DECISION SUPPORT SYSTEM FOR ECOLOGICAL RISK ASSESSMENT

Mesman M., Jensen J., Rutgers M. and Bierkens J.

3.1 Introduction

Ecological Risk Assessment is often a complex process with many variables to take into account. ERA involves many stakeholders and all have to be dealt with in a clear and consistent way. A stepwise or tiered approach is therefore useful to overcome the complexity of an ERA. In order to structure all the information collected, a Decision Support System (DSS) can be used. Each tier will lead to a decision to proceed or to stop. As mentioned in Chapter One, a number of decisions supporting systems or frameworks have already been developed in other countries, e.g. UK, the Netherlands and the USA. The DSS presented here is based on basic principles also common in the methodologies used in the USA and UK. However, in the present DSS measures of bioavailability and the use of the Triad approach may be build into the system more systematically.

This chapter introduces the overall framework of a novel DSS including the Triad approach and the challenge to weight and scale results used in that process. Chapter 4 gives more details to the Triad approach and Chapter 5 introduces the actual decision trees and flowcharts.

Risk of groundwater pollution

3.2 Framework for ecological risk assessment

Rutgers et al. (2000) developed a basic flowchart for Ecological Risk Assessment, which is used as the backbone of the decision support system (DSS) presented in this book (Figure 3.1).

The DSS found in this book is separated in three different stages. All of these are described in Chapter 4, i.e.

- Stage I. Site characterisation and description of land-use.
- Stage II. Determination of ecological aspects.
- Stage III. Site-specific tiered assessment (the Triad):
  - Tier 1. Simple screening
  - Tier 2. Refined screening
  - Tier 3. Detailed assessment
  - Tier 4. Final assessment.

Each of these four tiers is based on a weight of evidence (WoE) approach combining three lines of evidence (Chemistry, (eco)Toxicology and Ecology) (Figure 3.1).

Boundaries of the DSS
The DSS in this book is not a full and comprehensive document for managing risk of contaminated land. It focuses strongly on supporting decisions made when considering risk to the terrestrial environment. Therefore it addresses only indirectly the risk to ground water and associated (connected) fresh water systems. Nevertheless information about e.g. reduced bioavailability may be useful when assessing potential risk for leaching of contaminants to ground water or fresh water. Furthermore, it is important to realise that the management of a contaminated site is more than assessing ecological risk. Issues like for example risk for humans, availability and cost of remediation solutions, development plans for the vicinity or the region are equally important.

3.3 Stage I. Site characterisation and land-use definition

The first step in the DSS is to establish what is often referred to as a Conceptual Site Model, e.g. Weeks et al. (2004). It aims at involving as many stakeholders as possible in order to describe site characteristics and to review all available information from the site, e.g. historical information about land-use, investigation of whether the site may be regulated under specific directives, obvious data gaps and urgency for reaction and data collection.

The spatial borders of the site should be defined and the current and the future land-use have to be defined. Consultation between administrators, planners and experts therefore has to take place as early as possible in the process.

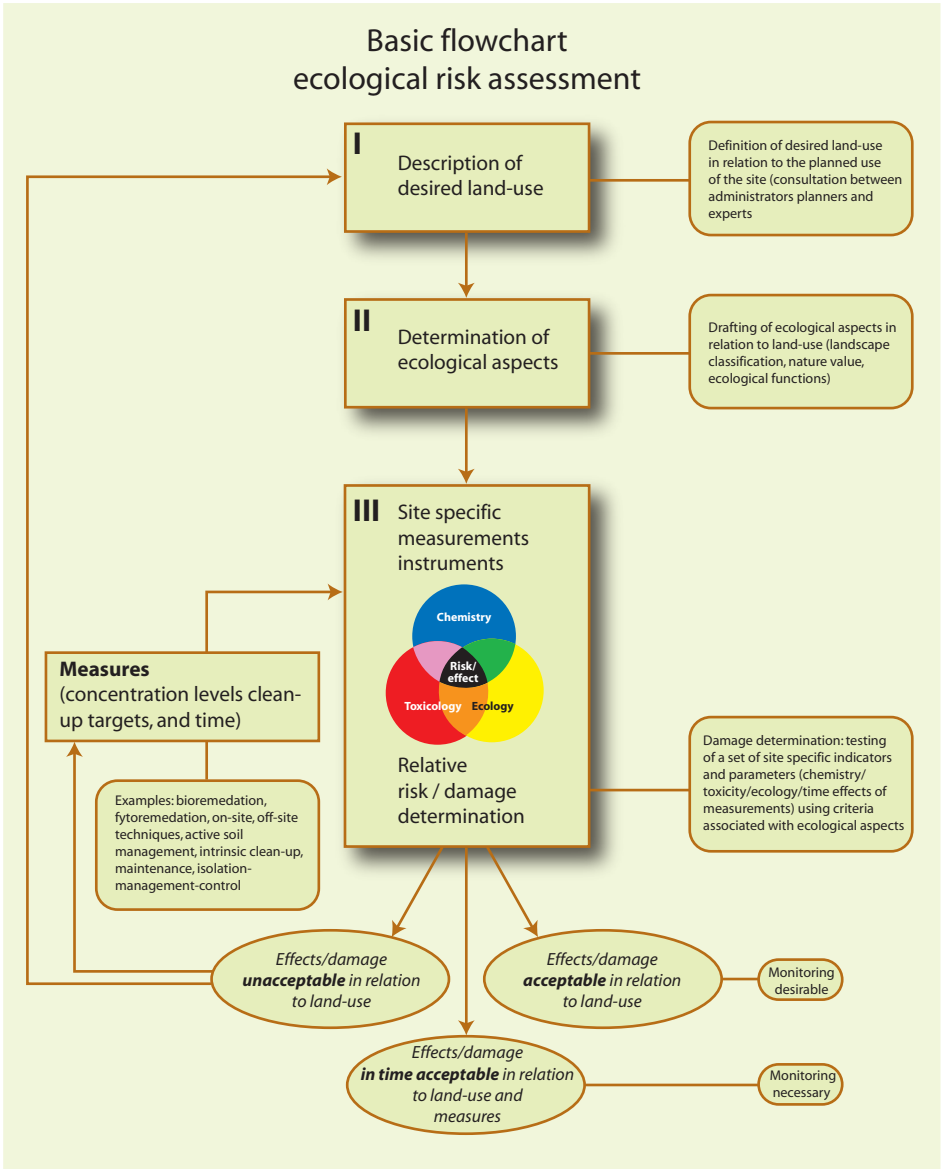

Figure 3.1 Basic flowchart for ecological risk assessment. Adapted from Rutgers et al. (2000).

3.3.1 Initial requirements in the DSS

An inquiry among all stakeholders should be conducted as one of the first initiatives. The aim should be to collect as much information about soil characteristics as possible. Historically information about past land-use, e.g. gas work, petrol station, factory, agriculture, is normally required in order to enter the DSS. It will in most cases reveal information about soil characteristics like pH, organic matter, soil type and the type of contaminants likely to be found at the site, such as organic pollutants,

metals, pesticides etc. If historically information about concentration of the most relevant contaminants is available it should be considered whether these are sufficient for a first screening of ecological risk or if the process would benefit from new chemical analyses of the entire area or only part of the site.

3.3.2 Defining land-use

One of the first actions to be taken among all stakeholders is to decide which land-use is required for the site, as this will determine the required data collection and testing. Many land-uses may be defined, but generally the four following overall categories of land-use classes are used:

- industrial area (including infrastructure and pavement).
- urban/residential area (including recreational and green areas).
- agricultural area.
- nature area.

By defining the land-use it is possible to narrow down the ERA. As different land-uses involve different requirements, or different soil functions, focus can be put on ecological aspects important for the site-specific land-use. Some soil functions may be totally absent for some land-uses or the requirement varies. For example, although the ecological function is important for both residential areas and areas such as nature reserves, the requirement is typically higher for the latter. Specific habitat functions may be relevant if the site is included in a special protection area or is covered by various Directives. Other, non-ecological, soil functions including physical function, groundwater reservoir function and more specific soil functions like natural attenuation, should also be considered as they may directly or indirectly influence the ERA process.

Some examples of ecosystem functions or services and associated ecological parameters and land-uses can be found in Table 3.1 and 3.2.

The DSS in this book is designed for ecological risk assessment, but is also possible to conduct a human health risk assessment with a similar approach. This can run parallel with ERA since the basic ideas are similar.

3.3.3 When is an ecological risk assessment needed?

Most often a site specific ERA will be initiated only when soil concentrations exceed soil screening levels. However, this may not in it self be a sufficient criterion to go through the entire ERA procedure. Some boundary conditions, based on the present and future type of land-use, the level of contamination and various ecological considerations have to be met in order to rationalise an ERA. The experts and the rest of the stakeholders should answer a number of simple questions in order to conclude whether the required boundary conditions are fulfilled.

Table 3.1 Examples of ecosystem services or function and important ecological parameters associated with these. Adapted from: "Ecological proxies for ecosystem functions. Vital Soil", Chapter 10: Ecological soil monitoring and quality assessment. Breure (2004).

Ecosystem service	Important ecological parameters
Supply of nutrients	Food web including earthworms Primary production Ratio of bacteria/fungi (De)nitrification
Water regulation	Earthworms Abundance and ratio bacteria/fungi pH, content of soil organic matter, groundwater level
Soil Structure	Earthworms Abundance and ratio of bacteria/fungi pH, content of soil organic matter Nematode Channel Ratio
Supply of clean shallow groundwater	Specific activity of bacteria and fungi Clean soil (concentration of pollutants lower than a maximum concentration) Extent of leaching of nitrogen, phosphate, and halogenated pollutants (EOX) Activity of the nitrogen cycle
Supply of clean deep groundwater	Amount and biodiversity of bacteria and fungi Clean soil Extent of washout of nitrogen and phosphate
Pest control in agriculture	Plant Parasitic Index of nematodes Amount and ratio of bacteria and fungi Mycorrhiza fungi
Changeability of soil use	Diversity of soil organisms Concentration of nitrogen and phosphate in the soil
Resilience and resistance	Diversity (within functional groups)

Table 3.2 Examples of land-uses and important ecosystem services or functions associated with these. From "Ecosystem services with different types of land-use. Vital Soil", Chapter 10: Ecological soil monitoring and quality assessment. Breure (2004).

	Nature area	Agricultural area	Residential area	Industrial area
Supply of nutrients	x	x	x	
Water regulation	x	x	x	x
Structure	x	x	x	x
Supply shallow ground water	x	x	x	x
Supply deep ground water	x	x		
Pest regulation in agriculture		x		
Changeability of soil use		x	x	
Resilience and resistance	x	x	x	

These questions are for example:

1. **Is ERA relevant for the site based on a) type of land-use and b) nature of pollutants?**
   a. Although in principle a healthy ecosystem could be a requirement for all sites, current practice shows that in some cases (land-use) a requirement of certain ecological criteria are of no practical use. For example, many industrial sites are covered with hardened surfaces. When no change in land-use is foreseen and no threat to the groundwater and/or an adjacent water body can be identified there is typically no need to conduct an ERA. Biological Valuation Maps, that geographically assign different labels to different zones based on criteria such as rarity, vulnerability and replaceability, may be useful for evaluating the relevance of ERA for more sensitive land-use like nature, park areas and agricultural land.
   b. If all or most of the relevant substances are found at levels below their soil screening values (SSL), ERA is generally not considered. However, even in cases where this is not the case, ERA may be redundant given knowledge on the nature of the pollutants present in combination with site specific soil characteristics; for example concentrations of highly hydrophobic substances slightly above the SSL in a peat soil might indicate a reduced risk.

2. **Is the extent and level of pollution sufficient to require and ERA?**
   When the first questions have been answered positively it should be considered whether the costs entailed in performing an ERA are justified, i.e. whether excavation and/or remediation options are available at an acceptable cost. Surface criteria in combination with pollutant levels may guide the decision about further actions. Examples are given Table 3.3. The values in Table 3.3 are based on the Dutch methodology to prioritise polluted sites and are used in this context as the upper limits below which no ERA should be performed given a certain type of land-use.

Table 3.3 Surface criteria below which no ERA should be performed (partly adapted from Koolenbrander, 1995)

Type of land-use	Extent of pollution	
	$1 \leq \frac{C_{environment}}{SSL} \leq 3$	$3 < \frac{C_{environment}}{SSL}$
Natural resource, agriculture and residential areas	100 m^2	50 m^2
Park areas	1000 m^2	500 m^2
Industrial sites	0,5 km^2	5000 m^2

SSL: Soil screening level; $C_{environment}$ = Concentration in soil samples

3. **Are there strong criteria for or against performing an ERA?**
   Additional arguments for and against performing an ERA may exist. These have to be balanced by the stakeholder before the final decision is made. The presence of endangered species or a rare ecotype at the site could for example be arguments against an ERA. In fact the contamination may, on its own or at least partly, be one of the main drivers in creating a special habitat for rare species. These may disappear as a result of changed soil conditions and/or species competition after a clean up. The site being an important transit zone for migratory animals could be one argument in favour of an ERA.

3.4 Stage II. Determination of ecological aspects

At stage II, site-specific ecological features and receptors relating to the land-use defined in Stage I need to be outlined. This includes aspects like key species and life support functions.

The potential ecological receptors should be identified in order to determine whether potential source-pathway-receptor linkages can be established. This includes not only ecological receptors directly linked to the site but also those linked indirectly e.g. through leaching of contaminants to connected fresh water systems or (migrating) birds or mammals feeding in the area.

In Table 3.4 some examples are given of land-use and related ecological aspects. This table can be used as a starting point for the selection of ecological aspect. Experts from ecotoxicology and ecology should be involved in the selection of ecological aspects.

3.5 Stage III. Site specific instruments (the Triad)

If after finalising Stage I and Stage II it is still considered that there is a need for a site specific evaluation of ecological risk the process continues to Stage III using the weight of evidence approach described below.

3.5.1 Weight of evidence approaches

In order to deal with conceptual uncertainties in a pragmatic way, it has been proposed to use weight of evidence (WoE) approaches for ERA (Long and Chapman, 1985; Den Besten et al., 1995; Suter et al., 2000; Rutgers et al., 2000; Hall and Giddings, 2000; Chapman et al., 2002; Burton et al., 2002). The rationale is, like in justice, that many independent ways to arrive at one conclusion will provide a stronger evidence for ecological effects, making ERA less uncertain.

Table 3.4 Some examples of land-use and ecological aspects related to it. Adapted from Rutgers et al. (2005).

Land-use	Ecological aspects
Nature	Species (key species, target species, predators, etc.) Interspecies relationships Ecosystem processes Nutrient cycles Natural attenuation
Agriculture	Sensitive crops, cattle Mycorrhiza Decomposition Groundwater quality Nutrient cycles Natural attenuation
Recreational area and parks	Plant species Nutrient cycles Natural attenuation Specific fauna Groundwater quality
Residential area with kitchen garden	Sensitive crops, ornamental plants Nutrient cycles Natural attenuation Pets
Residential area with garden	Ornamental plants Nutrient cycles Natural attenuation Pets
Residential area without garden Infrastructure Industrial areas	Roadside flora Grass species Nutrient cycles Natural attenuation Groundwater quality

In the sediment research area the application of WoE started at an early stage and was called the Sediment Quality Triad (Long and Chapman, 1985). For terrestrial ecosystems WoE approaches and the Triad are still in a developing stage (Suter et al., 2000; Rutgers et al., 2001; Mesman et al., 2003). The Triad approach is based on the simultaneous and integrated deployment of site-specific chemical, toxicological and ecological information in the risk assessment (Figure 3.2). The major assumption is that WoE in three independent disciplines will lead to a more precise answer than an approach, which is solely based on, for example, the concentrations of pollutants at the site. A multidisciplinary approach will help to minimise the number of false positive and false negative conclusions in ERA. It also gives acknowledgement to the fact that ecosystems are too complex to analyse in one-factorial approaches. As the Triad approach was chosen as a suitable weight of evidence approach in this book a more detailed description can be found in Chapter 4.

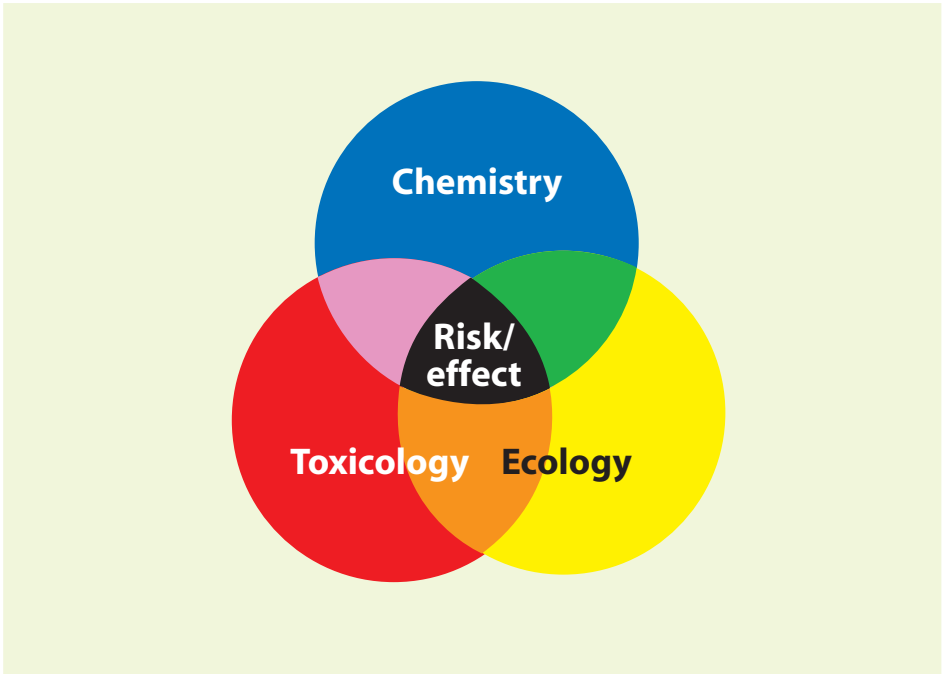

Figure 3.2 Schematic presentation of the integration of three fields of research according to a Triad:

- **Chemistry:** The concentration of contaminants in the environment (totals, bioavailable), accumulated in biota, or modelled via food-chains is used for calculation of risks on the basis of toxicity data from the literature.
- **Toxicology:** Bioassays with species across genera are carried out in order to measure the actual toxicity present in environmental samples from the site.
- **Ecology:** Field ecological observations at the contaminated site are compared to the reference site. Deviations from the reference site, which can be plausibly attributed to the contamination levels, are funnelled into the Triad.

CHAPTER 4 USING THE TRIAD IN SITE SPECIFIC ASSESSMENT OF CONTAMINATED SOIL

Mesman M., Rutgers M. and Jensen J.

4.1 The Triad

As described in the previous chapter, the Triad is a powerful weight of evidence approach originally developed in order to evaluate sediment quality (Long and Chapman 1985). In the terrestrial compartment less experience is available on the practical use of the Triad. This chapter describe the use of Triad in more detail and gives an insight into some of the important decisions risk assessors have to make when conducting the Triad in practise, e.g. how to scale, weight and integrate the outcome of the various investigations.

4.1.1 Lines of evidence

The Triad approach exists of three lines of evidence (LoE), the so-called Triad "legs", i.e. chemistry, (eco)toxicology and ecology. The Triad approach includes a tiered system in which each consecutive tier is increasingly fine-tuned to the site-specific situation. In the first tier the research is simple, broad and generic. In later tiers more specific and complex tests and analyses may be used.

For each of the LoE in the Triad there is a variety of analyses or tests that can be chosen. Some examples are:

- Chemistry: Measurement of total concentrations, bioavailable concentrations, bioaccumulation, etc.
- Toxicology: Bioassays (in field and/or in lab), biomarkers etc.
- Ecology: Field observations of vegetation, soil fauna, micro-organisms, etc.

In Chapter 6, a number of tests or tools that are for suitable for use in each tier are presented for the chemistry, toxicology and ecology LoE.

4.1.2 Triad tiers

The tiered approach is chosen for several reasons, the most important of which is cost-effectiveness. If no inconsistency is found in the first tier of the Triad, then the ecological risk assessment may be finished and actions taken if needed (Figure 4.1). If there are conflicting results, more investigations are desirable in a higher tier. The information from previous tiers can be used in the assessment of the next one. At the end of each tier a final judgement is made (see section 4.2 for details). In this final assessment all available results will be used including the results from previous tiers. Data should be deleted only when further research has shown that a result is not reliable e.g. when the validity criteria are not met due to low quality of the test organisms or high temperature fluctuations in a climate chamber.

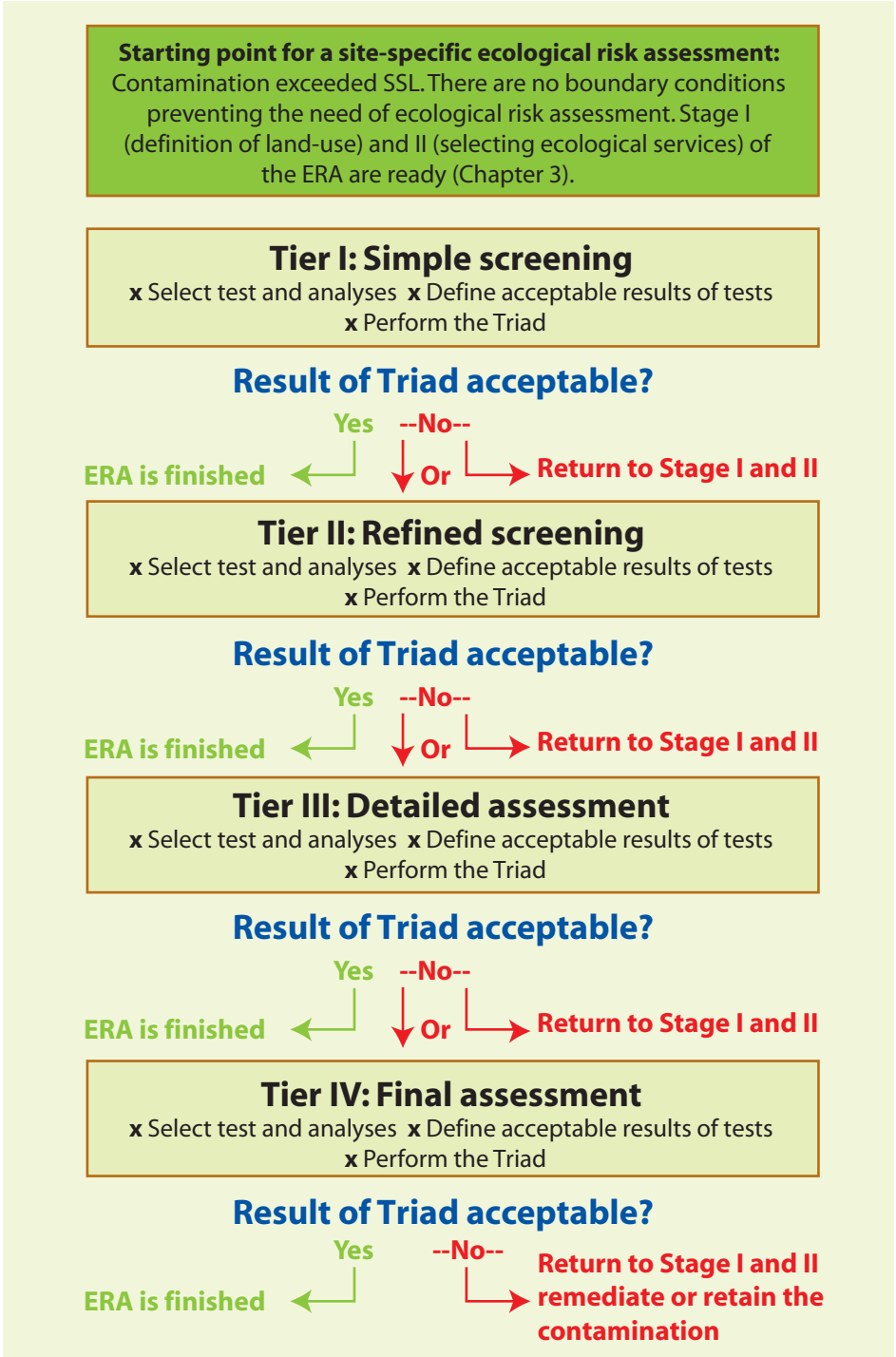

Figure 4.1 Overall construction of the tiered approach of the Triad used in this book (Chapter 5 gives more details). If considered most cost-effective, it is always possible to stop further investigations after each tier and either re-define the land-use or if needed take necessary actions to remediate or prevent dispersion of contaminants.

4.2 Scaling, weighting and integrating results

4.2.1 Quantification of results from terrestrial tests

Essentially, the results from all tests should be funnelled into the risk assessment framework. To be useful for risk assessment, the outcome from all tests in a WOE approach should therefore be made comparable across the various LoE, e.g. by a uniform scaling method. This should preferably be done without losing quantitative information (Smith et al., 2002; Burton et al., 2002). The primary aim is to maximise the utilisation of the results of particular tests as quantitative as possible, and to use results from all tests together in a transparent and integrative scheme, e.g. in a decision matrix. Burton et al. (2002) reviewed several possibilities for disseminating final WoE findings, and concluded that tabular decision matrices are the most quantitative and transparent.

In order to derive a quantitative decision matrix for easy evaluation and integration of results from different tests in the Triad, it is proposed to use an effect scale running from zero to one, corresponding to no effect up to maximum effect. The results from each parameter (e.g. bioassay, biomarker or ecological field survey) should be projected on this effect scale, according to best available knowledge or best professional judgements.

Different tests will obviously require different approaches. For instance, for a growth test the percentage of inhibition can be used as the unit for effects directly. For ecological field monitoring, the results should be scaled relatively to the ecological state of the reference site (= 0), and a (theoretical) state indicating 100% effects. Projection of test results on this effect scale requires some experience and expertise. Fortunately, the WoE approach will help to address and to correct mismatches of specific scaling methods due to wrong assumptions (Chapman et al., 2002).

Once all results are scaled into a uniform effect value, the overall response of a set of methods, e.g. the chemical LoE, can be calculated. For this, the geometrical mean is used of the 'reverse' effect (1 – effect). Back transformation of this value gives one integrated effect value for each of the LoE in the Triad. In this way extra weight is put on results from tests which demonstrate a positive ecological effect. The rationale is that biological methods, especially on the screening level, might be relatively insensitive and sometimes produce false negative results.

4.2.2 Scaling in practise

As mentioned above, a paramount issue when selecting tools for use in the Triad approach is the ability to scale the outcome of an assay. **If the outcome of a method can not be scaled from 0 to 1 it is not applicable in the context of the Triad approach presented in this book.** However, it should in principle be possible to scale any tool, which has ecological relevance and ability to serve as an indicator of toxic

stress, from 0 to 1. It may nevertheless sometimes need strong expert judgement to do so, wherefore basic knowledge of ecological risk assessment is an advantage.

Scaling of results is not part of the description in standard guidelines or publications. Therefore some effort must be given to this before initiating and conducting the studies. Examples, and it is important to stress that these are only examples, on how to scale the results of different types of studies are given in seven text boxes on the following pages.

Text Box 1. Examples on how to scale the results from two types of toxicity tests.

Scaling. Example 1. Results in percentages.
This method can be used as default when the results from the test are expressed as percentages (%), e.g. mortality (negative effect) or survival (positive effect). Note: the results have to lie between 0 and 100%.

Scaling method 1A. Negative response in reference/control sample
Test Example: Algae light inhibition

Data:	Reference	Site A	Site B
Test results (%):	4.0	46	71

Step 1. Divide data by 100. R1=X /100

	Reference	Site A	Site B
Result (R1)	0.04	0.46	0.71

Step 2: Scale difference between X and reference. R2 = (X – Ref) / (1 – Ref)

	Reference	Site A	Site B
Result (R2)	0.0	0.44	0.70

Scaling method 1B. Positive response in reference/control sample
Test Example: Survival of earthworms

Data:	Reference	Site A	Site B
Test results (%):	98	40	10

Step 1. Subtract from 100 and then divide by 100. R1=(100-X)/100

	Reference	Site A	Site B
Result (R1)	0.02	0.60	0.90

Step 2. Scale difference between X and reference. R2 = (X – Ref) / (1 – Ref)

	Reference	Site A	Site B
Result (R2)	0.0	0.59	0.90

Text Box 2. Example how to scale the results from field surveys.

Scaling. Example 2. BKX_Triad.

The BKX_Triad method can be used to scale several results from one (biological) survey or test. This method was developed to combine results from ecological observations into one number (Schouten et al., 1999). The results can be on very different effect scales, e.g. the Maturity Index for nematodes is on a scale from 1-5, while the number of earthworms can be between 0 and 1000. Also lower **and** higher values than the reference can be used. The BKX_Triad takes these differences in scales into account. Besides calculation of ecological observations it is also possible to use this formula for ecotoxicological results. In case one wants to combine results from several endpoints into one number (e.g. earthworm survival, number of offspring and growth).

BKX_Triad = 1 - 10 ^ ((-Σ | log x_n |) / n), where x is the result from the sample divided by the result from the reference sample, and n= the number of results (endpoints).

N.B.: The BKX-Triad calculates a geometric average value, i.e. using a log transformation. Consequently, very high values will not contribute too strong to the average observation. This is also why the formula does not work with "0" or other very small numbers for your reference. Therefore it may not be possible to use it for some ecotoxicological tests.

Example: Survey of nematodes

	Reference	Site A	Site B
Taxa (No.)	10	8	5
Individuals (No.)	957	750	233
Herbivores (%)	50	38	10

Step 1. Ratio between site x and reference. R1 = X/Ref

	Reference	Site A	Site B
Taxa (R1)	1	0.80	0.50
Individuals (R1)	1	0.78	0.24
Herbivores (R1)	1	0.76	0.20

Step 2. Calculate absolute values of log (R1)

	Reference	Site A	Site B
Taxa (R2)	0	0.097	0.301
Individuals (R2)	0	0.106	0.614
Herbivores (R2)	0	0.119	0.699

Step 3. Calculate sum of all values and multiply with –1. R3 = -1 * Σ (R2)

	Reference	Site A	Site B
Result (R3)	0	-0.32	-1.61

Step 4. Calculate number of endpoints. R4 = N

	Reference	Site A	Site B
Result (R4)	3	3	3

Step 5. Use results from step 3 (R3) and 4 (R4) in the BKX_Triad formula

	Reference	Site A	Site B
Results (R5)	0	0.22	0.71

Text Box 3. Example on how to scale results from chemical extractions.

Scaling. Example 3. Chemical extraction techniques I. Sorption.
Exposure through pore water is assumed to be an important factor for predicting the impact of the contaminants on terrestrial species. Pore water concentrations (PWC) can be measured via e.g. SPME. The sorption will be higher and the pore water concentration is lower in an aged soil especially in soils with strong sorbing matrices like for example soot. A theoretical example is given for pyrene, log K_{OW} = 5.18, log K_{OC} = 4.97 according to a model of Karickhoff (1979).

Example: Difference between modelled and measured sorption coefficient

Step 1. Model the sorption coefficient ($K_{OC} = C_{soc}/C_{pore\ water}$) with e.g. QSAR

	Site A	Site B
Result (R1)	93,000	93,000

Step 2. Measure sorption coefficient ($K_{OC} = C_{soc}/C_{pore\ water}$) in aged soil with e.g. PDMS coated fibres

	Site A	Site B
Result (R2)	125,000	780,550

Step 3. Calculate the ratio between modelled and observed sorption coefficient. R3 = R1/R2

	Site A	Site B
Result (R3)	0.74	0.12

Any results higher than 1 is set to 1.

Text Box 4. Example on how to scale results from chemical extractions.

Scaling. Example 4. Chemical extraction techniques II. Mild solvent extraction.
The concentration (mg kg^{-1} dry soil) extracted by solvents from the contaminated soil samples is compared directly to the SSL and the result is scaled from 0-1 in order to be used in the Triad (Chapter 2 gives more details on potential extraction methods). The example presented here is for the individual effects of contaminants found in a site. However, it can also be used to calculate a combined risk for all chemicals identified at the site by including Step 6. Alternatively see Text Box 6-7 for calculations of toxic pressure based on contaminant concentrations and SSD or SSL.

Step 1. Measure extractable fraction using mild organic solvent (mg kg^{-1})

	Reference	Site A	Site B
Result (R1)	0.8	4.5	35.5

Step 2. Collect or calculate individual soil screening values (SSL)

	Reference	Site A	Site B
Result (R2)	5.0	5.0	5.0

Step 3. For each contaminant, calculate ratio between R1 and R2. R3 = R1/R2

	Reference	Site A	Site B
Result (R3)	0.16	0.9	7.1

Step 4. For each contaminant, calculate the scaled risk number. R4 = 1 – (1 / (1 + R3))

	Reference	Site A	Site B
Result (R4)	0.14	0.47	0.88

Step 5. Correct for background concentrations. R5 = (R4 – R4ref) / (1 – R4ref)

	Reference	Site A	Site B
Result (R5)	0.0	0.38	0.86

Step 6. Calculate the combined risk of *n* chemicals. R6 = 1- $((1\text{-}R5)_1 \times (1\text{-}R5)_2 \times (1\text{-}R5)_3 \ldots\ldots\ldots (1\text{-}R5)_n)$

Result (R6)	No example is given here

Text Box 5. Example on how to scale results from chemical extractions.

Scaling example 5. Chemical extraction techniques III. Pore water concentration.
Pore water concentrations (µg/L) can be measured via e.g. SPME or cyclodextrin. The measured pore water concentrations (PWC) are compared to water quality objectives (WQO) and the results scaled in order to enter the Triad (Chapter 2 gives more details). WQO can for example be obtained from Verbruggen et al. (2001). The example presented here is for the individual effects of contaminants found at a site. However, it can also be used to calculate a combined risk number for all chemicals identified at the site by including Step 6. Alternatively see Text Box 6-7 for calculations of toxic pressure based on contaminant concentrations and SSD or SSL.

Step 1. Measure pore water concentration (µg L^{-1}) with PDMS coated fibres

	Reference	Site A	Site B
Result (R1)	0.01	0.47	3.9

Step 2. Collect or calculate water quality objectives (µg L^{-1})

	Reference	Site A	Site B
Result (R2)	1.5	1.5	1.5

Step 3. For each contaminant, calculate ratio between R1 and R2. R3 = R1/R2

	Reference	Site A	Site B
Result (R3)	0.01	0.31	2.6

Step 4. For each contaminant, calculate the risk number. R4 = 1 – (1 / (1 + R3))

	Reference	Site A	Site B
Result (R4)	0.01	0.24	0.72

Step 5. Correct for background concentrations. R5 = (R4 – R4ref) / (1 – R4ref)

	Reference	Site A	Site B
Result (R5)	0.0	0.23	0.72

Step 6. Calculate the combined risk for *n* chemicals. $R6 = 1- ((1-R4)_1 \times (1-R4)_2 \times (1-R4)_3 \ldots\ldots\ldots (1-R4)_n)$

Result (R6) No example is given here

Text Box 6. Example on how to scale results from the calculation of toxic pressure (TP) using species sensitivity distributions.

Scaling example 6. Calculation of toxic pressure of toxicant mixtures using Species Sensitivity Distribution (details in Section 6.3.4).

Total, extractable or pore water concentrations can be measured via different techniques (Chapter 2 gives details). Total concentrations of the investigated soil can be used together with SSDs based on total soil concentrations. Pore water concentrations of the investigated soil can be used with SSDs for groundwater or surface water organisms. Parameters for the SSD curve (alpha and beta, in which alpha is also known as HC_{50}, the Hazardous Concentration for 50% of the species) are used to calculate the toxic pressure of one contaminant. Subsequently the estimated effect from a mixture of contaminants with assumed different toxic modes of action (TMoA) (details in section 6.3.4) can be calculated. The toxic pressure of the mixture is quantified in the parameter combi-PAF or multi-substance PAF, depending on the aggregation protocol used to aggregate toxic pressures per compound (PAF) to the toxic pressure caused by the mixture, with PAF = Potentially Affected Fraction of species. In combi-PAF, all compounds are assumed to represent different Toxic Modes of Action, in msPAF. Toxic Modes of Action are pooled for compound groups, and within-group msPAFs are calculated according to the Concentration Addition model. In this report, the combi-PAF approach is used for pragmatic reasons, regarding data availability.

Step 1. Measure the (total, extractable or pore water) concentration

	Reference	Site A	Site B
Result (R1)	24	126	697

Step 2. Collect or calculate the parameters of the SSD curves (e.g. HC_{50} and β)

	Reference	Site A	Site B
Result (e.g.HC50)	210	210	210
Result β	0.45	0.45	0.45

Step 3. For each contaminant, calculate toxic pressure (PAF) per compound.
R3 = 1 / (1 + exp^ ((log HC_{50} – log R1)/β))

	Reference	Site A	Site B
Result (R3)	0.09	0.38	0.70

Step 4. Correct for background concentrations. R4 = (R3 – R3ref) / (1 – R3ref)

	Reference	Site A	Site B
Result (R4)	0.0	0.32	0.67

Step 5. Calculate the combined risk number of *n* chemicals with the different TMoA's according to the rules of the applicable Response Addition model. R5 = 1- $((1\text{-}R4)_1 \times (1\text{-}R4)_2 \times (1\text{-}R4)_3 \ldots\ldots\ldots (1\text{-}R4)_n)$

Result (R5) No example is given here

Text Box 7. Example on how to scale results from the calculation of toxic pressure (TP) using soil screening values derived by the use of assessment factors.

Scaling example 7. Calculation of toxic pressure of toxicant mixtures using environmental quality standards (details in section 6.3.4).

Total, extractable or pore water concentrations can be measured via different techniques (Chapter 2 gives details). These concentrations can be used in combination with environmental quality objectives like soil screening values or water quality criteria. Subsequently the effect of a mixed contamination can be calculated. In this toxic pressure calculation with default slope, and with the use of the valid EQO instead of the alpha = HC_{50} of the SSD, the principles of the procedure are the same as in the previous example (Text Box 6). Note that the approach implicitly assumes dissimilar toxic modes of action for all compounds, given the final calculation (R5). A value for β of 0.4 is useful as default for a wide range of tests. An alternative and simpler approach for using EQO is also described in Text Box 4-5.

Step 1. Measure the (total, extractable or pore water) concentration

	Reference	Site A	Site B
Result (R1)	0.8	4.5	35.5

Step 2. Collect or calculate EQO (e.g. SSL),

	Reference	Site A	Site B
Result (R2)	5.0	5.0	5.0

Step 3. For each contaminant, calculate toxic pressure (PAF) per compound.
R3 = 1 / (1 + exp^ ((log R2 – log R1)/0.4))

	Reference	Site A	Site B
Result (R3)	0.12	0.47	0.89

Step 4. Correct for background concentrations. R4 = (R3 – R3ref) / (1 – R3ref)

	Reference	Site A	Site B
Result (R4)	0.0	0.40	0.88

Step 5. Calculate the combined risk number of *n* chemicals with different TMoA's according to the rules of the applicable Response Addition model. R5 = 1- $((1\text{-}R4)_1 \times (1\text{-}R4)_2 \times (1\text{-}R4)_3 \ldots\ldots\ldots\ldots (1\text{-}R4)_n)$

Result (R5) No example is given here

4.2.3 Weighting

Besides the issue of scaling, attention should also be paid to the issue of weighting different tests, tiers and Triad LoE. Some general principles can be put forward:

1. The different LoE in the Triad should be equally weighted in the risk assessment, unless special considerations demand for a differential weight. The Triad is divided into three parts, each parts has its own weaknesses and strengths. Together they form a strong starting point for the risk assessment according the principles of a balanced WoE approach.
2. Within one LoE attention should be given to different aspects of the ecosystem. According to the SSD approach the starting point can be equal weights for all organisms and processes, applying the following statement: “all organisms are unequal, but equally important”. Another possibility is to give important ecological functions or life support functions equal weights. A balanced Triad approach should address all the important functions of a soil ecosystem like production, decomposition and consumption.

In specific cases, differential weighting between the different LoE in the Triad may be needed. For instance with strongly disturbed sites, the ecological field surveys may be hampered by exhibiting a completely different ecosystem, but not because of the presence of the contaminants. Another example would be if a chemical assessment is difficult because of very complicated exposure routes.

Within an individual LoE of the Triad, differential weighting of tests may be applied for three possible reasons:

1. First, differential weights on the endpoints can be applied because of ecological considerations. This differential weighting should be defined in the conceptual model, which serves as the basis for the ERA. This allows extra attention to specific (functional) groups, key species, and endangered or “charismatic” species.
2. The second reason for applying differential weights is to account for the uncertainty or variety within the end-points. Tests with a high level of uncertainty, or with a high variety in results, may be given a smaller weight in the ERA (Menzie et al., 1996).
3. The third and last reason for differential weight might correct for bias in measured and calculated effects. For instance the geometric mean of the inverted effect value gives extra weight to those observations giving a positive response. This acknowledges the fact that many bioassays of ecological field surveys are not able to demonstrate ecological effects, although in reality these effects are present, for instance in highly dynamic ecosystems. In such systems money may be too tight to collect and analyse the necessary number of replicates to demonstrate a significant effect.

Den Besten et al. (1995) used differentiated weights in the ERA for aquatic systems following a multi-criteria decision analysis. Effects on e.g. top predators and benthos received a higher weight than parameters such as mentum deformities. This information was used to rank different sites according to their possible risk for ecosystem health. For the terrestrial system less experience is available. Therefore no weighting is applied in this book (or in other words every test is weighted equal).

4.2.4 Integration of results

Once the results have been scaled for each test it is possible to integrate the results of the different tests in each of the line of evidence (LoE). Finally the integrated results from all three LoE are further integrated into one "risk number" of the Triad.

It may be argued that as well the integration within (intra) and between (inter) the various lines of evidence in principle are "comparing apples and oranges". However, for the moment it is the best approach available, although it is still open for improvement and adjustment.

The first integration process, i.e. within one LoE, aims to get a sufficient and complete set of information for estimating the risk of contamination. Different pieces of information are used together for this evaluation. For instance, the application of SSD adopts the reasoning that all organisms are important although they have a different sensitivity towards the contamination (Posthuma et al., 2002). Furthermore, estimates of effects based on different exposure scenarios may be used together to account for species-specific differences in bioavailability.

In the second integration step, the independent pieces of information from the three LoE are incorporated into one number of risk. Here it is also evaluated to what extend the three LoE indicate the same risk, wherefore a measure of deviation between the three LoE is added. A high deviation between the results of the three LoE could also trigger further research, as more insight is necessary to draw a final conclusion on the ERA. Below is presented one example of integration within one LoE of the Triad and one example of the final overall integration. Chapter 7 includes a full case-specific evaluation of risk.

Chemical LoE.

Data (estimation of effect from)	**Reference**	**Site A**	**Site B**
Sum TP org. Chemicals	0.00	1.00	1.00
Sequential Supercritical Fluid Extraction	0.00	0.20	0.24
Leaching test in hand -packed columns	0.00	0.01	0.03
Sorption according to SPME measurements	0.00	0.00	0.56
Concentration in plant shoots (mg/kg)	0.00	0.24	0.68
Step 1. Calculate log to (1-scaled result). R1 = log(1-X)			
	Reference	Site A	Site B
Sum TP org. Chemicals	0.00	-3.00	-3.00
Sequential Supercritical Fluid Extraction	0.00	-0.10	-0.12
Leaching test in hand -packed columns	0.00	0.00	-0.01
Sorption according to SPME measurements	0.00	0.00	-0.36
Concentration in plant shoots (mg/kg)	0.00	-0.12	-0.50
Step 2. Average all log values. R2= Average (X1…X*n*)			
	Reference	Site A	Site B
Result (R2)	0.00	-0.64	-0.80
Step 3. Transform log values into values. R3=1-(10^X)			
	Reference	Site A	Site B
Result (R3)	**0.00**	**0.77**	**0.84**

A similar exercise is then performed for the two other LoE. All results are hereafter combined into one integrated number of risk.

Integrated risk.

	Reference	Site A	Site B
LoE – Chemistry:	0.00	0.77	0.84
LoE – Toxicology:	0.00	0.23	0.34
LoE - Ecology:	0.00	0.21	0.29
Step 1. Calculate log to (1-scaled result). R1 = log(1-X)			
	Reference	Site A	Site B
LoE – Chemistry:	0.00	-0.64	-0.80
LoE – Toxicology:	0.00	-0.11	-0.18
LoE – Ecology:	0.00	-0.10	-0.15
Step 2. Average all log-values to one integrated log value. R2 = Average (X_1…X_n)			
	Reference	Site A	Site B
Result (R2)	0.00	-0.29	-0.38
Step 3. Transform log-values into integrated risk (IR) values. R3 = 1-(10^R2)			
	Reference	Site A	Site B
Result (R3 = Integrated Risk)	**0.00**	**0.48**	**0.58**
Step 4. Calculate standard deviation (Std) of the integrated results for each site, i.e. three LoE			
	Reference	Site A	Site B
Result (R4 = Std)	**0.00**	**0.55**	**0.53**

Table 4.1 Example how to interpret the outcome of the risk analysis in the Triad. It is highly recommended that stakeholders produce a similar table before the start of the Triad process. "Not acceptable" land-use does not necessarily have to imply remediation or soil management, but could also lead to more investigations. N = nature, A = agricultural, R = residential, I = industrial land-use.

Deviation (D)	Integrated Risk (IR)	Conclusion (land-uses)	
		Acceptable	Not Acceptable
D < 0.4 *	0.00 < IR < 0.25*	N, A, R, I	-
	0.26* < IR < 0.50*	A, R, I	N, A (with targets of concern)
	0.51* < IR < 0.75*	I, (R)	N, A, R (with "green" functions)
	0.76* < IR < 1.00*	I (with sealed soils)	N, A, R, I (with "green" functions)
D > 0.4* further studies or alternatively:	0.00 < IR < 0.25*	A, R, I	N, A (targets of concern)
	0.26* < IR < 0.50*	I, (R)	N, A, R (with "green" functions)
	0.51* < IR < 1.00*	I (with sealed soils)	N, A, R, I (green zones)

* These numbers are arbitrarily chosen, and can be part of the negotiation process between stakeholders, authorities and risk assessors. The goal of this table is to demonstrate the common sense of choosing criteria for interpreting Triad results in the decision-making process.

The major advantage of this integration method is the use of quantitative numbers, instead of the more qualitative "+" and "– " symbols used by e.g. Chapman (1996). By using risk numbers instead of risk symbols less information is lost and information about the magnitude of the risk (high effect, small effect) is given.

No definite limit for acceptable risk (or deviation of risk) can be given. This may vary according to the land-use of the site as well as the decisions made by stakeholders. Table 4.1 gives examples on how the risk numbers could be interpreted. In this Table a primary distinction is between low and high deviation (uncertainty of the results). In case of high deviation two approached can be taken. More research is conducted to lower the uncertainty or the high uncertainty is accepted but as a result of this a less sensitive land-use should be chosen. By using only four types of land-uses it sometimes needs further specification. For example agricultural land-use can also have a nature function, e.g. protection of black-tailed godwits in grasslands, and gardens can be with or without home grown vegetables. In Table 4.1 some suggestions for specifications are made.

CHAPTER 5 DECISION CHARTS IN ECOLOGICAL RISK ASSESSMENT OF CONTAMINATED SITES

Jensen J. and Mesman M.

5.1 Flowcharts

This book is an attempt to present a decision support system, which can guide risk assessors in their assessment of site-specific ecological risk. As presented in earlier chapters a number of site-specific questions need to be answered before a final decision on performing an ecological risk assessment can be made.

This chapter introduces a flow chart for ecological risk assessment of contaminated sites. The flowchart is presented as decision trees (Figure 5.1 and 5.2) together with a more in-depth introduction to the relevant questions that needs to be addressed and answered when performing a site-specific ecological risk assessment (Section 5.2).

As mentioned in earlier chapters, this decision support system relates to similar systems already in use in some countries or published in the open literature. However, to our knowledge this is the first coherent attempt to include bioavailability measures in practical risk assessment of contaminated sites. Although, fully recognising that a lot of issues are not yet entirely resolved and explained in this matter, a pragmatic approach is taken in order to use a number of relatively simple extraction methods in ecological risk assessment. It is the hope that by moving away from total concentration to (rough) estimates of bioavailable concentrations, the risk assessment will be made more realistic and less conservative. Furthermore, more practical experience will enable the production of more site-specific information that, together with scientific research, is needed to improve and validate these methods in the future.

5.2 Decision making in ERA

The assessment of ecological risk, as presented in this book, is performed stepwise in tiers. Higher tiers represent gradually more and more complex studies, but also more expensive and laborious studies. The full site-specific risk assessment covers four tiers, i.e.

- Simple screening: Tier 1.
- Refined screening: Tier 2.
- Detailed assessment: Tier 3.
- Final assessment: Tier 4.

The main principle in going from a simple screening over a more refined screening to a detailed assessment of the contaminated site is to minimise time and effort.

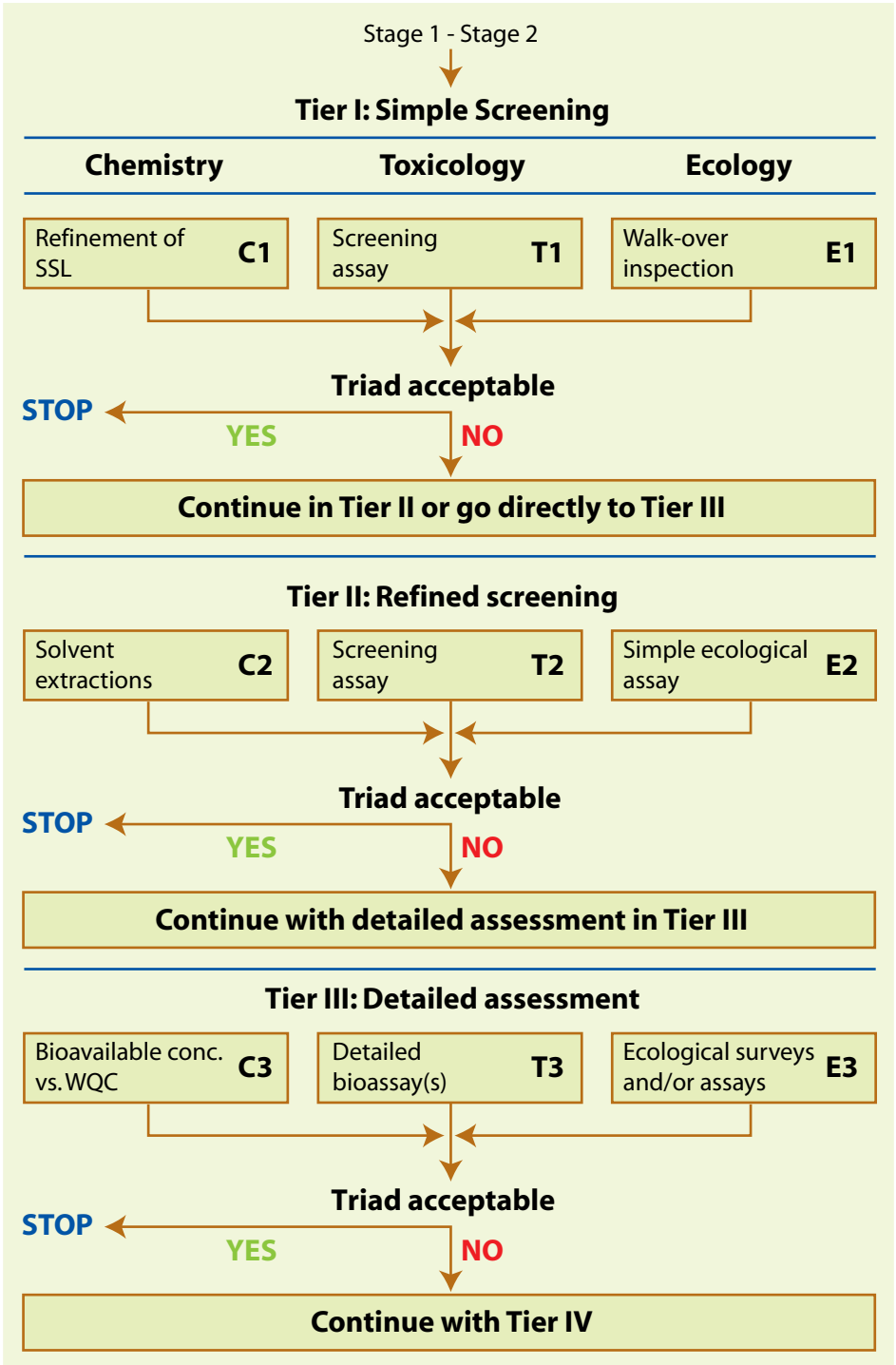

Figure 5.1 Flowchart for the decision making in the screening assessment (Tier 1 and 2) and detailed assessment (Tier 3 and 4) of contaminated sites. Relevant toolboxes (Chapter 6) are indicated in bold.

The actual performance of the risk assessment and use of the various tiers may be very site-specific. For example the land-use may be so sensitive, e.g. nature reserve or national parks, that it is decided by all stakeholders that a detailed assessment is needed in all cases, and so no initial screening is undertaken. Alternatively, the outcome of the simple screening may be so conclusive that it is decided to be more efficient to go directly to a more detailed assessment instead of performing a refined screening in between. Finally, the outcome of the screening may indicate that the planned land-use, e.g. parks or gardens, will not be feasible, whereas the ecological services may be sufficient for a less sensitive land-use such as industrial activity. In that case it may be decided to change the land-use instead of validating the outcome of the screening tests by spending more money performing a more detailed assessment.

In any case, after each Tier it is recommended to reconsider the conclusions made in stage 1 and 2 of the ERA process, i.e. consider the land-use and the ecological services associated with this, before continuing the site specific risk assessment in a higher tier.

The various tiers are presented in more detail below. As the entire DSS is based on the Triad approach, i.e. creation of three lines of evidence (LoE) covering information from chemistry, toxicology and ecology, the various LoE are presented separately for each Tier. The various tools are organised in toolboxes in Chapter 6. Here more details are found regarding the tests themselves as well as on the consideration behind including the tests in specific tiers. The toolboxes include

- Toolbox C1. Chemistry tools for simple screening.
- Toolbox T1. Toxicology tools for simple screening.
- Toolbox E1. Ecology tools for simple screening.

- Toolbox C2. Chemistry tools for refined screening.
- Toolbox T2. Toxicology tools for refined screening.
- Toolbox E2. Ecology tools for refined screening.

- Toolbox C3. Chemistry tools for detailed assessment.
- Toolbox T3. Toxicology tools for detailed assessment.
- Toolbox E3. Ecology tools for detailed assessment.

- Toolbox IV. Various tools for the final (Tier 4) assessments.

5.2.1 Tier 1. Simple screening

After deciding in the two first stages of the ERA (Chapter 3, section 3.3 and 3.4) that ecological concern needs special consideration, the risk assessment starts typically with a simple evaluation at the screening level. This is done in order to minimise costs until new information indicates the need for further assessment and more sophisticated studies. Therefore, the tools used in the first screening need not only to be reasonably quick and easy, but also relatively cheap.

The tools for use in Tier I are described in more detail in the toolboxes C1, T1 and E1 (Chapter 6).

Chemistry
At the very first stage of the Triad, total concentrations of all relevant chemicals are used (Chapter 2) in order to evaluate the need for conducting a site-specific assessment. Total concentrations of the contaminants are individually compared to soil screening levels (SSL), also known as soil screening values, soil quality objectives or criteria target values etc.

In the screening phase of the ERA the generic SSL is tailored to a more site specific need as described in more details in Toolbox C1. This is for example done by deriving new site-specific benchmarks or by calculating the potentially fraction of affected species (PAF).

Toxicology
Select one of the tools from Toolbox T1. The main objective of the selected bioassay should be to screen the soil samples for presence of toxic compounds. This includes toxic degradation products or compounds, which are not routinely included in various national analytical programs for contaminated sites.

Ecology
Select one of the tools from Toolbox E1. The main objective of the ecological studies at this level should be to get a quick and first impression of the ecological structure and functioning of the soil, i.e. is there any visible damage or may the overall functioning be hampered?

Stop or continue?
On the basis of the results of instruments used in Tier 1 it is decided to either stop further assessment or continue to a higher tier. This may be as a more refined screening (Tier 2) or if it is considered to be more cost-effective as a more detailed assessment (Tier 3).

A continuation of the assessment is normally recommended unless the outcome of the Triad is acceptable (see Chapter 4 for a discussion on the interpretation of Triad results). However, it should always be considered whether more detailed studies are likely to change the conclusion from the previous Tier(s). It could be more efficient, on the basis of the information already available, to either change the land-use or initiate a remediation of (parts of) the site.

5.2.2 Tier 2. Refined screening

Tier 2, still considered being at the screening level, aims at refining the measurement of exposure and at the same time to provide further insight into the toxicological and ecological properties of the contaminated soil.

Tier 2 deviate from the conservatism normally associated with the use of total concentration in the risk assessment by taking (rough) estimations of bioavailability into consideration in the chemical LoE. A better screening of the toxicological and ecological properties of the soil compensates for the reduced conservatism in the Chemistry LoE of the Triad.

The tools for use in Tier 2 are described in more details in the toolboxes C2, T2 and E2 (Chapter 6).

Chemistry
Select one of the tools from Toolbox C2. The main objective of the selected instruments in Tier 2 is to obtain a (rough) estimate of the exposure concentration of contaminants found at the site and to make the site-specific exposure estimation and the exposure situation found in most laboratory studies more comparable (see Chapter 2).

Toolbox C2 contains a number of non-exhaustive extraction procedures with the similar aim to give a closer estimate of the actual exposure, i.e. the bioavailable fraction, rather than the total concentration. The risk assessment in Tier 2 is still on a screening level. The extraction procedures should therefore give a more realistic estimate of the actual exposure compared to total concentrations, but at the same time be relatively conservative as only limited information about toxicological and ecological properties of the soil is obtained in this phase of the ERA.

The extracted concentration (i.e. mg chemical kg^{-1} dry soil) is compared to the SSL and the result is used in the Triad after scaling.

Toxicology
Select one of the tools from Toolbox T2. The main objective of the selected bioassay at Tier 2 should be to screen the soil for presence of toxic compounds and to give further insight into the toxicological properties of the soil. By adding a toxicological screening test to the one used in Tier 1, the uncertainty in the assessment is reduced.

Ecology
Select one of the tools from Toolbox E2. The main objective of the ecological studies at this level should be to get a quick and first impression of the ecological structure and functioning of the soil, i.e. is the overall functioning or structure of the soil hampered?

Stop or continue?
On the basis of the results in Tier 2 a decision should be made to either stop further assessment or continue to a higher Tier. A continuation of the assessment is normally recommended unless the outcome of the Triad is acceptable (see Chapter 4 for a discussion on the interpretation of Triad results). However, it should always be

considered whether more detailed studies are likely to change the conclusion from the previous tiers. It could be more efficient, on the basis of the information already available, to either change the land-use or initiate a remediation of (parts of) the site.

5.2.3 Tier 3. Detailed assessment

The tools in Tier 3 differ from the ones used in Tier 1 and Tier 2 in that they are more laborious, costly and may take longer. On the other hand they are (often) more realistic and/or ecological relevant in order to give a more comprehensive assessment of the ecological risk at the specific site.

The stakeholders should beforehand negotiate a minimum set of tests. Is it for example necessary to consider all trophic levels in the toxicological and ecological LoE? Or does the land-use suggest otherwise? Is it necessary (or possible) to estimate the bioavailability of all the substances exceeding their SSL? If not, how are the non-investigated substances dealt with?

The tools described for use in Tier 3 are described in more details in the toolboxes C3, T3 and E3 (Chapter 6).

Chemistry
Tools from Toolbox C3 are selected. Common for the methods found in Toolbox C3 is that they all aim at estimating the freely dissolved and/or easily desorbing fraction of contaminants either by a non-depleting or a depleting extraction technique. They are therefore useful for assessing the bioavailable fraction of pollutants in historically contaminated soils. For further insight to the problems about ageing and assessment of bioavailability please see Chapter 2.

The various methods have different strengths and weakness depending on the substances in question. None of these tools exist currently as international guidelines. The original papers or laboratories with practical experience must therefore be consulted. This book gives some recommendations and suggestions. However, this may be disputed and more information will be generated over time on this relatively new research topic, which may change the conclusions presented here. Negotiations between stakeholders must therefore take place before starting the experiments.

The estimated pore water concentration (µg L^{-1}), i.e. freely dissolved or extracted concentration, is compared to water quality standards (see for example Text Box 5, Chapter 4).

Toxicology
The tools used in the detailed assessment of the Triad (Tier 3) are normally long-term studies focusing on chronic endpoints like reproduction and growth or more specific mineralisation processes. Depending on land-use, a suite of bioassays from Toolbox T3 should be selected. Such suites of bioassays could be:

Sensitive land-use:
Growth study with one or more plant species, reproduction tests with one or more soil invertebrates and microbial activity measured as specific N- or C-mineralisation, e.g. potential ammonium oxidation.

Agricultural land-use:
Growth study with one or more crop species, earthworm reproduction test and microbial activity measured as specific N- or C-mineralisation, e.g. potential ammonium oxidation.

Industrial land-use:
Plant growth study with one common grass species and a soil induced respiration (CO_2 production) test.

Ecology
Tools from Toolbox E3 or comparable studies are selected. The main objective of the ecological studies conducted at this stage of the risk assessment is to get a more detailed insight to the possible impact of contamination on the populations and communities of fauna and flora at the site. This may include changes in diversity and populations of e.g. earthworm, microarthropod, nematode and/or plant communities or development of pollution induced tolerance of the microbial community. Typically these kinds of surveys are more time consuming and may require specific knowledge not always present at e.g. private consultants. Expertise, specialist and good planning are therefore necessary in order to reach firm conclusions. Special attention should be paid to:

- Finding a suitable reference site or reference situation.
- Finding information about the natural variation and fluctuation of populations in similar ecosystems in order to optimise the sampling strategy.

It is not likely that the survey will lead to firm conclusions unless it is possible to find a reference site resembling the contaminated site in at least the most critical parameters and there is sufficient resources to collect and perform a taxonomically characterisation of the adequate number of samples.

Stop or continue?
Depending on the results from Tier 3 a decision should be made to either stop further assessment or continue with an even more detailed assessment in Tier 4. However, it should always be considered whether more detailed studies are likely to change the conclusion from the previous Tiers. It could be more efficient, on the basis of the information already available, to either change the land-use or initiate a remediation of (parts of) the site.

5.2.4 Tier 4. Final assessment

In Tier 4, the aim of the studies is to answer any remaining questions and to decrease existing uncertainties and this may often require more in-depth research. Tools in Tier 4 can be similar to tools of Tier 3, but more focus has to be on site-specific circumstances. For example bioassays should be done with organisms, which normally occur at the site. Furthermore, it may be more relevant to consider ecological effects outside the contaminated area on e.g. predators or herbivores feeding in the area or effects in adjacent fresh water systems.

This Tier requires specialised knowledge and experience with ERA, which implies that costs can be high and only a limited number of people may be able to perform the tests. Generally only on a very limited number of site evaluations will include investigations at this level. The solutions and choice and design of investigations should therefore be done on a case-by-case basis. No detailed guidance is therefore given in this book. However, a short list of examples on Tier 4 investigations is presented below.

Chemistry
TP calculations can be made with key or target species or with dominating groups of species at the site.

Long term bioaccumulation studies, determination of bioconcentration factors, and monitoring data from biota or even target organs of biota can be collected in order to model food web effects and dispersion of the pollutants.

Toxicology
Bioassays can be performed with organisms collected from the site. For instance several species of earthworms can be used in a reproduction test. Endpoints in bioassays in Tier 4 have to be sensitive (like reproduction and growth) and long term exposure covering for example a complete life-cycle (or even life-cycles) gives good measure of possible effects on population level.

Mesocosm studies
Contaminated soil from the site can be collected in larger containers in order to expose several plant and animal species simultaneously in the laboratory or in the open over a longer period of time (weeks/months).

Ecology
Vegetation or fauna surveys can be conducted every season for a successive number of years.

Survey of the food transfer of pollutants can be investigated (e.g. earthworm ➔ small bird (or mammal) ➔ predatory bird).

Site remediation

Stop or continue?

If the results of Tier 4 still indicate risk there are basically two possible solutions. Accept the risk and leave the contamination or remove (parts of) the contamination. Both involve risk management options and are not therefore discussed further in this book.

CHAPTER 6 A TRIAD-BASED SELECTION OF TOOLS FOR SITE-SPECIFIC ASSESSMENT OF ECOLOGICAL RISK

Jensen J., Sorokin N., Dirven-van Breemen E.M., Bogolte T., Erlacher E., Ehlers C., Ter Laak T., Hartnik T., Bierkens J., Rutgers M. and Mesman M.

6.1 Triad based selection of methods

For each of the three Lines of Evidence (LoE) in the Triad various methods or tools are available. Table 6.1 gives an overview of some of the methods and tests that are available and have been used in ecological risk assessment. Table 6.1 is not exhaustive, but gives a reasonable overview of the current situation. On the basis of this list a number of the most commonly used tests have been selected as the most appropriate tests for conducting an ERA. This chapter gives an overview of some of these tools.

In order to facilitate the selection of appropriate tools in the right context, the tools have been compiled in subclasses or toolboxes. Each of these is a collection of tools considered to be potentially useful in the designated tiers and LoE of the Triad, i.e. chemistry, toxicology and ecology. Furthermore, the tools are arranged according to their complexity, price and practicability or in other words depending on whether they are most useful for screening or detailed assessment, i.e.

- Toolbox C1. Chemistry tools for simple screening.
- Toolbox T1. Toxicology tools for simple screening.
- Toolbox E1. Ecology tools for simple screening.
- Toolbox C2. Chemistry tools for refined screening.
- Toolbox T2. Toxicology tools for refined screening.
- Toolbox E2. Ecology tools for refined screening.
- Toolbox C3. Chemistry tools for detailed assessment.
- Toolbox T3. Toxicology tools for detailed assessment.
- Toolbox E3. Ecology tools for detailed assessment.
- Toolbox IV. Various tools for the final (Tier 4) assessments.

6.2 Overview of toolboxes

A number of the most commonly used tests have been selected as the most appropriate tests for conducting an ERA. The selection, and thereby also the toolboxes, may be challenged as time and development will expand our insight and (hopefully) lead to new tests and abandon of old ones. The various toolboxes may therefore be expanded and/or reduced according to the discussion and negotiation between stakeholders.

Table 6.1 Selected tools and their potential application in various land-uses.

Triad LoE							
Indicator (instrument)	**Tier**	**Nature**	**Agri-cultural**	**Parks etc.**	**Garden - food**	**Garden**	**Indus-trial**
Chemistry							
Site-Specific benchmarks	1	•	••	••	••	•••	•••
Toxic pressure (TP), combi-PAF	1	••	••	••	••	•••	•••
Toxic pressure, multi-substance PAF	1-2	••	••	••	••	••	•••
Toxic pressure, specific target species PAF	1-2	•••	••	••	••	•	•
Toxic pressure, specific soil functions PAF	1-2	•••	•••	••	•••	••	•
Modelling bioavailability	3-4	•••	••	••	•••	•	•
Modelling bioaccumulation	3-4	•••	••	•	•••	•	•
Modelling effects on populations	4	•••	••	•	•	•	•
Bioaccumulation measurements	4	•••	•••	•	•••	•	•
Toxicology							
Microtox (elutriate)	1	••	••	•••	••	•••	•••
Rotoxkit (elutriate)	1	••	••	•••	••	•••	•••
PAM-algae test (elutriate)	1	••	••	•••	••	•••	•••
Emergence test with plant seeds	2	•	•	•	•	•	••
Earthworm: avoidance test, acute toxicity test	2	••	•••	•••	•••	•••	•••
Plant growth test	3-4	•••	•••	••	•••	•••	••
Nematodes: survival, growth, reproduction	3-4	•••	•••	••	••	••	•
Enchytraeids: growth, reproduction	3-4	•••	•••	••	••	•	•
Earthworms: growth, reproduction	3-4	•••	•••	••	••	••	•
Snails: reproduction	3-4	••	••	••	••	•	•
Isopods: survival, growth and reproduction	3-4	•••	••	••	••	•	•
Springtails: survival and reproduction	3-4	•••	••	••	••	•	•
Mites: survival, growth and reproduction	3-4	•••	••	••	••	•	•
Ecology							
Floristic survey	1-4	•••	••	••	••	•	•
Micro-organism: C- and N-mineralisation	2	•••	•••	••	••	••	••
Bait-lamina: feeding activity	2	••	••	••	••	••	•••
Substrate Induced Respiration (SIR) test	2	•	•	••	••	••	•••
Nematodes survey	3-4	•••	•••	••	••	•	•
Micro-organisms: number and biomass	3-4	•••	•••	••	••	••	••
Micro-organisms: Specific syntheses rate	3-4	•••	•••	•	•	•	•
Earthworms survey	3-4	•••	•••	•••	•••	••	•
Fauna: survey (butterflies, birds, mammals)	3-4	••	••	••	••	•	•
Micro-organisms: genetic diversity	3-4	•••	•••	••	•	•	•
Micro-organisms: metabolic diversity (Biolog)	3-4	•••	••	••	•	•	•
Enchytraeids: survey	3-4	•••	••	••	••	•	•
Microarthropods survey	3-4	•••	•	••	••	••	•
Decomposition (litterbag method)	2-3	••	••	•	•	•	•
Decomposition (wheat straw method)	2-3	••	••	•	•	•	•
Decomposition (cotton strip method)	2-3	••	•	•	•	•	•
PICT micro-organisms	3-4	••	••	••	••	••	••

••• Highly applicable; •• Reasonably applicable; • Moderately applicable.

Any choice of method should be well documented and motivated and it should reflect the desired land-use and the ecological services associated with this (see Chapter 3). It is therefore recommended to include experts from the various fields of research in the discussion.

The intention is not to describe all selected tools in details. Instead a useful review is presented together with references to documents where more details can be obtained. Emphasis is instead on identifying some of the advantage and some of the drawback of each of these tools. This can hopefully be a valuable help for the risk assessors in their selection of tools. Tools should be tailored to the land-use and the selection of tools may hence vary depending on the current or the future land-use. The right tools for the right job, no more - no less, is the key issue when selecting. Aspects like reproducibility, sensitivity, costs, performance, time frame, applicability and ecological relevance are, among others, important issues to consider.

The tools listed below and presented later in this chapter are the outcome of a selection made by the authors. It is partly based on already existing practise in national frameworks and partly on the personal experience and knowledge of the authors. The selection of tools is in many cases limited to the ones most commonly used in ERA. However, what is most common vary between countries and regions and may depend on the expertise available for a specific ERA. Alternative methods may be included in the various steps as long as it is scientifically justified, motivated and agreed upon by all stakeholders.

6.3 Toolbox C1 (Chemistry tools for screening)
- Refinement of soil screening levels.
- Calculation of toxic pressure.

6.4 Toolbox T1 (Toxicology tools for screening)
- Acute luminescent bacteria test (Microtox®).
- Chronic luminescent bacteria test.
- Invertebrate toxicity kits, e.g. the Ostracod test.

6.5 Toolbox E1 (Ecology tools for screening)
- Ecological screening by a simple survey.

6.6 Toolbox C2 (Chemistry tools for refined screening)
- Selective organic solvent extractions.

6.7 Toolbox T2 (Toxicology tools for refined screening)
- Earthworm acute test.
- Invertebrate avoidance test.

6.8 Toolbox E2 (Ecology tools for refined screening)

- Bait lamina test.
- C and N mineralisation tests.
- Soil induced respiration test.

6.9 Toolbox C3 (Chemistry tools for detailed assessment)

- Solid phase micro extraction (SPME).
- Tenax extraction.
- Cyclodextrin extraction.

6.10 Toolbox T3 (Toxicology tools for detailed assessment)

6.10.1 Solid phase bioassays

- Plant growth test.
- Earthworm reproduction test.
- Springtail reproduction test.
- Enchytraeid reproduction test.
- Microbial metabolic diversity tests, e.g. BIOLOG.

6.10.2 Liquid phase bioassays

- Algae test.
- Aquatic plant test, e.g. *Lemna minor*.
- *Daphnia* tests.

6.11 Toolbox E3 (Ecology tools for detailed assessment)

- Higher tier assessment of the impact on biological activity and organic matter breakdown.
- Higher tier assessment of the impact on the microbial community.
- Higher tier assessment of the impact on the plant community.
- Higher tier assessment of the impact on the soil invertebrate community.

6.12 Toolbox for Tier IV

- Accumulation in biota.
- Sequential supercritical fluid extraction (SSFE).

6.3 Toolbox C1. Chemistry tools for simple screening

At the very first stage (Stage I, Chapter 3) of the ERA process, total concentrations of all relevant chemicals are individually compared to soil screening levels (SSL) in order to evaluate whether there is a need for a site specific assessment of ecological risk. In the current Stage III of the ERA, this first generic evaluation of risk is followed by a more site-specific screening of risk including information from all three lines of evidence in the Triad. In the Chemistry part of the Triad more site-specific information is collected by:

- Refining and targeting the comparison of soil concentrations with soil related benchmarks for site-specific purposes.
- Incorporation of the accumulative risk of a mixture of contaminants by calculating the toxic pressure (TP) of a mixture and by doing so generating more site-specific insight to the potential ecological impact of a contaminated site.

Each of these steps can be done separately or in combination, e.g. the TP can be calculated using existing SSL or using new developed benchmarks based on either NOEC or EC_{50} values or site-specific benchmarks can be compared to soil concentrations individually. The approach entirely depends on the strategy taken by the stakeholder group and the availability of data.

6.3.1 Site-specific modification of soil screening levels

Typically national soil screening levels, also known as soil screening values, soil quality objectives or criteria, target values etc., are used in Stage I if available. If national SSL are not available, e.g. for some of the contaminants found at the site, SSL from other countries may be used. A number of countries have developed national SSL, e.g. the Netherlands, Canada, Germany, USA.

Below is described a set of possibilities that enables the risk assessors, in agreement with the stakeholders and authorities, to develop new SSL or to tailor the existing SSL to more site-specific purposes.

To avoid misinterpretation such site-specific criteria for assessing ecological risk is named benchmarks in this DSS. Benchmarks should not be considered as generic soil screening levels, as they are derived for site-specific use only. They are hence not necessarily applicable at all contaminated sites. It is important to distinguish between nationally accepted generic criteria applicable at all sites and these more case-by-case derived benchmarks. Nevertheless in some countries or regions development of benchmarks may not be acceptable al all since this may open up for an undesirable difference in the initial evaluations of ecological risk between various provinces or local communities.

Site-specific benchmarks
Some of the existing SSL were developed many years back and new information may now be available for a recalculation of the SSL. Substances found at the site may not even have a corresponding SSL, as data can have been unavailable when the SSL were published. In these cases, new benchmarks can be derived provided that (enough) ecotoxicological data is available and there is acceptance from the authorities to use such “case-by-case” derived benchmarks. Benchmarks are not directly comparable to generic soil screening levels, as they are derived for site-specific use only.

For some groups of chemicals, a default set of chemicals may routinely be chosen for ecological risk assessment, although the original selection was based on e.g. risk to

humans. This may for example include highly volatile substances or compounds with carcinogenic properties. One example of this could be the set of PAHs routinely used for risk assessment in many countries, e.g. the 16 US-EPA PAHs. Such sets of PAHs (sum of PAHs) normally include substances poorly associated with ecological risk, e.g. benzo(a)pyrene and other high-molecular weight PAHs. In some cases it could target the site-specific investigation if attention is limited to substances relevant for ecological risk. See e.g. Jensen and Sverdrup (2003), Kapustka (2004ab) or Maliszewska-Kordybach (2004) for a discussion about soil screening levels for PAHs.

A few ecotoxicological databases with public access can be found. The US-EPA has collected a large number of ecotoxicological data for numerous substances in the ECOTOX database. The database are public available via the homepage of the US-EPA. The RIVM institute in the Netherlands has also compiled a large set of ecotoxicological data in the database named "e-toxBase" (Wintersen et al., 2005), which can be used when deriving new benchmarks. This database is available for the public at: www.e-toxBase.com.

Using EC_{50} values in the calculation of site-specific benchmarks

The Weigh of Evidence approach should ideally address only independent Line of Evidence (LoE). However, the outcome of the various LoE should enable a direct comparison of results across LoE in order to reach a final decision for the site (Rutgers and Den Besten, 2005). The key to this is a suitable scaling method to project accurately the results from the three LoE into one integrated risk number. Theoretically, with an infinite amount of information for all three LoE in the Triad (chemistry, toxicology and ecology), uncertainty is minimised and the differences between the outcome of the individual LoE should hence disappear.

In order to apply and compare the results for benchmarks (chemistry LoE) and bioassays (toxicology LoE) in the Triad, it may be necessary to evaluate the scaling process. SSL are normally derived from NOEC values e.g. by using Species Sensitivity Distributions (SSD) (Posthuma et al., 2002). For most land-uses, intervention is often based on the estimations of significant impact, e.g. the level corresponding to 5 or 50% of the species exposed above their NOEC value (HC_5 or HC_{50}). When calculating the total toxic pressure (see 6.3.4) for the kind of mixtures typically found at severely contaminated sites, it becomes obvious that using NOEC values to calculate SSL will not produce information, which is optimally distributed on the integrated effect scale running from 0 to 1. In other words even moderate levels of contaminants will, when considered together, typically result in integrated risk values close to 1. For these reasons it may be suggested to use EC_{50} or LC_{50} values instead of NOEC values to calculate the combined effect of all the contaminants found at the site.

Another argument for the use of EC_{50} or LC_{50} values in the derivation of site-specific benchmarks is to be found in the other LoE of the Triad. In comparison to standard toxicity testing natural variations are normally significantly higher in field surveys (Ecology LoE) and most bioassays (Toxicology LoE). It is therefore often practically or economically unfeasible to include sufficient number of replicates to identify effects at the NOEC level, i.e. statistically different from the control, whereas this is done

routinely in the ecotoxicity studies used for SSL or benchmark calculations (Chemistry LoE). Effects in field studies or bioassays are instead interpreted in distinct terms, e.g. percentage of inhibition compared to a reference soil. These observations are often congruent with marked effect levels, e.g. 50% effect. From a theoretical point of view, the Ecology and Toxicology LoE at severely contaminated sites should therefore regularly provide equivalent answers to the Chemical LoE when EC_{50} and LC_{50} values are used instead of NOEC values to calculate site-specific benchmarks. This will result in risk numbers, which are projected more optimally at a scale running from 0 (no effects) to 1 (full effects of the pollution) (See Chapter 4 for more details on how to scale results).

6.3.3 Calculating the potentially affected fraction of species (PAF)

SSL are calculated by various mathods (e.g., the lowest of a set of NOELs divided by a safety factor), or by the use of species sensitivity distributions, e.g. by estimating the maximum concentration which potentially affects a predefined fraction of the species, e.g. 5%, also referred to as the HC_5 or the hazardous concentration affecting 5% of species (see more details in e.g. Posthuma et al., 2002). It may be very useful in a site specific risk evaluation to shift the focus from estimation of the "protective" concentration, which affects a predefined fraction of all species (SSL or HC_5), to estimation of the fraction of species potentially affected by a (set of) site specific soil concentrations (PAF) (Figure 6.1). The benefits of doing so may be many. For example:

- Whereas a comparison of local soil concentrations to a SSL gives a "good" or "no-good" judgement, the PAF estimation may provide the assessor with a better insight of the magnitude of the problem, since it is scaled from zero to one, with the scale proportional to probable ecological impact (fraction of species affected).
- The PAF may be calculated for various trophic levels, i.e. micro-organisms and soil function characteristics, plants and soil invertebrates, or even on sub-groups of these if enough data is available.
- Acceptable PAF can be defined to judge severity of impact for different land-use classes. In other words: where a maximum of 5% of all potentially affected species may be acceptable for natural areas, it may be considered acceptable that 50% of soil invertebrate and plant species are potentially affected at industrial areas as long as essential microbial processes are protected. Preferably the stakeholders should negotiate the set of criteria for acceptable PAF before initiating the data collection and calculations. Or nationally such differentiated trigger values for toxic pressure in this respect could be established or be defined based upon current PAF-levels calculated from pertinent monitoring data of such sites.

Site-specific PAF values can, just like site-specific benchmarks, be generated by the use of EC_{50} values instead of NOEC values. Using NOEC values to calculate combi-PAF (the PAF of a chemical mixture as calculated assuming dissimilar TMoA's for all compounds) at severely contaminated sites will not produce information, which is optimally distributed on the integrated effect scale running from 0 to 1. Pollutants

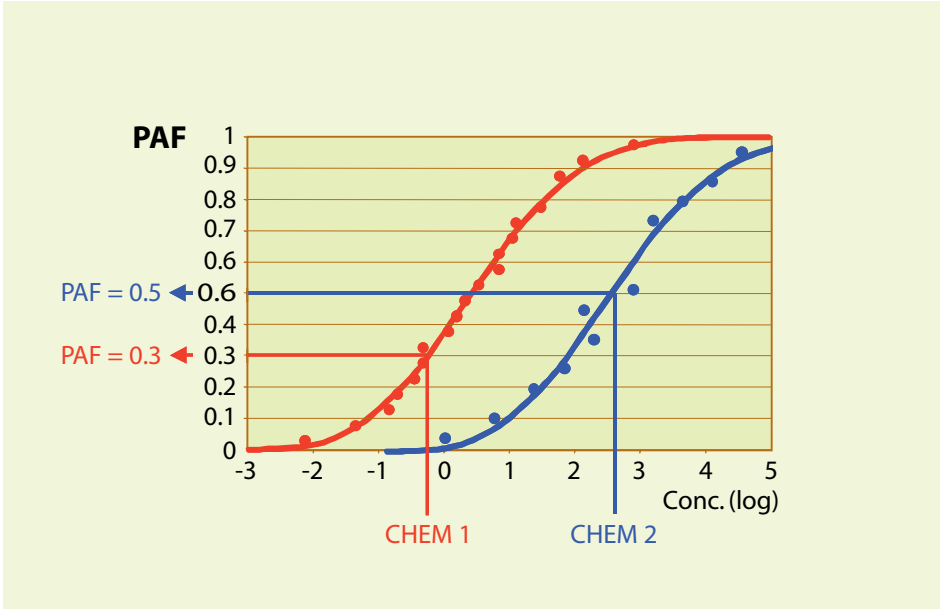

Figure 6.1. The concentration in soil can be used to assess the Potentially Affected Fraction (PAF) of species in ecosystems, e.g. for chemical one the estimated PAF is 0.3, and for chemical two it is 0.5. The combi-PAF (assuming different toxic modes of action) is calculated as 1- (1-0.3)(1-0.5) = 0.65.

tend to be present in mixtures and by adding all individual PAF values, the final PAF of the mixture, combi-PAF, based on NOELs (see 6.3.4), regularly approaches the value of 1 even at moderate polluted sites. Calculation of combi-PAF based on EC_{50}s would provide better discrimination and ranking across a set of moderately to highly contaminated sites. Moreover, it would provide a better ecological impact quantification, since acute effects beyond the 50% level for a certain proportion of species would be clearly visible anyway.

The RIVM institute in the Netherlands has developed a software programme (ETX2.0), which can calculate normal distribution based hazardous concentration and fraction of species affected (Van Vlaardingen et al 2004). These are useful for derivation of SSL and PAF. This software, which is a companion-software to the theoretical papers of Aldenberg and Jaworska (2000) and Aldenberg and Luttik (2002), is available for non-commercial purposes from RIVM free of charge (Van Vlaadingen et al., 2004). Furthermore, more relevant information about how to calculate SSL can be found in e.g. Wagner and Løkke (1991), Aldenberg and Slob (1993) and Posthuma et al. (2002). A graphic example of a PAF calculation is shown in Figure 6.1.

6.3.4 Toxic pressure of contaminant mixtures

To calculate the toxic pressure (TP) of a mixture of contaminants different methods can be used. The methods are all based on the same principle, i.e. the use of toxicity

data from laboratory tests to predict ecosystem effects at a contaminated site, but the different methods have different data requirements.

The PAF method

The field concentration of a contaminant can be used to calculate the toxic pressure of the compound, expressed by the parameter PAF at the Y-axis from a Species Sensitivity Distribution (SSD) (Figure 6.1). Once the PAF values for all locally occurring contaminants are known, the combi-PAF or the multi-substance PAF method can be used to quantify the overall toxic pressure of the whole mixture of contaminants (see e.g. De Zwart and Posthuma, 2005 for more details). The combi-PAF and ms-PAF methods are conceptually different, since the former assumes different TMoA's for almost all compounds (formally except for narcotic compounds, for which concentration addition is assumed) and the latter assumes sub-groups of compounds in the local mixture that share the same TMoA. In both methods, response additivity is applied to aggregate single-compound PAFs to the aggregate parameter, but only in the ms-PAF-method, this is preceded by concentration addition based sub-aggregation within the sub-groups of compounds. In short this is done as described below.

- Concentration addition (CA) is applied to contaminants, which have the same toxic mode of action in order to aggregate a single PAF (PAF_{CA}) for all contaminants (see Text Box 6, Chapter 4).
- Response Addition is then applied to the set of PAF_{CA}-values (one value for each toxic mode of action). The aggregated value corresponds then to the final combined fraction of species potentially affected at the site, i.e. ms-PAF.

In the case of the combi-PAF method, all compounds are assumed to have different toxic modes of action, which reduces this process to the application of the response addition model to all compounds seperately. To use this method, sufficient toxicity data must be available for all contaminants measured.

The SSL method

A second method to calculate the toxic pressure (TP) is by means of available Soil Screening Levels or Soil Quality Criteria like for example the Eco-SSL from the US-EPA. These criteria can be derived from SSD's, like for example the Dutch SRCeco values, which represent the HC_{50} concentration from the SSD-curve. In cases of insufficient toxicity data, SSL can be derived by the use of assessment factors (also known as safety factors) (Traas, 2001). An example how to calculate TP from SSL or similar standards is shown in Text Box 7, Chapter 4. Again one can distinguish (when possible) between chemicals with the same toxic mode of action (concentration addition) and groups of substances with different toxic mode of action (response addition) (see above). Please note that the algorithms of combi-PAF and ms-PAF calculations do differ substantially, both regarding concepts, mathematics and data needs. However, the numerical values of combi-PAF and ms-PAF for a case study may be quite similar, especially when the slope factors of the SSDs are moderate (beta approxemately 0.4).

6.4 Toolbox T1. Toxicology tools for simple screening

The main objective of the selected toxicity tests or bioassay at Tier 1 should be to screen the soil for presence of toxic compounds. This includes toxic degradation products or compounds, which are not routinely included in various national analytical programs for contaminated sites. This Tier is the first screening level of the ERA and the cost in form of manpower and money should hence be relatively low.

Equipment for measuring luminescence of Vibrio fischeri

Acute luminescent bacteria test (Microtox®, inclusive the solid phase test).

Description

The inhibitory effects of potentially contaminated soil elutriates on bacterial luminescence are measured using the marine bacteria *Vibrio fischeri*. Bacteria are added to a suspension of the test sample (typically pore water or leachate). The mixture is then filtered using a device supplied with the kit. The bacteria produce light as a by-product of metabolism and this is measured using a Microtox photometer analyser. The median inhibitory concentration (EC_{50}) is determined after 5, 15 or 30 minutes, relative to a control. In the solid phase test, bacterial populations are exposed for a period of 5-30 minutes to a suspension of the solid test substance. Results measure a change in rate of a continuous response by changes in light emission from the supernatant.

References

- ISO 11348. 1998. Water quality – Determination of the inhibitory effect of water samples on the light emissions of *Vibrio fischeri* (Luminescent bacteria test). Part 1-3, ISO, Geneva, Switzerland.
- Dorn, P.B., Vipond, T.E., Salanitro, J.P., Wisniewski, H.L. 1998. Assessment of the acute toxicity of crude oils in soils using earthworms, Microtox®, and plants. Chemosphere 37:845-860.
- Doherty, F.G. 2001. A review of the Microtox (R) toxicity test system for assessing the toxicity of sediments and soils. Water Quality Research Journal of Canada 36:475-518.
- Environment Agency. 2004. Biological Test Methods for Assessing Contaminated Land. Stage 2 – A demonstration of the use of a framework for the ecological risk assessment of land contamination. Environment Agency Science Report P5-069/TR1, United Kingdom. http://publications.environment_agency.gov.uk.

Limitations

- Specific equipment such as luminometer and cooling device is required.
- The test is based on photometric measurement and may hence be influenced by colour and turbidity of the sample.
- The test species isolated from a marine animal.
- Low sensitivity to some PAH, and pesticides with specific modes of action.

Benefits

- Rapid and cheap test.
- Well defined test protocol.
- Repeatable and reproducible results.
- Respond with sensitivity to a wide spectrum of contaminants.
- Solid phase test with aqueous suspensions of soil is more sensitive than tests with aqueous leachates.
- Generally an efficient screening tool.

Chronic luminescent bacteria *(Vibrio fischeri)* test.

Description
Inhibitory effects of potentially contaminated soil elutriates on bacterial luminescence (and growth) are measured after chronic exposure. The bacteria used are *Vibrio fischeri* and the exposure duration is 22-24 hours. Two test kits are available; Microtox measuring percentage bioluminescence inhibition and LUMIStox measuring bacterial growth.

References

- ISO 11348-1. 1998. Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) -- Part 1: Method using freshly prepared bacteria. ISO, Geneva, Switzerland.
- ISO 11348-2. 1998. Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) -- Part 2: Method using liquid-dried bacteria. ISO, Geneva, Switzerland.
- ISO 11348-3. 1998. Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria. ISO, Geneva, Switzerland.
- Guzzella, L., Mingazzini, M. 1994. Biological assaying of organic compounds in surface waters. Water Science and Technology 30:113-124.

Limitations
- Specific equipment such as luminometer and cooling device is required.
- The test is based on photometric measurement and may hence be influenced by colour and turbidity of the sample.
- The test species is isolated from a marine animal.
- Low sensitivity to some PAH, and pesticides with specific modes of action.

Benefits
- Rapid and cheap test.
- Well defined test protocol.
- Repeatable and reproducible results.
- Respond with sensitivity to a wide spectrum of contaminants.
- Solid phase test with aqueous suspensions of soil is more sensitive than tests with aqueous leachates.
- Generally an efficient screening tool.

Ostracod test.

Description
The 6-day Ostracodtoxkit F™ makes use of the neonates of the ostracod crustacean *Heterocypris incongruens* hatched from dormant eggs (cysts). The test gives information about mortality and growth inhibition of the test organism after 6 days of direct contact to undiluted contaminated soil.

References
The OSTRACODTOXKIT F™ is a registered trademark of MicroBioTests Inc., Nazareth, Belgium.

- Plaza, G., Nalecz-Jawecki, G., Ulfig, K., Brigmon, R.L. 2005. The application of bioassays as indicators of petroleum-contaminated soil remediation. Chemosphere 59:289-296.
- Blaise, C., Gagne, F., Chevre, N., Harwood, M., Lee, K., Lappalainen, J., Chial, B., Persoone, G., Doe, K. 2004. Toxicity assessment of oil-contaminated freshwater sediments. Environmental Toxicology 19:267-273.
- Chial, B.Z., Persoone, G., Blaise, C. 2003. Cyst-based toxicity tests. XVIII. Application of ostracodtoxkit microbiotest in a bioremediation project of oil-contaminated sediments: Sensitivity comparison with *Hyalella azteca* solid-phase assay. Environmental Toxicology 18:279-283.
- Latif, M., Licek, E. 2004. Toxicity assessment of wastewaters, river waters, and sediments in Austria using cost-effective microbiotests. Environmental Toxicology 19:302-309.
- Chial, B., Persoone, G. 2003. Cyst-based toxicity tests XV - Application of ostracod solid-phase microbiotest for toxicity monitoring of contaminated soils. Environmental Toxicology 18:347-352.

Limitations
- Limited practical experience from the terrestrial environment.
- Relevance of organism to soils may be questioned although sediment dwelling species most likely are better indicators than aquatic species.

Benefits
- The test was originally developed for direct contact sediment testing, but can be applied to soils.
- Available as a test kit, including the availability of standardised biological material.
- Total test performance time is short (2 hrs performance).
- Results compare to other invertebrate tests.
- Cheap and simple test.
- No need of “in-house” culture.

Various commercial invertebrate test kits.

Description
CERIODAPHTOXKIT F™: 24 hr acute toxicity test using the cladoceran crustacean *Ceriodaphnia dubia*. The test measures mortality after 24 hours.

THAMNOTOXKIT F™: 24 hr acute toxicity tests using the anastracan crustacean *Thamnocephalus platyurus*. The test measures mortality after 24 hours.

ROTOXKIT F™: short chronic test using the species *Brachionus calyciflorus*. Test measures mortality and reproduction after 24 and 48 hours, respectively.

PROTOXKIT F™: 24 hr chronic test measuring growth inhibition on the ciliate protozoan *Tetrahymena thermophila*. Test measures biomass.

References
Read more for example on the homepage of the Canadian company EBPI (Environmental bio-detection products Inc.). EBPI is specialised in development, manufacture and distribution of toxicity, genotoxicity and mutagenicity colorimetric bioassays. http://www.ebpi-kits.com/.

Limitations
- Little practical experience in terrestrial ERA.
- Relevance of using aquatic organisms for screening of effects to soils dwelling species may be questioned.

Benefits
- Available as commercial test kits.
- Fast and cheap tests.
- Adheres to USEPA and/or AFNOR Test Guideline.
- Sensitive to a wide range of toxic chemicals.

6.5 Toolbox E1. Ecology tools for simple screening

Ecological surveys or monitoring studies are generally considered a time consuming effort performed by experts. This is in most cases true, wherefore detailed surveys normally take place in higher tier assessment. However, in order to ensure that also ecological information is collected and used in the Triad already in the screening phase, it is recommended to perform a limited examination of the site.

A survey of the area with special focus on visible changes in e.g. plant cover or presence or absence of specific plants, trees or scrubs may indicate ecological damage, which can be associated to contaminants present at the site. If any aerial pictures are available from the area these may give valuable information about the plant cover also historically, which may be helpful in identifying parts of the site where the impact may be highest (hot spots).

At this stage the conclusion can in most cases only be indicative. Therefore if the results from the other line of evidence may cause any doubt or the survey indicated potential impact, it is recommended to either continue with a more refined screening in Tier 2 or go directly to the detailed assessment in Tier 3.

Simple survey of the site

6.6 Toolbox C2. Chemistry tools for refined screening

Selective solvent extraction

As described in Chapter 2 it may be considered useful to adjust the estimate of exposure by taking bioavailability into consideration and hereby deviating from the conservatism normally associated to the use of total concentration in the risk assessment. The principle in this refinement of the ecological risk assessment is to extract a more ecotoxicologically relevant fraction of the contamination than the total concentration. The latter generally tends to overestimate the risk of historically contaminated soils. In this screening phase no attempt is made to estimate the freely dissolved or readily bioavailable concentration of contaminants. Instead the fraction of the contaminants is extracted, which can be directly compared to the existing soil screening levels. This is considered to be a relatively simple and quick method to screen for potential risk of contaminants in a more realistic way than using total concentrations.

The extracted concentration (mg kg^{-1}) is compared to the SSL and the result used in the Triad. It is therefore a prerequisite of this comparison that the extractability in the tests (with spiked soils) used for deriving SSL is close to 100% by the methods used. In most short-term tests (< four weeks) it will be reasonable to assume that only little "true" ageing or strong sequestering occurs and hence a majority of the spiked chemicals are still extractable with mild organic solvents. However, for most methods this still has to be fully validated (see Chapter 2 for a discussion).

Table 6.2 Outline of principal studies that employed chemical extractants to evaluate bioavailability.

Contaminant (Ref)	Solvent	Bioassay	Operation	Comments
Atrazine Phenanthrene (1)	Methanol/water, n-Butanol	Earthworm uptake Degradation	25 ml extractant, 10 g solid. Shaking for 2 h	Methanol/water best predictor for atrazine, n-butanol for phenanthrene.
DDT, DDE, DDD PAH (mixture) (2)	THF Ethanol	Earthworm uptake	15-20 ml extractant, 1 g soil, 10 sec mixing	Good correlation with earthworm accumulation.
Anthracene, Fluoranthene, Pyrene (3)	n-Butanol Propanol Ethyl acetate	Plant retention Earthworm uptake Degradation	25 ml extractant, 1-2 g soil, 5 sec mixing	Reasonable correlation with bioassays.
Phenanthrene Pyrene Chrysene (4)	n-Butanol	Earthworm uptake Degradation	15 ml extractant, 5-10 g soil, Mixing: 5 sec (worm) or 120 sec (degradation)	Applicable for bioavailability prediction.

References: 1. Kelsey et al. (1997); 2. Tang et al. (1999, 2002); 3. Tang and Alexander (1999); 4. Liste and Alexander (2002)

Organic solvents most frequently used include methanol/water in different ratios, n-butanol, ethanol, propanpol, ethyl acetate and tetrahydrofuran (THF) (Table 6.2). The method establishes preferential partitioning of hydrophobic contaminants to the extractant by increasing their solubility in the aqueous phase whilst removing pollutant compounds from soil surfaces establishing equilibrium conditions.

No standard protocol has been adopted for mild chemical extractions in relation to bioavailability testing. Common methodology in literature primarily includes a soil sample to which a volume of chemical extractant is added (generally 1 – 10 g soil, 15 – 25 ml extractant). This is followed by a period for mixing, e.g. vigorous mixing for 10 – 120 seconds or shaking by orbital shakers for up to 2 hours.

The extraction studies have mostly involved PAH and insecticides (including DDT, DDE, DDD and atrazine) (Kelsey et al., 1997; Wahle and Kördel, 1997; Tang and Alexander, 1999; Tang et al., 1999, 2002; Liste and Alexander, 2002). Studies that have related extractability with results from bioassays have generally focused on uptake and accumulation (% taken up by earthworms or plants) and bacterial degradation (% removed). Therefore, since convincing relationships between the chemical and biological tests were found it may indicate a potential for such extraction methods to predict bioavailability.

6.7 Toolbox T2. Toxicology tools for refined screening

In the first simple screening of Tier I focus was on marine bacteria and aquatic/sediment living species. In Tier 2 relatively simple tests with soil dwelling species are used for a more refined screening of the soil samples, i.e. the earthworm survival tests and avoidance tests using soil invertebrates.

The habitat function of soils is often assessed using the reproduction test with *Eisenia fetida*. The avoidance test with *Eisenia fetida* is a suitable screening test, which is less cost-intensive in terms of duration and workload than the reproduction test, and at the same time (normally) more sensitive than the acute test with the same species.

Earthworm acute toxicity test.

Description

Test organisms used are *Eisenia fetida* (compost worm) or *Eisenia andrei* (red worm). The principle of the test involves determining the mortality of adult earthworms placed in potentially contaminated soil for a period of 7 or 14 days at a temperature of 20°C +/- 2°C.

References

- ISO 11268-1. 1993. Effects of pollutants on earthworms *(Eisenia fetida).* Part 1: Determination of acute toxicity using artificial soil substrate. ISO, Geneva, Switzerland.
- OECD 207. 1984. Guideline for testing of chemicals: earthworm, acute toxicity tests. OECD, Paris, France.
- Kula, H., Larink, O. 1998. Tests on the Earthworms *Eisenia fetida* and *Aporrectodea caliginosa*. In: Løkke, H., Van Gestel, C.A.M. (Editors) Handbook of soil invertebrate toxicity tests. John Wiley and Sons, Chichester, United Kingdom. pp 95-112.

Limitations

- *Eisenia fetida* is less ecological relevant as it is not a true soil-dwelling species, but a litter or compost-dwelling organism. Nevertheless, in most cases it has sensitivity comparable to truly soil dwelling earthworms.
- Needs specific equipment to maintain the temperature at 20°C +/- 2°C.
- Animals need to be sorted from soil before endpoint can be measured.
- The method does not take into account any volatilisation or degradation of pollutants during the test.
- Normally considered a relative insensitive test.

Benefits

- *Eisenia fetida* belongs to the composting worms and can be easily cultured in large quantities in the laboratory.
- Handling and breeding of worms are easier than more indigenous species like *Lumbricus terrestris* or *Aporrectodea caliginosa*.
- The test is cheap and relatively simple to perform.
- Standard methods and Quality Control procedures are published.

Soil invertebrate avoidance behaviour tests.

Description

The overall principle of the avoidance behaviour test, originally developed for earthworms, is that soil dwelling- or surface-living invertebrate species are exposed to contaminated and non-contaminated soil for a period. The soil is distributed in two-section chambers, which can be separated from each other by a removable split or divider. The animals are introduced to the system in the middle section or separating line between the two chambers. After an exposure period of typically two days the divider is introduced again and the number of animals incorporating each half of the vessel is counted separately. In addition to earthworms, test animals may be Isopods, springtails or enchytraeids.

References

- Lukkari, T., Aatsinki, M., Vaisanen, A., Haimi, J. 2005. Toxicity of copper and zinc assessed with three different earthworm tests. Applied Soil Ecology 30:133-146.
- Amorim, M.J.B., Römbke, J., Soares, A.M.V.M. 2005. Avoidance behaviour of *Enchytraeus albidus:* Effects of benomyl, carbendazim, phenmedipham and different soil types. Chemosphere 59:501-510.
- Loureiro, S., Soares, A.M.V.M., Nogueira, A.J.A. 2005. Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. Environmental Pollution 138:121-131.
- Schaefer, M. 2004. Assessing 2,4,6-trinitrotoluene (TNT)-contaminated soil using three different earthworm test methods. Ecotoxicology and Environmental Safety 57:74-80.
- Hund-Rinke, K., Achazi, R., Römbke, J., Warnecke, D. 2003. Avoidance Test with *Eisenia fetida* as indicator for the Habitat Function of Soils: Results of a Laboratory Comparison Test. Journal of Soil and Sediment 1:7-12.
- ISO/DIS 17512-1. 2005. Avoidance test for testing the quality of soils and effects of chemicals on behaviour -- Part 1: Test with earthworms (*Eisenia fetida* and *Eisenia andrei*). ISO, Geneva, Switzerland.

Limitations

- Variation between soil and laboratories may be relatively large.
- Still not run routinely by many commercial laboratories.
- Only sensitive to contamination perceived through chemo-receptors.

Benefits

- Cheap and fast.
- Relative high reproducibility.
- Ecological relevant endpoint.
- More sensitive than the survival test and sometimes even the reproduction test.
- International draft guideline is available.

6.8 Toolbox E2. Ecology tools for refined screening

In Tier 2 the observations from the survey may be expanded by simple on-site assessment of the overall soil functioning or biological activity of the soils.

Recommended tools include bait-lamina sticks and simple microbial tests using general endpoints like soil respiration or C/N mineralisation rates.

The main principle for tests at this level is to be relatively simple and cheap but at the same time to give valuable information whether or not the soil has lost some of its main services. Bait-lamina sticks for example have been demonstrated useful for describing biological activity of the soils in a general matter (Van Gestel et al., 2003).

Bait-lamina sticks

Ammonium oxidation test.

Description
The potential ammonium oxidation rate is determined for a period of 6 hours. Ammonium sulphate is added to the soil acting as a substrate for ammonium oxidising bacteria. The accumulation rate of nitrite is determined and used as an estimate for the activity. The test organisms are autotrophic ammonium oxidising bacteria in the soil.

References
- ISO 15685. 2004. Determination of potential nitrification and inhibition of nitrification – Rapid test by ammonium oxidation. ISO, Geneva, Switzerland.

Limitations
- Indigenous ammonium oxidising bacteria may already have adapted to the conditions in contaminated soil. Therefore a possible underestimation of toxicity should be considered when interpreting the results.

Benefits
- Ammonium oxidation is a vital process in the soil maintained by a limited number of bacterial species.
- Standardised ISO guideline.

Nitrogen mineralisation test.

Description
The test assesses the ability of the autochtoun populations of microorganisms in a soil to convert organic nitrogen to inorganic nitrogen. The extent of mineralisation in each contaminated soil sample is then compared to a control soil sample. The soils are sieved, adjusted for moisture, amended for nitrogen content, mixed and incubated in the dark for 28 days. The concentration of nitrate is measured after 14 and 28 days. Variation between replicates in the control must be <10%. If the mineralisation rate differs by more than 15% from the control the test is extended up to a maximum of 100 days.

References
- OECD 216. 2000. Soil Microorganisms, Nitrogen Transformation Test. OECD, Paris, France.
- ISO 14238. 1997. Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes. ISO, Geneva, Switzerland.
- Environment Agency. 2004. Biological Test Methods for Assessing Contaminated Land. Stage 2 – A demonstration of the use of a framework for the ecological risk assessment of land contamination. Environment Agency Science Report P5-069/TR1, United Kingdom. http://publications.environment_agency.gov.uk.

Limitations
- Relative long duration, which can be up to 100 days.
- Soil samples need to be incubated at 20°C.
- Sensitivity of the test to different contaminants is not well documented.
- Often variable results, reflecting natural variability of soil.

Benefits
- Nitrogen mineralisation is a vital process in the soil environment.
- Standardised international guidelines.

Carbon mineralisation test.

Description
The test assesses the effects of toxic substances on the carbon mineralisation activity of soil microorganisms. The soils are sieved, adjusted for moisture, amended for glucose content, mixed and incubated in the dark for 14 and 28 days. The respiration rates of the soil are monitored for 12 hours. Variation between replicates in the control must be <10%. If the respiration rate differs by more than 15% from the control the test is extended up to a maximum of 100 days. Individual data on carbon dioxide evolution or oxygen consumption is analysed.

References
- OECD 217. Soil microorganisms, Carbon Transformation Test. OECD, Paris, France.
- Environment Agency. 2004. Biological Test Methods for Assessing Contaminated Land. Stage 2 – A demonstration of the use of a framework for the ecological risk assessment of land contamination. Environment Agency Science Report P5-069/TR1, United Kingdom. http://publications.environment_agency.gov.uk.

Limitations
- Long duration study, which can extend up to 100 days.
- Soil samples need to be incubated at 20°C.
- Often variable results, reflecting natural variability of soil.

Benefits
- Carbon mineralisation is a vital process in the soil environment.
- Standardised OECD guideline.

Substrate Induced Respiration (SIR) method.

Description
The substrate-induced microbial respiratory activity (CO_2 production) of soil is determined for up to 24 hours. The test organisms used are aerobic and facultative anaerobic microorganisms in the soil.

References
- ISO 14240-1. 1997. Determination of soil microbial biomass. Part 1: Substrate-induced respiration method. ISO, Geneva, Switzerland.
- ISO 16072. 2002. Laboratory methods for determination of microbial soil respiration. ISO, Geneva, Switzerland.

Limitations
- A continuous supply of oxygen is advised.
- May be relative insensitive as less susceptible bacteria may take over or even benefit from the loss of more sensitive bacteria.

Benefits
- Standardised ISO guidelines.
- In many cases or land-uses overall microbial activity is the most relevant parameter to describe generic soil functioning.

Bait-lamina sticks.

Description

Bait-lamina strips are PVC strips containing small holes filled with a suitable bait substrate used to examine feeding rates of invertebrates (and microorganims) in the soil. The strips (including food material) are deployed in the field, inserted into the soil and left for approximately 2 weeks. Perforation rates depend on the activity and density of soil community, especially earthworms and to a lesser extend springtails, enchytraeids and microorganims. A lightbox is used to score the bait strips as either 'fully pierced' or 'partially pierced' by the soil fauna.

References

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- Van Gestel, C.A.M., Kruidenier, M., Berg, M.P. 2003. Suitability of wheat straw decomposition, cotton strip degradation and bait-lamina feeding tests to determine soil invertebrate activity. Biology and Fertility of Soils 37:115-123.
- Larink, O., Sommer, R. 2002. Influence of coated seeds on soil organisms tested with bait lamina. European Journal of Soil Biology 38:287-290.
- Geissen, V., Brummer, G.W. 1999. Decomposition rates and feeding activities of soil fauna in deciduous forest soils in relation to soil chemical parameters following liming and fertilization. Biology and Fertility of Soils 29:335-342.
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- Kula, C., Römbke, J. 1998. Evaluation of soil ecotoxicity tests with functional endpoints for the risk assessment of plant protection products - State-of-the-art. Environmental Science and Pollution Research 5:55-60.
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Limitations

- Environmental conditions other than contaminants may impact feeding of invertebrates, which may influence the results of the test. Therefore spatial (and temporal) controls should be included.
- The need for a series of pre-deployments of bait strips can be a time consuming process. However, if a suitable local site is available to act as a surrogate for the on-site control, this would reduce the time needed.
- Bait removal may also be influenced by soil water content. Could provide false positive result.

Benefits

- Easily applied in the field.
- A large number of bait strips can be deployed which reduces the variability in results.
- Differences in feeding rates of invertebrates can be identified.
- The assay is a rapid tool for measuring the abundance and activity of soil fauna.
- Requires only limited manpower, scientific expertise and training.
- Cheap test, particularly if sticks are refilled and reused.
- Statistical methods to interpret results are simple.

6.9 Toolbox C3. Chemistry tools for detailed assessment

The objective of the tools found in this toolbox is to assess the bioavailable and freely dissolved fraction of pollutants found in pore water of soils from contaminated sites. The methods should (in principle) be able to mimic the fraction of organic pollutants available for uptake in biota.

The collection of methods includes various non-depleting and depleting pore water extractions. A more detailed description about the problems and solutions associated to absorption of organic pollutants in soils over time, i.e. ageing, and the methodologies for measuring or estimating bioavailability is found in Chapter 2.

Very few terrestrial ecotoxicity data are yet expressed as e.g. pore water concentrations. Instead, the outcome of the methodologies in this toolbox is compared with water quality standards. An underlying assumption in this comparison is that terrestrial and aquatic species have similar sensitivity to organic contaminants when exposure, i.e. biological uptake, is the same (Chapter 2).

Negligible-depletive solid phase microextraction (nd-SPME).

Description

nd-SPME is a simple, cheap and reliable method that can be used to measure freely dissolved concentrations of hydrophobic organic contaminants in complex matrices, e.g. soil, sediment and biological matrices. A very small hydrophobic phase (a PDMS coated SPME fibre) is exposed to a sample of a contaminated soil until equilibrium is reached. The amount sampled by the fibre is negligibly small, so the original freely dissolved concentration in the soil is not affected.

References

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Limitations

- The fibres can only sample hydrophobic (organic) compounds.
- The fibres need to be equilibrated with the matrix, which needs to be monitored and generally takes longer with increasing hydrophobicity of a compound.
- Extractability does not account for metabolism in an organism.
- The polymer-water partition coefficient is needed to calculate the freely dissolved concentration in a matrix.
- Commercial laboratories do not (yet) use this method routinely.

Benefits

- The reproducibility is high, generally within 10%, which can be improved further.
- The soil sorption coefficients of spiked soils, determined with the nd-SPME technique, are comparable to literature data and model predictions.
- Aid to demonstrate if field-contaminated soils have higher sorption coefficients than predicted by models.
- Method is very sensitive, e.g. pore water concentrations in the ng/L range for PAHs.

Tenax extraction.

Description

Tenax extraction uses a solid-phase sorbent [poly(2,6-diphenyl-*p*-phenylene oxide)]. It is a simple, cheap, and fast method to estimate the amount of hydrophobic contaminants in soil and sediment that is absorbed in amorphous organic matter without extracting the amount adsorbed onto the hard carbon constituents. A mixture of Tenax beads and soil or sediment in water is shaken for 6 h. Tenax is then easily separated from soil/sediment. The organic contaminants sorbed by Tenax are solvent extracted in a few minutes at room temperature.

References

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Limitations

- Tenax extraction for 6 h extracts about 50% of the amount in amorphous organic matter: low molecular weight compounds are extracted to a higher extent, high molecular weight compounds are extracted less. The fraction extracted ranges from about 25% to 100%.
- Extractability does not account for metabolism in an organism.
- The method has not been tested on compounds with log Kow<3.
- Only a few commercial laboratories have yet implemented the method.
- In very rare occasions, the separation of Tenax and soil can be difficult.
- Not suited for a very accurate determination, for scientific purposes, of the fraction absorbed by amorphous organic matter. To that end a consecutive Tenax desorption technique has to be used.

Benefits

- Costs are virtually equal to a total content measurement by solvent extraction (less clean-up needed).
- No additional skills are required. Inter-laboratory variation for Tenax extraction of PAHs proved to be comparable to total content determination by solvent extraction in a test conducted among commercial laboratories with no previous experience.
- The method may be used to extract mixtures from soil or sediments.
- Method can be used to predict extent of microbiological degradation of organic compounds.
- Reproducibility is high, about 5%.

Non-exhaustive extraction with β-cyclodextrin.

Description

β-cyclodextrin is a water-soluble, synthetic polymer that encapsulates hydrophobic compounds that are released into the aqueous phase. The results of this method are expressed as extractabilities (in percent) of a hydrophobic compound in soil. Non-exhaustive extraction of soil with β-cyclodextrin can therefore be used to determine the bioavailable fraction of hydrophobic compounds (e.g. PAH, PCB, non-ionic pesticides) in soil.

References

- Crini, G., Bertini, S., Torri, G., Naggi, A., Sforzini, D., Vecchi, C., Janus, L., Lekchiri, Y., Morcellet, M. 1998. Sorption of Aromatic Compounds in Water Using Insoluble Cyclodextrin Polymers. Journal of Applied Polymer Science 68:1973-1978.
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Limitations

- Uptake in soil living organisms correlates only to β-cyclodextrin extractability until water solubility of the compound in pore water is reached.
- β-cyclodextrin extractability does not account for metabolism in an organism.
- The partition coefficient between compound in water and in cyclodextrin has to be sufficiently high to extract the bioavailable fraction from soil. For a lot of compounds the partition coefficient (alternatively the stability constant) has not yet been determined.
- β-cyclodextrin extraction can be used to qualitatively rank different soil samples with respect to their bioavailability. However, it can not be used to quantitatively estimate uptake in soil living organisms unless uptake is calibrated against cyclodextrin extractability for a certain compound and organism.
- Extraction with β-cyclodextrin can only be used for compounds with a log $K_{OW} > 3$.
- Spatial and temporal variations in bioavailability are normally not monitored by the method.

Benefits

- Relatively low standard deviations, i.e. below 10%.
- Chemicals that are not membrane permeable are not taken up by β-cyclodextrin.
- The tool is shown applicable to assess bioavailable amounts of hydrophobic compounds like PAHs, PCBs and chlorobenzenes for microorganisms.

6.10 Toolbox T3. Toxicology tools for detailed assessment

The objective of the tools found in this toolbox is to evaluate the potential impact of contaminated soils to fauna and plants and hereby the entire ecosystems.

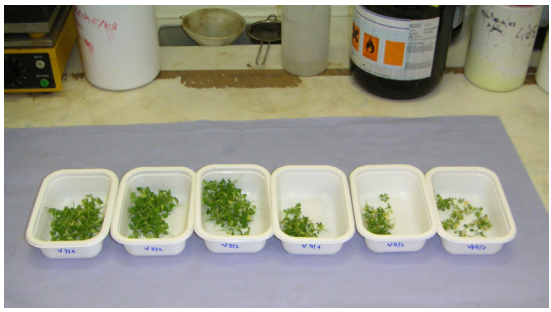
Some of the methods use introduced, and not intrinsic, species. The benefit of this is a higher degree of standardisation, as the species used in these bioassays is easy to maintain in laboratory cultures compared to naturally occurring species. The drawback may be that their ecological relevance is less obvious. For example the compost worm *Eisenia fetida* is used as a surrogate to evaluate risk to soil dwelling earthworms.

Two sets of bioassays are presented. One for directly assessing potential risk for soil dwelling species, including micro-organisms, plants and soil invertebrates, and one for assessing indirectly risk to aquatic species through e.g. leaching of contaminants. It is often anticipated that soil organisms are exposed to pollutants mainly through uptake from pore water. Therefore it may also be possible to evaluate, or at least to compare or rank, the risk of contaminated soil samples to soil dwelling organisms on the basis of the outcome of the aquatic test using elutriate or pore water.

The choice of bioassays depends on a number of variables, e.g.:

- The current and future land-use, i.e. targets of protection.
- The size of the contaminated area.
- The potential for ground water or surface water contamination.
- The need of many simple tests or fewer more complicated tests.

Useful information when selecting the set of bioassays in ecological risk assessment of contaminated sites may be found in Suter et al. (2000), Lanno (2003) and Thompson et al. (2005).



Simple plant test

6.10.1 Solid phase bioassays

Plant test.

Description
A multitude of test species can be used (75 species) with the number used recommended from 2 to 10. The species include both monocotyledon and dicotyledons. The test evaluates the inhibition of germination, emergence and early plant growth resulting from contact to potentially contaminated soil or aqueous soil extracts. The exposure time depends on the species but can extend up to 28 days.

References

- OECD 208. 1984. Guideline for testing of chemicals: terrestrial plants, growth test. OECD, Paris, France.
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- US EPA. OPPTS 850.4250 Vegetative vigor, Tier II. US EPA, United States of America.
- ASTM E1598-94. 1994. Standard practice for conducting early seedling growth tests. American Society of Testing and Materials, West Conshohocken, United States of America.
- Wang, W., Freemark, K. 1995. The use of plants for environmental monitoring and assessment. Ecotoxicology and Environmental Safety 30:289-301.

Limitations
- Can require large laboratory space glasshouse and/or plant growth chamber.
- Cultivated species may lack ecological relevance.
- Can be a time consuming test.
- Dissipation of volatile substances and degradation due to biological activity during the exposure time can influence the results of the test.
- The selection of an appropriate reference (control) soil is crucial for obtaining correct results, as plants may be affected by general soil quality.
- The guideline does not stipulate operating temperature. Therefore absolute comparisons of responses between laboratories and different times of the year are not possible.
- Plant growth is sensitive to the nutrient status of the soil. One way of overcoming this potential interference may be to supplement all soil samples with nutrients, thereby removing nutrient limitation as a factor.
- Emergence can be a poor response endpoint as plants may only be exposed to contamination after root formation.

Benefits
- Test is simple.
- Results (measurement of wet and dry weights) are easy to interpret.
- Provides assessment of the biological effects of 'whole' soil sample (contamination, soil structure and nutrient conditions).
- OECD Test Guideline gives considerable latitude in the tests operation.

Plant test. Inhibition of root elongation.

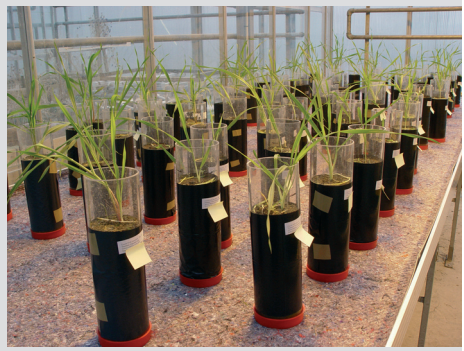
Description

The principle of the test involves inhibition of root lengths of seeds grown for a period of 7 days in potentially contaminated soil or aqueous soil extracts. The ISO guideline focuses on using seeds of barley (*Hordeum vulgare* L.) as the test species. However, there is a wide range of other test species recommended.

References

- ISO 11269-1. 1993. Determination of the effects of pollutants on soil flora. Part 1: Method for the measurement of inhibition of root growth. ISO, Geneva, Switzerland.
- USEPA OPPTS 850.4200.1996. Seed germination/root elongation toxicity test. US EPA, United States of America.
- Wang, W. 1987. Root elongation method for toxicity testing of organic and inorganic pollutants. Environmental Toxicology and Chemistry 6:409-414.

Limitations



- Can require large laboratory space, glasshouse and/or plant growth chamber.
- Cultivated species may lack ecological relevance.
- Dissipation of volatile substances and degradation due to biological activity during the exposure time can influence the results of the test.
- The selection of an appropriate reference (control) soil is crucial for obtaining correct results, as plants may be affected by soil quality.
- Plant growth is sensitive to the nutrient status of the soil. One way of overcoming this potential interference may be to supplement all soil samples with nutrients, thereby removing nutrient limitation as a factor.

Benefits

- Test is simple and results easy to interpret.
- Provides assessment of the biological effects of 'whole' soil sample (contamination, soil structure and nutrient conditions).
- Ecologically relevant endpoint.

Earthworm reproduction test *(Eisenia sp.)*.

Description

Test organisms used are *Eisenia fetida* (compostworm) or *Eisenia andrei* (red worm). The principle of the test involves exposing cocoons and juveniles of adult worms to potentially contaminated soil. After 4 weeks, adult worms are removed from soil and the cocoons are counted. After 8 weeks the juveniles are counted.

References

- ISO 11268-2. 1998. Effects of pollutants on earthworms *(Eisenia foetida)*. Part 1: Determination of effects on reproduction. ISO, Geneva, Switzerland.
- OECD 222. 2004. Earthworm Reproduction Test *(Eisenia fetida/Eisenia andrei)*. OECD, Paris, France.
- Kula, H., Larink, O. 1998. Tests on the Earthworms *Eisenia fetida* and *Aporrectodea caliginosa*. In: Løkke, H., Van Gestel, C.A.M. (Editors) Handbook of soil invertebrate toxicity tests. John Wiley and Sons, Chichester, United Kingdom. pp 95-112.
- Spurgeon, D., Hopkin, S.P. 1995. Extrapolation of the laboratory-based earthworm toxicity test to metal-contaminated sites. Ecotoxicology 4:190-205.
- Spurgeon, D.J., Weeks, J.M., Van Gestel, C.A.M. 2003. A summary of eleven years progress in earthworm ecotoxicology. Pedobiologia 47:588-606.

Limitations

- *Eisenia fetida* has limited ecological relevance as it is a litter-dwelling rather than soil-dwelling organism. Nevertheless, it has sensitivity comparable to soil dwelling earthworms.
- Long duration test.
- Locating and counting of juveniles can be time consuming.
- The method does not account for volatilisation or degradation of pollutants during testing.
- Limited use (to date) in assessing contaminated soils as opposed to spiked soils.

Benefits

- *Eisenia fetida* belongs to the composting worms and can be easily cultured in large quantities in the laboratory.
- Handling and breeding of worms are easier than more indigenous species.
- The test is relatively inexpensive and simple to perform.
- Standard methods and quality control procedures are published.
- Sensitive to both metals and complex chemical mixtures.

Earthworm reproduction test (soil-dwelling species).

Description

Earthworm reproduction is assessed in the laboratory using soil dwelling earthworms like *Lumbricus rubellus, Aporrectodea caliginosa,* or *Dendrobaena octaedra*. The worms are exposed to contaminated soils for a period of 42 days in a 6 hour light/8 hour dark regime at 15°C. Throughout the period suitable food is added. Every 7 days, earthworm mortality is assessed. The rate of cocoon production is determined at the end of the study and may be compared to survival data.

References

- Environment Agency. 2004. Application of sublethal ecotoxicological tests for measuring harm in terrestrial ecosystems. Environment Agency R & D Technical Report P5-063/TR2. United Kingdom. http://publications.environment_agency.gov.uk.

Limitations

- Long duration test.
- Soil-dwelling earthworm species are normally not kept in culture. As worms are collected from natural soils prior to testing their generic variation and historically exposure to e.g. pesticides is not fully controlled.
- Reproduction may be low in some soil types.
- Requires regulation of temperature and light conditions.
- Locating, collecting and counting of organisms can be time consuming.

Benefits

- Environmentally relevant species, which has greater representative value than e.g. compost species.
- The test is relatively simple to perform.
- The test requires basic equipment and can be inexpensive.
- Robust test.
- Greater sensitivity than the acute OECD test method.
- Sensitive to both metals and complex chemical mixtures.

Earthworm – The Neutral Red Retention Time (NRR-T) biomarker assay.

Description

The NRR-T assay investigates earthworm lysosomal membrane stability by making use of the fact that only lysosomes in healthy cells permanently can retain the cationic dye after initial uptake. Cells are isolated from coelomic fluid of the earthworms and placed on microscope slides suspended in earthworm physiological Ringer solution. The dye is then added and the slide covered with a cover slip. When 50% of the cells are stained this is registered as the neutral red retention time.

References

- Booth L.H., Heppelthwaite V.J., O'Halloran K. 2005. Effects-based assays in the earthworm *Aporrectodea caliginosa* - Their utilisation for evaluation of contaminated sites before and after remediation. Journal of Soils and Sediments 5: 87-94.
- Maboeta M.S., Reinecke S.A., Reinecke A.J. 2004. The relationship between lysosomal biomarker and organismal responses in an acute toxicity test with *Eisenia fetida* (Oligochaeta) exposed to the fungicide copper oxychloride. Environmental Research 96: 95-101.
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- EA (2004). Application of sublethal ecotoxicological tests for measuring harm in terrestrial ecosystems. Environment Agency R & D Technical Report P5-063/TR2. http://publications.environment_agency.gov.uk.

Limitations

- For a number of substances no clear link is established between individual biomarker response and ecological parameters on population level.
- Seasonal effects, water regime and other environmental stressors may influence retention time. Therefore appropriate controls need to be used.
- The assay uses cells collected from live worms from experimental soil. Consequently, only a maximum of 30 worms can be analysed per day and careful resource planning is required to obtain real time analysis.

Benefits

- Indigenous earthworm species can be used.
- Assay uses a simple light microscope.
- Reproducibility of results is good.
- The test is cheap.
- Sensitive to metals and likely to be sensitive to organic compounds.
- Useful for both site screening and more detailed risk assessment.

Collembola reproduction test.

Description

Test organisms used are the springtail *Folsomia candida* or *Folsomia fimetaria*. The reproduction of springtails is determined after exposure to contaminated soil for a 4- or a 3-week period depending on the species. The juveniles produced can be counted manually under microscope after floatation or automatically after heat extraction using digital image processing.

References

- ISO 11267. 1999. Inhibition of reproduction of Collembola *(Folsomia candida)* by soil pollutants. ISO, Geneva, Switzerland.
- Wiles, J.A., Krogh, P.H. 1998. Tests with the Collembolans *Isotoma viridis, Folsomia candida* and *Folsomia fimetaria*. In: Løkke, H., Van Gestel, C.A.M. (Editors) Handbook of soil invertebrate toxicity tests. John Wiley and Sons, Chichester, United Kingdom. pp 131-156.
- Fountain, M.T., Hopkin, S.P. 2005. *Folsomia candida* (Collembola): A "standard" soil arthropod. Annual Review of Entomology 50:201-222.
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- Crouau, Y., Gisclard, C., Perotti, P. 2002. The use of *Folsomia candida* (Collembola, Isotomidae) in bioassays of waste. Applied Soil Ecology 19:65-70.
- Sverdrup, L.E., Kelley, A.E., Krogh, P.H., Nielsen, T., Jensen, J., Scott-Fordsmand, J.J., Stenersen, J. 2001. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria* L. (Collembola, Isotomidae). Environmental Toxicology and Chemistry 20:1332-1338.
- Axelsen, J.A., Holmstrup, M., Krogh, P.H. 1998. Simulation of development and reproduction of Collembola sampled from synchronized cultures. Pedobiologia 42:1-9.
- Krogh, P.H., Johansen, K., Holmstrup, M. 1998. Automatic counting of collembolans for laboratory experiments. Applied Soil Ecology 7:201-205.

Limitations

- Time consuming when counting juveniles manually and hence subject to error.
- Heat extraction and digital image analysis could be used, but may require method development.
- Fungal growth on/in soil can restrict survival and reproduction of exposed springtails.
- Certain soil conditions not suitable for this species, e.g. low pH.

Benefits

- Ecologically important group of species.
- Breeding and maintenance of *Folsomia* species in the laboratory is comparatively simple.
- Relatively rapid life cycle.
- The test is well established and relatively simple and cheap.
- International ISO standards published.

Enchytraeid test.

Description

Test organisms used are typically *Enchytraeus albidus* or *Enchytraeus crypticus* sp. (potworms). The test determines the effect on adult survival and surviving juveniles produced over either a six or a three week period depending on the choice of species. Adults and juveniles are counted manually under microscope after floatation.

References

- OECD 220. 2004. Enchytraeid Reproduction Test. OECD, Paris, France.
- ISO 16387. 2004. Effects of pollutants on Enchytraeidae (Enchytraeus sp.). Determination of effects on reproduction and survival. ISO, Geneva, Switzerland.
- Rundgren, S., Augustsson, A.K. 1998. Tests on the Enchytraeid *Cognettia sphagnetorum* (Vejdovsky) 1977. In: Løkke, H., Van Gestel, C.A.M. (Editors) Handbook of soil invertebrate toxicity tests. John Wiley and Sons, Chichester, United Kingdom. pp 73-94.
- Weyers, A., Römbke, J., Moser, T., Ratte, H.T. 2002. Statistical results and implications of the enchytraeid reproduction ringtest. Environmental Science and Technology 36:2116-2121.
- Römbke, J., Moser, T. 2002. Validating the enchytraeid reproduction test: organisation and results of an international ringtest. Chemosphere 46:1117-1140.
- Römbke, J. 2003. Ecotoxicological laboratory tests with enchytraeids: A review. Pedobiologia 47:607-616.

Limitations

- Long duration test.
- Adult survival is normally a very insensitive endpoint.
- Counting of juveniles is time consuming.

Benefits

- Ecologically important group of species.
- Enchytraeids are easy to culture and maintain.
- Easy and reproducible test.
- International guidelines are published.

6.10.2 Liquid phase bioassays

Algae test.

Description
The growth inhibition of algae exposed to soil elutriates is determined. Algae species used include; *Selenastrum capricornutum* ATCC 22662, *Scenedesmus subspicatus* 86.81 SAG, *Chloressa vulgaris* CCAP 211/11b. The exposure time of the test is 72 or 96 hours.

References
- ISO 8692. 2004. Freshwater algal growth inhibition test with unicellular green algae. ISO, Geneva, Switzerland.
- OECD 201. 2002. Freshwater Alga and Cyanobacteria, Growth Inhibition Test (Draft Revised Guideline, July 2002). OECD, Paris, France.
- USEPA OPPTS 850.5400 1996. Algal toxicity, Tiers I and II. US EPA, United States of America.
- ASTM E1218-04. Standard Guide for Conducting Static Toxicity Tests with Microalgae. American Society for Testing and Materials, United States of America.

Limitations
- Samples with high concentrations of nutrients may lead to a promotion of growth and toxic effects of pollutants may be masked.
- Coloured samples may influence the growth due to reduced light intensity and limit the use of optical density and fluorescence as a parameter for cell concentration.
- Requires specific equipment such as a device for determining cell concentration (e.g. electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, colorimeter).

Benefits
- Available as a testkit "ALGALTOXKIT FTH" (MicroBioTests Inc., Nazareth, Belgium).
- The application of a 96 well microtiter plate facilitates the measurement of a high number of samples.
- Quick sample time.
- Cheap test.

Plant *(Lemna minor)* growth inhibition test.

The effect of soil elutriates on plant growth is determined using *Lemna minor* (duckweed) and measuring growth over a 4 or 7 day period.

References

- OECD 221. 2002 Lemna sp. Growth Inhibition Test (Draft Revised Guideline July 2002). OECD, Paris, France.
- ISO 20079. 2005. Determination of the toxic effect of water constituents and waste water on duckweed *(Lemna minor)* -- Duckweed growth inhibition test. ISO, Geneva, Switzerland.
- ASTM E1415-91. 2004. Standard Guide for Conducting Static Toxicity Tests With *Lemna gibba* G3. American Society for Testing and Materials, United States of America.
- USEPA OPPTS 850.4400. 1996. Ecological Effect Test Guidelines 850 Series (Public Draft): Aquatic plant toxicity test using *Lemna* spp. Tiers I and II. US EPA, United States of America.

Limitations

- Problems with algae contamination of test system can occur.
- *Lemna* may require sterilisation prior to testing.
- Recording frond numbers can be time consuming and laborious.

Benefits

- Ecologically relevant species for aquatic systems.
- Robust species.
- Sensitive to a wide range of chemicals.
- Standardized test.
- Integrated and automatic systems are commercially available.
- As the plants float coloured leachates are less of a problem to test.

Immobilisation test with *Daphnia magna.*

Description
The mortality, i.e. the immobilisation, of crustaceans exposed to soil elutriate or leachate is determined. The test organism used is *Daphnia magna* (water flea) and the test duration is 24 or 48 hours.

References
- OECD 202.1984. *Daphnia* sp. Acute Immobilisation Test (Updated Guideline, adopted 13th April 2004). OECD, Paris, France.
- ISO 6341. 1996. Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) -- Acute toxicity test. ISO, Geneva, Switzerland.
- US EPA OPPTS 850.1300 Daphnid chronic toxicity test. US EPA, United States of America.

Limitations
- The test must be undertaken in a temperature controlled environment at 20°C.
- Relevance of aquatic species to soil elutriate is questionable.
- Possible issues of physical immobilisation with leachates with high dissolved particulates.
- Leachates with high Biochemical Oxygen Demand (BOD) may cause toxic effects, interfering with determination of chemical effects.
- Opaque leachates may make identification of *Daphnia* difficult.

Benefits
- The test is available as a test kit DAPHTOXKIT FTH MAGNA, MicroBioTests Inc., Nazareth, Belgium.
- Cheap test with a short duration.
- Sensitive species to a range of chemicals.

Reproduction test with *Daphnia magna.*

Description
The reproductive effects of soil elutriate or leachate to crustaceans is determined. The test organism used is *Daphnia magna* (water flea) and the test duration is 21 days. In the test both survival of parent organisms and the number of offspring are registered.

References
- OECD 211. 1998. *Daphnia magna* Reproduction Test. OECD, Paris, France.
- ISO 10706. 2000. Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea). ISO, Geneva, Switzerland.
- US EPA OPPTS 850.1300. Daphnid chronic toxicity test. US EPA, United States of America.

Limitations
- The test must be undertaken in a temperature controlled environment at 20°C.
- Possible issues of physical immobilisation with leachates with high dissolved particulates.
- Leachates with high Biochemical Oxygen Demand (BOD) may cause toxic effects, interfering with determination of chemical effects.
- Opaque leachates may make identification of *Daphnia* difficult.

Benefits
- The test is available as a test kit DAPHTOXKIT FTH MAGNA, MicroBioTests Inc., Nazareth, Belgium.
- Relative cheap test with relative short duration.
- The test simulates chronical exposure, i.e. it is sensitive and has a higher ecological relevance than the immobilisation test.
- Sensitive species to a wide range of chemicals.

6.11 Toolbox E3. Ecology tools for detailed assessment

In this late tier of the Triad, the objective of the activities is community or population response analysis, typically by conducting field surveys. As these studies (most often) are time consuming, costly and dependent on ecologically, taxonomically and statistical expertise they are most frequently done on large-scale sites with a long-term-remediation perspective.

In fresh water ecosystem community surveys have been widely used with relative success. The absence of species from places where they would be expected to occur could be a strong identification of unacceptable levels of contaminants. However, this type of studies have only seldom been used for the terrestrial environment. The reasons for this are many. One of the dominants may be the lack of a concentration gradient and obvious “upstream” reference sites at most contaminated areas.

No world-wide accepted guideline on how to plan and perform a terrestrial field survey is available, and hence no straight-forward and easy-to-follow description can be given. The decision on when, where and how to conduct field surveys depends on a number of issues, e.g. the size of the area, the land-use, the type of contaminants present, time of the year and last but not least the time and money available to perform the study. Nevertheless, a number of general considerations have to be made in the planning phase of a successful field survey. These include (but are not limited to):

- Identify the targets of concern and the species to monitor.
- Elucidate the natural temporal and spatial variation before initiating a field study.
- Use statistical (power) analyses to determine the minimum number of samples or replicates needed to demonstrate the decided difference, e.g. 25% change.
- In order to establish a cause-effect relationship, a number of confounding parameters need to be characterised both at the reference and the test site, e.g. soil type, pH, salinity, hydrology, nutrient- and organic matter content and the presence of other contaminants.

As no single description on how to perform ecological surveys for contaminated sites can be given, some general considerations and useful references for this tier of the ecological risk assessment are given below for:

- Assessing impact in the overall biological activity and organic matter breakdown.
- Assessing impact on the microbial community.
- Assessing impact on the plant community.
- Assessing impact on the invertebrate community.

If **terrestrial wildlife** or ecosystems in adjacent **freshwater systems** are considered to be at risk these have to be assessed as well. It is, however, considered, to be outside the scope of this book to present detailed information on these matters. More

information may be found from institutions outside Europe such as the US-EPA or Environment Canada, just as the open literature contains numerous studies.

Reference data from reference sites, reference samples and literature

A crucial factor in a risk assessment is the quality of reference data, because the results of the site-specific ecological measurements or calculations are compared against these data. This is true for as well chemical information (i.e. background levels in that region), toxicological data from bioassays (i.e. site relevant reference soil and well characterised control soil in order to verify the test performance) and ecological field surveys. The reference soil should in principle resemble the contaminated soil in all relevant parameters, e.g. texture, pH, organic matter, water-holding capacity, nutrient content. In practice, these ideal spots are difficult to find. If there is no or inadequate reference information, effects can only be determined in relative terms by comparison with other sites. This is usually adequate for determining the degree of urgency and/or the need for remediation.

Reference data can be obtained by including reference sites (preferably more than one) in the sampling scheme, including reference measurements in the experimental set-up, or by obtaining reference data from the literature (e.g. Bailer et al., 2002; Didden, 2003) or by expert based judgement (Chapman et al., 2002).

Litter-bags

6.11.1 Higher tier assessment of the impact on biological activity and organic matter breakdown

In addition to the general information about biological activity in soils generated in Tier 2 from the bait-lamina test, other, slightly more laborious, tests may give additional information about the overall biological activity in soil, e.g. wheat straw decomposition (litter bag test) and cotton strip degradation.

A review paper from Van Gestel et al. (2003) concluded that while the bait-lamina gave the best reflection of the biological activity of soil animals, e.g. earthworms, springtails and enchytraeids, the litter bag test and the cotton strip test are more indicative of the microbial activity in the soil. Knacker et al. (2003) reviewed the usefulness of five different litter decomposition tests and concluded that the litter-bag test had distinct advantages over the others.

All of these simple tests only give insight into the overall activity in soils and the breakdown of organic material. They are hence most suitable on their own in cases of land-use with low sensitivity, e.g. industrial land. For land-uses where structural endpoints, e.g. biodiversity or specific species, are the target of protection other endpoint(s) should be monitored as well.

Litter-bags.

Description
A defined amount of organic material (e.g. leaf litter, straw or cellulose paper) is enclosed in bags of non-degradable and flexible material with a size of up to 600 Cm^2 and a mesh size of 2µm to 10 cm. The bags are buried into the soil at depth of 5-10 cm for periods that may go up to years. The mass loss between bags placed in contaminated soil and bags placed in reference soils are compared by the end of the study. In addition microbial and faunal endpoints in the bags may be assessed.

References

- Bocock, K.L., Gilbert, O. 1957. The disappearance of leaf litter under different woodland conditions. Plant and Soil 9:179-185.
- Cortet, J., Pointsot-Balaguer, N. 2000. Impact of phytopharmaceutical products on soil microarthropods in an irrigated maize field: The use of litter-bag method. Canadian Journal of Soil Science 80:237-249.
- Hendrix, P.F., Parmalee, R.W. 1985. Decomposition, nutrient loss and microarthropod densities in herbicide-treated grass litter in a Georgia Piedmont agroecosystem. Soil Biology and Biochemistry 17:421-428.
- Knacker, T., Förster, B., Römbke, J., Frampton, G.K. 2003. Assessing the effects of plant protection products on organic matter breakdown in arable fields – litter decomposition test systems. Soil Biology and Biochemistry 35:1269-1287.
- Paulus, R., Römbke, J., Ruf, A., Beck, L. 1999. A comparison of the litterbag-, minicontainer- and bait-laminia methods in an ecotoxicological field experiment with diflubenzuron and btk. Pedobiologia 43:120-133.
- Römbke, J., Heimbach, F., Hoy, S., Kula, C., Scott-Fordsmand, J., Sousa, P., Stephenson, G., Weeks, J. 2003. Effects of plant protection products on functional endpoints in soil (EPFES): Lisboa 24-26 April 2002. Society of Environmental Toxicology and Chemistry (SETAC), Pensacola Florida, USA.

Limitations
- Only functional and not structural endpoints are measured.
- May be time-consuming.
- Exposure time may be long.
- The test mainly covers the first phase of the decomposition process and to a lesser degree the final phase, i.e. mineralisation.

Benefits
- The test is relatively well developed and standardised.
- Use natural organic matter.
- Large experience is available and result described in the literature.
- It can be used at all sites provided a suitable reference can be found.
- It is relatively robust and easy to conduct.
- Data evaluation needs minimum of specific expertise.
- It has relative high reproducibility.

Cotton strips.

Description
The cotton strip method is used to assess the cellulolytic activity in soil. Strips of cotton cloth (pure cellulose impregnated with due) are inserted into the soil. When collected, the tensile strength of the strips is determined as a measure of decomposition. Large strips of cotton with a size of e.g. 30 x 10 cm may be inserted vertically into the soil and cut into sub-strips when retrieved. The decomposition in various depths of the soil can then be determined.

References

- Howson, G. 1991. The cotton strip assay. Field applications and global comparisons. In: Wilson, W.S. (Editor) Advances in soil organic matter research: The impact of agriculture and the environment. Royal Society of Chemistry, Cambridge, United Kingdom. pp. 217-228.
- Knacker, T., Förster, B., Römbke, J., Frampton, G.K. 2003. Assessing the effects of plant protection products on organic matter breakdown in arable fields – litter decomposition test systems. Soil Biology and Biochemistry 35:1269-1287.
- Kutniar 1950. New methods of determinations of forest soil activity. Sylwan 94:49-57.
- Latter, P.M., Howson, G. 1977. The use of cotton strips to indicate cellulose decomposition in the field. Pedobiologia 17:145-155.
- Latter, P.M., Bancroft, G., Gillespie, J. 1988. Technical aspects of the cotton strip assays in soils. International Biodeterioration 24:25-47.

Limitations
- Only functional and not structural endpoints are measured.
- The test does not use natural organic matter and evaluate only effect on breakdown of one substance (pure cellulose).
- The test mainly covers the first phase of the decomposition process and to a lesser degree the final phase, i.e. mineralisation.
- Exposure time may be long.
- Specific technique is required to assess tensile strength.
- Little practical experience is available.

Benefits
- It can be used at all sites provided a suitable reference can be found.
- It is (assumed to be) relatively robust and easy to conduct.
- Data evaluation needs minimum of specific expertise.

6.11.2 Higher tier assessment of the impact on the microbial community

The number of microorganisms, especially bacteria, in soil is extremely large. They differ widely in their function and sensitivity to chemicals. Besides more classical (and simple) measures of the microbial community like total bacterial biomass, the number of colony forming units and substance induced respiration rate (SIR), more advanced methods for assessing the impact of contamination on soil microorganisms have recently been made available. This include microbial "fingerprinting" like phospholipid fatty acid analysis (PLFA) and community-level physiological-profiling (CLPP) based on metabolic response using BIOLOG plate systems, and the use of pollution induced community tolerance (PICT). These are therefore described in more detail below.

CLPP (community-level physiological profiling): analysis of microbial communities with Biolog plates.

Description

Community-level physiological profiling is a fingerprinting method covering a part of the microbial community in the soil. The method is based on incubation of microbial community extracted from soil samples in mulit-well plates with a broad suite of carbon and energy substrates. Plates with 95 or 31 different substrates are commercially available. Each well also contains nutrients and a redox dye. This redox dye indicates the intensity of substrate oxidation by bacteria through the irreversible formation of purple formazon, which can be detected using a plate reader.

References

- Van Elsas, J.D., Rutgers, M. (red.) 2006. Soil microbial diversity and community composition. In: Bloem, J., Hopkin, D.W., Beneditti, A. (Editors) Micribiological Methods for Assessing Soil Quality, CAB International, Wallingford, United Kingdom. pp. 183-227.
- Rutgers, M., Breure, A.M. 1999. Risk assessment, microbial communities, and pollution –induced community tolerance. Human and Ecological Risk Assessment 5:661-670.
- Winding, A., Hund-Rinke, K., Rutgers, M. 2005. The use of microorganisms in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety 62:230-248.
- Boivin, M.-E.Y., Breure, A.M., Posthuma, L., Rutgers, M. 2002. Determination of field effects of contaminants. The importance of pollution-induced community tolerance. Human and Ecological Risk Assessment 8:1035-1055.

Limitations

- The main problems for reproduction lie in biological variation arising from inconsistent soil extracts. Plate inoculation follows Poisson distribution and this should be taken into account.
- As with all ecological surveys, causal relationships between potential stressors (contaminants) and responses may be weak.
- All microbial profiling methods including CLPP have limitations because only a small part (limited diversity, limited features) of the community is studied.
- The physiological information, which is revealed from Biolog studies, cannot be directly used to explain events under natural circumstances as the conditions in the plates are artificial.

Benefits

- The sensitivity of this method is theoretically high, because the microbial community from contaminated soil is directly exposed, not mobile and highly diverse.
- Biolog plates are commercially available.
- Numerous laboratories can conduct CLPP.
- Measurements are made on the indigenous organisms. Although the method relies on extracted micro-organisms, it is applicable at most sites if enough soil is present to allow such extraction, and the density of the bacteria is high enough.

Pollution Induced Community Tolerance (PICT): Analysis of microbial communities with Biolog plates.

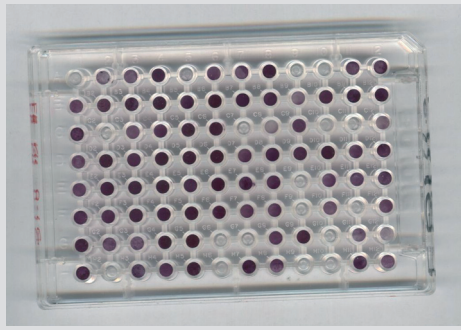
Description

Application of multi-well Biolog plates to determine PICT provides information on the tolerance of the microbiological community for the tested compounds. This method is based on incubation of the microbial community extracted from soil samples in 96 well plates containing 95 or 31 different carbon sources and a control. In laboratory controlled conditions the microorganisms are exposed to a range of added concentrations of the test substance. Each well also contains nutrients and a redox dye. This redox dye indicates the intensity of substrate oxidation by bacteria through the irreversible formation of purple formazon, which can be detected using a plate reader. In this way 95 or 31 different dose response relationships can be obtained, providing information about the sensitivity of the microbial community (e.g. in EC_{50} values) for the toxicant. Increased PICT indicates previous exposure and pollutant caused selection or stress.

References

- Van Beelen, P., Wouterse, M., Postuma, L., Rutgers, M. 2004. Location Specific Ecotoxicological Risk Assessment of metal-polluted soils. Environmental Toxicology and Chemistry 23:2769-2779.
- Winding, A., Hund-Rinke, K., Rutgers, M. 2005. The use of microorganisms in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety 62:230-248.

Limitations



- The method is not standardised but the procedure is used and published by at least three research groups.
- Only the effect of soluble compounds can be measured.
- Careful assessment of the toxicant gradient is necessary, because some chemicals will precipitate with the phosphate buffer in the plates.
- Some toxicants will adsorb to the plastic wall, and some toxicants will evaporate or degrade rapidly by microbial activity.
- Determination of community tolerance is dependent on the density of the inoculum. The density of the inoculum should therefore be carefully standardised.
- Biological variation arising from inconsistent soils extracts and Poisson-like distribution of features in the plates may be high.

Benefits

- The sensitivity of this method is theoretically high, because the microbial community in soil is directly exposed, and has a high diversity.
- The influence of confounding factors is low.
- Because plates are commercially available, there are not many specific facilities required.
- Measurements are made on the indigenous fauna.
- It is shown that development of tolerance by bacterial communities was accompanied by shifts in the composition of microbial and invertebrate communities (genetic and physiological). This ecological relevance is a key to appraisal of the technique in ERA, but further validation is needed.

6.11.3 Higher tier assessment of the impact on the plant community

Plants interact dynamically with the physical and chemical characteristics of soils. Soil types and site characteristics, therefore, greatly influence the occurrence of plants and their total aboveground biomass (also called, Net Primary Production, i.e. NPP) within given climatic conditions and human management.

Vegetation cover is an important indicator of soil quality and a diverse plant community is normally a good indication of essential soil functions such as the decomposition process, the mineralisation rate, and the occurrence of soil dwelling animals associated to (fresh) organic matter. Vascular plants are easy to sample. They are immobile and hence associated to soil contamination (and airborne pollution). Plant community parameters like plant cover, aboveground plant biomass, plant shoot/root ratio, species diversity and the binary occurrence (presence/absence) of specific indicator species like metal-tolerant species may be used successfully in ERA.

Information on plant species sampling and community analysis has been published in several papers. Two journals, Journal of Vegetation Science and Applied Vegetation Science, are entirely devoted to this subject and have from 1990 onwards published a number of case studies. These papers, too many to be quoted here, may be useful when designing and evaluating plant surveys. One of the first books (and still one of the most complete surveys) on the possibilities of vegetation analysis in environmental science is provided, to our knowledge, by Kent and Coker (1992).

Plant survey

6.11.4 Higher tier assessment of the impact on the soil invertebrate community

Survey of soil biota in order to evaluate the effect of various sources of pollution on soil communities on historically contaminated sites have not yet been used on a larger scale by e.g. consultants. However, numerous (monitoring) studies by various research groups can be found in the open literature. Methods of surveying include:

- Collection of soil samples followed by extraction in the laboratory.
- Extraction or collection of organisms in the field, e.g. by hand-sorting or by the application of mustard or formalin.
- Trapping (surface dwelling) animals by the use of e.g. pit-falls.

Monitoring species includes earthworms, snails, oribatid mites, nematodes, springtails, ants, ground-living beetles and spiders. Most of the studies have been done on metal contaminated sites (see references below). A substantial amount of work has been put into the challenge of developing a soil invertebrate system for evaluating risk of pollutants. The only soil invertebrate system that is used on a regularly basis in the context of ecological risk assessment of contaminated soils is most likely the nematode Maturity Index (MI). The system is based on the evidence, that rapid colonising species dominate nematode communities in disturbed ecosystems. In the Netherlands experience with surveys of soil invertebrates from the monitoring programme Biological Indicator for Soil Quality (BISQ) has also been used in ERA (Rutgers et al., 2001; Schouten et al., 2003ab). At this moment several ISO Drafts are being developed on the sampling of soil invertebrates (see selected references below).

Soil fauna sampling

Supporting references:

Bengtsson, G., Rundgren, S. 1988. The Gusum case: a brass mill and the distribution of soil Collembola. Canadian Journal of Zoology 66:1518-1526.

Blakely, J.K., Neher, D.A., Spongberg, A.L. 2002. Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination. Applied Soil Ecology 21:71-88.

Bongers, T. 1990. The Maturity Index – an ecological measure of environmental disturbance based on nematode species composition. Oecologia 83:14-19.

Fauntain, M.T., Hopkin, S.P. 2004a. Biodiversity of Collembola in urban soils and the use of *Folsomia candida* to assess soil ´quality´. Ecotoxicology 13:555-572.

Fauntain, M.T., Hopkin, S.P. 2004b. A comparative study of the effect of metal contamination on Collembola in the field and in the laboratory. Ecotoxicology 13:573-587.

ISO 11268-3. 1999. Soil quality – Effects of pollutants on earthworms - Part 3: Guidance on the determination of effects in field situations. ISO, Geneva, Switzerland.

ISO 23611-1. 2004a. Draft: Soil quality - Sampling of soil invertebrates Part 1: Hand-sorting and formalin extraction of earthworms. ISO, Geneva, Switzerland.

ISO 23611-2. 2004b Draft: Soil quality - Sampling of soil invertebrates Part 2: Sampling and extraction of microarthropods (Collembola and Acarina). ISO, Geneva, Switzerland.

ISO 23611-3. 2004c. Draft: Soil quality - Sampling of soil invertebrates Part 3: Sampling and soil extraction of enchytraeids. ISO, Geneva, Switzerland.

ISO 23611-4. 2004d. Draft: Soil quality - Sampling of soil invertebrates Part 4: Sampling, extraction and identification of free-living stages of nematodes. ISO, Geneva, Switzerland.

Jansch, S., Römbke, J., Didden, W. 2005. The use of enchytraeids in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety 62:266-277.

Markert, B.A., Breure, A.M., Zechmeister, H.G. (Editors) 2003. Bioindicators and Biomonitors. Principles, Concepts and Applications. Elsevier, Oxford, United Kingdom.

Mulder, Ch., Schouten, A.J., Hund-Rinke, K., Breure, A.M. 2005. The use of nematodes in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety 62:278-289.

Read, H.J., Wheater, C.P., Martin, M.H. 1987. Aspects of the ecology of carabidae. Environmental Pollution. 48:61-76.

Römbke, J., Jansch, S., Didden, W. 2005. The use of earthworms in ecological soil classification and assessment concepts. Ecotoxicology and Environmental Safety 62:249-265.

Ruf, A., Beck, L. 2005. The use of predatory soil mites in ecological soil classification and assessment concepts, with perspectives for oribatid mites. Ecotoxicology and Environmental Safety 62:290-299.

Spurgeon, D.J., Hopkin, S.P. 1996. The effects of metal contamination on earthworm populations around a smelting works: quantifying species effects. Applied Soil Ecology 4:147-160.

Tranvik, L., Eijsackers, H. 1989. On the advantage of *Folsomia fimetarioides* over *Isotomiella minor* (Collembola) in a metal polluted soil. Oecologia 80:195-200.

6.12 Toolbox for tests in Tier IV

The final assessment in the ERA process is not likely to be initiated for many contaminated sites. The choice of additional tests or monitoring at this level of the ERA is bound to be very site-specific and hence an issue for negotiation between stakeholders and experts. Nevertheless a few alternative studies not yet mentioned in the previous Tiers are described here.

Accumulation in biota is included in this toolbox as the internal concentration in biota is believed, at least to some extend, to reflect uptake and then bioavailability. An alternative in this final tier could also be to model uptake in biota provided sufficient data is available (e.g. Trapp et al., 1990; Zhu and Gao, 2004).

Detailed field survey

Accumulation in biota.

Description
The uptake of pollutants by plants, soil invertebrates or even mammals is measured in controlled bioassays in the laboratory or in samples collected in the field. Test endpoint is the concentration in the e.g. plant shoots, earthworm tissue or mole liver. The uptake can be expressed as bioaccumulation factors (BAF) or bioconcentration factors (BCF).

References

- US EPA OPPTS 850.4800. Plant uptake and translocation test. US EPA, United States of America.
- Chiou, C.T. 2002. Uptake by root crops from different soils. In: Partition and Adsorption of Organic Contaminants in Environmental Systems. Wiley and Sons, Inc., Hoboken, New Jersey, United States of America.
- Gao, Y., Zhu, L. 2004. Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55:1169-1178.
- Jager T. 1998. Mechanistic approach for estimating bioconcentration of organic chemicals in earthworms (Oligochaeta). Environmental Toxicology and Chemistry 17: 2080-2090.
- Ryan, J.A., Bell, R.M., Davidson, J.M., O´Connor, G.A. 1988. Plant uptake of non-ionic organic chemicals from soils. Chemosphere 17:2299-2323.
- Simonich, S.L., Hites, R.A. 1995. Organic pollutant accumulation in vegetation. Environmental Science and Technology 29:2905-2914.
- Stephenson G.L., Wren C.D., Middelraad I.C.J., Warner J.E. 1997. Exposure of the earthworm, *Lumbricus terrestris*, to diazinon, and the relative risk to passerine birds. Soil Biology and Biochemistry 29: 717-720.
- Van Brummelen T.C., Verweij R.A., Wedzinga S.A., Van Gestel C.A.M. 1996. Polycyclic aromatic hydrocarbons in earthworms and isopods from contaminated forest soils. Chemosphere 32: 315-341.
- Wang, W., Freemark, K. 1995. The use of plants for environmental monitoring and assessment. Ecotoxicology and Environmental Safety 30:289-301.

Limitations
- If uptake studies are performed in the laboratory it may be time consuming and costly. Besides the equipment and facilities like green house in plant uptake studies, special equipment is required for the extraction and analysis of contaminants in tissues (Soxhlet, HPLC).
- Plant growth and uptake is very depending on various physical-chemical properties of soils, especially nutrient status. Excess nutrients should be added and water supplied in accordance to the water-holding capacity of each test soil.
- If (larger) fauna is collected from the site for analyses it may be difficult to assess the actual time spend in the area and hence the fraction of food obtained from the contaminated site.

Benefits
- Plants are essential for most land-uses and plant uptake is often a critical parameter useful also in human risk assessment.
- By knowing the BAF in biota secondary risk to predators like raptors or moles can be estimated.
- Site-specific uptake from biota collected from the area can be determined directly.

Sequential supercritical fluid extraction (SSFE).

Description
SSFE is a fast and reliable chemical mimetic method to determine bioavailability of organic pollutants in soils. The results are expressed as extractabilities (in percent) of contaminants over 16 successive extraction steps.

SSFE is conducted using carbon dioxide as extraction fluid. The supercritical fluid extraction involves five successive extraction phases with increasing extraction strength (from "very mild" to "very harsh") by subsequently raising the temperature and density of the fluid.

References

- Björklund, E., Bøwadt, S., Mathiasson, L., Hawthorne, S.B. 1999. Determining PCB sorption/desorption behaviour on sediments using selective supercritical fluid extraction. 1. Desorption from historically contaminated samples. Environmental Science and Technology 33:2193-2203.
- Björklund, E., Nilsson, T., Bøwadt. S., Pilorz, K., Mathiasson, L., Hawthorne, S.B. 2000. Introducing selective supercritical fluid extraction as a new tool for determining sorption/desorption behaviour and bioavailability of persistent organic pollutants in soil. Journal of Biochemical and Biophysical Methods 43:295-311.
- Hawthorne, S.B., Poppendieck, D.G., Grabanski, C.B., Loehr, R.C. 2002. Comparing PAH availability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. Environmental Science and Technology 36:4795-4803.
- Loibner, A.P., Holzer, M., Gartner, M., Szolar, O.H.J., Braun, R. 2000. The use of sequential supercritical fluid extraction for bioavailability investigation of PAH in soil. Bodenkultur 51:279-287.
- Szolar, O.H.J., Rost, H., Hirmann, D., Hasinger, M., Braun, R., Loibner, A.P. 2004. Sequential supercritical fluid extraction (SFFE) for estimating the availability of high molecular weight polycyclic aromatic hydrocarbons in historically polluted soil. Journal of Environmental Quality 33:80-88.

Limitations
- The technology is a time consuming process.
- Special equipment is required for the extraction as well as for the analysis.

Benefits
- Useful for elucidating the adsorption of contaminants and is providing useful information about bioavailability in general.
- Low standard deviation (typically below 10%).
- Extraction profiles show clearly different extractabilities between e.g. low and high molecular PAHs, also for different soils.
- Bioaccumulation in plants and microbiological degradation have been shown to be in good accordance with the first extraction phase of the SSFE.

CHAPTER 7 THE CASE STUDY SKAGEN, DENMARK

Dirven-van Breemen E.M., Mesman M., Rutgers M., Jensen J., Sorokin N., Bierkens J., Ter Laak T., Erlacher L. and Bogolte T.

7.1 Introduction

The EU project Liberation decided to use a contaminated site in Skagen located in the northern part of Denmark as a case study. Until the mid-sixties the site was used for drying fishing nets after coating with heavy tar. The nets were transported from the sea to the tar-site in-land and soaked in warm tar before they were left drying on a relatively large area. During the drying process part of the tar leached to the soil below the nets, which left the area with PAH levels considerable above the background concentrations (Table 7.1).

The location of the site in Denmark is shown in Figure 7.1. The site is now a combination of *Calluna* heather and small pine plantations. Pictures of the original tar container and the study area are found in Figure 7.2.

A set of limited field studies was conducted and soil samples collected in order to carry out a number of different ecotoxicity and bioavailability assays in the laboratory. No detailed historical data regarding PAH levels were available. To get an overview of the contaminated area, concentrations of PAH were therefore determined in 36 soil samples taken with a core sampler from marked micro-plots in the area (Table 7.1, Figure 7.2). Before soil sampling, the same plots were used as sampling points in the plant- and microarthropod survey. On the basis of the chemical data from the 36 soil samples, larger samples of more than 100 kg of soil where subsequently collected from each of three larger plots within the investigated area aiming for three distinguished contamination levels: low, medium and high. After thorough mixing subsamples of the collected soil were distributed to all partners in the Liberation project and stored cold until use. Results from the chemical analyses of the sub-samples revealed, however, only minor differences in the level of PAHs between the low and

Table 7.1 Concentration (mg kg^{-1} dry weight) of PAH in 36 different plots from the Skagen site in Denmark. The concentrations are a sum value of 16 different PAH.

Plot	1	2	3	4	5	6	7	8	9
mg kg^{-1}	11546	9551	10842	1220	678	660	1716	225	448
Plot	10	11	12	13	14	15	16	17	18
mg kg^{-1}	395	12527	90	3269	750	263	46	244	39
Plot	19	20	21	22	23	24	25	26	27
mg kg^{-1}	31	38	81	50	136	37	35	117	113
Plot	28	29	30	31	32	33	34	35	36
mg kg^{-1}	126	128	138	98	634	210	246	184	1165

Figure 7.1 Location of the Skagen field site in Denmark

medium samples (Table 7.2). The relatively small difference between Skagen Low and Medium is most likely due to a heterogeneous distribution of PAHs at the site. Therefore, the medium level of PAH detected in the small sub-sample (0.1 kg) may have been diluted by less polluted soil surrounding the centre of the large plot (>100 kg).

The three, four and five ring PAHs dominated with fluoranthene, phenanthrene, pyrene and benzo(b,k)fluoranthenes were found in the highest concentrations. However, although it was more than 40 years since the last tar coated nets were dried at the site, two ringed PAHs like naphthalene were still detected in relatively high amounts at the site, i.e. from below the detection limit to more than 300 mg kg^{-1}.

Figure 7.2 The field site in Skagen with original tar container and white marking sticks for sampling plots.

Table 7.2 Soil characteristics and PAH levels in soil (mg kg^{-1}) and pore water (µg L^{-1}) in Skagen samples. The sample "Skagen Low" could be considered as the local reference sample.

	Skagen Low soil	Skagen Low pore water*	Skagen Medium soil	Skagen Medium pore water*	Skagen High soil	Skagen High pore water*
Organic carbon (%)	1.6		2.6		3.4	
Pyrene	0.71	0.00627	1.87	0.00691	158	0.73
Fluorene	0.00		0.06		6.2	1.22
Benzo(b)fluoranthene	0.55	0.00025	1.34		72	0.0101
Phenanthrene	0.52	0.04055	1.22	0.07433	698	4.92
Anthracene	0.03	0.00241	0.16	0.00803	16.8	0.875
Fluoranthene	1.13		2.77		176	
Benzo(a)anthracene	0.41	0.00054	1.07	0.00058	70	0.06405
Chrysene	0.43	0.00210	1.06		69	0.133
Benzo(ghi)perylene	0.34		0.83		46	0.0023
Benzo(k)fluoranthene	0.25	0.00010	0.67		36	0.0062
Benzo(a)pyrene	0.36	0.00008	1.00		63	0.0085
Indeno(123cd)pyrene	0.50		1.37		77	
Napthalene	0.14		0.53		27.7	
Acenaphtene	0.25		0.56		65	
Dibenzo(ah)anthracene	0.10		0.20		15	0.0015

* As determined by solid phase micro extractions

7.2 Triad assessment

The results from a number of studies in the LIBERATION project are used in order to illustrate the practical use of the Triad presented in the previous chapters of this book. Parts of the results are not yet fully processed or analysed and should therefore be taken as preliminary results only. Furthermore, data was originally generated as part of various research activities and not as an example on how to use the Triad. This has a number of implications. First of all, data collection does not necessarily follow the framework presented in this DSS. Therefore data gaps are found, and data are generally not optimised for e.g. scaling purposes. Despite of this, we have included the case study in order to present the practical use of the Triad even in a case where data collection could have been more optimal with regard to a site-specific ERA.

7.2.2 Chemistry LoE

Chemistry tools for simple screening (Toolbox C1)

The toxic pressure (TP) of PAHs in Skagen soil samples was calculated as described in Text Box 6 and 7, Chapter 4. First, the measured total concentrations of 16 PAHs in the Skagen soil samples were converted to concentrations in a standard soil with 10% organic matter (Swartjes, 1999; Rutgers and Den Besten, 2005). The equation for the conversion to standard soil is:

$$C_{PAH\ st} = \frac{(C_{PAH}) * (C_{om})}{10}$$

where C_{om} is the organic matter expressed in % of dry soil.

Table 7.3 Serious Risk Concentrations (SRC) of PAH for ecosystems used to calculate toxic pressure in soil and soil pore water (Verbruggen et al., 2001).

PAH	SRC soil (mg kg^{-1})	SRC groundwater (µg L^{-1})
Pyrene	-	-
Fluorene	-	-
Benzo(b)fluoranthene	38	-
Phenanthrene	31	30
Anthracene	1.6	1.4
Fluoranthene	260	-
Benzo(a)anthracene	2.5	1.0
Chrysene	35	-
Benzo(ghi)perylene	33	-
Benzo(k)fluoranthene	38	-
Benzo(a)pyrene	7	-
Indenopyrene	1.9	-
Naphtalene	17	-
Acenaphtylene	-	-
Acenaphtene	-	-
Dibenzo(a)anthraceen	-	-

- = No data available

Serious Risk Concentrations (SRC) of PAH were used to calculate the TP (Table 7.3). In the Netherlands SRC's are derived from the lowest HC_{50} value of either a SSD based on toxicity data for terrestrial organisms or a SSD based on toxicity data for terrestrial processes (Verbruggen et al., 2001). However, for most PAH the SRC's could not be derived with SSD's due to of lack of terrestrial data. In those cases assessment factors were applied or aquatic data were used (Verbruggen et al., 2001).

The toxic pressure is first calculated for each of the single PAH where both soil concentrations and SRC values are available. Subsequently the individual TPs are used to calculate the toxic pressure for the combinations of PAHs (combi-PAF) (see section 6.3.4).
The toxic pressure (TP) is calculated using the equation (Posthuma et al., 2002):

$$TP = \frac{1}{1 + e^{\left(\frac{\log SRC - \log C_{PAH\ st.}}{\beta}\right)}}$$

where β is a slope parameter of the species sensitivity distribution (SSD), which describes the standard deviation of the collected NOEC data used for the SSD. The β-values are not given by Verbruggen et al. (2001) but can be derived from literature data. We assumed a β of 0.4 as a reasonable default value for calculation of the TP.

Chemistry tools for detailed assessment (Toolbox C3)
The toxic pressure (TP) of the PAHs detected in pore water of Skagen samples was determined according to the description in Text Box 6 and 7, Chapter 4. In this way, bioavailability was pragmatically addressed by assuming that only the concentration

Table 7.4 Results of the chemistry LoE in soil samples and pore water from Skagen by calculating toxic pressure (TP). Skagen Low is the local reference site.

	Skagen Low	Skagen Medium	Skagen High
TP (soil)	0.998	1.00	1.00
TP (pore water)	0.14	0.19	0.71

in pore water is responsible for effects. The pore water concentrations were measured with the solid phase micro extraction (SPME) technique (see Toolbox C3 for details). Instead of SRC values for soil, SRC values for ground water were used to calculate the TP. Unfortunately ground water SCRs are only available for very few PAHs (Table 7.3).

Table 7.4 shows results of the calculations of toxic pressure for soil and pore water. The TP for the soil indicates no difference between Skagen Low, Medium and High as they all have very high TP. On the contrary, the TP for the pore water discriminates between the three locations with a significant elevated risk at Skagen High.

7.2.3 Toxicology LoE

Four bioassays (toxicity tests) were applied to determine the toxicity in the soil samples collected from the Skagen site.

Toxicology tools for simple screening (Toolbox T1)
The Microtox® and the Ostracod tests were carried out. More details about the tests can be found in Toolbox T1 in Chapter 6. The results are shown in Table 7.5.

Toxicology tools for detailed assessment (Toolbox T3)
The reproduction test with springtails and the acute immobilisation test with *Daphnia* were conducted using soil and soil pore-water, respectively. Details about the tests can be found in Toolbox T3 in Chapter 6. The results of the bioassays are summarised in Table 7.5.

The results indicated that Skagen High generally was more toxic than Skagen Medium and especially Skagen Low. However, the results also demonstrate that no single test is certain to give conclusive results, wherefore the use of a battery of bioassays is preferred above the use of a single bioassay.

Table 7.5 Results of four bioassays with soil samples from Skagen. Skagen Low is the local reference site.

Bioassay	Skagen Low	Skagen Medium	Skagen High
Microtox (solid phase). IC50 (%) of leachate	4.4	>10	>10
Ostracod test. Mortality (%)	65	70	95
Springtail reproduction test (number of juveniles)	332	403	209
Daphnia acute test (number of surviving adults)	7	4	4

Table 7.6 Scaled ecological observations in soil samples from Skagen or from plot with soil concentration corresponding to these samples. Sample Skagen Low is the local reference site.

	Skagen Low	Skagen Medium	Skagen High
Microarthropods (BKX_Triad)	0.00	0.13	0.30
Plant community (No. species)	0.00	0.17	0.34
Biolog (CLPP)	0.00	0.19	0.18

7.2.4 Ecology LoE

Ecology tools for detailed assessment (E3)
A metabolic diversity analysis of the microbial communities (the BIOLOG CLPP) was carried out. Furthermore, a survey was made of the plant- and microarthropod communities. Here only parts of the total information from these surveys are shown. Details about the CLLP can be found in Toolbox E3 in Chapter 6. The results are summarised in Table 7.6.

The results of the PCA from the microbial metabolic diversity test (BIOLOG CLPP) showed a significant difference between soil Skagen Low and the pooled results of Skagen Medium and Skagen High ($P<0.001$). Plant and microarthropod community surveys were conducted in 36 plots in the area. The data from these are compiled in three sub-classes covering approximately the same levels of PAHs as found in the three excavated large soil samples. An overall decreasing trend in the number of plant species could be found in plots corresponding in concentration ranging from Skagen Low to Skagen High. Concerning microarthropods, mites and springtails responded differently, the latter being stimulated. If microarthropod data was used according to the BKX_Triad method described in Text Box 2, Chapter 4, the plots covering the concentration ranges found in Skagen Medium and Skagen High did not pose a high risk to soil invertebrates (Table 7.6). However, more detailed multivariate analysis of the data may show differently.

7.3 Integration of results

Results from the Skagen case study
The selection of tools for the assessment was based on scientific and pragmatic arguments, for instance by focussing on readily available techniques for determination of the concentration of contaminants in pore water, and readily available biological tests such as simple bioassays and the monitoring of plants and soil organisms.

The three sub-locations (Skagen Low, Skagen Medium and Skagen High) demonstrated a trend in increasing contamination levels for most PAH, both for total and for pore water concentrations (Table 7.2). However, the sum-concentration of all PAHs in the Skagen Low sample was 25 mg kg^{-1} after correction to standard soil with 10% OM. This is significantly higher than the background level found in most non-pollut-

ed sites. Here soil concentrations are typically below 1-2 mg PAH kg^{-1}. However, this was balanced against the drawbacks of collecting soil with lower PAH levels from a remote area not resembling other site-specific parameters.

For the Microtox measurements, toxicity was lowest in Skagen Medium and High, and highest in the sample Skagen Low. For the Ostracod and *Daphnia* test, toxicity was observed in all soil elutriates. Slight toxicity was observed in the springtail reproduction test, the number of juveniles increased by 20% in samples Skagen Medium and decreased by 30% in samples Skagen High.

A few ecological parameters were determined (Table 7.6) and especially for the Skagen High difference from the "reference sample", Skagen Low, was observed for the composition of the plant and microarthropod community.

Scaling and integration of results

For integration purposes the results in the three LoE in the Triad were scaled on a 0 to 1 scale. In Text Boxes 1-7 in Chapter 4 different methods for scaling are described. For the "reference sample" Skagen Low, the risk values were set to zero (local reference). The scaled results within a LoE were integrated into one number for each LoE in the Triad and for the final judgement these three numbers were again integrated into one integrated number of risk. Table 7.7 gives an overview of the scaling and integration results of the Skagen study.

Integration of all results provides a clear indication of effects of PAHs at Skagen High and some indication of a potential risk at Skagen Medium. It is obvious that especially the chemical LoE indicates high risk. This is the main reason for the relatively large deviation found in the final risk number. In contrary to this, the Ecology LoE was unable to identify high risk in any of the sub-sets of soil from the Skagen site.

The toxic pressure and risk numbers found when comparing pore water ('bioavailable') concentrations with toxicity data from aquatic tests were notably lower than when comparing total soil concentrations and soil toxicity data. It is therefore an example of how bioavailability measures can improve the chemical LoE of the risk assessment. However, only a few examples are currently available and more practical experience is needed before it can be finally evaluated to use aquatic toxicity data for predicting effects from pore water concentrations of contaminants on soil dwelling species.

This case study clearly shows that information from three LoE gives more detailed information about the risk than the generic comparison of total soil concentrations and soil screening levels. In the latter even the toxic pressure of the Skagen Low samples indicated a risk of almost 1, i.e. 0.998 (Table 7.4).

On the basis of the results from the Triad, management decisions concerning the future of the site have to be made. According to the "decision matrix" found in Table

Table 7.7 Triad decision matrix showing the scaled results from tests conducted in relation to investigation from the Skagen site in Denmark on a quantitative effect scale (see text for more details). No screening test for the Ecology LoE was performed wherefore the relative simple analysis of plant community is included as a first Tier assessment. Toolboxes are indicated in brackets.

Tier 1		
Chemistry	**Skagen M**	**Skagen H**
Sum TP soil (C1)	0.94	1.00
Toxicology		
Microtox solid phase (T1)	0.05	0.05
Ecology		
Plant community analyses (E3)	0.17	0.34
Integrated risk number	**0.64**	**0.60**

Tier 2 + 3		
Chemistry	**Skagen M**	**Skagen H**
Sum TP soil (C1)	0.94	1.00
TP porewater (SPME) (C3)	0.06	0.66
Risk number	0.76	0.98
Toxicology		
Microtox solid phase (T1)	0.05	0.05
Ostracodtoxkit mortality (T1)	0.14	0.86
Springtail reproduction test (T3)	0.18	0.37
Daphnia survival (T3)	0.43	0.43
Risk number	0.21	0.53
Ecology		
Microarthropods (E3)	0.13	0.30
Plant community analyses (E3)	0.17	0.34
Biolog (CLPP) (E3)	0.19	0.18
Risk number	**0.16**	**0.28**
Final assessment		
Risk number - Chemistry	0.76	0.98
Risk number - Toxicology	0.21	0.53
Risk number - Ecology	0.16	0.28
Integrated risk number	**0.46**	**0.82**
Deviation	**0.58**	**0.62**
Risk Indicators:		
(IR = Integrated Risk)	$0.00 < IR < 0.20$	no risk
	$0.21 < IR < 0.50$	low risk
	$0.51 < IR < 0.75$	moderate risk
	$0.76 < IR < 1.00$	high risk

4.1 it would be recommended either to re-evaluate the existing studies, to do more studies or to change the land-use to industry. It can be noted that the investigated area is located in one of the most treasured parts of Denmark with high cultural and nature value. Further action in order to restore the area, e.g. remedial actions, could therefore destroy more than it would ameliorate. If this had been a “true” case, physical remediation would therefore most likely have been unacceptable whereupon further discussion most likely would have focused on the following:

1. Reduce the weighting of the TP of total concentrations.
2. Include measurement of the bioavailable fraction from more sub-samples.

3. Identify the most sensitive group of species and calculate TP by using SSDs (for the few PAHs where sufficient data are available).
4. Use EC_{50} values in the TP calculations.
5. Conduct additional bioassays with the most sensitive group(s) of species.
6. Include more sophisticated analysis of ecological field data.
7. Investigate the potential for (monitored) natural attenuation.

It is the hope that the example of the use of the Triad has "given some flesh and blood" to the theoretical description found the previous six chapters. It is only by gaining more practical experience that we will be able to improve the way contaminated sites are assessed in reality.

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ABBREVIATIONS

ABACUS	Availability to biota for organic compounds ubiquitous in soils and sediments
AFNOR	Association Française de Normalisation
ANOVA	analysis of variance
APAT	Agency for Environmental Protection and Technical Services
BAF	bioaccumulation factor
BCF	bioconcentration factor
BISQ	biological indicator for soil quality
BKX	bodemkwaliteitsindex (Dutch, soil quality index in English)
CCME	Canadian Council of Ministers for the Environment
CEC	cation exchange capacity
CLARINET	Contaminated land rehabilitation network for environmental technologies in Europe
CLPP	community-level physiological-profiling
CSM	conceptual site model
DQO	data quality objectives
DSS	decision support system
EC_{50}	median effective concentration
EQO	ecological quality objectives
ERA	ecological risk assessment
EU	European Union
ERAMANIA	Ecological risk assessment methodology and application to the site of national interest Acna
HC_p	hazardous concentration to some percentage p of species
HOC	hydrophobic organic contaminant
HPLC	high performance liquid chromatography
ISO	International Organisation for Standardisation
Koc	partitioning coefficient or sorption coefficient to organic carbon
Kow	octanol-water partitioning coefficient
LIBERATION	Linking bioavailability, ecological risk and ground water pollution
LC_{50}	median lethal concentration
LoE	line of evidence
MI	maturity index
ms-PAF	multi substance potentially affected fraction
NERI	National Environmental Research Institute (Denmark)
NGO	non-governmental organisation
NOEC	no-observed effect concentration
NPP	net primary production
OECD	Organisation for Economic Co-operation and Development
PAF	potentially affected fraction
PAH	polycyclic aromatic hydrocarbons
PAM	pulse amplitude modulation

PCA	principal component analysis
PCB	polychlorinated biphenyl compounds
PDMS	polydimethylsiloxane
PEC	predicted environmental concentration
PICT	pollution induced community tolerance
PLFA	phospholipid fatty acid analysis
PNEC	predicted no effect concentration
POP	persistent organic pollutant
PVC	polyvinyl chloride
PWC	pore water concentration
RIVM	National Institute for Public Health and the Environment (in Dutch: RIVM)
SIR	substrate induced respiration
SMDP	scientific-management decision point
SOM	soil organic matter
SPME	solid phase microextraction
SRC	serious risk concentration
SSD	species sensitivity distribution
SSFE	sequential supercritical fluid extraction
SSL	soil screening level
THF	tetrahydrofuran
TME	terrestrial model ecosystem
TMoA	toxic mode of action
TP	toxic pressure
UK	United Kingdom
USA	United States of America
US EPA	Untied States Environmental Protection Agency
VROM	Ministry of Housing, Spatial Planning and the Environment (the Netherlands)
WoE	weight of evidence
WQO	water quality objectives
XAD2	a synthetic resin (nonionic polystyrene divinylbenzene resin)

This book is the outcome of the EU-funded research project 'Liberation' which looked to the development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution – focussing particularly on organic contaminants. The aim is to provide guidance to risk assessors and stakeholders of contaminated land in their decision making process. The book is organised in two parts.

Chapters 1-3 give short introductions and an overview of relevant topics, whilst Chapters 4-7 give more detailed guidance on how to perform an Ecological Risk Assessment of contaminated sites using the Triad approach. Chapter One gives a brief introduction to the overall principles and concepts in ecological risk assessment and decision support systems. Chapter Two presents the challenges and solutions for including bioavailability of organic pollutants in an ecological risk assessment framework. There is a more detailed presentation in Chapter Three of decision support systems for evaluating the environmental risk of contaminated soil including a description of the different stages of the Ecological Risk Assessment (ERA) process. Chapter Four gives a more detailed description of the principles in the Triad approach (a weight of evidence approach originally developed in order to evaluate sediment quality), which is recommended as a powerful tool in the final stage of the ERA. Chapter Five provides guidance for a tiered assessment of the ecological risk of contaminated soil, including decision charts and selecting of site-specific assays in the various tiers. Chapter Six contains a presentation of the most common and useful tools for assessing fate and effect of pollutants found at contaminated sites. The tools are organised in various toolboxes each allocated to a specific tier of the ERA process. Finally, Chapter Seven is a short review of the outcome of using the Triad approach in a case-study with a contaminated site in Denmark.

ISBN 90-6960-138-9
ISBN 978-90-6960-138-0