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Bioavailability of Organic Chemicals in Soil and Sediment

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The Handbook of Environmental Chemistry

Volume 100

Founding Editor: Otto Hutzinger

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Bioavailability of Organic Chemicals in Soil and Sediment

Volume Editors: Jose Julio Ortega-Calvo · John Robert Parsons

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Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last four decades, as reflected in the more than 150 volumes of *The Handbook of Environmental Chemistry*, there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry*, the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Series Editors

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Introduction Setting of the Scene, Definitions, and Guide to Volume

Jose J. Ortega-Calvo and John R. Parsons

Abstract The bioavailability of potentially hazardous organic chemicals (persistent organic pollutants, pesticides, biocides, pharmaceuticals, and others) in soil and sediment has a major impact on the environmental and human health risks of these chemicals and is an important area of scientific research. However, this area remains only partially recognized by regulators. Based on the positive experiences from the previous implementation for metals, regulatory frameworks have recently started to include bioavailability within retrospective risk assessment (rRA) and remediation for organic chemicals. In this regard, realistic decision-making in terms of hazard definition and priority setting will ensure the protection of environmental and public health, in contrast to the established approach of using total extractable concentrations, which has been shown to be inappropriate. Moreover, by addressing bioavailability reduction instead of only pollutant removal as a paradigm shift, new remediation strategies become possible. However, the implementation of bioavailability for rRA remains difficult because scientific developments on bioavailability do not always translate into practical approaches for regulators, thus requiring specific measures. For the same reason, bioavailability remains largely unexplored within prospective regulatory frameworks (e.g., REACH, pesticide RA) that address the approval and regulation of organic chemicals.

Keywords Bioaccumulation, Bioavailability, Methods, Persistence, Remediation, Risks, Sorption, Toxicity

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The bioavailability of potentially hazardous organic chemicals (persistent organic pollutants, pesticides, biocides, pharmaceuticals, and others) in soil and sediment has a major impact on the environmental and human health risks of these chemicals and is an important area of scientific research. However, this area remains only partially recognized by regulators. Based on the positive experiences from the previous implementation for metals, regulatory frameworks have recently started to include bioavailability within retrospective risk assessment (rRA) and remediation for organic chemicals. In this regard, realistic decision-making in terms of hazard definition and priority setting will ensure the protection of environmental and public health, in contrast to the established approach of using total extractable concentrations, which has been shown to be inappropriate. Moreover, by addressing bioavailability reduction instead of only pollutant removal as a paradigm shift, new remediation strategies become possible. However, the implementation of bioavailability for rRA remains difficult because scientific developments on bioavailability do not always translate into practical approaches for regulators, thus requiring specific measures. For the same reason, bioavailability remains largely unexplored within prospective regulatory frameworks (e.g., REACH, pesticide RA) that address the approval and regulation of organic chemicals.

This handbook provides an updated introduction to existing bioavailability concepts and methods, options for their innovative application and standardization, as well as pathways for the justifiable implementation of bioavailability into risk assessment and regulation. The main idea behind this handbook started from a series of scientific sessions on bioavailability of organic chemicals that we both chaired since 2010 in the annual meetings of the Society of Environmental Toxicology and Chemistry Europe (SETAC Europe), from a symposium on the topic [1], and a position paper published in 2015 in *Environmental Science and Technology* [2] . We are proud to see that this effort has already resulted, 5 years later, in the publication of this handbook, with individual chapters from the main actors in their respective fields. We believe that this book will constitute an excellent precedent for bringing this effort towards the definitive application of bioavailability into national and transnational regulations. With special emphasis on the latest advances from the last 5 years, this handbook examines comprehensively the three major coordinates defining in the chemical space of bioavailability: the physicochemical characteristics of the chemical(s), the composition of the soil/sediment matrix, and the eco-physiological, morphological, and metabolic complexities of the organisms exposed to soils and sediments that are contaminated by organic chemicals. These coordinates are discussed in the first part of this handbook, either by focusing on the chemical distribution in soil and sediment (Sect. 1), on bioaccumulation (Sect. 2), or on toxicity, persistence, and remediation (Sect. 3).

Section 1 starts with the chapter "Importance of Soil Properties and Processes on Bioavailability of Organic Compounds," which provides an overview of sorption processes, reviewing soil properties that are key for understanding sorption and examining the relationship between sorption and bioavailability to microorganisms, animals, and plants. The chapter "Sorption of Polar and Ionogenic Organic Chemicals" provides a summary of recent studies that aim to systematically uncover

how the interactions between polar and ionic chemicals and soil components are influenced by sorbate descriptors, sorbent composition, and aqueous phase conditions. The two other chapters in this section give separate attention to, respectively, non-extractable residues (NER) and dissolved organic matter (DOM) in the context of bioavailability. The contribution "Environmental Fate Assessment of Chemicals and the Formation of Biogenic Non-extractable Residues (bioNER)" describes the general microbial degradation processes of organic chemicals as related to the formation of NER and summarizes the state of the art on NER analytics with particular focus on biogenic NER. Section 1 ends with "Impact of Sorption to Dissolved Organic Matter on the Bioavailability of Organic Chemicals," which examines how sorption to DOM can modify the distribution, biological uptake, accumulation, and biodegradation of hydrophobic chemicals.

Section 2 includes three chapters on, respectively, plants, invertebrates, and vertebrates. The chapter "Measuring and Modelling the Plant Uptake and Accumulation of Synthetic Organic Chemicals - with a Focus on Pesticides and Root Uptake" discusses the different experimental approaches and predictors for the uptake and bioaccumulation of organic chemicals by plants. The focus changes in the chapter "Bioaccumulation and Toxicity of Organic Chemicals in Terrestrial Invertebrates," which covers how terrestrial invertebrates are impacted by organic chemicals, focusing on up-to-date information regarding bioavailability, exposure routes, and general concepts on bioaccumulation, toxicity, and existing models. Bioavailability to humans exposed to contaminated soils and sediments is then discussed in the chapter "Assessment of the Oral Bioavailability of Organic Contaminants in Humans."

Section 3 starts with "Carbon Amendments and Remediation of Contaminated Sediments," by introducing the most common sediment remediation methods through monitored natural recovery and environmental dredging and capping, as well as activated carbon-based sediment amendment technologies. The chapter "Why Biodegradable Chemicals Persist in the Environment? A Look at Bioavailability" turns the reader's attention to the contradictions caused by bioavailability in persistence assessments, discussing how biodegradable chemicals may become persistent due reductions in their bioavailability, thereby impacting on the rate and extent of biodegradation in soils and sediments. Finally, "Bioavailability as a Microbial System Property: Lessons Learnt from Biodegradation in the Mycosphere" summarizes the recent research on microbial ecology of contaminant biodegradation in the microhabitat surrounding and affected by mycelial fungi.

The second part of this handbook is composed of outreach chapters towards methodological and regulatory aspects of bioavailability. In Sect. 4, the chapter "Bioavailability, Bioaccessibility of Hydrophobic Organic Contaminants in Soil and Associated Desorption-Based Measurements" discusses the fate of hydrophobic chemicals in soils, the bioavailability and bioaccessibility of organic contaminants, and their associated desorption-based measurements. The contribution "Passive Sampling for Determination of the Dissolved Concentrations and Chemical Activities of Organic Contaminants in Soil and Sediment Pore Waters" explains how the bioavailability of organic chemicals in soils and sediments can be assessed by applying passive sampling. The last chapter of this Sect. 4, "Microbial, Plant and

Invertebrate Test Methods in Regulatory Soil Ecotoxicology," provides an overview on ecotoxicological effect tests, covering standard methods for the main soil organism groups (microbes, invertebrates, and plants). The single chapter in the last book Sect. 5, "Implementation of Bioavailability in Prospective and Retrospective Risk Assessment of Chemicals in Soils and Sediments", analyzes the common approaches in prospective and retrospective risk assessment and offers options for inclusion and implementation of the encompassing bioavailability assessment in these schemes.

We provide, in the last summarizing chapter, our overall perception on these advances, explaining why bioavailability science is ready for use in regulation of organic chemicals.

We would like to thank all authors in this handbook for their generous effort in providing the best of their writing skills for these individual contributions and the positive reactions always received during our editorial work. We also thank those individuals who contributed intellectually during the last years to this handbook idea but did not directly contribute as chapter authors. Special thanks to Joop Harmsen and Michael D. Aitken, who, in addition to their intellectual contributions, went beyond that by offering their personal support and friendship during all these years. The facilitating role of SETAC Europe in being the home of many of these discussions is gratefully acknowledged.

Jose Julio Ortega-Calvo & John Robert Parsons

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Part I
Chemical Distribution in Soil and Sediment

Importance of Soil Properties and Processes on Bioavailability of Organic Compounds

Joseph J. Pignatello and Sara L. Nason

Contents

Abstract Soil properties and processes play an important role in determining the availability of organic contaminants to environmental receptors. In this chapter, we provide an overview of sorption processes, review soil properties that are key for understanding sorption, and examine the relationship between sorption and bioavailability to microorganisms, animals, and plants. Traditionally, contaminant-soil systems are assumed to be controlled by equilibrium-driven processes. We review these aspects but also include information about non-equilibrium soil processes such as

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high desorption resistance and receptor-facilitated bioavailability. Understanding the full breadth of soil processes that impact bioavailability is necessary for making accurate toxicological predictions and risk assessments. We conclude the chapter by recommending areas for future research that will help improve our understanding of these complex systems.

Keywords Bioaccessibility, Bioavailability, Organic contaminants, Soil, Sorption

1 Introduction

Bioavailability is a critical factor governing the hazards of chemicals associated with particles to which they are attached. The focus of this chapter is on the processes and geochemical conditions in soil systems that influence the bioavailability and bioaccessibility of organic compounds to receptors of concern that contact contaminated soil. The term soil or soil system will be used to refer inclusively to terrestrial soil and aquatic sediment, usually accompanied by its entrained pore fluids (water and air). Relevant receptors include soil-dwelling biota such as microorganisms, plants, and earthworms as well as soil visitors who frequently contact soil via their diet or activities.

By convention, the *bioavailable fraction* is defined as the percent of total contaminant initially present in a parcel of soil that crosses the *critical biological membrane (CBM)* of the receptor under the exposure conditions. The CBM is the membrane through which molecules must pass in order to enter the organism and potentially exert a toxic effect. Depending on the receptor and mode of uptake, the CBM may be the cell membrane (as with microorganisms), the root exodermis (plant root uptake), the skin (dermal contact), the intestinal lining (ingestion), the pulmonary lining (inhalation), or other barrier. Contaminant present in soil is measured based on an exhaustive extraction process, and the amount that has crossed the CBM is usually measured in vivo.

The *bioaccessible fraction*, on the other hand, is the percent of total chemical initially present that is *potentially* available to cross the CBM under the exposure conditions and is usually estimated using in vitro experiments. The bioaccessible fraction includes the fraction of contaminant present in the fluids surrounding the CBM and the fraction sorbed to the CBM. Due to the expense and difficulties of conducting in vivo tests for many receptors, bioaccessibility is often what is studied, and a central issue in risk analysis is establishing the relationship between bioavailability and bioaccessibility.

Relative to a soil-free benchmark, the soil matrix imparts resistance to bioavailability and bioaccessibility. This resistance is primarily due to *sorption*, which inhibits the transport of molecules from their microscopic locations in the soil matrix to the CBM. Transport may be limited for thermodynamic and/or kinetic reasons.

In addition to limiting contaminant transport, soil may alter the speciation of a chemical in ways that affect the chemical's bioavailability/bioaccessibility. The physical properties of the soil system, such as particle size or moisture content, may influence contaminant molecule accessibility. Sorbed molecules can exist in states that equilibrate with the fluid at different rates, ranging from rapid to extremely slow relative to the receptor exposure timeframe. A major issue in risk analysis is whether it is possible to reliably quantify a fraction of the total analytical concentration that can confidently be considered bio-inaccessible, and therefore protective of the receptor(s) [1–3].

This chapter will focus on the properties and processes that control sorption and bioaccessibility of organic molecules. We cover foundational processes, with a focus on connections to recent literature. It is written from the authors' perspective rather than intending to be an exhaustive review of the literature and focuses primarily on the qualitative aspects of sorption and bioavailability. Some recent reviews have covered aspects of bioavailability/bioaccessibility for specific types of organic compounds and organisms in soil [4–7].

2 General Considerations

2.1 Types of Sorbates

For convenience we can categorize organic contaminants into compounds described as *apolar* (weakly polar groups with no significant hydrogen bond capability), *polar*, *multipolar* (more than one region of polarity), *ionizable* (one or more pK_a within the normal environmental pH range), *ionic* (pH-independent charge), and *zwitterionic* (opposing charges in the same molecule). These categories can exhibit distinct sorptive behaviors. We may speak of apolar and polar regions of molecules, as well — for example, the apolar hydrocarbon "tails" and polar "heads" of surfactants.

2.2 Sorption Fundamentals

Sorption is the net removal of molecules from the bulk fluid phase by solid particles. For many soil and sediment environments, a large percentage of a compound's molecules will be in the sorbed state at any given time. Thus, sorption is a key process regulating the fluid phase concentration, and thus the bioaccessibility, of a contaminant. The tendency of a contaminant to sorb (and later desorb) depends on its molecular structure, its concentration, the nature of the soil particles, the type of the sorptive interactions, the solution-phase composition, and temperature. Sorption is a dynamic process because local equilibrium seldom exists and can be disturbed by the receptor itself.

Sorption encompasses *physisorption* and *chemisorption*. Physisorption, which by far is the most common mode of sorption for anthropogenic organic compounds, involves weak intermolecular forces and leaves the electronic structure of the sorbing molecule largely unperturbed. The weak forces include London (known as dispersion), Debye (induction), and Keesom (electrostatic, encompassing dipole-dipole, quadrupole-quadrupole, charge-dipole, and charge-charge) forces. The hydrogen bond is mainly controlled by the dipole-dipole force. However, certain very strong hydrogen bonds [8] have covalent character, although they are still weak compared to ordinary covalent bonds. A comprehensive discussion of the weak forces appears in Israelachvili [9] and of the hydrogen bond in Gilli and Gilli [8]. Another major driving force for physisorption is the *hydrophobic effect*. The hydrophobic effect is not a distinct force, but rather an effect resulting from the net free energy loss upon removal of apolar molecules (or parts of molecules) from the aqueous to the sorbed phase. It is due principally to disruption of the cohesive energy of water, not any special attractive force between the sorbate and condensed phase nor any special repulsive force between the solute and water. Physisorption is generally reversible, although certain physical properties of the solid may render it slow or even to appear irreversible on the experimental timeframe (vide infra).

Chemisorption includes covalent bond formation with SOM and coordination bond formation with metal ions present at mineral or SOM surfaces. Chemisorption involves significant orbital overlap and/or atomic rearrangement. Covalent bond formation is not usually reversible, either because the activation energy for bond breakage to regenerate the original molecule is too high to proceed at an appreciable rate or because bond breaking leads to a different compound altogether. Coordination bonds are inherently reversible, but disassociation may be slow and require the presence of a displacing ligand.

The simplest equation relating equilibrium sorbed concentration (C_s, mol kg^{-1}) and equilibrium solution-phase concentration (C_w, mol L^{-1}) under a given set of conditions is the linear isotherm (Eq. 1).

$$C_s = K_d \cdot C_w \tag{1}$$

where K_d is the *sorption distribution coefficient*. For volatile compounds, partitioning between water and the gas phase may be calculated using the *Henry's law coefficient*. The percentage of compound sorbed is dependent on the ratio of fluids to solid. Ignoring the gas-phase component, a compound having a K_d equal to 1 L/kg will be 90% sorbed at equilibrium at 10% moisture by weight, but only 50% sorbed at 50% moisture [10].

Most compounds in most soils will exhibit nonlinear sorption behavior, meaning that K_d is concentration-dependent. Typically, sorption weakens as concentration increases because "site" filling progresses from the highest to the lowest energy sites. Sorption is linear only over a relatively narrow range in concentration (in which case, Eq. 1 may include an intercept) or generally as the solute concentration approaches zero. Sorption may or may not level off at very high concentrations, where all sites become occupied, but in any case ceases at the aqueous-phase solubility limit of the

compound. Various *sorption isotherm models* have been derived that account for nonlinearity and provide potentially meaningful fitting parameters, for example, the Langmuir, Freundlich, Toth, Polanyi-Manes, and related models [10–13]. Sorption nonlinearity may be a significant consideration in bioavailability models when the concentration range of interest is wide because the fluid-phase concentration determines bioaccessibility. Typically, a smaller percentage of total contaminant present in a parcel of soil will be in the fluid phase at low than at high total concentration. Desorption kinetics are also concentration-dependent. Normalized to the mass finally desorbed, the appearance of mass in the fluid phase is slower at lower concentration where the sorption energy is greater. This has implications for bioaccessibility in cases where desorption from soil is rate-limiting.

Sorption and desorption branches of an isotherm may not follow the same path. This is known as *hysteresis*, or non-singularity, and can result in less desorption than expected when the fluid-phase solute concentration is reduced, whether by receptor uptake or some other process. Hysteresis observed in laboratory experiments is often due to experimental artifacts such as non-equilibrium or unaccounted mass loss from the system (e.g., degradation, evaporative loss) during the observation. However, hysteresis can also be true in the thermodynamic sense. True hysteresis, known as thermodynamic irreversibility, can occur when the sorbate and sorbent interact to form a metastable complex. Two types have been identified: capillary condensation hysteresis in mesopores, in which the compound initially condenses as a metastable film on pore walls [14], and pore deformation hysteresis, in which the incoming solute causes inelastic expansion of the occupied pore (i.e., incomplete relaxation when the solute leaves) [15–18]. The latter occurs in pores that have flexible walls, usually associated with organic matter materials. Non-singularity means that a given solute concentration corresponds to two different sorbed concentrations! Which branch of the sorption isotherm is relevant to bioaccessibility estimation is a question that has not been satisfactorily addressed. Sometimes the desorption branch can appear to intersect the sorbed concentration axis at a non-zero level, suggesting little or no bioaccessibility of this fraction.

3 Properties of Soil Particles Important for Bioavailability

Nonliving natural soil particles encompass sesquioxide minerals, layer silicate clays, partially decomposed plant material and microbial cells, *pyrogenic carbonaceous material (PCM)*, and recent and ancient non-pyrogenic *soil organic matter (SOM)*. These materials usually exist in complex heterogeneous aggregates that may display sorption behavior not necessarily the sum of the behaviors of the individual materials. In addition, the aqueous phase may include organic matter that stays suspended in the aqueous phase known as *dissolved organic matter (DOM)* that can act as a sorbent. Figure 1 shows a schematic of contaminant distribution among different soil components and phases.

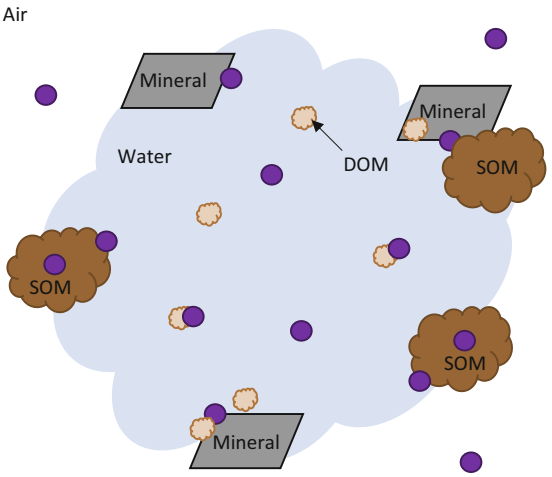

Fig. 1 Contaminants in soil (purple dots) can be found dissolved in pore fluids (water and air) and sorbed to soil components such as minerals, soil organic matter (SOM), dissolved organic matter (DOM), or complex conglomerates. Other materials such as black carbon and anthropogenic products (not pictured) can also interact with contaminants

3.1 Solid and Dissolved Organic Matter

On a mass basis, natural organic matter (OM) is the predominant sorbent of most organic compounds in soil because it presents a relatively hydrophobic phase for escape from water of molecules that are hydrophobic or have hydrophobic parts. OM molecules can exist in the "dissolved" (DOM) or solid (SOM) states. DOM, which includes molecules that are truly dissolved and those that are present in non-settling aggregates or colloids, is usually operationally defined as OM passing through a 0.45 μm filter. The current paradigm for DOM is that of a supramolecular aggregate of molecules (as small as a few hundred Daltons) held together by weak forces and metal ion bridges between coordinating groups [19, 20]. SOM may be layered on mineral surfaces or exist as patches on mineral surfaces or as discreet particles. The cohesive forces holding SOM and SOM coatings are presumably the same as for DOM, with additional forces involved in their attachment to surfaces. Most SOM/DOM molecules have net pH- and ionic strength-dependent charge due to the presence of dissociable hydroxyl and carboxyl groups and so are negatively charged at normal soil pH. Thus, OM has appreciable cation exchange capacity but little anion exchange capacity.

As a sorbent, DOM in aggregates or colloids is best described as a flexible, gel-like phase. Sorption to the gel phase occurs by solid-phase dissolution, commonly called *partitioning*. Partitioning is the cooperative intermingling of sorbate molecules and gel phase strands, such that the sorbate is more or less free to migrate among the strands within the gel. Thus, in the partition concept, the "sites" are ephemeral, and sorption is closely linear with solute concentration (as in Eq. 1). DOM can compete with the solid phases for organic solutes, especially for highly hydrophobic compounds, raising the apparent liquid-phase solute concentration. DOM may also compete for sorption sites on the solid phases.

At the microscopic level, SOM is best described as a material with both flexible-chain (soft, rubbery) regions and stiff-chain (hard, glassy) regions [15–18, 21–24]. Although sorption to it is still commonly called "partitioning," the stiff-chain regions have open voids (unrelaxed free volume) that provide specific sites for sorbate molecules to rest, thereby imparting some nonlinearity to the sorption isotherm. Molecular migration to and from these sites in SOM requires diffusion through both more flexible and less flexible regions. Typically, sorption intensity increases in the order, DOM $\leq$ extracted SOM reconstituted in particulate form $<$ unextracted SOM in whole soils $<$ ancient SOM particles (such as coaly material and kerogen). SOM phases and pores are inaccessible to even the smallest organisms. Chemisorption to SOM/DOM is possible for certain types of compounds (vide infra). Bioavailability of freshly added compounds often varies inversely with the total organic carbon (TOC) fraction of the soil (reviewed in Yu [4]). However, this relationship is not so straightforward for historically contaminated soils or for soils differing widely in composition. Many other factors come into play including polarity, charge, concentration, presence of competing solutes, SOM composition, fraction of OM composed of PCMs, nanoporosity, exposure conditions, and history of the contaminated sample.

3.2 Pyrogenic Carbonaceous Materials

PCMs, often called "black carbon," include atmospheric soot deposits, chars from natural and set fires, and carbonaceous materials deliberately added to soil for agricultural or environmental management, such as biochar and activated carbon. PCMs are regarded as ubiquitous at levels of a few percent in soils of nonimpacted areas due to natural fires. PCMs are strong sorbents by virtue of their high nanoporosity and surface area. During heating, the structure of woody or cellulosic material evolves from a transition phase consisting primarily of biopolymers with cellulose crystallinity largely preserved, an amorphous phase of thermally altered biomolecules, a composite phase of clusters of graphene (polyaromatic) sheets randomly mixed with the amorphous phase, and lastly to a turbostratic state comprised of short stacks of disordered graphitic microcrystallites [25]. The polyaromatic sheet size increases with heating temperature [26], and sheets are rimmed by polar (mainly oxygen) functional groups. The microcrystallite structure creates a network of micropores (up to 2 nm in width), mesopores (2–50 nm), and macropores ($>$50 nm). Pore size distribution and surface area depend on the pyrolysis conditions and subsequent aging processes in the environment in ways that are not completely understood or predictable [27]. Solutes undergo weak interaction with the faces and edges of PCM rings and can condense in the pores via capillary forces into liquid-like or disordered crystalline phases. Depending on their source and formation conditions, PCM typically sorbs hydrophobic contaminants more intensely than other forms of OM, often by several orders of magnitude. Thus, PCMs may dominate sorption in a soil if present in significant concentration

relative to SOM, such as at fire-impacted or industrial sites. Sorption to PCM is usually much more nonlinear than to SOM. Aging in soil typically reduces the sorptive affinity of PCM for hydrophobic compounds due to competition from deposited humic and other substances on sorption sites or in pore domains, as well as by abiotic and/or biotic oxidative processes that change the surface chemistry of PCMs after long-term exposure in the soil environment [27]. Therefore, it may be expected that environmental weathering would reduce the ability of PCM to suppress contaminant bioavailability. This was observed in the field for activated carbon added to marine sediments to reduce bioavailability of PCBs to benthic organisms [28].

3.3 Mineral Phases

Minerals commonly found in soil include the oxyhydroxides and carbonates of Ca, Mg, Al, and Fe, as well as the layer silicate clays. The surfaces of oxyhydroxides and carbonates and the edges of silicate clays generally terminate in hydroxyl groups, which are strongly hydrated. Most neutral organic compounds, especially hydrophobic ones, have low affinity for oxyhydroxide surfaces compared to the surfaces and interstices of SOM and PCM. The most important interactions of solutes at oxyhydroxide surfaces are ion exchange and coordination bonding [29]. Ion exchange can occur at surface hydroxyl groups, which may exist in positively or negatively charged form ($\equiv M^{n+}\text{-}OH_2^+ \rightleftharpoons \equiv M^{n+}\text{-}OH \rightleftharpoons \equiv M^{n+}\text{-}O^-$), depending on the metal (M), underlying mineral composition, pH, ionic strength, and local surface charge density. Coordination bonding on oxyhydride surfaces is available to organic compounds having functional groups that can displace an H_2O or OH^- ligand from the underlying metal ion (e.g., $\equiv M^{n+}\text{-}OH + RCO_2^- \rightleftharpoons \equiv M^{n+}\text{-}O_2CR + OH^-$) – especially carboxyl, phosphonate, sulfonate, phenolate, amino, and sulfhydryl groups. Complexation is greatly enhanced by the presence of adjacent groups on the same molecule that can lead to chelation of the metal, for example, salicylic acid. Organic ions face direct competition from naturally occurring ions for charged sites and coordination sites on minerals. Complicating an evaluation of the role of minerals in sorption in natural soils is that their surfaces may be coated with OM, which masks the effect of the underlying mineral.

Layer silicate clays present edge and interlayer surface environments for sorbing molecules. Clay interlayer surfaces generally have permanent negative charges distributed over a siloxane surface composed of Si-O-Si groups. Each charge is delocalized over a few O atoms and may serve as a site for ion exchange of the "natural" cation for an organic cation. The local uncharged regions of the siloxane surface are hydrophobic by nature. The interlayer space is only a few nanometers wide and packed with water, metal ions, and possibly natural organic molecules, meaning that contaminant molecules may be subject to size exclusion or retarded diffusion within the interlayer.

3.4 Anthropogenic Substances

Soils may contain anthropogenic substances that can influence bioavailability through their effects on contaminant sorption. Examples include surfactants originating from personal care products and agrochemicals; microplastics; soil amendments such as biochar, activated carbon, ash, compost, biosolids, etc.; atmospherically deposited soot particles; and nonaqueous phase liquids (NAPLs) such as coal tar and fuels. Through their micelle, hemimicelle, and admicelle forms, surfactants can influence bioavailability by their effects on apparent water solubility and interactions with soil or CBM surfaces (vide infra). Microplastics are sorptive themselves – although not powerfully so – but are usually present in low concentrations. However, they may contain or accumulate potentially toxic contaminants that can be bioaccessible when ingested. Organic soil amendments may increase the sorptive capacity of the soil. NAPLs may act as partition domains [4].

3.5 Other Soil Features Affecting Bioavailability

Soil physical-structural features, including particle size, porosity, and pore size, have a large effect on sorption and bioavailability. Smaller particles tend to have higher OM contents, larger surface areas, greater nanoporosity, and higher concentrations of contaminants. In regard to dermal exposure, particle size affects adherence to skin and mass transfer to the skin. Fine particles preferentially adsorb to skin [30, 31].

Micropores and mesopores are abundant in geological media and may account for the vast majority of total surface area of both SOM and mineral [32] phases. Sorption of hydrophobic contaminants is favored in hydrophobic nanopores – those found in SOM, PCM, and some minerals – due to the absence of strong competition from water there. Pore condensation by capillary forces in nanopores imparts a high degree of nonlinearity to a compound's isotherm. Steric size and shape can limit or prevent pore diffusion if the pore or pore throat is narrow relative to molecular size. Significant effects on the effective molecular diffusion coefficient begin to appear when the minimum critical diameter of the molecule reaches about 10% of pore diameter [33]. Since pore sizes are broadly distributed, molecules of different size will each have access to a different subset of pores. Such "molecular sieving" effects have been shown experimentally [34–36]. Nanopores are impenetrable to cells (bacteria are larger than about 1 μm) as well as many extracellular enzymes that might contribute to contaminant degradation. Duan [37] found that relative bioavailability of benzo[a]pyrene spiked in soils fed to swine decreased with increasing proportion of pores smaller than 6 nm, as determined by N_2 adsorption porosimetry.

Soil temperature and moisture content can also affect sorption. Because sorption is typically slightly exothermic, an increase in temperature generally decreases sorption affinity and therefore can be expected to increase bioaccessibility. Temperature also has a generally positive effect on molecular diffusivity. Moisture content

can affect sorption both thermodynamically (water suppresses sorption by competing for sorption sites and pore space) and kinetically (moisture facilitates diffusion by increasing connectivity between grains). The effects become exponential as moisture content decreases toward zero. Wetting-drying cycles appear to reduce bioavailability; it has been suggested that this is due to structural changes in pores or SOM phases that lead to deeper penetration of contaminant molecules [38].

4 Sorption and Bioavailability: Thermodynamic Controls

4.1 Chemical Speciation

Chemical speciation is an important factor to consider in contaminant sorption. As discussed earlier, both contaminant molecules and soil particle surfaces can have permanent or pH-dependent charge. While some contaminants of emerging concern are permanently charged (e.g., some per- and polyfluoroalkyl substances (PFAS), antibiotics, surfactants, and pharmaceuticals) under normal environmental conditions, others have pH-dependent charge because they have functional groups with pK_a values near the soil pH. Whereas sorption and mobility of neutral contaminants is largely controlled by hydrophobic interactions with organic matter, cationic and anionic contaminants have charge-based interactions that also need to be considered. Sorption behavior of cationic contaminants is particularly complicated and difficult to predict because of the variety of negatively charged surfaces in soil such clay minerals, metal oxides, PCM, and SOM, as well as direct competition for sorption sites by inorganic cations such as NH_4^+, Na^+, Ca^{2+}, and Mg^{2+}. Organic anions will meet competition from common inorganic anions in solution (e.g., sulfate, carbonate, chloride, etc.), as well as from DOM, which is a polyanionic electrolyte. Organic ions are also affected by electrostatic repulsion from surface charges and charge screening provided by ions in solution.

4.2 Partition Models and Structure-Activity Relationships

Ultimately, the way that chemicals partition in soils controls their availability to receptor organisms. The vast majority, if not all, receptors can directly access only contaminant molecules that are present in the fluid phases (gaseous or aqueous) contacting the CBM. For example, plants accumulate the highest levels of benzodiazepines in soils with the lowest amount of sorption [39]. For soil dwellers, such as plants [40] and invertebrates [6, 41], the chemical concentration in the liquid phase in soil is directly linked to adverse effects, and pore water-mediated uptake is generally the dominant pathway. The same is largely true for sediment dwellers (benthic organisms).

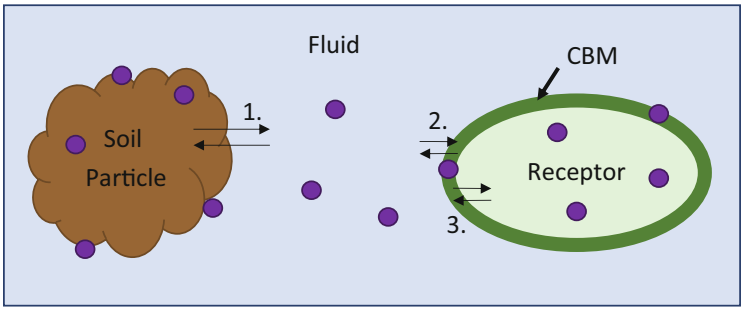

Fig. 2 Partitioning of contaminant x (purple dots) between soil and receptor organisms can be pictured as a set of equilibrium processes between (1) soil particles and pore fluids, (2) pore fluids and the critical biological membrane (CBM), and (3) the CBM and the receptor

The sorption distribution coefficient, K_d (defined above) can be used to approximate pore water concentration and thus bioaccessibility. A generalized phase diagram illustrating bioavailability of contaminant x in a soil-containing environment is given in Fig. 2.

The components are the soil particles, the surrounding fluid phases, and the CBM. Depending on the organism and mode of uptake, the fluid phases may be the in situ soil pore water and air, the digestive fluids, the pulmonary fluids, or the dermal surface lipid film. Consider the situation in which partitioning of a contaminant x between soil, fluid, and the CBM is always at equilibrium during the exposure. Bioaccessibility under thermodynamic control can be thought of as essentially a "push-pull" competition for the aqueous contaminant, x_{aq}, between soil and the CBM (Eq. 2).

$$\text{Soil}\cdots x \rightleftharpoons x_{aq} \rightleftharpoons \text{CBM}\cdots x \tag{2}$$

There are too many current and potential organic contaminants for all to be studied individually, so soil sorption and bioavailability prediction models are necessary for risk assessment. Models use physical and chemical properties of the soil matrix, receptor, and contaminant to predict how much of a contaminant will be present in various compartments such as those depicted in Fig. 2. To a first approximation, sorption affinity of the soil will be dominated by the SOM + PCM components, which can be represented by the total organic carbon (OC) fraction. Single-parameter linear free energy relationships (LFER) have been established between OC-water partitioning (K_{OC}) and *n*-octanol-water partitioning (K_{OW}) [42]. They do well for hydrophobic compounds or compounds of similar structure. Poly-parameter LFERs that take into account multiple driving forces for sorption are more accurate for diverse sets of polar and apolar compounds but still have limited predictive ability in some scenarios (vide infra) [43–46]. Since partitioning to real CBMs is experimentally difficult to measure, most work has been done using surrogate membranes like bilayer phospholipid vesicles ("liposomes") [47–51]. LFERs between octanol-water

partitioning and liposomes-water partitioning (K_{lip}) have been established for a number of compound sets [47–53].

Establishing structure-activity relationships between soil sorption and bioaccessibility is more problematic. Combining the OC-OW LFER with the liposome-OW LFER gives the liposome-OC LFER relationship with octanol-water partitioning:

$$\log K_{\text{lip-OC}} = \left(a_{\text{lip}} - a_{\text{OC}}\right) \cdot \log K_{\text{OW}} + \left(b_{\text{lip}} - b_{\text{OC}}\right) \tag{3}$$

where $K_{\text{lip-OC}}$ ($= K_{\text{lip}}/K_{\text{OC}}$) is the liposome-OC distribution coefficient, and a and b are the regression fitting parameters of the OC-OW or liposome-OW LFER. The slope of this relationship, however, is found to be quite shallow [54] due to the parity in "push" and "pull" forces represented by Eq. (2). In other words, an incremental increase in solute affinity for the soil may correspond to a similar increase in affinity for the liposome. Uptake by plants has been correlated with solute hydrophobicity, but these relationships have been developed mostly for hydroponic systems.

LFERs developed for neutral solutes are less successful for charged compounds whose sorption and partitioning is more affected by Coulombic forces. Organic cation behavior in soil has proven especially difficult to predict. The 2013 sorption model by Droge and Goss is considered a good model for predicting cation sorption. It combines estimates of partitioning to organic matter and cation exchange capacity to predict charge-based interactions with clay minerals [55]. However, this model has limited accuracy, and there is newer literature that tries different approaches to cation sorption prediction, such as the use of probe compounds that act similarly to contaminants in soil systems [9]. There is also active research focusing on methods to efficiently collect the large amounts of data that are necessary for sorption modeling. For example, Jolin et al. [56] developed a chromatography column-based method for determining sorption isotherms for cationic compounds that requires less time and labor than conventional batch experiments. However, it assumes equilibrium transport conditions. Cation behavior in soil will continue to be an important research topic as concern about cationic pharmaceuticals, surfactants, and PFAS in the environment increases. Attempts to model the relationship between soil bioavailability and receptor accumulation of ionizable contaminants have been published, but these models have received minimal validation [57, 58]. This will be an important research area moving forward.

However, even the most thorough thermodynamically driven models fail to encompass the entire relationship between sorption and bioavailability. Bioavailability is commonly modified by soil-specific speciation effects of the chemical and aging processes, non-equilibrium, and specific factors of the receptors, including contaminant metabolism abilities, morphology, feeding habits, routes of water and food uptake, and nutritional status [6]. Non-equilibrium-based aspects of sorption and bioavailability are discussed at length in Sect. 5.

4.3 Competitive Effects

Co-solutes may compete with contaminant molecules for sorption sites in soil particles, thereby increasing bioaccessibility. Competitive sorption between contaminants in soil and sediment systems is a widely reported phenomenon [4, 23, 59–64] and can be predicted by established models [24, 60, 65]. Competition is greatest between compounds of similar size due to better overlap of accessible pore sizes [59, 60]. Shared functionality may also be important if the competing solutes engage in some specific interaction with the sorbent, such as electrostatic interaction, π-π electron donor-acceptor interaction [66], or very strong hydrogen bonding [67]. Competitive effects have also been observed between contaminants and natural small molecules such as plant exudates, between contaminants and humic substances for sites on PCM, and between contaminants and polyvalent metal ions for sites on PCM [27, 61, 68]. A schematic of competition between charged species for limited sorption sites is shown in Fig. 3.

The most successful competitive model for physisorbing compounds is ideal adsorbed solution theory (IAST) [69, 70]. Equations giving individual sorbed concentrations as a function of the independently obtained single-solute isotherm parameters of each solute have been derived for the Freundlich and Langmuir models [69, 70]. Sorption of each solute depends on its own concentration and affinity for the solid at that concentration and is inversely dependent on the corresponding concentrations and affinities of the competing solutes. Compounds showing single-solute linearity are noncompetitive in the multisolute system. For a contaminant that sorbs nonlinearly alone, addition of a competitor, in proportion to competitor concentration, renders the contaminant's isotherm in the mixed system more linear; this happens because a given concentration of a competitor becomes less effective at displacing the contaminant as contaminant concentration increases [66]. This effect toward greater linearity is merely an apparent result of competition and does not signify a change in the sorption mechanism of the contaminant.

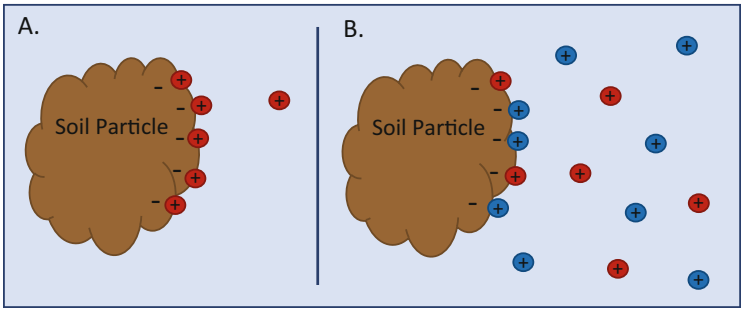

Fig. 3 In a system with one charged ion (red dots), the ion can occupy all of the sites on an oppositely charged soil particle (**a**). In a system with multiple components, those with similar properties (blue and red dots) may compete for the same sorption sites, resulting in lower sorption of each component (**b**)

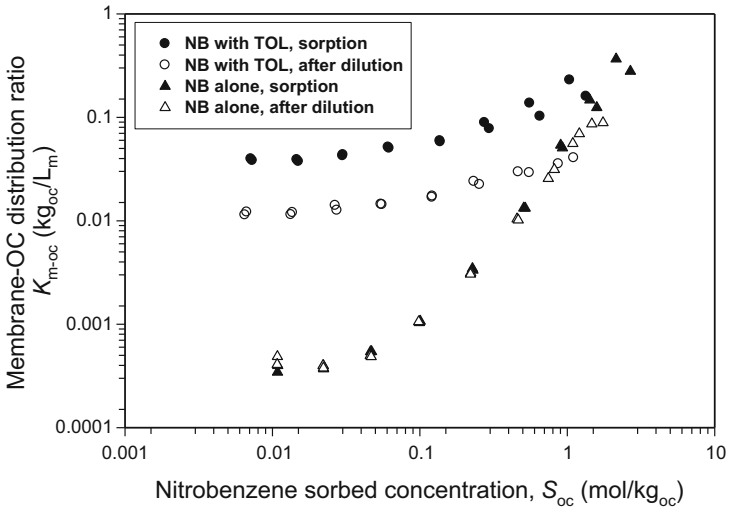

Fig. 4 Competitive effect on nitrobenzene (NB) partitioning between a liposome and a char in aqueous medium with and without a competing co-solute, toluene (TOL) at 4.23×10^{-3} mol/L initial concentration. $K_{m\text{-}OC} = K_m/K_{OC}$, where K_m is the membrane-water distribution ratio ("m" is the DMPC liposome [49]) and K_{OC} divided by the "OC" is organic carbon content of hardwood char [50, 53]. Open symbols represent experiments carried out after a tenfold dilution of the aqueous phase. Adapted from ref. [54]

Some features of competitive sorption are illustrated for nitrobenzene distributed between a phospholipid bilayer vesicle (liposome) representing a cell membrane and a wood char [54]. The single-solute sorption isotherm of nitrobenzene on the liposome is close to linear, but on the char is highly nonlinear. The liposome-char distribution ratio as a function of nitrobenzene concentration in the presence or absence of toluene competitor at two different concentrations is plotted in Fig. 4. In the absence of toluene, the distribution ratio is strongly concentration-dependent, reflecting the large difference between the two sorbents in the degree of nonlinearity of their respective isotherms. Addition of toluene suppresses sorption of nitrobenzene, (a) more strongly to the char than to the liposome; (b) in relation to toluene concentration; and (c) less effectively with increasing nitrobenzene concentration. This has the effect of flattening out the distribution ratio curve. The implication is that a competing solute can increase bioaccessibility and reduce its concentration-dependence.

Evidence for bioaccessibility enhancement by a competing co-solute has been reported. Mineralization of phenanthrene in two different soils by a *Pseudomonas* spp. enrichment culture was enhanced after adding pyrene, a non-biodegradable substrate for this organism [62]. Sterile controls showed that pyrene partially displaced phenanthrene into solution, reducing its K_d by up to 83% [62]. The presence of multiple contaminants enhanced mineralization of ^{14}C-hexadecane [71]. To the author's knowledge, no biological experiments have been performed

to validate the converse competitive hypothesis – that bioavailability decreases after removing a competing co-solute.

5 Sorption and Bioavailability: Non-equilibrium

5.1 General Considerations

Many bioaccessibility estimates and models for organism accumulation of contaminants are equilibrium-based, but equilibrium is a questionable concept for real soils, and by definition, a living organism is not at equilibrium. When non-equilibrium prevails, exposure to a toxicant will be subject to diffusion and advection processes governing transport of molecules within soil particles and from soil particles through a fluid phase to the CBM. There are many studies showing biodegradation by soil microbiota in stirred systems to be rate limited by desorption [62, 72–74]. Plant uptake is subject to diffusion both within particles and diffusion/advection through the soil column to the root [75–77]. Diffusion depends on molecular structure, the nature and geometry of the diffusing medium, the chemical potential gradient, interfacial boundary conditions, and temperature. Soil heterogeneity complicates the application of mathematical diffusion models [33, 78–80].

Intraparticle mass transport involves diffusion through pore fluids (pore diffusion), along pore walls (surface diffusion), and through the solid matrices of organic matter (solid-phase, or matrix diffusion). While diffusion is length scale-dependent, soil particles are not homogeneous, and the observed soil grain size may not represent the characteristic length scale for contaminant diffusion through particles [22]. Diffusion through tight aggregates of smaller particles may be hindered by the need to cross numerous grain-grain and grain-water interfaces to reach the edge, exacerbated by low moisture content. Diffusion through mineral aggregate pores may be slowed by sorption to particles/coatings of organic matter occluded within them [81]. Diffusion through pores is retarded by the tortuosity of pore network pathways, sorption on pore walls, and (in pores of molecular dimensions) steric hindrance. In studies of porous solids, steric effects become noticeable when molecular diameter reaches 10% of pore diameter and become severe as the diameter approaches the pore diameter [33]. Water in nanopores has restricted translational and rotational mobility, providing resistance to diffusion of small molecules compared to the bulk water phase (reviewed in [82]). Matrix diffusion in SOM requires cooperative flexing or movement of organic matter macromolecules as the molecule jumps from place to place [79]. Intra-organic matter diffusion coefficients for SOM are difficult to measure but are estimated to be a few orders to many orders of magnitude smaller than in water [79, 83]. Diffusion of an organic ion is hindered relative to its neutral form because the ion has a larger hydration shell and because counterions must diffuse simultaneously to maintain charge balance [34].

In the laboratory, sorption of a freshly added compound is often found to be slower than its desorption. There are a number of possible reasons: the intrinsic

effect of sorption nonlinearity on diffusion kinetics (desorption is slower from higher-affinity than lower-affinity sites) [80]; insufficient time allowed for equilibrium during sorption, such that during desorption some contaminant is still diffusing to "deeper" sites within the particles; or thermodynamic irreversibility in the sorption isotherm [84]. In addition, the presence of a competing solute can accelerate desorption, a result that has been demonstrated experimentally [24, 85]. These phenomena are profoundly important for predicting bioavailability based on sorption behavior and should be kept in mind when employing newly added spikes to assess bioaccessibility.

5.2 *High Desorption Resistance and Its Effects on Bioavailability*

Many reports have documented that a significant fraction of the chemical residue in a historically contaminated soil determined after exhaustive extraction strongly resists desorption and microbial degradation [22, 79, 86–88]. High desorption resistance may also be found in freshly spiked samples after even only a few hours of contact [88, 92]. Such behavior can be exhibited by many different kinds of compounds, including small hydrocarbons [89–91] and halogenated hydrocarbons [88, 90–95] capable only of nonspecific weak forces. Biodegradation of added chemicals that had been pre-equilibrated with sterilized soil prior to inoculation with a degrading culture often tails off to leave a small bio-resistant, desorption-resistant fraction [22, 62, 74]. The term "resistant," and its converse "labile," is not rigorously defined but depends on the experimental timeframe and methodology of the observer.

A number of studies using isotope labeling techniques have shown that the observed distribution ratio between soil and water after apparent equilibrium is often much greater for historical residues than freshly added chemicals, presumably because the historical residue has a high fraction of its molecules in slowly reversible sorption domains [22, 96]. Gan and co-workers proposed an isotope dilution technique to measure the bioaccessibility of historical residues of hydrophobic compounds in soil (pyrethroid insecticides, DDT, PCB derivatives) after a given short exposure period [97, 98]. They find that the bioaccessible fraction of the historical residue is as much as 80% less than that of the spiked isotope-labeled version of the same chemical.

Proposed mechanisms for formation of highly desorption-resistant fractions are described below.

1. *Formation of covalent or strong coordination bonds with the matrix.* Covalent bonding is possible for certain contaminants capable of undergoing 2-electron (nucleophilic, electrophilic) or free-radical reactions with soil substances. First, both SOM and PCM are known to contain electrophilic moieties that can react with nucleophiles to form a covalently attached product. For example, the α-β-unsaturated keto group, including the quinone group, can react with aromatic

primary amines (Ar-NH_2) or alkyl or aryl sulfhydryl compounds (R-SH) via nucleophilic attack followed by loss of H_2O to form the Schiff base or Michael-type addition product [99]. Such reactions are reversible in principle, but only difficultly so. Second, some compounds (e.g., phenols) are inherently reactive toward PCMs by oxidation and reduction pathways; the exact functional entities of PCM responsible for its reactivity are not well established, and it is not clear how much compound becomes chemically bound [100]. Third, some compounds can be converted by microbial oxidative enzymes (e.g., laccases) to reactive intermediates that can covalently bind to OM [101, 102]. Fourth, many compounds can act as growth substrates for soil microorganisms; as such, their C and N can be incorporated into complex cell biomolecules that, after death, become incorporated into the soil organic matter fraction [103]. Lastly, coordination bonding to metal ions may be slow if coordination is especially favorable and ligand detachment is the rate-limiting step.

2. *The normal process of retarded diffusion in and out of "remote" sorption domains within particles.* As discussed above, diffusion can be slowed when molecules must traverse tortuous and narrow pore networks, grain-grain boundaries, or highly viscous organic matter phases to reach the liquid phase. The normal process of retarded diffusion can often explain the observed "aging effect," in which bioavailability decreases with soil-chemical contact time prior to exposure to the receptor [104] (Fig. 5). The greater the progress toward equilibrium achieved in the prior contact step, the less will leak out during the exposure step. Due to the "random walk" nature of diffusion, molecules will diffuse both inward and outward of the particle during exposure. Thus, even when the prior contact time is short such that only the "skin" of the particle had been penetrated, some of the contaminant nevertheless will be driven inward of the particle and appear to the observer to be resistant to desorption after exposure occurs.
3. *Entrapment of molecules in closed pores*. Entrapment may occur during particle synthesis or weathering. Polycyclic aromatic hydrocarbons (PAHs) are commonly found in fuel soots and biomass chars because they are key intermediates in gas-phase reactions leading to soot condensation in flames [105]. Such PAH residues can resist desorption even under extreme conditions [106, 107], and they may poorly equilibrate with isotope-labeled PAH compounds when placed in aqueous suspension [108]. It is proposed that high desorption resistance is due to entrapment of PAHs in closed pores formed during soot condensation; escape is possible only under harsh conditions that cause swelling or flexibility of the matrix [108–111].

   Sorbed molecules may also become trapped as a result of natural weathering processes. It has been suggested that small pores can become irreversibly clogged with organic matter or mineral deposits [95]. It has also been proposed that molecules can be trapped via the adsorption-desorption process itself. According to this hypothesis, molecules at relatively high concentration approaching a local region of stiff matrix may force themselves into voids between the strands via a plasticization (softening) effect on the local matrix. When the concentration

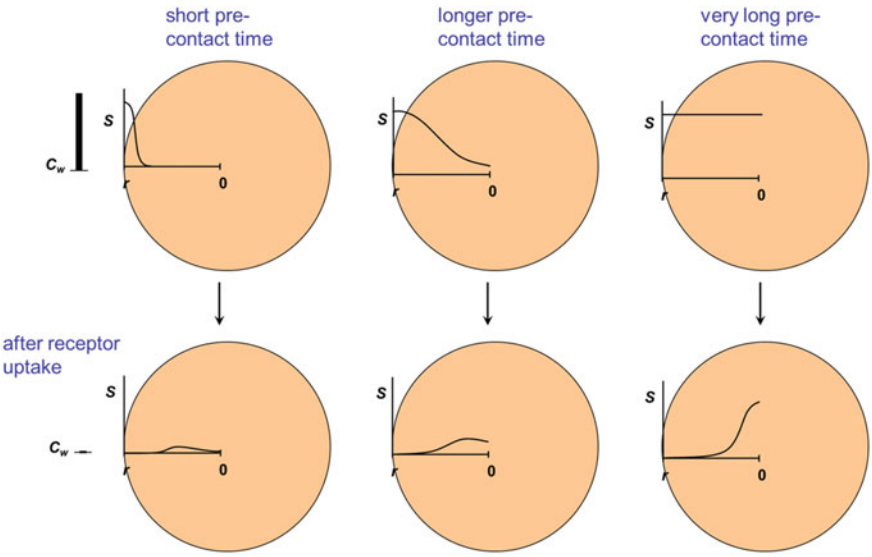

Fig. 5 Diffusion explains the "aging effect" on bioavailability. Consider a hypothetical uniform spherical soil particle of radius r in a bath at constant solution concentration C_w. Increasing the precontact time leads to deeper penetration until the sorbed concentration S reaches equilibrium. When the particle is subsequently exposed to a receptor that nearly depletes the solution phase, contaminant leaks both outward and further inward of the particle. The longer the precontact time, the more is left in a bio-inaccessible state

declines, the local matrix shrinks and stiffens around some molecules before they have a chance to escape [109, 112, 113].

Desorption resistance obviously has critical implications for bioavailability of soil contaminants to complex organisms, as well as to microorganisms that are involved in natural attenuation and bioremediation [22, 104, 114, 115]. It has become a fundamental concern of many investigators. Simply put, it relates to the question of "how clean is clean?" If some fraction of a contaminant's molecules is observed to be unable to desorb within the timeframe of exposure to a receptor, it is argued that the hazard associated with a soil containing that fraction is equivalent to that of a pristine soil [3], and thus remediation of the soil is necessary only to that level. This argument rests on the assumption that the highly resistant contaminant is truly irretrievable and will not slowly re-populate more labile states that can be bioaccessible in the future.

Several non-exhaustive, chemically based or physiologically based extraction techniques (CBET or PBET) have been developed for the purpose of predicting bioaccessibility in specific kinds of situations. One CBET approach includes the addition of a large excess of granular activated carbon or a strong polymeric adsorbent such as Tenax beads or XAD resin to the soil-water mixture to absorb nearly all potentially available molecules. This approach is relevant to situations in which the visiting receptor quickly and efficiently depletes the solution-phase

concentration. Cornelissen et al. [116] introduced an empirical exponential desorption model (Eq. 4) intended to be used in the presence of a strong third-phase sink such as Tenax that assumes "fast" and "slow" compartments of the soil,

$$\frac{S_t}{S_0} = F_{\text{fast}} e^{-k_{\text{fast}} t} + F_{\text{slow}} e^{-k_{\text{slow}} t} \tag{4}$$

where S_0 is the initial sorbed concentration, F is mass fraction, k is a desorption rate constant, and t is time. In some cases a third term for a "very slow" compartment is included. The sink is removed periodically and solvent-extracted to determine S_t. This model has been used for predicting bioavailability of historically present hydrophobic compounds with respect to microbial degraders [116–119] or soil invertebrates [120, 121]. Another CBET approach is to use extractability by an aqueous solution of hydroxypropyl-β-cyclodextrin [122], whose molecules reversibly bind contaminant molecules in a hydrophobic cavity, the binding strength of which depends on size and hydrophobicity of the contaminant.

Oral ingestion of environmental particles (including soil) resulting from hand-to-mouth activities is a contributing pathway of human exposure to some contaminants, particularly in children. A number of studies have investigated the oral bioaccessibility of particle-borne contaminants using PBETs that mimic gastrointestinal conditions (reviewed by [123]). The gastrointestinal bioaccessibility of PAHs in fuel soot particles was investigated using an in vitro human digestion model [124–127]. It was hypothesized that PAHs in the soot initially existed in either a labile or resistant state with respect to the assay conditions; the resistant fraction of individual PAHs ranged from 38% to 69% and was not correlated with molecular size.

5.3 Receptor-Facilitated Bioavailability

Facilitated bioavailability refers to the ability of the receptor itself to induce changes in its environment, actively or passively, that favor uptake. Facilitated bioavailability has been attributed in different situations to (1) receptor behavior; (2) the "surface depletion" effect; (3) physical-chemical alteration of soil structure or interfacial chemistry; (4) release of biosurfactants that enhance solubilization thermodynamically or kinetically; (5) release of substances that competitively displace contaminants from sorption sites; or (6) "direct mining" of sorbed molecules. These will be considered in turn.

5.3.1 Receptor Behavior

In many cases, the rate at which receptors consume pore water and/or soil has a large effect on how much of a contaminant accumulates in the receptor. For example, models for plant accumulation of polar and ionizable organic contaminants that

account for water uptake kinetics [57, 128, 129] tend to be more successful than those based solely on chemical properties and partitioning [130]. Similarly, for sediment-dwelling worms, those that could feed took up more triclosan than those that could not, implying that the kinetics of water and soil intake both are important for worm accumulation of some contaminants [131]. Developing a better understanding of the kinetics of contaminant molecules movement from soil into organisms is important for improving terrestrial bioaccumulation estimations.

5.3.2 The Surface Depletion Effect

Any time a receptor draws down the contaminant concentration in the soil pore water, the steepened concentration gradient induced at the interface between water and the particle "skin" will favor outward diffusion of molecules from the particle interior to the particle skin, thus favoring desorption. In in vitro bioaccessibility studies, this effect is often mimicked by the addition of a third-phase sorptive sink such as Tenax [123] or silicone polymer phases [124–127]. For example, in the study of human gastrointestinal bioaccessibility of PAHs in soot mentioned above, a silicone sheet was used to mimic solution depletion resulting from passive transfer of molecules from lumen fluids across the small intestinal epithelium (the CBM). The silicone sheet increased the apparent bioaccessible fraction, accounted for by a corresponding decrease in labile fraction still sorbed to the soot, indicating that uptake by the CBM will promote desorption from particles [126]. Other studies of hydrophobic compounds in environmental dusts also have found that inclusion of a sorptive sink enhances in vitro oral bioaccessibility [132] by promoting desorption. James et al. [133] found that inclusion of a third-phase sink in in vitro tests better predicted in vivo bioavailability of PAHs in swine. A major issue that has not yet been settled is which type of sink, if any, correlates best with in vivo gastrointestinal bioavailability.

Bacteria may demonstrate enhanced surface depletion capability because they tend to live in biofilms located close to particle surfaces. Through uptake and degradation, the biofilm may deplete the contaminant concentration in this boundary layer, driving diffusion out of the particle more effectively than an external third phase that may not penetrate the boundary water layer. Another consideration is the exopolysaccharide (EPS) mucus that many bacterial strains produce to bind cells together in a biofilm and assist biofilm attachment to surfaces [134]. This film provides a moderately effective sorptive medium, as well as a potential kinetic barrier to release of contaminants into bulk solution [135–137]. Li et al. [118] found that PAH biodegradation by native microorganisms in a historically coal tar-contaminated soil correlated roughly 1:1 with the Tenax-desorbable fraction, but when a suite of macro- and micronutrients was added, biodegradation exceeded the Tenax-desorbable fraction. It was concluded that biodegradation was nutrient-limited and that the nutrient-stimulated biofilm helped draw out the desorption-resistant PAH molecules via the surface depletion effect. It was proposed that the Tenax method may have underestimated the bioaccessible fraction due to the

inability of Tenax particles to approach the surface as closely as biofilms and to enter pores that could be colonized. Attachment of bacterial cells to surfaces has been noted to affect their ability to degrade contaminants [138, 139]; it is likely attachment gives them closer approach to the sorbed fraction while maintaining their access to the dissolved fraction.

5.3.3 Alteration of Soil Matrix or Interfacial Chemistry

Possible sources of chemical alteration induced by the receptor are a change in pH, input of chelating agents, and modification of the physical structure of particles. The pH of the 2–3 mm of soil directly surrounding plant roots (the rhizosphere) can differ from that of the bulk soil by up to 2 units in either direction due to proton uptake and excretion by root cells [140, 141]. A change in pH can shift the speciation of weak acids and bases, potentially altering their affinity for particles and/or membranes. The effects of acid-base speciation on oral absorption of drugs in physiologically based pharmacokinetic models are well-known [142]. The availability of different forms of nitrogen nutrients can result in differential plant uptake of lamotrigine, a cationic pharmaceutical. The variation in nutrient availability causes the plant to change the pH in the area directly around its roots, which causes changes in lamotrigine speciation, sorption, and bioavailability [143]. Changes in pH have far less effect on distribution of neutral, non-ionizable compounds [144].

Metal complexing or chelating agents originating from microbes or plant root exudates may accelerate desorption of soilborne contaminants by solubilizing polyvalent metal ions that cross-link OM or tether OM to mineral surfaces [118, 145, 146]. Removal of cross-links and tethers can disrupt the cohesive and adhesive forces of OM, promoting liberation of contaminant molecules entrained in the OM. Recent literature documents the effects of root exudate compounds, such as low-molecular-weight acids and on sorption and bioavailability of organic contaminants. Simple aromatic acid root exudates were found to displace 1,3-dichlorobenzene and 2,4-dichlorophenol sorbed to soil [61]. Exudation of natural chelating agents by plant roots was offered as one explanation for facilitated uptake of residual chlorinated hydrocarbon insecticides by plants [147]. LeFevre et al. [148] found that root exudates collected from various species reduced sorption of naphthalene to soil. In a similar type of test, Ren et al. [149] collected wheat root exudates, fractionated them based on charge, and found that the anionic component was responsible for most of the desorption effect [149]. Additionally, low-molecular-weight organic acids that are common in root exudates (citric, malonic, oxalic) promote desorption of pyrene [150], phenanthrene [151], sulfamethoxazole [152], BDE-28 [, 153], and BDE-47 [153] from soils, sediments, and chars. While it is clear that chemicals present in root exudates can affect contaminant sorption in soils, there is still much research to be done in this area. Differences in root exudate collection method can cause differences in the product obtained [154]. Additionally, exposure to contaminants can affect root exudate composition [155, 156], which in turn, may affect contaminant sorption.

Bioaccessibility can also be affected by changes in the physical structure of particles induced by actions of the receptor. The gizzard present in birds, reptiles, earthworms, many fish species, gastropods, and crustaceans grinds food, which can serve to break up soil aggregates and release their contaminants. However, little work has been done in this area [157]. In the gastric fluids of a human gastrointestinal model, vegetable oils included as food components promoted mass transfer of sorbed PAHs from resistant to labile states in soot particles [126]. It was suggested that lipids penetrate pores and extract contaminants there, analogous to the action of an organic solvent in analytical methodology.

5.3.4 Release of Biosurfactants

Bacteria may produce glycolipid-, lipopeptide-, phospholipid-, fatty acid-, and neutral lipid-biosurfactants. Synthetic surfactants have been studied for many years in efforts to promote bioavailability for the purpose of aquifer bioremediation [158]. Biosurfactants such as bile acids are produced in the digestive systems of humans and many animals to facilitate uptake of food substances and nutrients. Secreted surfactants increase the total liquid-phase concentration of contaminants via formation of micelles, microemulsions, or similar forms that serve as micropartition domains; they also aid in transport of contaminants across the epithelial membrane. The hydrophobic domains of surfactants compete with soil sorbents and the CBM phases for contaminant partitioning [159–162]. Surfactants can also form admicelles and hemimicelles on soil and/or CBM surfaces, potentially affecting contaminant partitioning and diffusion kinetics at the surface-water interface [158].

Experimentally, surfactants added to soil-water systems can stimulate or inhibit microbial biodegradation of contaminants, depending on the surfactant, surfactant concentration, and conditions [163, 164]. Added below the critical micellar concentration (cmc) in water, surfactants appear to have little effect on dissolution mass transfer rate coefficients with respect to nonaqueous phase liquids (NAPL) [165] or crystals [137, 166]. However, they may help separate NAPLs from soil particles by lowering surface tension [167]. There seems to be no evidence, however, that surfactants below the cmc affect interfacial mass transfer coefficients of (dilute) sorbed soil contaminants. In an interesting case, synthetic surfactants added below the cmc actually *reduced* the bioavailability of sorbed contaminants to bacteria by disrupting biofilm attachment [164]. Added above the cmc, surfactants actually *lower* the NAPL-[165, 168] or crystal-[166] to water mass transfer coefficient of chemicals, although the observed dissolution rate increases due to enhanced solubilization. PAH degrader biofilms can grow directly on the surfaces of PAH crystals [137, 169, 170]. Nevertheless, added bacterial EPS do not increase mineralization rates of contaminants despite boosting their water solubility [137]. Hemimicellular phases on cell surfaces seem to facilitate chemical entry into the biomembrane [161, 162, 171, 172]. A recent review [173] summarizes literature that finds that

rhamnolipids can modify bacterial membrane properties in ways that increase permeability to hydrocarbons.

Food ingestion triggers secretion of bile acids in mammalian digestive systems. Bile acids in human in vitro digestion models generally increase bioaccessibility of hydrophobic compounds in soil, black carbon particles [126, 174], and environmental dusts [132]. However, bile acids alone at realistic concentrations had no systematic effect on the distribution of native PAHs between labile and resistant fractions in a fuel soot [126]. The addition of soybean oil representing dietary lipids increased PAH bioaccessibility in soot in an in vitro gastrointestinal model [126], and lipids are also known to increase bioavailability of PAHs in grilled meat [175]. Apart from the organic solvent extraction effect mentioned above, this may be due to the formation of mixed lipid-bile acid micelles that have expanded hydrophobic domain [176], which helps solubilize hydrophobic compounds relative to the pure bile acid micelles [177, 178].

Inhalation bioaccessibility of organic compounds has received relatively limited attention [179, 180]. Human lung fluids contain a surfactant soup of phospholipids (>90%) and proteins (<10%), as well as smaller amounts of organic acids, amino acids, antioxidants, and metal ions [181, 182]. PBETs representing inhalation bioaccessibility of organics have employed simple model phases such as phospholipid vesicles or 1-octanol or the more complex liquids mimicking human extracellular or intracellular lung fluids [179, 180]. The fluids might be expected to have a surfactant effect on contaminant desorption from environmental particles. However, a recent study found little release of PAHs from biochar in complex simulated lung fluids [180].

The natural lipids present in or on the external skin epidermis may affect dermal bioavailability. While it has been shown that epidermal lipids can facilitate intercellular diffusion in the skin [31, 183], the question of whether lipid films can themselves transfer to attached soil particles and facilitate mass transfer of contaminants out of the particles has apparently received no attention.

5.3.5 Direct Mining

It has been suggested that bacteria are capable of directly accessing sorbed molecules. This "direct mining" hypothesis stems from findings that biodegradation rates sometimes exceed maximum desorption rates obtained by exhaustive physical stripping from the aqueous phase, such as by gas sparging or the addition of polymer resin beads [118, 184–188]. However, the hypothesis that cells can pluck molecules off the surface requires careful scrutiny because bacteria are too small to enter locations in the solid phase where the vast majority of sorbed molecules reside – in micropores and small mesopores or within SOM phases. More likely, the observed rate enhancements are due to biosurfactant production or the surface depletion effect. The latter is supported by a comparison of dissolution rates of nonaqueous phase liquids (NAPLs) [189, 190] and PAH crystals [137] in the presence versus the absence of bacterial degraders, as discussed above. Singh

[191] reports that bacteria can degrade fenamiphos molecules sorbed in the interlayers of cetyltrimethylammonium-exchanged montmorillonite clay much faster than the molecules can desorb to an activated carbon third-phase sink in the external aqueous phase. They provide evidence for the involvement of an extracellular enzyme produced by the bacterium that is capable of adsorbing to the organoclay while still remaining active and suggest the enzyme penetrates the interlayer space. Given the narrow width of the measured interlayer space in the presence of the pesticide – about 1.80 nm – it would seem far easier for fenamiphos molecules to diffuse out than enzyme to diffuse in. A simpler explanation is the creation of a surface depletion condition induced by enzyme adsorption to the external surfaces [139, 185]. Regardless of whether the "direct mining" hypothesis turns out on further research to be valid, it will almost certainly remain generally true that the dissolved state is more bioaccessible than the sorbed state.

6 Conclusions and Future Directions

Soil sorption is a major factor controlling bioavailability and bioaccessibility. Sorption is a complex phenomenon, and an understanding of the processes that underlie sorption is necessary for conducting accurate risk assessment for chemical exposures as well as for developing technologies used to contain and remediate sites with organic chemical contamination. The standard way of thinking about sorption focuses on bulk properties and equilibrium conditions, but as we have described, this is not sufficient for fully encompassing the sorption-bioavailability relationship. For example, Duan [37] found no correlation between soil properties (including total organic carbon, clay, silt, pH, electrical conductivity, or cation exchange capacity of soils) and relative bioavailability of a PAH to swine. Future research is necessary for incorporating sorption dynamics as well as receptor effects into sorption and bioavailability models and predictions. Our understanding of the influence of soil properties and contaminant structure on oral, pulmonary, and dermal bioaccessibility in humans and vertebrates is still in infancy. In these environments soil particles are isolated from the soil matrix and surrounded by biological fluids. Efforts are needed in the development of in vitro models. Topics of focus should include identifying appropriate mimicking fluids; identifying appropriate agitation conditions (in the case of gastrointestinal bioaccessibility); developing free energy relationships for partitioning of contaminants between soil particles and lung, gastrointestinal, and dermal fluids; and developing free energy relationships for partitioning of contaminants between water and surrogate biomembranes. A critical need is models or protocols that can relate in vitro bioaccessibility to in vivo bioavailability.

It will also be necessary to expand the scope of chemicals targeted for study. Much current and previous research focused on the legacy contaminants, such as PAHs and PCBs, which are neutral and hydrophobic. More effort should be directed toward polar, ionic, and ionizable compounds in classes such as pharmaceuticals, personal care products, pesticides, per- and polyfluoroalkyl substances (PFAS), and

other commercial compounds, which can be both highly water soluble and bioaccumulative and whose behavior cannot be as easily predicted using K_{OC} and K_{OW}. An additional concern is the degradation byproducts of such compounds which can be numerous and potentially hazardous.

Although sorption to soil per se has received a lot of attention historically, there are still many aspects of sorption behavior whose effects on bioaccessibility remain unclear or undocumented. While it is well-known that sorption and sorption rate are concentration- and moisture-dependent, the influence of the same on bioaccessibility has not been systematically investigated. Sorption studies are usually carried out under saturated conditions in a flask, but the vadose zone can fluctuate widely in moisture content. Further research on sorption of charged and ionizable compounds in soils is essential. Sorption hysteresis is an important topic in sorption science. Yet serious questions remain about how to interpret a non-singular isotherm in the context of bioavailability. The aging of chemicals in soil and the weathering of sorbents introduced to soil such as PCMs have received a fair amount of attention, but CBET models that can predict bioaccessibility in historically contaminated soils are still lacking. The role physical entrapment plays in high desorption resistance has not been satisfactorily resolved. The effects of water uptake and soil ingestion on bioaccessibility of contaminants to soil dwellers are incompletely understood. Further studies are needed to address bioaccessibility of contaminants to plants, as contaminants are introduced via irrigation water and biosolids applications, and plants are known to take up a variety of compounds. Chemical changes in the rhizosphere affecting contaminant speciation and bioaccessibility to plant roots (pH, root exudates) require further attention.

Connections between sorption, bioavailability, and bioaccessibility will remain a necessary and fascinating research topic for the foreseeable future, and we look forward to learning about future advances in the field.

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Sorption of Polar and Ionogenic Organic Chemicals

Steven T. J. Droge

Contents

Abstract The sorption process of polar chemicals to soil is in general similar to that of nonpolar chemicals and is in most cases still dominated by interactions with soil organic matter. In contrast, the sorption process for ionogenic organic chemicals (IOCs) is very different from that of nonpolar chemicals, particularly for IOCs where >90% is ionized as a cation, anion, or zwitterion. Organic ions in soil sorb to different parts of organic matter, by different processes, and often also to different soil components, such as minerals. This chapter provides a summary of several relatively recent studies that aimed to systematically uncover how the interactions between polar chemicals and ionic chemicals and soil components are influenced by (a) sorbate descriptors, (b) sorbent composition, and (c) aqueous phase conditions. The sorption data in several of these studies were collected on a single type of soil organic matter, micronized Pahokee peat, by a single method, dynamic high-pressure flow-through column studies using controlled aqueous medium. This

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chapter collected these consistent K_{OC} values obtained for a structurally diverse range of (non)polar, cationic, (perfluorinated) anionic, and zwitterionic chemicals, which could serve as a (growing) reference database for environmental scientists, modelers, regulators, and registrants.

Keywords Linear free energy relationships, Minerals, Organic matter, Polyparameter relationships, Sorption mechanisms

1 Sorption of Polar (Nonionic) Chemicals

The sorption process of polar chemicals to soil is not too different from that of nonpolar chemicals. In most cases it is still dominated by the chemical's hydrophobicity, the disruption of the cohesive energy of water, making it more favorable to be absorbed in the far less cohesive matrix of soil organic matter (SOM). Polar interactions such as hydrogen bonding typically weaken the sorption process, because they result in more favorable chemical interactions with water molecules relatively to SOM [1]. Whereas the octanol-water partitioning coefficient does include hydrogen bond interactions, it has been shown that when multiple polar functional groups are present in a chemical, the overall set of interactions with SOM is significantly from those with octanol [2, 3]. Only for a few neutral chemical classes such as anilines and alcohol ethoxylates do other sorption processes and soil components become more dominant in controlling their sorptive properties, thereby controlling their environmental fate [4–6].

1.1 Classical Linear Free Energy Relationships

The classic hydrophobic sorption model dating back to the 1981 paper of Karickhoff [7] is a simple single-parameter relationship between the OC-normalized soil partition coefficient (K_{OC}) and the octanol-water partition coefficient (K_{OW}). It was carefully evaluated with a relatively large dataset, but it should be kept in mind that it was basically based on a K_{OC}-K_{OW} relationship for a series of only five polycyclic aromatic hydrocarbon (PAH) components: benzene, naphthalene, phenanthrene, anthracene, and pyrene.

The first starting point of Karickhoff's approach was to define that the OC content was the dominant soil binding component. For each of the five PAHs, a strong correlation was found between the sorption coefficient and the OC content of a set of 17 sediments and soils. Convincingly, a constant K_{OC} could be derived for each evaluated compound that explained sorption to all these environmental substrates.

From this set of five PAH structures, the now famous equation was derived using K_{OW}:

$$\text{Log } K_{OC} = 0.989 \cdot \text{ log } K_{OW} - 0.346 \quad (1)$$

As Karickhoff noted, the near-unity coefficient for log K_{OW} "substantiates the constancy of the ratio of fugacity coefficients in the organic phases" (i.e., octanol and organic matter) for this series of chemicals, which allows for the linear form of:

$$K_{OC} = 0.411 \cdot K_{OW} \quad (2)$$

Of course, these five PAH compounds are structurally not very diverse, so it was questionable from the start how this relationship applies to polar and ionogenic compounds. Karickhoff [7] already evaluated this equation against an extensive set of K_{OC} values derived for pesticides, which included a wide variety of polar features. Compounds for which solute speciation was expected (such as organic bases with $pK_a > 3$) were, wisely, already excluded by Karickhoff. From this dataset evaluation, Karickhoff found that the calculated K_{OC} deviated in most cases not more than a factor of 3 (or 0.48 log units), which could be considered adequate for risk assessment modeling. Phenyl ureas (e.g., diuron) presented an interesting exception, however, with K_{OW} calculations consistently more than an order of magnitude lower than measured K_{OC} values. Since octanol is also closely related to physicochemical parameters such as water solubility, early "linear free energy relationships" to predict K_{OC} were also derived with water solubility as a parameter. Water solubility, however, relates to the interactions between the solute with itself in a crystalline form, and for many chemicals these are often less accurate than the interactions with octanol in describing the interactions with organic matter. Karickhoff already presented the example of the chloro-S-triazines, where the least soluble (simazine) is also the least sorbed. Another well-known example for PAHs is the three-ringed isomer pair of anthracene and phenanthrene, for which both the log K_{OW} and log K_{OC} are nearly equal (4.53/4.48 and 4.3/4.2, resp.) while the maximum aqueous solubility of anthracene is a factor of 20 lower than that of phenanthrene [8].

A major source of uncertainty in deriving, modeling, and predicting the K_{OC} for more complex polar chemicals is that for collecting a sufficiently large database, data is extracted from multiple sources that performed experiments with different soil types and different experimental setups and in different labs. Bronner and Goss (2011) derived their own independent and highly consistent set of K_{OC} values for a systematic series of organic chemicals with high structural variability, including a broad series of pesticides, as listed in Table 1 [2, 3]. They used dynamic column binding studies with a single batch of micronized Pahokee peat as a purified form of soil organic matter [2, 3]. This systematic evaluation of how nonpolar and polar functionalities influence the sorption to soil confirmed the strong relationship between K_{OW} and K_{OC} for nonpolar organic chemicals, following the same trend line as defined by Karickhoff, within a window of 1 log unit with the Karickhoff line as a maximum level (Fig. 1 – Left). Nonpolar chemicals were defined as having a

Table 1 Sorption coefficients for neutral chemicals on micronized Pahokee peat [2, 3]

Chemical name	Log K_{oc}	Chemical name	Log K_{oc}	Chemical name	Log K_{oc}
C_8-based neutral		*Other neutral*		*Neutral pesticides*	
2,2,4-Trimethylpentane	3.60	1-Heptene	2.84	Alachlor	1.84
1-Octene (unsaturated)	3.33	1-Nonene	3.84	Atrazine	1.82
1-Chlorooctane (halogen)	3.83	1-Decene	4.39	Azoxystrobin	2.44
2-Octanone (ketone)	1.24	1-Chloropentane	2.47	Bensulide	3.47
2-Ethyl-1-hexanol (hydroxy)	1.51	1-Chloroheptane	3.36	Bromacil	1.4
Di-*n*-butyl ether (ether)	1.65	Di-*n*-pentyl ether	2.78	Carbamazepine	2.29
1-Nitrooctane (nitro)	2.47	Di-*n*-hexyl ether	3.60	Carbaryl	2.31
Ethylbenzene (aromatic)	2.08	Ethyl tert-butyl ether	0.64	Carbendazim	2.96
		Ethyl tert-pentyl ether	0.99	Chlorobenzilate	3.26
Simple cyclic neutral structures		3-Ethyl-3-hexanol	1.05	Chlorothalonil	2.96
Cyclohexene	1.84	4-Ethyl-3-hexanol	1.28	Clothianidin	2.05
1-Methylcyclohexene	2.08	2-Nonanone	1.87	Cyanazine	1.76
Benzene	1.32	2-Decanone	2.37	Cymoxanil	1.22
Toluene	1.77	2-Undecanone	2.82	Cyproconazole	2.3
Chlorobenzene	2.08	1-Nitropentane	1.4	Desethylatrazine	1.56
4-Chlorophenol	1.94	1-Nitrohexane	1.95	Diazinon	2.46
1,4-Dichlorobenzene	2.88	Isoflurane	1.26	Dichlofluanid	1.75
2-Chloroaniline	1.59	Enflurane	1.19	Dimethenamid	1.75
Propiophenone (ketone)	1.99	Halothane	1.46	Diuron	2.56
Anisole (ether)	1.46	Methoxyflurane	1.44	Endosulfan	2.89
Methyl benzoate (ester)	1.55	2-Nitroanisole	1.89	Ethofumesate	1.84
2,6-Dimethylaniline	1.38	3-Nitroanisole	2.11	Fenthion	3.44
Indole	2.04	4-Nitroanisole	2.28	Fluazinam	1.95
Benzofuran	2.04	2-Nitrotoluene	1.82	Flumioxazin	2.29
Thiophene	1.33	2-Chloronitrobenzene	2.08	Flusilazole	3.02
1,2-Dicyanobenzene	1.69	2,4-Dinitrotoluene	2.23	Fosthiazate	1.27
Nitrobenzene	1.90	2,4,6-Trinitrotoluene	1.92	Imiprothrin	2.18
Naphthalene	2.48			Irgarol	2.73
1,2-Dimethylnaphthalene	3.64	*Neutral personal care products/ drugs*		Isoproturon	1.65
Acenaphthene	3.22	Estradiol	2.76	Metamitron	1.99
Fluorene	3.63	Testosterone	2.24	Metazachlor	1.69

(continued)

Table 1 (continued)

Chemical name	Log K_{oc}	Chemical name	Log K_{oc}	Chemical name	Log K_{oc}
Phenanthrene	4.26	Deoxycorticosterone	2.53	Methidathion	2.16
Propylbenzene	2.53	Hydrocortisone	1.79	Metolachlor	1.87
Butylbenzene	2.99	Progesterone	3.02	Metoxuron	2.04
Pentylbenzene	3.51	Phenylbutazone	2.08	Metribuzin	1.48
Hexylbenzene	3.93	Ibuprofen (neutral form)	2.63	Monuron	2.06
1,2,4-Trichlorobenzene	2.95	Bisphenol A	2.49	Napropamide	2.70
1,2,3,4-Tetrachlorobenzene	3.51	Triclosan (neutral form)	4.02	Nitrofen	3.98
1-Naphthol	2.36			Octhilinone	2.75
2-Chlorophenol	1.85	*Neutral mycotoxins* (Schenzel et al.) [9]		Orbencarb	3.19
3,4-Dichlorophenol	2.40	Aflatoxin M1, B1, B2, G1, G2	2.6–3.2	Parathion	3.23
2,4,5-Trichlorophenol	2.90	Alternariol	2.1	Phenmedipham	2.16
2-Methylbenzofuran	2.37	Altenuene	2.6	Procymidone	2.23
Dibenzofuran	3.51	Tentoxin	1.4	Propachlor	1.35
Pentanophenone	2.32	Zearalenone	3.3	Propiconazole	2.77
Heptanophenone	3.16	α-Zearalenol and β-zearalenol	2.8	Propoxur	1.53
Ethyl benzoate	1.92	Verrucarin A	2.2	Sulfentrazone	1.52
Diethyl phthalate	1.53	Verrucarol, DON	<0.7	Tebutam	1.91
Di-*n*-propyl phthalate	2.01	T-2 toxin/HT-2 toxin	1.0	Terbutryn	2.55
Di-*n*-butyl phthalate	3.20	Patulin	1.2	Thiazopyr	2.33
Di-*n*-pentyl phthalate	3.73	Diacetoxyscirpenol	<0.7		
		Daidzein	3.0		
		Equol	2.6		

mass fraction of oxygen + nitrogen atoms in the molecule $\leq$ 12%, so this also includes simple monofunctional organic chemicals that are relatively hydrophobic. However, for the polar chemicals, mostly multifunctional compounds, the K_{OC}-K_{OW} relationship is actually very weak (Fig. 1 – Right), with much wider deviations observed both higher and lower than the Karickhoff trend line. This uncertainty margin may not be considered desirable from a risk assessment point of view, and improved modeling of the sorption interactions with OM is required to more accurately assess the sorptive affinity of polar (nonionic) chemicals.

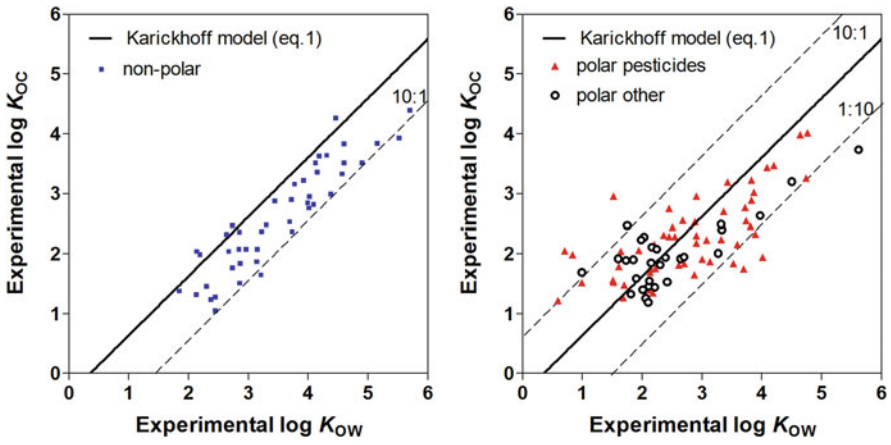

Fig. 1 Left: K_{OC}-K_{OW} plot for nonpolar sorbates. Right: Plot of log K_{OC} values for polar sorbates from this work (including pesticides, pharmaceuticals, and hormones) versus the respective log K_{OW} values. Definition of nonpolar: mass fraction of oxygen + nitrogen atoms in the molecule $\leq$ 12%. Redrawn from data from Bronner and Goss [2]

1.2 Using a Systematic Polyparameter Approach to Account for all Nonionic Sorptive Interactions

The key assumption in the K_{OW} approach as a single descriptor for K_{OC} for neutral molecules, as indicated by Karickhoff already, is that a solute's chemical interaction with octanol molecules represents that with soil organic matter (SOM). This may indeed be true for nonpolar chemicals and many chemicals with a relatively simple polar moiety, with clear exceptions such as phenyl ureas. Many pesticides, however, are often multifunctional and highly polar. The solvation interactions between molecules in octanol may substantially differ with those in SOM, and it becomes more unlikely that the single parameter K_{OW} approach to derive K_{OC} results in an accurate prediction [1]. From a mechanistically sound approach of the K_{OC} of the compound, it is thus more important to derive the average properties of SOM itself, rather than relying on octanol, that are involved with sorption interactions with the full spectrum of polar chemicals. The polyparameter linear free energy relationship (pp-LFER) approach is based on a concept that considers all interactions involved in partitioning by separate parameters, calibrated with a sorption dataset for the partitioning phases. The minimal set of five parameters should cover the prevalent nonpolar and polar chemical interactions between the whole solute molecules and average SOM structures and are also ideally derived experimentally, to avoid accumulated predictive uncertainties. One of the most comprehensive sets of the five pp-LFER includes molecular volume (V_x) and hexadecane-air partitioning (L), to cover nonpolar interactions, two hydrogen bond descriptors that relate to the capacity to act as an H donor (A) or H acceptor (B) in a hydrogen bond, and a residual polar interaction term (S), all scaled to standardized ranges [10]. Based on these five

Table 2 Examples of pp-LFER parameters for some C_8-based chemicals and two polar chemicals

	Vx	*L*	*S*	*A*	*B*
2,2,4-Trimethylpentane	1.24	3.11	0	0	0
1-Chlorooctane	1.36	4.77	0.40	0	0.10
Ethylbenzene	1.00	3.78	0.51	0	0.15
2-Octanone	1.25	4.26	0.68	0	0.51
Di-*n*-butyl ether	1.29	3.92	0.25	0	0.45
2-Ethyl-1-hexanol	1.29	4.38	0.39	0.37	0.48
Bisphenol A	1.86	8.95	1.56	0.99	0.91
Estradiol	2.20	11.11	1.77	0.86	1.10

descriptors, the coefficients (italics small font) for each descriptor (capital font) the pp-LFER can be derived based on multiple linear regression of high-quality sorption coefficients and the five descriptors:

$$\text{Log } K_{\text{sorbent}-\text{water}} = v \cdot V_x + l \cdot L + s \cdot S + a \cdot A + b \cdot B + c \tag{3}$$

whereas V is readily calculated via standardized methods and the other four parameters L, A, B, and S are best derived experimentally for each chemical using four of five substantially different sorbent phases, for which chromatographic columns provide sufficient discriminative power and consistent results [11]. These descriptors are becoming available for large sets of pesticides too [12, 13]. An online database of these descriptors is available [14]. Fitting the pp-LFER equation to the K_{OC} obtained for 79 chemicals resulted in the equation:

$$\begin{aligned}\text{Log } K_{OC} = {} & 1.2 \cdot V_x + 0.54 \cdot L - 0.98 \cdot S - 0.42 \cdot A - 3.34 \cdot B \\ & + 0.02 \left(\text{SE} : 0.24, n = 79, R^2 = 0.929\right)\end{aligned} \tag{4}$$

The pp-LFER descriptors are shown in Table 2 for several chemicals tested by Bronner and Goss [2]. Nonpolar compounds have values of 0 for polar descriptors S, A, and B, but chlorine increases S to 0.4 and B to 0.1, and similarly an aromatic ring increases S and B. Ketones and ether are only hydrogen bond acceptors and only have increased B descriptors while A remains 0. A hydroxy moiety adds to both A and B. The more polar and bulky bisphenol A and estradiol accordingly have higher V and L values and higher S, A, and B.

As shown in Fig. 2 for the training set of 79 chemicals, which included as diverse a range of properties to cover the range of descriptors, this pp-LFER approach provides for a good description of the interactions involved in the SOM sorption process. Whereas the diverse set of pesticides showed a poor correlation with K_{OW} (Fig. 1), an evaluation set of 56 pesticides and pharmaceuticals showed a relative mean standard error (rmse) of 0.4 log units, within the factor 3 recommended by Karickhoff, which corresponds also to the variation observed between K_{OC} values reported. In addition to the large set of reference neutral molecules, pesticides, and drugs, determined by Bronner and Goss [2, 3], Schenzel et al. [9] used the same

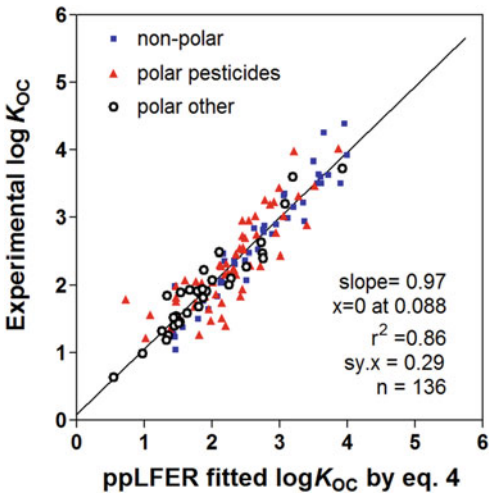

Fig. 2 Left: experimental K_{OC} values for 79 chemicals plotted against fitted K_{OC} values using the pp-LFER approach in Eq. (4). Redrawn with data taken from Bronner and Goss 2011 [2]

micronized peat and dynamic column retention setup to study the sorption affinity for a series of (mostly neutral) mycotoxins (both sets of K_{OC} values are listed in Table 2). Many of these mycotoxins represent complex polar structures, and the authors noticed that the K_{OW} values calculated with various commonly used algorithms (KowWin, ACDLabs, Marvin, etc.) often ranged over two orders of magnitude. For verrucarin A, which includes a large ring structure composed of 15 carbon atoms and 3 ester bonds, the calculated K_{OW} varied by up to four orders of magnitude. In the absence of (accurately) measured K_{OW} values, this clearly makes a K_{OW}-based estimation of K_{OC} highly uncertain, and experimental approaches or refined modeling efforts would be strongly preferred.

The key to understanding the sorption of polar chemicals to soils is thus to adequately capture the chemical interactions driving the affinity for binding to SOM over staying in water, using chemical descriptors that encompass the complexity of a multifunctional structure. Other soil components, such as black carbon phases (soot), clay minerals, and metal oxides, may also be involved in the sorption process for specific types of polar organic compounds. For example, alcohol ethoxylates, the group of mostly used nonionic detergents, sorb mostly to clay minerals in sediment because of their extensive chains of ethylene oxide units that allow for strong hydrogen bonding with silica surfaces [6]. Aniline moieties may even form (irreversible) covalent bonds with quinone moieties of SOM [4, 5]. Whereas black carbon itself is a highly variable sorbent type, typically adsorbing planar chemical structures (containing aromatic rings with little functionalities attached) more effectively than more bulky chemicals, the influence of other soil solids on overall sorption is highly specific for certain chemical classes, or even few chemicals within a class, and only applies to certain soil types (e.g., with relatively low OC content). Only systematic screening may elucidate which relevant chemical descriptors and soil properties should be included in soil sorption models and how to quantify these. It is important to notice that such additional sorbent

components in soil hamper the calculation of a K_{OC} based on soil sorption data, because the sorption is not only related to the fraction organic matter/carbon.

2 Sorption of Ionogenic Chemicals

The sorption process for ionogenic organic chemicals (IOCs) is very different from that of (non)polar chemicals, particularly for IOCs where >90% is ionized as a cation, anion, or zwitterion. Upon ionization of an IOC, the aqueous solubility is typically enhanced by orders of magnitude. However, whereas water molecules are still neutral molecules that may engage in dipole-charge interactions, many environmental substrates are also full of charged or ionizable moieties, which may strongly attract oppositely ionized molecules by various charge-charge-based electrostatic interactions. Organic ions in soil sorb to different parts of soil organic matter than neutral chemicals, by different processes, and often also to different soil components, such as minerals [15–17]. Therefore new molecular rules apply to adequately describe these sorption processes, requiring carefully calibrated new sets of models specific for each type of IOC. Most environmental substrates are predominantly negatively charged, causing organic cations to be attracted and organic anions to be somewhat repulsed from the diffusive aqueous layers surrounding these surfaces. This includes clay minerals and weathered organic matter, although commonly present metal oxides and clay mineral edges do provide for positive surface potentials. This indifferent electrostatic attraction/repulsion is strongly influenced by the aqueous chemistry. The actual interactions of these attracted or repulsed organic ions with a wide variety of charged and neutral surface functionalities are nearly always still in a hydrated phase and are influenced both by specific nonionic molecular features and by competitive inorganic and organic sorbates.

2.1 Relevance of Ionogenic Chemicals for Risk Assessment

An ionic, ionogenic, or ionizable organic chemical (IOC) is a substance that is or can become an ion in water under relevant conditions. The respective ionic species has a negative charge (anion), a positive charge (cation), or multiple charges. IOCs also include ions that have both negative and positive charges in the molecular structure but with the zero net charge (i.e., zwitterions). The terms that are most often associated with IOCs are of course “acids” and “bases.” In the context of environmental risk assessment (ERA), the terms acids and bases principally follow the Brønsted-Lowry definition. In this instance, an acid is defined as a chemical that releases a proton (H^+), and a base accepts H^+. A chemical that acts as both acid and base is referred to as an amphoteric chemical. Amphoteric chemicals with acidic dissociation constant (pK_a) which is lower than its basic pK are present primarily as zwitterion at intermediate pH.

Ionogenic organic chemicals (IOCs) represent an important group of chemicals that are widely used in commerce and industry. For instance, based on an analysis of industrial chemicals that have been preregistered at the European Chemicals Agency, Franco et al. [18] suggest that a significant fraction are IOCs (51% neutral; 27% acids; 14% bases; 8% zwitterions/amphoterics). Largely in agreement with this screening effort, a more extensive review of 5,530 substances registered with the REACH legislation in 2014 [19] indicated that 50.5% were neutral, 41.1% ionizable, and 8.4% ionic. Within the pH range 4–10, 15.3% were acidic, 14.8% basic, and 16.7 amphoteric. Some of these substances are produced and applied in high tonnages per year, and detailed risk assessment on environmental fate is essential. Furthermore, a survey of more than 900 active pharmaceutical ingredients (APIs) listed in the *Australian Medicines Handbook* found that the majority of APIs were found to be ionizable (64.2%), with the remainder comprising compounds that had a high molecular weight (14.9%) or were neutral (12.4%), always ionized (4.7%), miscellaneous (2.4%), or inorganic salts (1.3%) [20, 21]. When mixtures, salts, and high-molecular-weight chemicals are removed from the list, 85% of small-molecular-weight (<1,000 Da) APIs are estimated to be IOCs. The high relevance of these APIs is of course that they are often designed to be bioactive and often have specific effects and often unintended side effects, at relatively low exposure levels. For chemicals used in personal care products, examination of a dataset of 254 chemicals [22] suggests that approximately 35% of these chemicals may be ionized within an environmentally relevant pH range. Many of such chemicals are applied on a regular basis by large fractions of the human population. Lastly, many agricultural pesticides (e.g., glyphosate), biocides (e.g., quaternary ammonium cations), herbicides (e.g., acidic 2,4-D and related structures), and fungicides (e.g., propamocarb) are IOCs. Given the propensity of IOCs used in commercial and industrial practices, it is thus prudent to develop robust tools for assessing their environmental fate, and transport, an improved understanding of which will lead to an improved assessment of environmental exposure.

The release of ionogenic organic chemicals into the environment presents risk assessors with multiple challenges. This is because the fundamental principles underlying the risk assessment of organic chemicals have been primarily developed based on relationships largely associated with the behavior of neutral organics [23]. Consequently, concerns regarding the domain of applicability with respect to the physical and chemical space defined for the tools, models, and algorithms currently used are likely to be limited and not necessarily appropriate for chemical substances that are subject to ionization at environmentally relevant pH. In addition, particularly in instances where environmental fate and behavior are influenced by changes in pH and ionic strength, models for neutral chemicals fall short.

Analogous to Karickhoff's approach for neutral chemicals described above, for ionogenic chemicals the sorption to soils much also first be systematically studied before any relationship with soil properties and chemical descriptors can be achieved. Two distinctions need to be made first:

1. If ionizable organic chemicals are mostly neutral in the common soil pH range, the sorption to soils is most likely dominated by the neutral form partitioning into

soil organic matter. Depending on the required accuracy, the K_{OW} approach or the pp-LFER approach may be applied to predict the K_{OC}. The sorption coefficient of a partially ionized chemical may be best considered as a summed contribution of both the neutral species fraction (f_N), sorbing via the K_{OC}, and the ionized species fraction ($1 - f_N$), sorbing via its own sorption coefficient.

2. Regarding the much higher densities of acidic groups in soil organic matter, relative to basic moieties, and the predominantly negatively charged surfaces of most mineral, it makes a huge difference if the ionizable chemical is speciated into an organic cation or an organic anion. Likewise, the sorption interactions for neutral chemicals with SOM will most likely strongly differ from those between SOM and ionic species. It is therefore not appropriate to aim for a single descriptor sorption model that could magically include neutral, anionic, and cationic organic structures and aim to derive specific sorption models for organic cations and organic anions, apart from their neutral species. For describing and predicting the soil sorption process of organic ions, it is critical to understand how the sorption sites in soil may look like and which properties of soil, chemical, and aqueous phase influence the sorption process.

2.2 Chemical Speciation for Ionogenic Chemicals

The critical chemical parameter describing the chemical's ability to ionize is the acid dissociation constant (pK_a). The pK_a defines at which pH 50% of the IOC is in either the neutral or ionic form by releasing an H^+ from the neutral molecule acids (AH to anion A^-) or accepting an H^+ onto the neutral molecule base (B to cation BH^+). Strictly speaking, an acid is the chemical species before releasing H+ (e.g., phenol, Ph-OH), and the corresponding anion is not an acid (e.g., phenolate Ph-O-). The same applies to bases. The equilibrium between neutral acid and dissociated form can thus be defined as:

$$[AH] \leftrightarrow [A^-] + [H^+] \tag{5}$$

where the chemical's equilibrium speciation is defined as:

$$K_a = \frac{[A^-] \cdot [H^+]}{[AH]} \tag{6}$$

which gives the pK_a as:

$$pK_a = -\log\ (K_a) \tag{7}$$

As a function of pH, the ratio of the acid and anion is defined by the Henderson-Hasselbalch equation as:

$$\mathrm{pH} = p\mathrm{K_a} + \log\left(\frac{[\mathrm{A}^-]}{[\mathrm{HA}]}\right) \text{for acids, and : } \mathrm{pH} = p\mathrm{K_a} + \log\left(\frac{[\mathrm{B}]}{\mathrm{BH}^+}\right) \text{for bases} \quad (8)$$

It is conventional to consider [BH+] as acid and use "pK_a" and other relationships for bases as well. The fraction of neutral species (f_N) for simple IOCs (one acidic or basic site) can be readily calculated with a derivatization of the Henderson-Hasselbalch equation:

$$f_N = \left(\frac{1}{(1 + 10^{\alpha}(-\mathrm{pH} + pK_a)}\right) \text{in which } \alpha = 1 \text{ for bases, and } -1 \text{ for acids.} \quad (9)$$

A complete, 100% ionization of an acid never happens in the strict sense, as can be demonstrated from the Henderson-Hasselbalch equation. For environmental risk assessment (ERA) purposes, strong acids/bases may be defined as those IOCs that are always >99% ionic (i.e., $f_N < 0.01$). Because the environmentally relevant pH range is 4–9 (see below), strong acids are those with $pK_a < 2$, and strong bases are those with $pK_a > 11$. For ERA, very weak acids/bases are those IOCs for which the neutral form will dominate nearly all relevant partitioning interactions, which we suggest is representative of systems where the ionic fraction is <10% at pH 4–9 (i.e., very weak acids $pK_a > 10$, very weak bases $pK_a < 3$). In the case of weak acids (i.e., pK_a between 2–9) and weak bases (i.e., pK_a between 3–11), the pH-dependent partitioning of both the ionic and neutral species should be considered to assess environmental fate and transport in specific environmental systems.

A "permanently charged chemical" means either of the following two:

1. An IOC that has only an ionic form. A neutral form does not occur by protonation or deprotonation. Examples are quaternary ammoniums, phosphoniums, and borates.
2. An IOC that is always charged for >99.99% within the relevant pH range. In this sense, permanently charged chemicals include "truly" permanently charged chemicals and very strong acids/bases. Examples may include organic sulfate and sulfonate anions ($pK_a < 0$), with the detergent ingredients sodium dodecyl sulfate (SDS) and linear alkylbenzene sulfonates as important representatives, but also include perfluorinated sulfonates and carboxylates such as PFOS and PFOA ($pK_a < 1$).

2.3 Sorbent Speciation Driving Surface Potentials

Soils and sediments can be composed of wide varieties of sandy (>63 μm), silty (2–63 μm), and clayish (<2 μm) particles that co-occur in various distributions. The natural organic matter fraction, and dissolved organic matter fractions, can also be of structurally very different compositions, depending on weathering status and types of organic input in the system. However, to understand the sorption of IOCs to environmental substrates, several features stand out:

1. Organic matter is not just a hydrophobic phase, and certainly not just a slightly polar solvent. In all cases, the weathered material that is left poorly degraded is rich in acidic functionalities such as carboxylic acids and phenolic groups. The typical cation exchange capacity (CEC) of dissolved organic carbon (DOC) such as humic acids and fulvic acids is 0.5–5 mol charge equivalent per kg dry weight (molC/kg dw) [24] or, in alternative units for the CEC, 50–500 meq/100 g. The carboxylic acids progressively dissociate in the pH range 3–5, while the phenolates progressively dissociate in the pH range 6–9, as shown by two bumps in the pH profile of charge development (see Fig. 4, HH-21). The anion exchange capacity (AEC) of DOC, in terms of residual amine groups, is often negligible because these valuable nitrogen sources are often actively reintegrated by microorganisms, in the order of a hundredfold lower than the CEC.
   - OM charge type A. [weathered organic matter]~C(=O)O^- . . .H^+ (pK_a range ~ 3–6)
   - OM charge type B. [weathered organic matter]~aromatic ring-O^- . . .H^+ (pK_a range ~ 8–10)
2. The surface area of sand particles is often negligible to that of the clay fraction, and therefore the sand fraction is often a negligible sorbent phase. Nearly all disk-like clay minerals (phyllosilicates) have a negatively charged surface (see Fig. 4), due to two features. Firstly, phyllosilicates are often reformed by weathering processes of larger mineral structures, and under specific conditions different clay minerals can form. Typically, the disks formed consist of a silica oxide layer on top of an alumina oxide layer (a 1:1 mineral) or have alumina oxide sandwiched in between two silica oxide layers (2:1). Often during clay formation, isomorphic substitutions take place in these crystal layers, e.g., Al^{3+} in place of Si^{4+} or Mg^{2+} in place of Al^{3+}. These substitutions create permanent charge defects that always create a negative surface charge on the outside of the particles. This may strongly contribute to the CEC of soils and sediments. Secondly, the external surfaces of stacked disks can have ionizable moieties. The silica oxide layer has some residual acidic hydroxyl moieties that give rise to an additional, pH-dependent, amount of negative charge that adds to the CEC. In contrast, alumina oxide is rich in hydroxyl groups that have a higher pK_a and which results in a +1 positively charged surface on the aluminum atom when a surface OH groups become protonated and detach as water molecules. This counters the surface potential influence of negative charges of dissociated silica hydroxyl moieties in 1:1 minerals and reduces the CEC in clays like kaolinite. Because the alumina layer is sandwiched in 2:1 minerals, these clays have a much higher CEC consisting of both permanent and pH-dependent charge types. The aluminum middle layer may contribute some positive charge sites at the disk edges. If the majority of the isomorphic substitutions are located in the alumina layer of 2:1 minerals, the charge defect is distributed over both silica surfaces, which creates only a rather weak attraction between stacked disks. As a result, such clays like montmorillonite are “expandable,” i.e., water and ions can penetrate and diffuse into the interlayers between each disk. This creates a strongly increased CEC. Non-expandable clays such as illite are kept tightly stacked with, e.g., potassium

ions kept non-exchangeable in between the disks, resulting in lower CEC than expandable clays.

- Clay charge type A. [isomorphic Al^{3+} in place of Si^{4+}, or Mg^{2+} in place of Al^{3+}]$^{-}$. . .K^{+} (permanent)
- Clay charge type B. [tetrahedral silicon oxide] -O^{-} . . .H^{+} (pK_a range ~ 3–6) (pH dependent)
- Clay charge type C. [basal octahedral aluminum oxide] Al_2-OH.H^{+} (pK_a range ~ 8) (pH dependent)
- Clay charge type D. [edge octahedral aluminum oxide] $AlOH^{-0.5}$.H^{+} (pK_a range ~ 10) (pH dependent)

3. Metal oxides based on iron (oxyhydroxide goethite, α-FeOOH; hematite, α-Fe_2O_3) and aluminum (gibbsite, $Al(OH)_3$) are the most common contributors to the anion exchange capacity in soils. The gibbsite surface is considered to have a positive surface potential with a pK_a of ~ 6 due to release of OH surface groups ($Al(OH)_2^{+}$ and $Al(OH)^{2+}$). At pH above 6, it is considerably neutralized to $Al(OH)_3$ but at elevated pH forms $Al(OH)_4^{-}$ (see Fig. 4 [25]). The similar protonation process of aluminum hydroxide occurs also in the 1:1 clay mineral kaolinite, although the overall net surface charge is negative due to excess dissociating silanol groups [26]. With iron oxides in water, hydroxylation occurs when Fe atoms on mineral surfaces complete their coordination with hydroxyl groups released by water molecules (Fig. 3). A hydroxyl group that coordinated with a

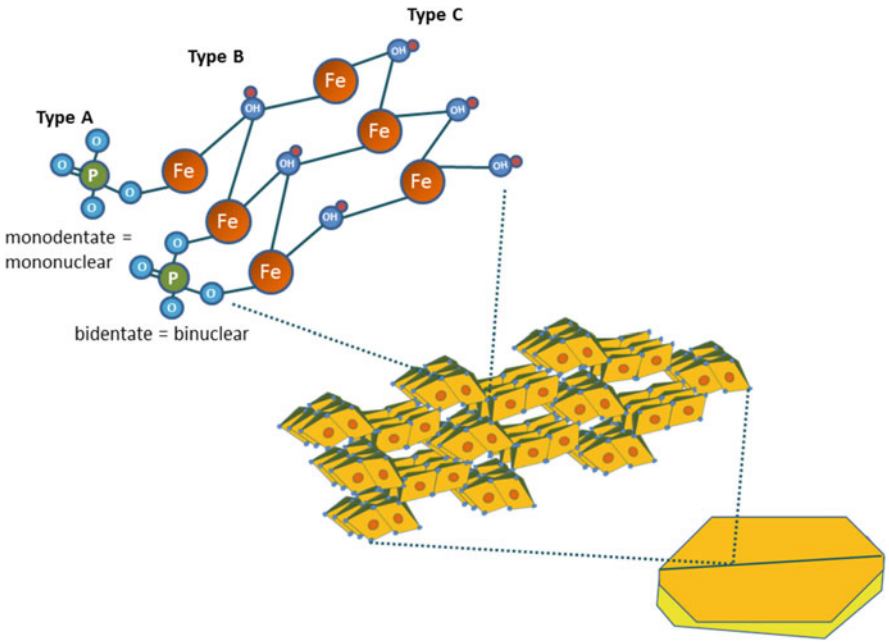

Fig. 3 Three different types of hydroxylated sites on the surface of iron hydroxide, whereby the single coordinated hydroxyl groups are replaced by phosphate in monodentate or bidentate coordination. Figure by S. Droge (2020)

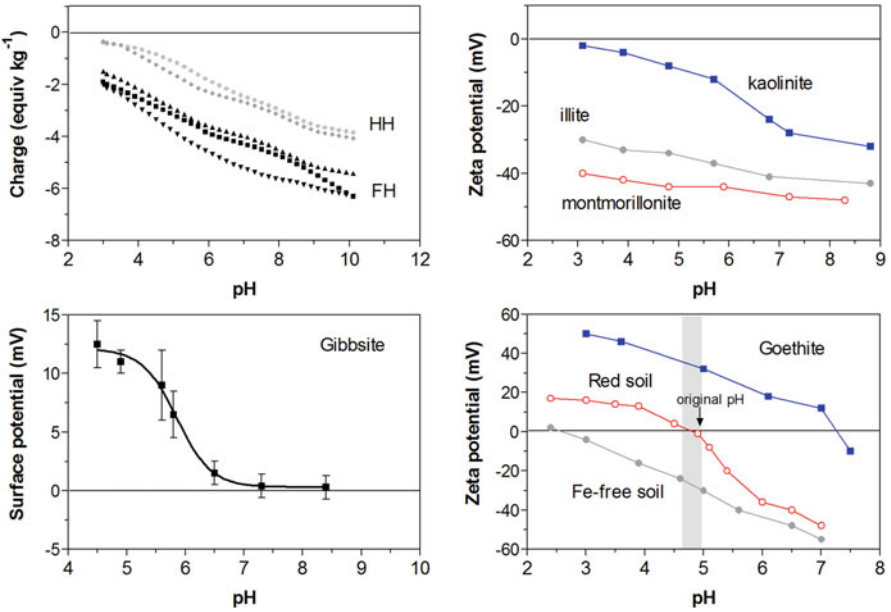

Fig. 4 pH-dependent surface charge progression on different natural substrates (*NB* charge depends also on ionic strength of the solutions): *top left* (redrawn from examples in [24]) negative charge on fulvic acids and humic acids in mol charge equivalents per kg dry weight; *top right* (redrawn from examples in [29]) the overall negative charge progression of different phyllosilicate clays in marine ionic strength solutions (0.56 M NaCl); *bottom left* (redrawn from examples in [25]) the positive surface potential progression for gibbsite (in 1 mM NaCl); *bottom right* (redrawn from examples in [28]) goethite and iron-rich/iron-depleted soil (in 1 mM NaCl)

single iron atom (type A) has a half negative charge $\equiv$Fe-OH$^{-0.5}$ which is easily protonated to get an overall +0.5 charge. In turn, this "protonated hydroxyl group" can be replaced by other inorganic anions, such as phosphate, in an inner sphere (covalent) bond. A hydroxyl group that coordinated with three iron atoms (type B) has a half positive charge $\equiv$Fe$_3$-OH$^{+0.5}$. The oxygen of the shared hydroxyl is much less electronegative than that in the type A oxide and is much less easily protonated. Goethite and hematite are thus positively charged in common soil pH, with a zero point of charge (ZPC) only at pH 7–9 [27, 28] (see Fig. 4). It depends on the number of surface iron atoms that coordinate with the hydroxyl groups how protonation occurs (type A–C). Of course, when high levels of iron oxides are mixed into soils, with a typical red coloration, the overall surface potential is lowered compared to the original goethite but may still be net positive overall at low pH (where high enough H$^+$ concentrations exist to protonate the surfaces).

- Iron oxide type A. Single iron atom coordinated hydroxyl $\equiv$Fe-OH$^{-0.5}$ (H$^+$ protonation)

- Iron oxide type B. Three iron atoms coordinated hydroxyl $\equiv Fe_3\text{-}OH^{+0.5}$
- Iron oxide type C. Two iron atoms coordinated hydroxyl $\equiv Fe_2\text{-}OH^{0}$
- Aluminum oxide surface groups with increasing pH: $Al(OH)^{2+}/Al(OH)_2^{+}/Al(OH)_3/Al(OH)_4^{-}$

2.4 Relevant Solvent Parameters for Ionogenic Chemicals

In a simplified view, the ionic moiety of an IOC can be thought of as being "attracted" to an oppositely charged sorbent, causing the IOC to be preferentially sorbed relative to being dissolved. The nonionic structure of an IOC can still be hydrophobic and for that reason gives the charged IOC molecules a preference to be sorbed into sorbent/onto a substrate surface rather than being fully dissolved. The ionic moiety of an IOC, however, mostly strongly prefers the molecule to be present in the aqueous phase. Not surprisingly, a dissociated acid anion has an orders of magnitude higher solubility compared to the neutral undissociated acid. The dielectric constant (symbol, ε) of a solvent is higher for more polar solvents, and this translates into a higher ability to dissolve ions. Water has an ε of 80.1, methanol 32.7, and acetonitrile 37.5. As a consequence, an ionizable acid will be more dissociated in water than in methanol. The ε of octanol is 10.3; the even less polar solvent dichloromethane has an ε of 8.5 and cyclohexane has only 2.02. In octanol, the partitioning coefficient of the neutral species is often more than a factor 1,000 higher than the dissociated anion or protonated cation. Due to the omnipresent acceptance of octanol as the prevalent descriptor of a chemical's sorption affinity to organic matter, it is often wrongly considered that ionic species of IOCs hardly sorb to environmental substrates. Organic cations have more recently been shown to sometimes sorb even as strongly as the deprotonated neutral base [15–17], while organic anions also have been shown to sorb substantially to natural colloids, soils, and sediments, as long as the nonionic structure is sufficiently hydrophobic [30–32].

The obvious fact that most environmental substrates and colloids are negatively charged particles results in that sorption of organic cations is a highly relevant process to describe in detail for adequate risk assessment purposes. An example of the higher than expected sorption of organic cations to organic matter has been presented by Sibley and Pedersen [16], who studied the parameters that influence the sorption of the base clarithromycin, a commonly used veterinary antibiotic, on dissolved Elliot soil humic acid. Clarithromycin is a base with a multiple polar moieties and a tertiary amine with a pK_a of 8.9 (see speciation diagram in Fig. 5). Illustrative for the underlying sorption process, this study clearly identified three different aspects of the aqueous solution composition that could influence the sorption affinity of ionizable bases.

1. When testing the pH dependency of the sorption affinity, as shown in Fig. 5, they showed that actually the protonated cation sorbed more strongly to dissolved

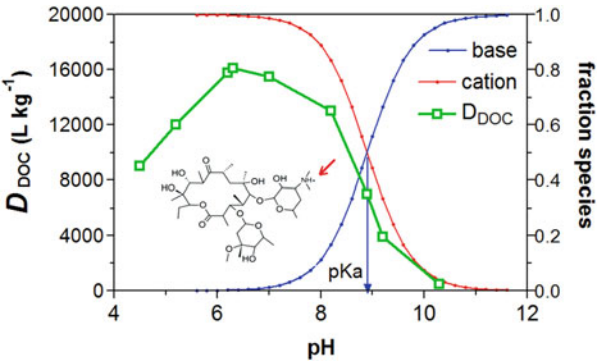

Fig. 5 pH-dependent sorption profile of clarithromycin on dissolved Elliot soil humic acid. The left *Y*-axis scales the DOC-water distribution coefficient (D_{DOC}), and the right *Y*-axis the speciation profile. The structure of clarithromycin is presented in the protonated form. Redrawn from example in [16]

organic carbon than the neutral base species, with a maximum distribution coefficient of 16,000 at pH 6, well below the basic pK_a of 8.9.

2. Furthermore, they found that when the ionic strength of the test solution was reduced by a factor 10, the sorption affinity of the protonated clarithromycin increased by a factor of 10.
3. Additionally, the sorption affinity of the protonated clarithromycin was twofold higher when the salinity was based on sodium phosphate buffer compared to a potassium buffer of equal ionic strength.

The main reason underlying this strong and variable sorption affinity of the organic cation to DOC is the abundance of negatively charged groups in DOC, such as carboxylic acids with a pK_a ~ 4–6 and phenolic acids with a pK_a ~ 8–10, that together give DOC its typical high cation exchange capacity (in the range of 0.5–5 mol charge/kg dry weight). The pH profile shown in Fig. 5 shows that the sorption affinity of the fully protonated clarithromycin increases in the pH range of 4.5–6, indicative of the increased dissociation of acidic sites on the DOC, which increases the cation exchange capacity. Another way of seeing this process is that the more abundant presence of H^+ cations in acidic solution is competing with protonated clarithromycin for the same dissociated DOC sites. It is often considered that the sorption of organic cations to DOC is an ion exchange process: the sorption of protonated clarithromycin releases a more weakly bound cation such as Na^+. K^+ cations (atomic mass 39) are larger than Na^+ cations (atomic mass 23), and K^+ consequently has a smaller hydrated radius, which translates into a higher sorptive affinity to anionic DOC sites than Na^+. At a ten times higher salinity, the solutions' cations that are competitive in binding with clarithromycin are thus also present at ten times higher levels, which would translate in the lower sorption affinity of clarithromycin.

However, this view of a mere competitive process is probably too simplistic to explain these phenomena. The sorptive capacities of DOC for metals have been described in more detail by more complex models that take into account both (competitive) electrostatic interactions at the actual sorption site (term A) and electrostatic attraction (term B) from the bulk water to the aqueous electrical double layer ("EDL") surrounding the organic DOC structure (also called diffuse water

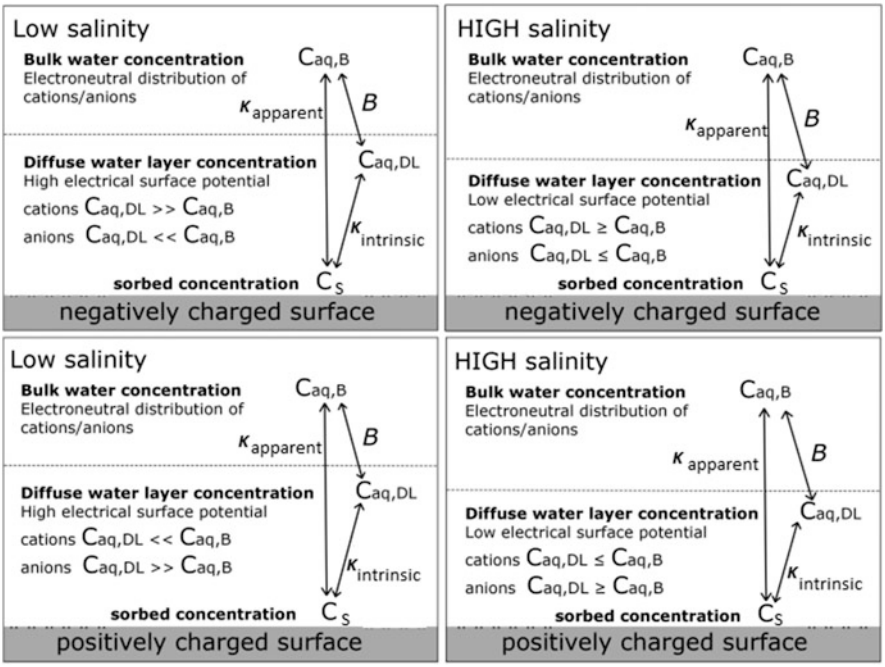

Fig. 6 Description of the sorption process between an ionic solute and a charged surface. Electrostatic attraction increases the dissolved concentration in the diffuse layer ($C_{aq,DL}$) compared to the concentration in the bulk water ($C_{aq,Bulk}$), by the Boltzmann factor B. The apparent sorption coefficient ($K_{apparent}$) should thus actually be accounting for the electrostatic attraction to identify the intrinsic sorption affinity for the surface ($K_{intrinsic}$). Extended figure from [33]

layer). The sorption affinity ($K_{sorbent\text{-}water}$) of a charged compound for a charged surface can thus be approached as an apparent affinity, which combines all effects, as well as an intrinsic sorption affinity, specific for the sorption site, as in the form below:

$$\text{apparent } K = \text{electrostatic attraction into EDL} + \text{interaction affinity with site} \tag{10a}$$

$$\text{intrinsic } K = \text{site interaction affinity, corrected for electrostatic attraction competition} \tag{10b}$$

Electrostatic attraction is described as the accumulation of oppositely charged molecules into a thin surface layer surrounding a charged surface (electrical double layer or diffuse layer) or, more relatable to dissolved organic matter, into the aqueous phase present in a wet matrix of charged organic matter structures. The *attracted* increase in a chemical's concentration in the diffuse layer is thus not due to any *interaction* with the sorption site. This electrostatic attraction can be

theoretically approached iteratively by the common Boltzmann potential equation, as is done, for example, in the Donnan term for the extensively parameterized NICA-Donnan model for metals [24, 34–36] and part of the WHAM model [37]. Ionic strength and the charge density of the sorbent material, and an adjustable sorbent property descriptor *b*, determine the influence on electrostatic attraction (see for details in Box 1).

The actual, *intrinsic*, sorption affinity of an ion for the "ion exchange site" is thus not due to the electrostatic attraction but only the competitive interaction affinity at the sorption site. What we often measure in a sorption study is the summed apparent overall sorption affinity. The electrostatic attraction strongly depends on the ionic strength and can be accounted for if one tests the influence of ionic strength on the sorption affinity. The difference between apparent and intrinsic sorption affinity is theoretically approached by the Boltzmann potential. The intrinsic sorption affinity is what is needed in the competitive sorption terms of a model like NICA (non-ideal competitive adsorption refers to the sorption process being exponentially nonlinear). These have been defined for a wide range of metal cations [35] for both the carboxylate and phenolate anion sites of DOC.

Box 1 provides a more detailed description given in Chen et al. [38] on the reasoning behind the NICA-Donnan equation which could be applied to describe (part of) the sorption affinity of cationic surfactants on DOC.

Droge and Goss [15] used dynamic column studies, and Chen et al. [38] batch sorption studies, to systematically evaluate the influence of ionic strength and main inorganic salt cation type (Na^+ and Ca^{2+}) on the sorption affinity of organic cations to micronized soil organic matter and dissolved humic acids. Both studies observed that divalent inorganic cations typically control the Boltzmann potential. At equal ionic strength, sorption affinity of organic cations is an order of magnitude lower in the presence of 5 mM Ca^{2+} compared to 15 mM Na^+:

$$\text{apparent } K_{(\text{DOC}-W)} \text{ in 15 mM } Na^+ = 10 \times \text{apparent } K_{(\text{DOC}-W)} \text{ in 5 mM } Ca^{2+} \tag{11}$$

At ten times lower divalent cation concentrations, the sorption affinity of organic cations decreases only by a factor of ~3 (0.5 log units), while in ten times lower monovalent cation concentrations, the sorption affinity of organic cations decreases by a factor of ~5 (0.7 log units):

$$\text{apparent } K_{(\text{DOC}-W)} \text{ in 15 mM } Na^+ = 5 \times \text{apparent } K_{(\text{DOC}-W)} \text{ in 150 mM } Na^+ \tag{12}$$

$$\text{apparent } K_{(\text{DOC}-W)} \text{ in 0.5 mM } Ca^+ = 3 \times \text{apparent } K_{(\text{DOC}-W)} \text{ in 5 mM } Ca^{2+} \tag{13}$$

As a result, even at a low hardness of 0.5 mM Ca^{2+}, sorption is stronger than that in the presence of 150 mM Na^+ (Fig. 7), so "hardness" controls the apparent $K_{(\text{DOC-}W)}$

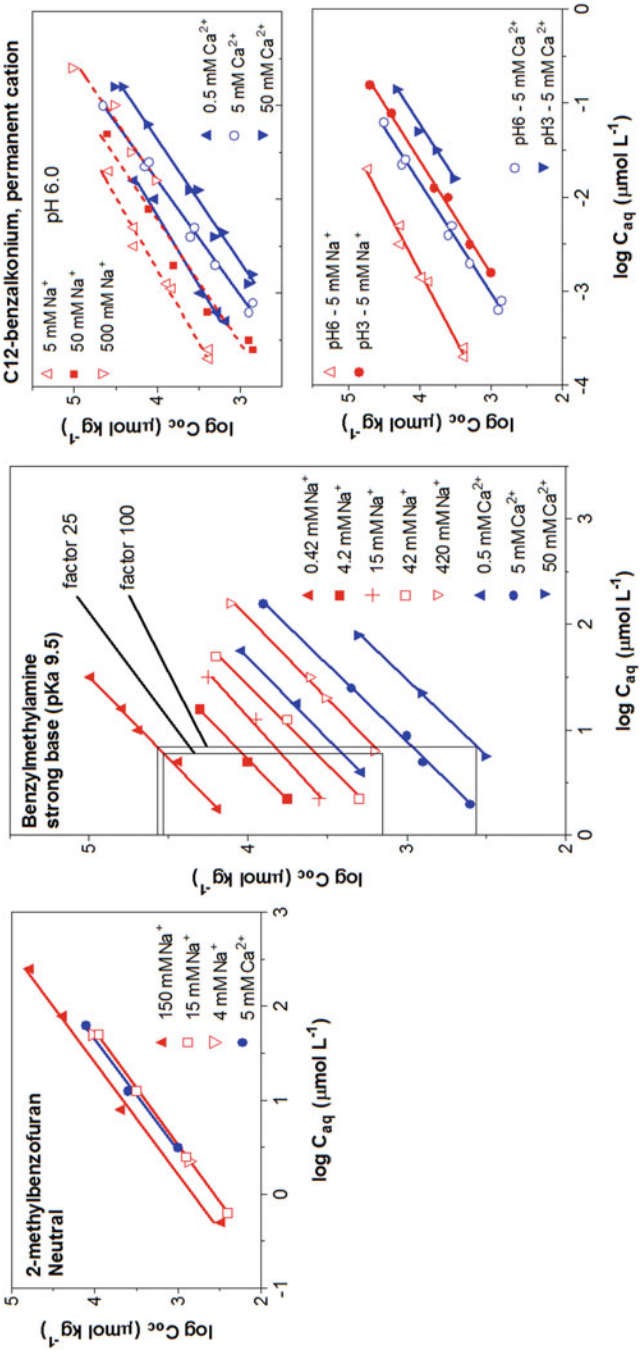

Fig. 7 Sorption isotherms in the presence of different ionic strength solutions for (left) a neutral chemical (redrawn from data in [15]), (middle) a small organic strong base at pH6 (redrawn from data in [15]), and a cationic surfactant C_{12}-benzalkonium (redrawn from data in [38])

over "ionic strength" in most environmental systems. It is important to take this into account when comparing laboratory studies performed under specific aqueous media.

Despite the obvious electrostatic repulsion that organic anionic have with negatively charged SOM/DOC and clays, with sufficiently hydrophobic structures, anionic surfactants also accumulate in organic phases of soils and sediment. Since electrostatic repulsion is reduced at higher salinity, this increases the sorption affinity. It is sometimes speculated whether the sorption of divalent cations such as Ca^{2+} forming a positive moiety [~carboxylate-Ca]$^{+}$ could directly bridge to sorb an organic anion solute, as increased Ca^{2+} concentrations somewhat increase organic anion sorption affinities, but this may also simply be due to the effect of surface potential screening. Tülp et al. [39] found only a small effect of the usual environmental Ca^{2+} concentration range (factor 2) on anion sorption; more importantly, this effect was independent of the anion molecular structure, i.e., there were no specific features observed that indicated specific calcium bridging for either phenolates, carboxylate, or complex anionic structure.

2.5 Relevant Chemical Parameters for Ionogenic Chemicals

Using a similar dynamic column setup with micronized Pahokee peat as Bronner and Goss [2] applied for 137 neutral chemicals, Tülp et al. [39] studied the sorption of 32 organic anion structures and corresponding neutral acid forms, and Droge and Goss [40] studied ~ 80 organic cations to Pahokee peat. Schenzel et al. [9] tested 25 mycotoxins and some phytoestrogens, including 2 cations and 1 anion. Zhi and Liu also used the dynamic column setup with micronized Pahokee peat to study perfluorinated chemicals, including 12 anionic and 3 amphoteric (betaine) structures [41]. All the K_{OC} values for ions obtained on Pahokee peat are listed in Table 3. This set of K_{OC} values for a single organic matter source, obtained with a similar experimental setup, is probably the most consistent sorption dataset available to investigate how structural features influence the sorption affinity. As discussed for polar organics earlier in this chapter, this allowed for the construction of pp-LFER type modeling based on the prevailing types of nonionic interactions. For ionic compounds, this is much less straightforward, since it was unknown which chemical descriptors could best be used to account for both the nonionic and ionic sorption interactions.

First of all, it still remains even unclear how ionic compounds are sorbed in the hydrated organic matter matrix. As discussed above, the first distinction to make is between electrostatic attraction/repulsion and the interaction of the ionic compound with the sorbent, which most likely for cations occurs through electrostatic interaction with the anionic moieties. The main question that still needs to be resolved is how the nonionic part of an ionic solute contributes to this sorption process: i.e., does it fully interact with the nonionic backbone of SOM surrounding the anionic moiety,

Table 3 Sorption coefficients for charged IOCs on micronized Pahokee peat

Chemical name	Log K_{OC} cation	Chemical name	Log K_{OC} cation	Chemical name	Log K_{OC} anion	Log K_{OC} acid
C_8-based cations [40] (pH 4.5, at 1 mmol/kg, 5 mM $CaCl_2$)		>99% *Cationic pharmaceuticals* [40] (pH 4.5, at 1 mmol/kg, 5 mM $CaCl_2$)		*Acidic chemicals* (fitted from pH profile, in 10 mM $CaCl_2$)		
1-octylamine	2.60	Serotonin	3.54	*Phenolic acids* [39]		
2-Phenylethylamine	2.45	Tryptamine	3.09	2,3-Dichlorophenol	n.d.	2.41
N-Benzyl-*N*-methylamine	2.31	Amphetamine	2.42	2,4,5-Trichlorophenol	n.d.	3.23
Octyltrimethylammonium	2.31	Methamphetamine	2.45	2,4,6-Trichlorophenol	1.65	2.88
Simple primary amine cations [40]		L-Adrenaline	0.90	2,3,4,6-Tetrachlorophenol	2.00	3.72
1-Hexylamine	2.11	Methylephedrine	2.16	Pentachlorophenol	2.86	4.26
1-Heptylamine	~2.35	(±)-Metoprolol	2.06	Chloroxynil	1.26	2.18
1-Octylamine	2.60	Propranolol	3.74	Bromoxynil	1.49	2.78
1-Decylamine	3.43	R-Atenolol	2.54	Bromoxynal	1.66	3.15
Benzylamine	2.30	Alprenolol	2.96	Ioxynil	1.91	3.15
4-Methylbenzylamine	2.72	Prilocaine	2.20	2-Nitrophenol	1.11	1.93
4-Butylbenzylamine	3.22	Lidocaine	2.20	4-Nitrophenol	1.11	1.98
4-Octylbenzylamine	4.06	Procaine	3.26	2,4-Dinitrophenol	1.63	2.62
2-Phenylethylamine	2.45	Atropine	3.06	Dinoseb	1.94	2.85
3-Phenylpropylamine	2.68	Scopolamine	2.27			
4-Phenyl-1-butylamine	2.95	Ropivacaine	2.40	*Carboxylic acids* [39]		
4-Chlorobenzylamine	2.84	Bupivacaine	2.50	2,4-D	1.32	n.d.
3,4-Dichlorobenzylamine	3.58	Fluoxetine	3.54	Mecoprop	1.04	n.d.
(±)-1-Aminoindane	2.54	Imipramine	3.56	2,4-DB	1.97	3.04
Naphthylamine	3.31	Codeine	2.50	2,4,5-T	1.68	n.d.
Simple secondary amine cations [40]		Clonidine	2.53	Ibuprofen	1.08	2.67
N-Benzyl-*N*-methylamine	2.31	(+/−) Verapamil	3.46	Ketoprofen	1.23	2.79

N-Benzyl-*N*-ethylamine	2.26	Nicotine (S)-(-)	2.60	Fenoprofen	1.34	2.99
N-Benzyl-*N*-butylamine	2.41			Naproxen	1.58	3.36
N-Benzylhexylamine	2.55	*Other amines with polar moieties* [40]		1-Methoxy-2-naphthoic acid	1.34	2.93
N-Benzyloctylamine	3.08	*N*-Benzylethanolamine	2.27	4-Fluoro-1-naphthoic acid	1.43	n.d.
3-methyl-*N*-methylbz.am	2.64	4-Amino-2-methylquinoline	4.10			
N-ethyl-M-toluidine	2.49	2-Phenylbenzimidazole	4.23	*Complex acids* [39]		
N-Methyl-phenethylamine	2.49	Benzimidazole	3.19	Warfarin	0.95	2.46
Dibenzylamine	3.16	Thiabendazole	3.65	Coumachlor	1.40	3.15
Simple tertiary amine cations [40]		*N*-Benzylamino-acetaldehyde diethyl acetal (2 ethers)	2.10	4′-Hydroxywarfarin	0.95	2.49
Pyridine	2.04			Coumafuryl	0.85	2.45
Quinoline	3.28	*N*-Benzyl-beta-alanine ethyl ester (ester)	2.31	Sulcotrione	0.90	n.d.
N,*N*-Diethylaniline	1.98			Mesotrione	0.90	n.d.
2-Ethylpyridine	2.19	3-Dimethylamino-propiophenone (ketone)	2.58			
2-Methylpyridine	2.08			*Anionic penicillium* (5 mM Ca^{2+}) [9]		
3,4-Dimethylpyridine	2.55	1-benzyl-3-acetamido-pyrrolidine (amide)	2.44	Citrinin	3.1	
2,6-Dimethylpyridine	1.87					
2,4,6-Trimethylpyridine	2.33	2,2′-(Benzylimino)-diethanol	2.18	*PFAS anions* (5 mM Ca^{2+}, pH 5.2–5.9) [41]		
N-Benzyldimethylamine	2.20	Acetylcholine	1.14	PFBA	1.27	
Quaternary ammonium cations [40]		Butyrylthiocholine	2.11	PFPA	1.24	
Phenyltrimethylammonium	2.12			PFHxA	1.26	
Benzyltrimethylammonium	2.24	*Cationic mycotoxins* (5 mM Ca^{2+}) [9]		PFHpA	1.35	
Benzydimethylhexylammonium	2.94	Ergocryptine	4.02	PFOA	1.54	
Benzydimethyloctylammonium	3.40	Ergocornine	3.88	PFNA	2.28	
Benzydimethyldecylammonium	3.88			PFBS	1.38	
Benzyltributylammonium	2.60	*Perfluorinated zwitterionic betaines*		PFHxS	1.47	
Benzyltripropylammonium	2.41	(5 mM Ca^{2+}, pH 5.2–5.9) [41]		PFOS	2.55	

(continued)

Table 3 (continued)

Chemical name	Log K_{OC} cation	Chemical name	Log K_{OC} cation	Chemical name	Log K_{OC} anion	Log K_{OC} acid
Benzyltriethylammonium	2.43	PFOAB	1.95	4:2 FTSA	1.42	
Difenzoquat methyl sulfate	3.16	PFOSB	2.40	6:2 FTSA	1.51	
		6:2 FTAB	2.11	8:2 FTSA	2.56	

or is it still partially/fully hydrated but strongly reduced in its entropic energy due to the sorptive interaction?

Although the electrostatic attraction can be deduced by the salinity effects, this attraction is equal for all monovalent organic cations [15]. As a result, as long as the same aqueous composition is applied, which is relatively easy to ensure in the chromatographic dynamic sorption setup with micronized peat, the relative differences in sorption affinity between different organic cations can still be examined to study the influence of a solute's nonionic structure. Droge and Goss [40] started their measuring series of organic cations with a wide variety of amines with a structure based on the formula $C_xH_yN^+$, thus lacking polar functionalities based on oxygen and nitrogen. Series of homologues with different alkyl chain length were included. From these series, it became apparent that a CH_2 unit in an organic cation contributes significantly less to the sorption affinity than a CH_2 unit in a neutral chemical, ~0.25 log units compared to ~0.5 log units per CH_2. Compare, for example, the alkylbenzenes in Table 2 with the alkylamine cations in Table 3. The reason for this must be due to the entirely different sorption site within the peat matrix between a neutral and a cationic chemical. The series of polar compounds tested by Bronner and Goss [2], for example, that in the selection of C_8-based chemicals in Table 2, clearly shows the influence of a single type of polar moiety on the K_{OC}, which can also be done for organic cations tested by Droge and Goss [40]. Whereas a hydroxyl group in a neutral chemical lowers the K_{OC} by ~2 log units (e.g., compare the C_8 chemical 2,2,4-trimethylpentane with 2-ethyl-1-hexanol), the organic cation *N*-benzylethanolamine has an equal sorption affinity as its "nonpolar analogue" *N*-benzyl-*N*-ethylamine (Table 3). From these examples, and also the strong influence of salinity on the sorption of organic ions, it thus also becomes clear that it is difficult to make a fair comparison between the sorption affinity of a neutral base and that of its protonated form: these species sorb to different sorption sites, which are governed by very different sorption processes, which operate by different structural contributions.

When the HPLC-measured, ion exchange-based, sorption affinity of organic cations to Pahokee peat (log $D_{OC,IE}$) was plotted against standard ways to predict K_{OC} from K_{OW} (Fig. 8 plot A, from [40]), virtually no relationships are obtained. Predicting the K_{OC} using a structural approach via EPISuite provides some trends for simple hydrocarbon structure-based cations (C_xH_yN in Fig. 8 plot D) but still a wide variation for organic cations with polar functionalities, and not relation for quaternary ammonium compounds.

Droge and Goss aimed to obtain a single consistent dataset that could identify the specific contribution of the most commonly present molecular functionalities to the relative sorption affinity to SOM. The first predictive step they suggested was to calculate the sorption affinity based on molecular size (McGowan's V_x index) and amine type (number of H on the protonated amine, *NAi*), which was defined by a set of 32 C_xH_yN cations, with average K_{OC} measured at pH 4.5–7, in aqueous solution with 5 mM Ca^{2+}:

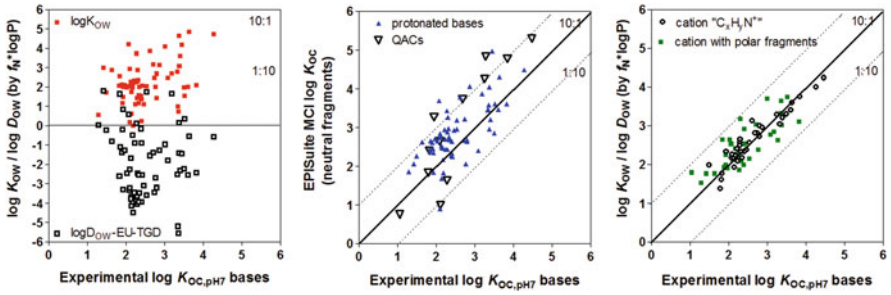

Fig. 8 Observed sorption affinities to SOM for cations (log $D_{OC,IE}$) compared to predictions of (*left*) either logKow (SPARC) or logDow (following EU-TGD approach of the neutral fraction fN multiplied by logKow), which excludes the 14 quaternary ammonium compounds (QAC), (*middle*) EPISuite predictions of logKoc based on the MCI structural fragment approach obtained with neutral structures (or QAC structure), (*right*) cation fragment-based approach developed by Droge and Goss [40], using Eq. (14) with McGowans volume Vx and number of hydrogen atoms on the charged amine (NAi) for the C_xH_yN backbone of each cation, and polar fragment corrective increments as listed in Table 4 if present in the cationic structure. Based on data presented in [40]

$$\text{Log } K_{OC}\left(\text{pH } 4.5-7,5 \text{ mM } Ca^{2+}\right) = 1.53 \cdot Vx + 0.32 \cdot NAi - 0.27\ \{+[\text{polar functionalities}]\} \quad (14)$$

As shown in Table 4, the presence of a polar functional groups would then be added (as [polar functionalities] in Eq. (14)) on this V_xNAi-based prediction. For example, as listed in Table 4, in four organic cations, an amide group was present next to a phenyl ring, and on average for these four compounds, this lowered the K_{OC} by 1.4 log units compared to the V_xNAi value. Table 4 also shows that the influence of a hydroxyl unit is minor in five evaluated organic cations, as indicated in the one example of the analogue structures mentioned above. Corrective increments on the V_xNAi model were derived for 16 different functional groups. As shown in Fig. 8 plot F, certainly for many of the simple C_xH_yN but also for the majority of the polar organic cations, the K_{OC} could be predicted within a factor of 3 this way. However, with a dataset of <50 molecules to define 16 polar functionalities, this is still rather limited to validate so many features, even though many compounds contained multiple functionalities. It is furthermore questionable if K_{OC} values derived from natural soils (see below that this is unlikely) or different types of organic matter could provide more input values to this dataset specifically derived on micronized Pahokee peat. Although the correction factor for ether units had to be modified to 0, Jolin et al. found that most other corrective increments in Table 4 were successful in predicting differences between organic chemicals in their relative sorptive properties [42].

Nevertheless, the description of how molecular structure influences the IOC sorption affinity is already much more advanced for cations than the current dataset for organic anions allow for (Tülp et al. [39] provides for the largest consistent dataset). All acidic chemicals include already multiple structural features, only

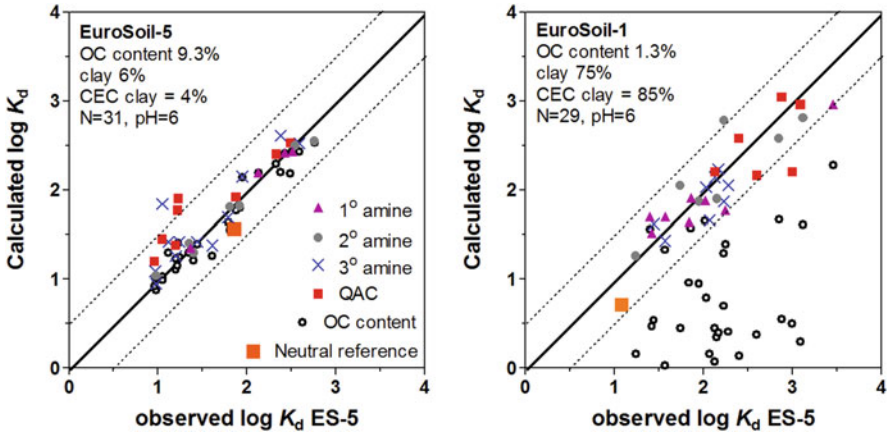

Fig. 9 Sorption coefficients for organic cations (1°, 2°, 3° amines, 4° QACs) and a neutral reference compound (N) on two reference Eurosoils and predictions with and without accounting for clay (OC content corrected only). Predictions for the soils are made using experimental sorption coefficients on reference OM (Pahokee peat) and reference clay (illite). The CEC_{soil} of Eurosoil-5 has only a minor contribution of CEC_{clay} (4%), while Eurosoil-1 has a major contribution of CEC_{clay} (85%). Based on data taken from [51]

Table 4 Empirical correction factors for polar fragments in addition to the average V_xNAi model [40]

Functional group	V_xNAi model correction factors (in log units)[b]
• Phenyl, or 3xF, or -ΞN, or S[c]	• 0
• -Cl	• +0.5 (3)
• Polycyclic aromatic ring	• +0.7 (2)
• Pyridine NH^+	• +0.7 (6)
• Aniline-NH_2	• +0.55 (1)
• -CNC- (HBDon)	• +0.6 (2)
• -CNC- (HBAcc or neutral)	• −0.1 (3)
• Benzimidazole	• +1.7 (3)
• -C-NH_2 (aniline)	• +1.2 (1)
• -OH	• −0.1 (5)
• -C(=O)NC- on phenyl	• −1.4 (4)
• -C(=O)NC- other	• −0.4 (1)
• -C(=O)OC-	• −0.8 (3)
• -COC-	• −0.6 (12)
• -C(=O)C-	• +0.1 (2)
• -C(=O)NH_2	• −0.65 (1)
• -Internal HB	• −1.3 (1)

[a]32 CxHyN compounds
[b]41 compounds used, no. of moieties (e.g., four ethers for verapamil) used in parentheses
[c]Phenyl was found to have negligible influence above that already covered in the V_xNAi model. 3xF, -ΞN, and S are largely neutral moieties and were assumed to be mostly covered by the size factor in V_xNAi model, and set to 0 when calculating correction factors for -COC- and benzimidazole

insight in the influence of chlorination on an aromatic ring, and a comparison between ketoprofen and fenoprofen (K_{OC} nearly equal, 17 and 22, with one having a ketone and the other an ether in between two phenyl rings) would allow for some comparison of how the neutral backbone of anionic chemicals influences their sorption affinity. In addition, chlorination on phenols not only influences the non-ionic part but also strongly influences the properties of the ionic moiety, which is reflected in the different pK_a values for the various chlorinated phenols included by Tülp et al.: pentachlorophenol has a pK_a of 4.8, while 2,3-dichlorophenol a pK_a of 7.6. Just as the affinity of the proton (H^+) to associate with the phenol unit, the charged phenolate moiety may also have a variable contribution to the sorption process in SOM. The good thing about the anion dataset is that in the same study, the K_{OC} for the corresponding neutral acids was also derived for 21 acids. This led to the surprising observation that the anionic form only had 7–60 times lower sorption affinities as the neutral forms, despite the obvious repulsion toward the sorption sites that anions probably are influenced by. This could allow for a rough K_{OW}-based calculation of the sorption affinity of anionic species (e.g., first calculating the K_{OC} for the neutral species and then subtracting an average of 1.3 log units) [43], but moreover it begs the question what process is enabling this sorptive affinity.

One possible reason for the unexpectedly strong apparent affinity of organic anions has in recent years been identified as (negative) charge-assisted hydrogen bonds or (−)CAHB. These (-)CAHB were first studied on the more simplified surfaces of functionalized black carbon [44] and carbon nanotubes[45]. More recently, (-)CAHB were discussed to also contribute significantly to OM cohesion itself [46], proving an important feature of the forces holding organic supramolecular structures together. The (−)CAHB can be exemplified by structures where an anionic sorption site approaching proton (H^+) connects two dissociated organic moieties, together still rendering a negatively charged group, for example, between two carboxylate structures:

$(\sim CO_2...H...O_2C\sim)^-$

or mixed moieties such as carboxylate and phenolate:

$(\sim CO_2...H...O\sim)-$,

It appears that the (-)CAHB forms between weak acids with similar proton affinity (similar pK_a) and is shorter, more covalent, and much stronger than ordinary hydrogen bonds. This may explain some of the observed K_{OC} differences between the anion-acid couples Tülp et al. [39] used, but since natural organic matter has many types of acidic sites with a wide variety in pK_a, the organic anions may always find optimal binding spots to form (-)CAHB with.

For the anions it was concluded [39] that the K_{OC} values of both the neutral and anionic species increased with increasing molecular size and decreased with increasing polarity. At a constant concentration of 10 mM Ca^{2+} over a pH profile, the investigated anions sorbed between a factor of 7 and 60 less than the corresponding neutral acid. A log unit lower Ca^{2+} concentration decreased the sorption affinity of the anions by 0.26 ± 0.05 log units. This was mainly explained as a reduced electrostatic repulsion at lower salinity.

Perfluorinated anions have been studied in sorption experiments too, but mostly with natural soils of widely different compositions and in various solution chemistries. As a result, widely ranging sorption values have been derived, and it is often not clear whether K_{OC} values can be obtained from such data because other soil components may have contributed to the sorption processes [47]. Campos Pereira et al. made a systematic summary of both literature reviews and own spiked experiments, taking the effect of solution chemistry on the net charge of OM into account [48]. Only in 2019 was the dynamic column setup used to determine the K_{OC} for PFAS structures on micronized Pahokee peat, from a separate batch as used by Bronner and Goss. No linear trends were observed, however, between perfluorinated chain length and K_{OC}, with minimal differences between C_4 and C_6 compounds. Also remarkable is the minor difference between perfluorinated carboxylates and analogue sulfonates (log K_{OC} (PFOS) − log K_{OC} (PFNA) = 0.27), whereas recent studies on phospholipid binding indicated a much larger difference (0.84 log units). This may reflect to the smaller effect of the hydration shell surrounding the charged anion group in binding to organic matter compared to that in phospholipids.

2.6 Relevant Sorbent Phases in Soils for Organic Cations

As discussed above, organic anions may be electrostatically attracted to positively charged surfaces such as mineral oxides and may coordinate to acidic sites on soil organic matter via (-)CAHB. Black carbon phases such as soot particles and biochar may also preferentially (ad)sorb acids in soils [49]. There is still no good model to distinguish between the sorption components in soil for organic acids.

For organic cations, a systematic sorption dataset has been established on three clay minerals, obtained in the same dynamic column sorption setup as used for micronized peat [50]. Similar effects of ionic strength were observed as for peat, indicating similar effects of dissolved ions on the surface charge, which induces electrostatic attraction. Differences between kaolinite (1:1), illite (2:1, non-expanding), and montmorillonite (2:1, expanding) clays could be reduced to within a factor of 3 when sorption coefficients are normalized to their CEC (log $K_{clay,cec}$). Apparently, the type of surface charge site does not make a large difference for sorption to these different clays. Ideal for modeling purposes, the log K_{OC} of organic cations to Pahokee peat, normalized to the CEC of peat, is within a factor of 10 of log $K_{clay,cec}$ for many organic cations. The polar amide moiety next to a phenyl ring reduced the clay sorption coefficient by 11.5 log units compared to a V_xNAi approach derived for clay, which compares well to the −1.4 log units for peat discussed above. Particular differences were observed however, in how the nonionic part and ionic group of organic cations influence sorption to clay relative to that in peat. For example, quaternary ammonium cations sorbed relatively more strongly to clays than to peat, while primary amines preferentially sorbed to peat compared to clays.

As a result of specific factors influencing sorption of organic cations to clay minerals and soil organic matter, these two sorbent phases should be accounted for separately in a soil sorption model. Droge and Goss [51] suggested a simple summed contribution model, based on the soil CEC and fraction organic matter to define the key soil parameters and the sorption affinities to reference organic matter (Pahokee peat) and reference clay (e.g., illite):

$$\text{Log } K_d(\text{soil}) = \text{log } K_{\text{OC,cation}} \cdot f_{\text{oc}} + \text{log } K_{\text{clay,cec}} \cdot \text{CEC}_{\text{clay}} \tag{15}$$

where CEC_{clay} represents the contribution of clay minerals to the soil CEC (CEC_{soil}), which is derived according to:

$$\begin{aligned} \text{CEC}_{\text{clay}} &= \text{CEC}_{\text{soil}} - \left(f_{\text{OC,soil}} \cdot f_{\text{OC,SOM}} \cdot \text{CEC}_{\text{SOM}}\right) \\ &= \text{CEC}_{\text{soil}} - \left(f_{\text{OC,soil}} \cdot 3.4\right) \end{aligned} \tag{16}$$

in which $f_{\text{OC,soil}}$ has units kg OC/kg dry soil, CEC_{SOM} is fixed at ~2 mol_{C}/kg organic matter [52], and $f_{\text{OC,SOM}}$ is fixed at the standard 1.7 kg organic matter/kg OC conversion factor [53]). The practical approach of this model is twofold: (1) it applies soil parameters for which standardized protocols exist already and which are well reported soil properties, and (2) it requires independently measured sorption coefficients on reference soil components, which can be further standardized. Jolin et al. [42] found that the value of 3.4 mol_{C}/kg organic carbon may be somewhat high for typical soil organic matter types other than peat and suggested a value of 1.75 mol_{C}/kg which provided a better fit to their set of soil sorption coefficients according to Eqs. (15) and (16). Still, Droge and Goss cross-validated the model of Eqs. (15) and (16) on two natural reference soils, one enriched in clay ($\text{CEC}_{\text{clay}} = 85\%$ of CEC_{soil}) and one enriched more in organic matter ($\text{CEC}_{\text{clay}} = 4\%$ of CEC_{soil}) [51]. Again, using the dynamic column setup, soil sorption coefficients were determined in controlled aqueous conditions, for a set of ~ 30 organic cations for which sorption coefficients on reference SOM and clay were determined. In the OM-enriched soil, sorption coefficients were explained by the f_{OC} and reference SOM log K_{OC} within 0.4 log units. In the clay-enriched soil, however, soil sorption coefficients were underestimated by a factor of 10−1,000 for most chemicals when only using f_{OC} and reference SOM log K_{OC}. However, when including the CEC_{clay} approach and reference log $K_{\text{clay,cec}}$ values, nearly all soil sorption coefficients were predicted within a factor ±3.

The CEC_{clay} approach is obviously a simplified model compared to the heterogeneous complexity of natural soils, where organic matter and clay minerals are also closely interacting. Nonetheless, it delivers adequate predictions that do take into account that organic cations bind to different surfaces, governed by surface-specific interaction rules. The V_xNAi model is obviously less effective in accurately predicting sorption affinities to natural soils than using experimental sorption coefficients to reference soil components but still provides a more realistic alternative compared to octanol-water-based approaches.

An evaluation of Eqs. (15) and (16) on 30 soils from across the USA covering six different classes of soils [54] confirms the strong contribution of the clay fraction to the total soil CEC. Binding to clay, therefore, will play a dominant role in the overall soil sorption affinity of organic cations. This leads to important insights in dealing with the soil sorption affinities of (strong) bases:

1. Deriving K_{OC} values for organic cations from soil sorption data will lead to strongly overestimated binding affinities to organic matter (e.g., in comparisons with sewage sludge)
2. Applying only the sorption affinities of organic cations to organic matter (or organic matter-enriched sorbent such as sewage sludge) can potentially result in substantive underestimation of the sorption affinities of organic cations to soils.

It is important to note that as an alternative to the V_xNAi model, the K_{OC} for a largely protonated basic IOC in soil could be derived as a proxy from sewage sludge sorption data [55, 56]. A first estimation of the sorption affinity to clay would be a similar sorption affinity between clay and OM normalized by CEC. Finally, the impact of Al^{3+} on both electrostatic attraction and competitive interaction in acidic soils may be stronger than that of Ca^{2+} and may lead to further refinement of K_d predictions [42].

2.7 Sorption of Amphoteric IOCs

Amphoteric pesticides (e.g., imidazolinones) typically exhibit a behavior that combines the processes previously described for acids and bases. A number of general rules can thus be drawn. For instance, sorption of amphoteric pesticides is generally positively influenced by organic carbon content and clay content (or CEC) and negatively influenced by pH [57]. In general, compounds with protonated basic functional groups at pH relevant to the environment strongly sorb to environmental matrices, due to strong electrostatic attractions (consistent with Fig. 10).

Nevertheless, zwitterions tend to be complex molecules and may also interact through mechanisms that cannot be extrapolated to other molecules. A remarkable example is that of the herbicide glyphosate, whose phosphonate group can bind directly to oxides through ligand exchange, resulting in the formation of inner sphere complexes [57]. A detailed study on the interactions between ciprofloxacin and soil and peat and aquatic humic substances also illustrates the variety and complexity of interactions that zwitterions may engage with environmental matrices [58]. Given the limited availability of datasets to build regression and/or fragment-based models, it is thus recommended to proceed on a case by case evaluation for those compounds on which positive and negative charges may coexist at environmentally relevant pH. The small set of perfluorinated betaines [41] provides some experimental data on the effect of speciation on K_{OC} but needs to be evaluated in more detail.

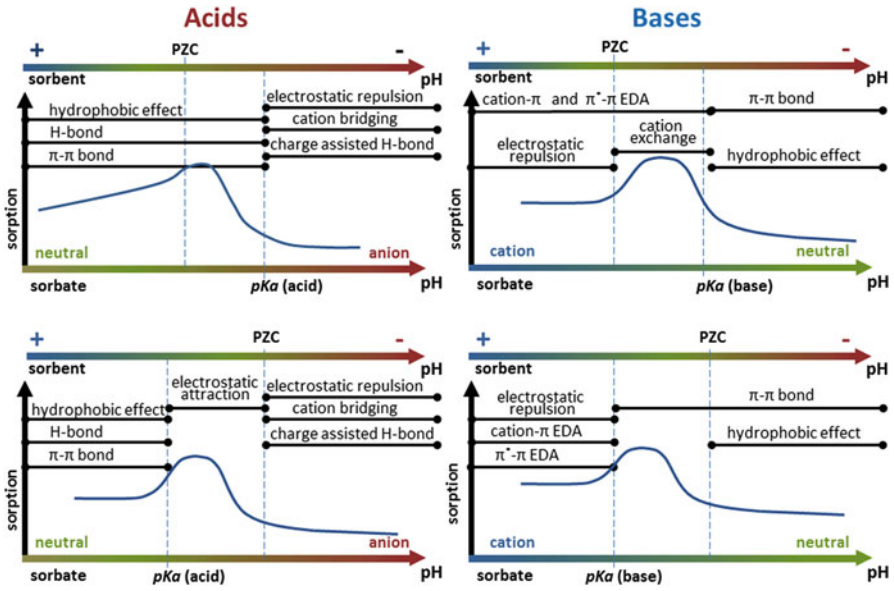

Fig. 10 Sorptive interactions governing the sorption processes of acids and bases in different pH ranges, under a situation with either the solute pK_a above the sorbent PZC (top row) or with the solute pK_a below the sorbent PZC (bottom row). Negative charge is red, positive charge is blue, neutral is green. The *X*-axis displays the sorbate speciation; the sorbent speciation is the arrow above. Adapted from a figure presented in [49]

Kah et al. [49] have reviewed the sorption processes of IOCs to various carbonaceous surfaces and provided an schematic overview of the governing sorptive processes for each IOC type in relation to its speciation properties (pK_a) relative to the speciation of the sorbent surface (pH where the surface has a point of zero charge (PZC), i.e., positive at lower pH, negative at higher pH). As, for example, shown in Fig. 10, an acid with a pK_a above the PZC is only dissociated when the surface is negative, while an acid with a pK_a below the PZC is partly negative while the surface is still positive. This overview sketches the summarized findings of this chapter on IOCs: neutral acids sorb more strongly than their corresponding dissociated anions on negatively charged sorbents, while neutral bases may sorb more weakly than their corresponding protonated cations on negatively charged sorbents. There are multiple scenarios possible to describe amphoteric chemicals that relate to the relative positions of the basic pK_a and acidic pK_a and the PZC of the sorbent. Typically, sorption of amphoteric compounds has a maximum near the neutral sorbent PZC.

Box 1 Assumptions in the NICA-Donnan Sorption Model for Organic Ions [38]

The higher cation concentration in the aqueous Donnan phase ($C_{i,D}$) compared to bulk medium phase ($C_{i,B}$) is thus not related to sorption to specific sites but is due to "indifferent accumulation." The difference in ion concentrations results in a Donnan potential (ψ_D). This potential ψ_D quantitatively accounts for the electrostatic attraction of all cations from bulk solution to the Donnan volume. The concentration ratio between Donnan phase and bulk phase is quantified by the Boltzmann factor (B) [59],

$$B = C_{i,D}/C_{i,B} = \exp\left(\frac{-z_i F \psi_D}{RT}\right) = \exp\left(\frac{-z_i e \psi_D}{kT}\right) \tag{17}$$

where z_i is the valency of the cation, F the Faraday constant, R the gas constant, and T the absolute temperature.

The first important assumption in the NICA-Donnan model approach is that the aqueous Donnan phase volume V_D depends on ionic strength (I, mol/L), which in a simplified form can be described by an empirical constant b [24, 34]:

$$\mathrm{Log} V_D = b(1 - \log I) - 1 \tag{18}$$

Maintaining electroneutrality requires that the enhanced cation concentration equals the charge density of the organic matter Q (mol charge/kg) in V_D (in L) [34, 36]:

$$Q/V_D = -\sum z_i(C_{i,D} - C_{i,B}) = -\sum z_i(B \cdot C_{i,B} - C_{i,B}) \tag{19}$$

By incorporating Eq. (17) in Eq. (19), ψ_D can be determined via $C_{i,B}$ and V_D, if Q and b are known (e.g., listed in Milne et al. [24] for humic acids). B can then be derived to calculate concentrations in the Donnan phase for each test condition. Note that Q depends on the number of unbound sorption sites, and therefore the Donnan potential ψ_D changes with higher specifically sorbed ion concentrations. Sorbed organic cation concentration on HS can then be replotted against $C_{i,D}$ instead of $C_{i,B}$. Such plots should explicitly reflect the specific ion binding, while the electrostatic effect caused by background salts is omitted.

Since V_D is related to b, $C_{i,D}$ is also dependent on b. Therefore, by adjusting b, the sorption isotherms obtained at different salt concentrations would merge to one "master curve" (MC) if the salt ions do not bind specifically to AHA [36, 60].

(continued)

Box 1 (continued)

The second important assumption in the NICA-Donnan model approach is that inorganic monovalent cations, except protons, do not bind specifically to ion exchange sites but only balance electroneutrality and thereby influence the Donnan potential (ψ_D).

In study of Chen et al. [38] with the cationic surfactant C_{12}-benzalkonium, the Donnan approach enables the isotherms measured at 5, 50, and 500 mM Na^+ successfully merging into one MC (Fig. 4 in Chen et al. [38]), resulting in an ion-specific log K_F (4.15 ± 0.05) where b is exclusively set to 0.59. The b is typically around 0.5 based on proton binding studies for different HA [24], but the fitted value agrees well with the value (0.63) obtained in the study using the same purified AHA as in this work [60]. The effect of Na^+ we observe on sorption of C12-BAC is thus only the result of variable electrostatic attraction, and just fitting a single b value (which corresponds to earlier findings for AHA) can explain this effect of Na^+.

One of the weak points of the second assumption in relation to tests with organic cations is that it does not explain why the sorption of organic cation clarithromycin to HA was more efficiently reduced for in solutions of K^+ compared to equal concentrations of Na^+ [16]. Such differences between the effect of various monovalent cations on sorption of organic cations were also found for polymers [61, 62], which suggests that specific sorption of some monovalent ions may not be negligible.

The third important assumption in the NICA-Donnan model approach is that divalent/multivalent cations and protons have a sufficiently high sorption affinity to both carboxylic groups on HS to compete with cationic surfactants, which is described with the non-ideal competitive ion binding (NICA) term. Monovalent organic cations appear to sorb much more strongly than most monovalent inorganic cations [63, 64], as a result of which organic cations also bind specifically to negatively charged sites in humic acids. The sorption affinity of organic compounds can therefore be regarded as the product of the (Boltzmann factor)∗(intrinsic sorption coefficient), where the intrinsic sorption coefficient is influenced by the concentration of competing ions.

Most sorption studies that wanted to understand the specific sorption affinities of divalent metals and apply NICA-Donnan have all tested under high background monovalent electrolyte concentrations, so that ionic strength was always constant and therefore also the Boltzmann factor was constant. In the study design of Chen et al. [38], Ca^{2+} influences both the electrostatic (Donnan) effect and the competition effect. The Donnan approach does not attain the same MC at different Ca^{2+} concentration using the same b, as shown in Fig. S5 of Chen et al. [38]. This is likely the reason why the MC for the Ca^{2+} data is lower than the MC for Na^+ data. The difficulty when applying the NICA-Donnan model for Ca^{2+} data is that Boltzmann factors are different for

(continued)

Box 1 (continued)
each different medium composition, which also affects the sorbed H^+ concentrations. However, different Boltzmann factors appeared to have only a minor influence when determining the Donnan parameter b with the Na^+ data, but it complicates calculations with the calcium data. This requires that the full NICA-Donnan is run for Ca^{2+} data, not only the Donnan model, which can be readily done with ECOSAT software.

A fourth important NICA-Donnan assumption is that sorption, and therefore also competition, occurs at two collections of sorption sites in HA: carboxylic acids and phenolic acids, which have a specific affinity distribution. At pH 6 and pH 3, however, phenyl groups are almost fully protonated and therefore hardly matters for cation binding in the test system of Chen et al. [38]. Thus, for a system at pH 6, the NICA equation [36] was simplified to include only carboxylic acids and considers specific sorption of H^+, cationic surfactant, and Ca^{2+}, namely

$$Q_i = \frac{n_i}{n_H} \cdot Q_{\max,H} \cdot \frac{\left(\overline{K}_i \cdot C_{i,D}\right)^{ni}}{\sum_i \left(\overline{K}_i \cdot C_{i,D}\right)^{ni}} \cdot \frac{\left[\sum_i \left(\overline{K}_i \cdot C_{i,D}\right)^{ni}\right]^p}{1 + \left[\sum_i \left(\overline{K}_i \cdot C_{i,D}\right)^{ni}\right]^p} \qquad (20)$$

where K_i is the median value for the "intrinsic" sorption coefficient for ion i to carboxylic acids (based on $C_{i,D}$, following from the MC); n_i the stoichiometry index of i, relative to n_H for protons; $Q_{\max,H}$ (Q in equation the total number of reference sorption sites; and p is the width of the affinity distribution for carboxylic acids for a specific HS, thereby accounting for sorption site heterogeneity.

The first quotient at the right-hand side defines the maximum ion exchange capacity of i, the second quotient is the fraction of covered sites occupied with i, and the third quotient indicates the total number of sites bound to an ion.

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Environmental Fate Assessment of Chemicals and the Formation of Biogenic Non-extractable Residues (bioNER)

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Contents

Abstract The approval of chemicals for placing on the market is subject to various regulations in many countries. Regulations often require the assessment of the environmental fate in simulation tests using isotope labels for facilitated analysis. Such tests simulate the turnover of a chemical in complex environmental systems such as soils, water-sediment or wastewater treatment systems. Non-extractable residues (NER) are formed during the turnover of organic chemicals in solid

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matrices. NER are the ‘black box’ in current risk assessments of organic chemicals since their chemical composition is largely unknown. NER can result from sorption of the parent compound or its primary transformation products to the solid matrix; this leads to xenobiotic NER formation (considered as ‘hidden hazard’). However, microbial biomass formed during microbial biodegradation of chemicals can also contribute substantially to NER formation (biogenic NER; considered as ‘safe sink’ of no environmental concern). Biogenic NER thus need to be distinguished from the toxic xenobiotic NER in improved risk assessments and registration procedures of chemicals. The formation and the analytics of NER have so far only been phenomenologically described. This chapter describes the general microbial degradation processes of organic chemicals and summarizes the state of the art on NER analytics with particular focus on biogenic NER. Knowledge gaps in the NER analytics and suggestions for improvement are presented.

Keywords Non-extractable residues, OECD tests 307, 308, 309, Pesticide fate

1 Fate of Chemicals in the Environment: Controlling Factors and Relevance for Risk Assessment

Xenobiotic organic chemicals are deliberately (e.g. pesticides) or unintentionally (e.g. polyaromatic hydrocarbons, chlorinated solvents, pharmaceuticals) released to nearly all compartments of the environment. Therefore, the use of chemicals in industrial economies is subject to regulation that often requires approval by the regulation bodies. Approval of a chemical in Europe depends on the results to be obtained within the environmental fate tests using isotope tracers. Such tests simulate the turnover in complex environmental systems such as soils [1], water-sediment [2] or wastewater treatment [3, 4] systems.

The fate of organic chemical in soils or sediments depends on many factors, for instance, environmental conditions, soil properties and availability of organic chemicals to potential microbial degraders [5]. Both environmental conditions (e.g. temperature) and soil or sediment physico-chemical properties (e.g. pH, organic matter [OM] content, humidity and texture) influence the metabolic activity of microorganisms, their abundance and phylogenetic diversity as well as the bioavailability of organic chemicals [5, 6]. Mesophilic temperatures (20–40°C) were reported to be the optimal for microbial degradation activity [7], whereas lower or higher temperatures may retard or even block chemical degradation [5, 8]. Organic chemicals in soils or sediments with higher OM contents can be degraded either faster because of the more abundant and diverse microbial communities [9] or slower due to sorption to the solid matrix in OM [10]. Beside oxygen contents, the pH of soil or sediment is considered to be one of the most influential factors controlling the activity and the phylogenetic

diversity of microorganisms [11]. In addition, pH also strongly influences the bioavailability of carbon, nutrients and organic chemicals [12], and exchangeable acidity was identified as the most influential soil factor for glyphosate mineralization [13]. Bioavailability of chemicals is, however, the key factor that controls the overall fate in complex environments, in particular microbial degradation and toxicity to biota [14].

Organic chemicals entering soil or sediment may undergo various turnover processes (see Fig. 1). They can be transformed chemically (e.g. photolysis), biologically by microorganisms or even mineralized, volatilized or leached to the groundwater or taken up by living organisms [15]. Alternatively, they can be immobilized as non-extractable residues (NER) [16], because soils and sediments as complex matrices provide a wide variety of binding sites promoting sorption of organic chemical or its transformation products to or within solid matrix [10, 17].

NER are the 'black box' in current fate and risk assessments in particular for pesticides, biocides and pharmaceuticals. It is anticipated that NER from toxic organic chemicals or primary transformation products may be remobilized and further exported to groundwater or taken up by food crops posing a delayed hazard for environmental and human health [15]. According to the IUPAC definition, the term NER is reserved for the parent organic chemical and primary transformation

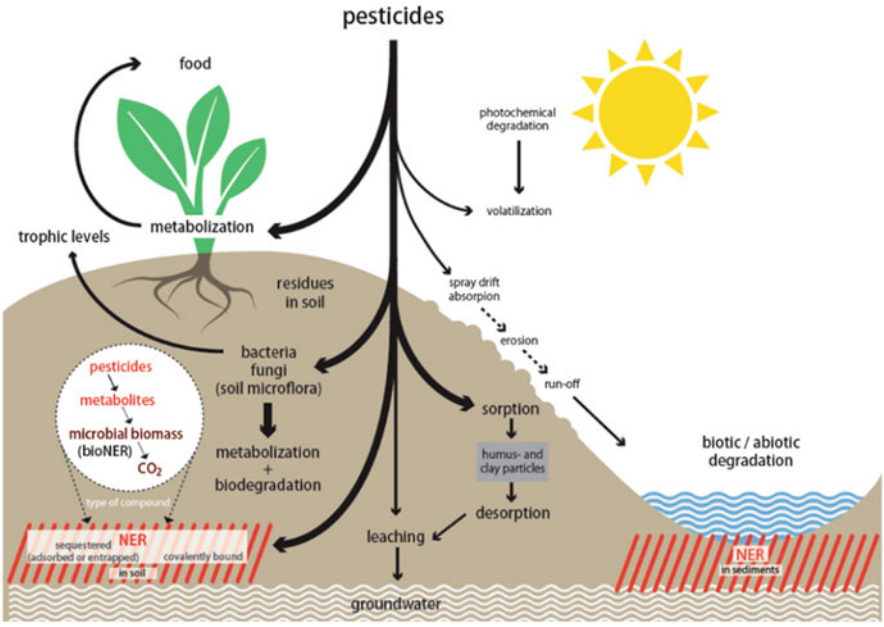

Fig. 1 Potential routes of chemicals, e.g. pesticides, in the environment. Fate-determining processes such as application, transport and degradation processes of pesticides in environmental compartments are well studied except the formation of non-extractable residues (NER) in soils and sediments that may be derived from parent compounds, metabolites, microbial biomass or re-fixed CO_2

products that are not extractable from soil or plant with appropriate solvents without destroying the matrix [18]. However, NER can also contain biogenic NER (bioNER) that is the result of chemical's carbon or nitrogen assimilation into microbial biomass (e.g. lipids or proteins) during microbial growth on these compounds as a carbon or nitrogen source [19, 20]. After the death of the organisms, their residues are subsequently stabilized in the OM [6, 19, 20]. As bioNER exclusively consist of biomolecules, they are generally considered not to pose any environmental risk. Furthermore, the biomolecules are explicitly excluded from the NER definition by IUPAC [18]. However, the NER are in most cases quantified as total NER in radiocarbon mass balances; thus bioNER are also included in the total NER causing a mismatch of definitions and regulation [21].

In the last decades, three types of NER types and the related differentiating analytics were proposed [6]. The total amount of NER after exhaustive extraction has been categorized as xenobiotic NER (xenoNER). A chemical or its transformation product(s) which is strongly adsorbed, sequestered or entrapped constitutes xenoNER type I, whereas covalently bound are xenoNER type II. A third type (III) refers to natural biogenic NER which are derived from biotic degradation accompanied by the anabolic use of the labelled atoms. These three NER types are formed by competing processes, and discriminating analytical methods have been described [21, 22]. More details on NER type and their analytics are discussed in detail in Sect. 8.

2 Environmental Fate and NER Risk Assessment in Regulatory Testing of Organic Chemicals

In order to assure the safe use of chemicals, each chemical (active compound) has to be tested in numerous regulatory tests prior to the approval by European Chemicals Agency (ECHA). The approval and application of pesticides, biocides, REACH (*r*egistration, *e*valuation, *a*uthorization and restriction of *ch*emicals) chemicals and human and veterinary products in Europe is regulated by various legislations: the REACH regulation EC_1907_2006 for industrial chemicals [23], regulation 528/2012 for biocidal products [24], EC 1107/2009 for plant protection products [25] and EC 726/2004 for human and veterinary medicinal products [26], respectively. These regulations include the assessment of the fate and of persistency (P), bioaccumulation (B) and toxicity (T) of compounds (PBT) which are specified in more detail in the guidance documents published by the ECHA [27–29].

The physico-chemical properties of active compound and its (bio)degradability are essential parameters for the fate and P assessment of the active compound and have to be determined in standardized tests according to the OECD guidelines [2, 30]. (Bio)degradability of active compound in complex environmental systems (e.g. soil or sediment) is usually balanced with radiocarbon (^{14}C)-labelled compounds [6]. The radiocarbon mass balance encompasses mineralization,

extractable active compound and its transformation products and NER at the remaining solid matrix [10]. Mineralization and NER formation results obtained in fate studies provide a baseline for the assessment of the potential environmental risks of active compound [27–29]. The instructions on incubation set-up and conditions (darkness, ±20°C) for testing active compound's (bio)degradability are available in OECD guidelines [1, 2, 30]. To date, available instructions on the extraction procedures of active compound from solid matrix and the analysis of NER speciation are still not satisfactory and lack comparability between different studies [22, 31]. The liquid-solid extraction method differentiating between the extractable residues of active compound and its non-extractable counterpart (NER) is crucial for the proper assessment of environmental risk. Different extraction methods, e.g. mild agitation of a solid sample using aquatic solvents or harsh extraction applying organic solvents and high pressure and temperature (e.g. Accelerated Solvent Extraction or Soxhlet), can be applied accordingly to the physico-chemical properties of target chemical [32]. The content of NER thus depends on the type of extraction method used, i.e. mild extraction results in higher NER, whereas harsh extraction yields in lower NER. After the extraction of active compound and transformation products, the remaining isotope carbon is then assigned to total NER, but not further characterized or categorized [21]. Due to limited knowledge about the structural composition, the NER were recently pre-cautionary considered in the risk assessments, and its enhanced formation (>70% of the initially applied ^{14}C) impeded the approval of active compound. Therefore, the guidance documents [27, 29] regarding the NER currently executed a paradigm shift from assuming NER as 'degraded residues' of no environmental concern (safe sink) in the regulation of pesticides [33, 34] to the new consideration. In this case, if the NER are below or the mineralization rate are above certain threshold values, the NER are considered as 'hidden hazard' in the P assessment [23, 27, 29], if no other specific information is available. This paradigm shift caused severe uncertainties in the assessment of pesticides and biocides.

Barriuso et al. [10] published a meta-analysis of mass balance data for the most relevant pesticides applied in Europe. The NER and mineralization in most mass balance studies of active compound show high variations between different soils [10]. Mineralization ranged from nearly negligible to very high values above 90%, and NER formation spans the full range from not detectable, very little to about 99% of the applied amounts [10]. According to this database, pesticide structural properties are obviously not per se indicative for the amount of NER formed. Furthermore, there is also no direct correlation of the mineralization and the NER formation. For instance, carbamates (including dithiocarbamates and bis-carbamates), urea herbicides (including sulfonylurea herbicides), hydroxybenzonitriles, arylalkanoic acids, strobilurines, pyrethroids and chloroacetamides cover the full range of NER formation; only benzamides, triazoles and organophosphates consistently form low amounts of NER [10, 35]. The authors stated that microbial activity of soils has a direct and significant effect on the NER formation. However, they did not consider a significant microbial contribution to NER formation, although they found indications for ^{14}C label incorporation from 2,4-D and glyphosate into cell constituents of bacteria

and fungi in pure cultures several years before [36]. Recent data indicate that we need much better methods to differentiate NER: the microbial conversion of four easily and readily biodegradable organic xenobiotics to carbon dioxide and microbial biomass leads to the formation of high amounts of non-toxic bioNER [19, 20, 37, 38]. The quantification approaches of NER assign the entire label remaining in soil or sediment to potentially hazardous xenoNER including natural compounds derived from bioNER. In this case, risk assessment based only on the total amount of NER amounts will definitely overestimate the environmental risk. Mineralization and NER data provided by the producers and compiled in the pesticides database of the EU [39] for approval of the active compounds need thus to be reconsidered for a proper risk assessment. For instance, easily and readily biodegradable pesticides could be the potential 'candidates' for extensive bioNER formation; vice versa pesticides with a high potential for forming hazardous xenoNER can now be identified and prioritized.

As a first approach to classify compounds in terms of the relative importance of bioNER and xenoNER formation, we compiled available databases based on mineralization and NER formation. The whole entire dataset comprised 222 data entries with 140 entries on 97 compounds from [10] and additional data from the registration dossiers available at the pesticides database of the EU covering 58 compounds and 82 entries. The compounds for which full information on both mineralization and NER formation were available (216 entries) were operationally divided based on turnover data into four groups in terms of NER formation (<30%, 30–50%, 50–70%, >70%) and three groups in terms of mineralization (<15%, 15–30%, >30%; Table 1; for the related compounds, see Annex for Table 2). This allows a rough estimate of potential environmental hazard:

1. Compounds with low microbial mineralization and very high NER formation rates (red cells in Table 1, in total 21.7% of the data entries) mainly form xenoNER.
2. Compounds with moderate microbial mineralization and moderate NER formation (brown cells, 36.9% of the data entries) show intermediate risk as they are prone to form both xenoNER and bioNER to a certain extent.
3. Compounds with high microbial mineralization and low NER formation production (green cells, 41.5% of the data entries) are expected to dominantly form bioNER derived from microbial biomass and only minor amounts of xenoNER.

Based on this assessment, the NER formed from group (1) compounds can be considered hazardous (mainly xenoNER), those of group (3) compounds can be regarded as harmless (major bioNER), whereas group (2) compounds need to be re-evaluated in risk assessment and approval procedures.

The first indication for bioNER formation from easily biodegradable herbicide 2,4-D using stable isotope carbon labelling and the detailed approach to differentiate NER types have already been published [6, 19, 20, 37, 38, 40]. However, bioNER have not yet been included in the fate assessments of the majority of organic chemicals. The main reason is the lack of cheap and fast method for quantitation

Table 1 Classification of chemicals

		Mineralization class			
		<15%	15-30%	>30%	sum
NER class	<30%	63	18	48	129
	30-50%	24	14	17	55
	50-70%	10	11	2	23
	>70%	6	2	1	9
	sum	103	45	68	216

Compounds of different mineralization and NER formation classes derived from the database presented by Barriuso et al. [10] extended by other relevant pesticides data of the EU database [39]. Numbers indicate the number of compounds falling in the various mineralization and NER categories

of bioNER using radio isotope tracer compounds. Therefore, for most chemicals, bioNER are included in the total NER and pre-cautionary considered as potentially harmful in the risk assessments. The ECHA has recently published a discussion paper in which some suggestions on extraction methods of active compound, NER analytics and 'screening' modelling approaches for potential of bioNER formation were included [21].

3 Microbial Degradation of Organic Chemicals

Assessing bioNER formation from chemicals requires detailed knowledge on the factors and processes involved, in particular microbial processes. Microbial growth and activity in environmental systems determine the fate of any organic compound and thus the extent of NER formation and the NER type (xenoNER versus bioNER). The extent of microbial degradation of an organic chemical and of NER formation depends not only on environmental conditions or solid matrix properties but also on the availability, concentration and structural complexity of carbon and nitrogen substrates that can be used for growth [41]. All living organisms, including heterotrophic bacteria and fungi in soils or sediments, must perform various metabolic steps to gain energy and building blocks from any organic substrate for sustaining cell maintenance and growth [42].

First of all, a growth substrate must be bioaccessible and provide sufficient energy for microbial use [43]. This substrate can be used as a carbon source. The same or a different substrate must also provide other elements, e.g. hydrogen, oxygen, nitrogen, sulphur and phosphorus, that are necessary for the formation of microbial biomass. Growth of microorganisms and thus formation of microbial

biomass can occur only when there is a proper stoichiometric ratio of the biomass elements similar to $C_4H_7O_{1,5}N$ with minor amounts of sulphur and phosphorus and a set of macro- and microelements (e.g. iron, potassium, calcium, magnesium) [44]. Microbes first consume the elements provided by their carbon source, but they will use additional organic substrates and nutrients from soil or sediment until the appropriate stoichiometric ratio is obtained. Thus, environmental systems must fulfil the minimal requirements for growth and biomass formation; otherwise growth is limited and the turnover kinetics is retarded.

The availability of carbon and nitrogen substrates in soil or sediment is usually limited, and it depends on environmental conditions and on properties of solid matrix and of substrates (see Sect. 1). Therefore, many microbes may take up and degrade any available substrate quickly to maintain their cell integrity and to gain energy and macroelements [44]. In certain cases, theoretically biodegradable organic compounds may not be biodegraded even if the potential degraders are present in high concentrations [45] or may be related to low energy gain for the degraders which does not allow effective degradation [43, 46]. However, substrate as a sole carbon source might be not sufficient to promote growth of microbial biomass. Several substrates are often needed to provide additional nutrients that are necessary for the formation of microbial biomass.

Energy metabolism relies on a chemical reaction sequence with two coupled redox reactions with the equation A + C → B + D. In the first reaction, an organic *substrate molecule A* is oxidized to a *product B* (often to CO_2). Then, the released electrons and protons (redox equivalents) are transferred in the living cells towards various terminal *electron acceptors C* (reduction), e.g. O_2, NO_{3-}, Fe^{3+} (or other oxidized metals), SO_4^{2-} or CO_2. The reduction of the electron acceptors to *product D* often occurs via respiration processes, which may also include the direct release of H_2, if H_2-consuming organisms are also present in the microbial community or the electron transfers to other specific organic molecules. Hence, the resulting energy available for the organisms in the redox reactions can be easily calculated [47, 48]. The various electron acceptor processes depend on their availability and concentrations, which overall determine the redox status of a given system. In other words, the availability of the electron acceptors determines the degradation processes of any organic molecule. However, a part of the carbon during the oxidation processes always converts to microbial biomass (=yield, not taken into account in the equation), whereas the other part oxidizes to CO_2. Also other elements (nitrogen, sulphur, phosphorus, etc.) are needed for growth, and they are typically recycled within the cells. They also can be obtained from organic or inorganic source if required to maintain cell stoichiometry but may also be released as metabolites in order to avoid a non-stoichiometric overflow of the cells, as shown for glyphosate [49].

Carbon and nitrogen substrates are degraded to intermediates, which are taken up into the microbial cells and used either for energy production (catabolism) or for biosynthesis (anabolism) of their cellular constituents (e.g. proteins, sugars, lipids; [50]; see Fig. 2).

The relationship between growth and substrate consumption/turnover follows saturation kinetic with initially nearly linear relation and decreasing growth rates

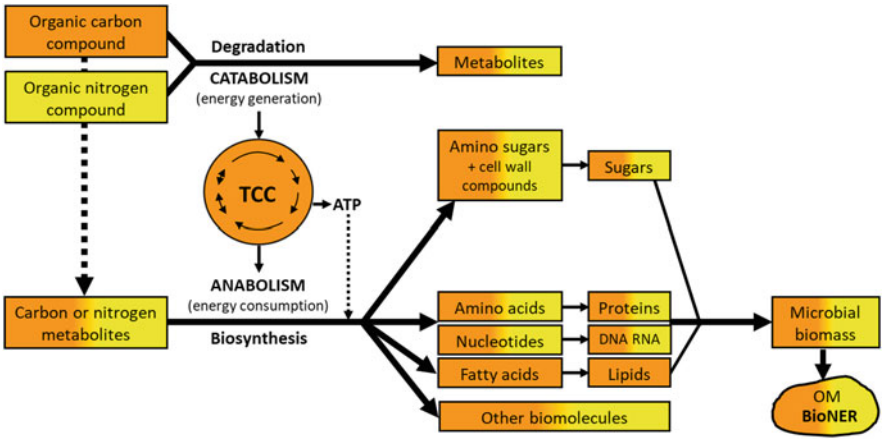

Fig. 2 Energy/matter fluxes and metabolic activities during microbial degradation of organic compounds in general. *TCC* tricarboxylic acid cycle provides the central building blocks for anabolism, *ATP* adenosine triphosphate, the energy transfer molecule, *DNA* deoxyribonucleic acid, *RNA* ribonucleic acid. CO_2 can be produced during TCC and degradation of microbial biomass and of bioNER. For sake of clarity, this is not presented in the figure

following higher concentrations above the half-saturation concentration (k_m) (Fig. 3A, [51]. However, it is often forgotten in textbooks that this relationship actually does not pass through the origin [52]. Microbes need a certain amount of carbon and energy for maintaining the cell integrity and function; thus a minimal substrate concentration is needed before any growth occurs. In other words, a substrate flux below this minimum threshold results in death and decay of the microorganisms. This threshold was determined for some aromatic compounds at low concentrations of degraders in the range of 10 mM [53].

In addition, turnover kinetics roughly depends on the relation of substrate concentrations and the concentrations of biocatalysts/microbes that are related to growth rates (Fig. 3B) [52, 54].

If the initial amount of degrading microorganisms in comparison to the substrate is sufficiently high, the kinetics is nearly linear of zero order with more or less constant elimination rates. If the number of degraders is higher in comparison to the substrate, the kinetics shifts to first order with increasing elimination rates. However, if the number of degraders is very low in comparison to the substrate, the microbes start growing after a lag phase, which results in increasing turnover rates, and a sigmoidal kinetic is observed. Finally, if the concentrations become very low, the turnover rates decreased significantly often resulting in apparently non-degradable residual concentrations.

No growth may occur, if microbes are degrading the compounds co-metabolically which means without significant gain of energy or carbon for their biomass. Such processes often occur accompanied by growth or maintenance on other substrates, and they are due to microbial enzymes that have multiple substrates [52, 55]

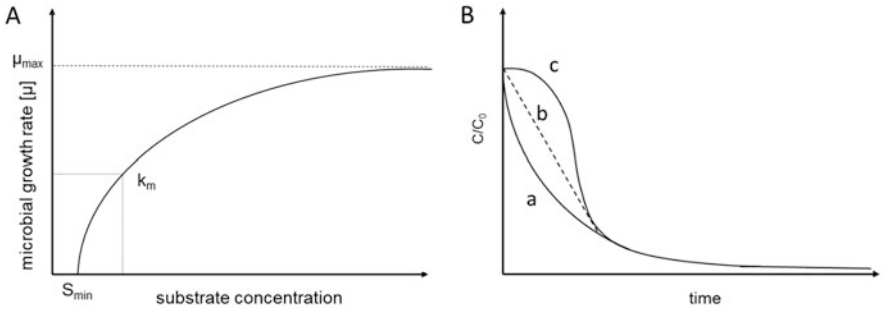

Fig. 3 Organic substrate turnover related to microbial growth. (**A**) Growth rate related to substrate concentration (extended Monod kinetics) [52]. Above a certain substrate concentration (maintenance threshold; >> 10 nM), the present amount of microbes starts to grow; k_m represents the substrate concentrations with half-maximum growth rate. (**B**) Degradation kinetics depending on the ratio of microbes (biocatalysts) to target substrates because growth rates depend on the initial amount of degraders when the growth is starting. The shape of kinetic gives information about the microbial status: (**a)** first order, degraders are present in sufficient amounts (relative to substrate concentration) to allow for immediate start of degradation depending on substrate concentration (= the abundance of degraders is not limiting) resulting in no or slow growth; (**b**) zero order/linear, can be (1) a transition between first order and sigmoidal (transition from degrader-limited to substrate-limited) or (2) low but constant flux to degraders (bioavailability driven); or (**c)** sigmoidal kinetics, initially, the degraders are present at low numbers, and degradation is limited by degrader abundance; over time, they grow and degradation rates increase correspondingly. Finally residual concentrations may remain depending on the maintenance threshold and the survival of the degraders at low concentrations

resulting in zero-order kinetics with relatively low turnover rates of the parent molecule to the respective metabolites.

As long as the flux of organic substrate, energy and nutrients exceeds the maintenance requirements, the microorganisms continue growing. When the flux is ceasing, the organisms start starving, and the cell maintenance requirements in terms of carbon, nitrogen or energy are no longer satisfied, causing the cells to eventually die and decay [56]. This results in cell fragmentation with the release of cytosol including enzymes and particulate cell debris representing the necromass which then is stabilized in OM [57]. If an isotope-labelled tracer compound is applied, labelled carbon derived from the organic chemical is partly oxidized to gain energy and partly used for growth and biomass synthesis. This in turn results in incorporation of the label into biomass compounds (e.g. proteins, cell envelope constituents) [19]. Similarly, labelled nitrogen from a ^{15}N-labeled substrate may be incorporated in nitrogen-containing biomolecules, e.g. proteins. After the cells decay, carbon and/or nitrogen label from the labelled compound can be traced in the stabilized necromass and ultimately in the bioNER [6].

4 Microbial Biomarkers for bioNER Analytics

The use of biomarkers allows straightforward analysis of bioNER in soil or sediment. The choice of appropriate biomarkers is guided by the composition of microbial biomass and analytical accessibility in OM of the compounds. Proteins and lipids fulfil both requirements [46]. Amino acids (monomers of proteins) and fatty acids (representative of lipids) are easily amenable biomarkers; they can be measured in two fractions, i.e. in the total OM and in the living microbial biomass [6, 19]. This allows estimating the amount of biomass residues or necromass in the OM by difference. Microbial necromass in OM was only rarely considered since soil microbiologists focus on living organisms, while soil chemists often do not properly consider the related microbial processes. The changes in biomarker contents in the living and non-living fractions over time enable to trace the flux from living biomass to necromass and decaying microbial residues stabilized in OM.

Fatty acids are generally used for tracing the carbon flux from stable isotope-labelled compounds through the microbial degrader communities [58, 59]. Phospholipid fatty acids (PLFA) are one of the constituents in membranes of living cells and make up around 5% of the microbial biomass [19, 42]. The PLFA pattern of the cell membranes can be used to distinguish between the main groups of microorganisms, i.e. the Gram-positive and the Gram-negative bacteria and the fungi [58, 60]. The turnover of fatty acids in soil or sediment is very fast as it has been shown in the mass balance studies with ^{13}C-labelled microbial biomass [61] and organic chemicals [19, 20, 37, 38, 40]. The use of fatty acids for bioNER assessment thus leads to a general underestimation of bioNER; therefore, this biomarker is inappropriate for quantification of total bioNER. Instead, fatty acid analysis is more appropriate for the identification of the microorganisms involved in organic chemical's turnover and for the tracking of microbial activity over time.

Amino acids in proteins are the most dominant microbial biomass components and constitute about 50% of the total microbial cell dry weight [19, 42]. In contrast to fatty acids, amino acid turnover in soil is relatively slow (half-lives in the range of decades) compared to the bulk carbon of microbial biomass (half-lives of much less than 1 year) [57]. Therefore, the analysis of the transfer of isotope-labelled carbon or nitrogen in particular into microbial proteins analysed in hydrolysed amino acids is the most powerful tool to reliably estimate the actual amount of bioNER formed in turnover studies [46]. However, analysis of amino acids does not provide any taxonomic information on the degrader community.

5 BioNER Explained Part of 'Black Box' NER in Several Fate Assessments

First quantifications of bioNER based on the amino acid contents of microbes were made in soil biodegradation studies with 1-[^{13}C]-labelled phenanthrene [62] and with $^{15}N_3$-trinitrotoluene [63]. The contributions of bioNER to the total NER were

low: 23% of total ^{13}C-NER and 11.3% of total ^{15}N-NER, respectively. The contents of amino acids and thus of bioNER might have been underestimated due to difficulty in the analytics of labelled amino acids. The quantification of amino acids in follow-up biodegradation experiments with different organic chemicals was optimized by an improved clean-up method [19].

Nowak et al. [19, 20] and Wang et al. [37, 38, 40] thoroughly balanced the formation of bioNER in fate studies of ^{13}C (and ^{15}N)-labelled readily biodegradable 2,4-D, ibuprofen, metamitron and glyphosate in soil or water-sediment microcosms according to the respective OECD tests [1, 2, 30]. They analysed the amount of ^{13}C (and ^{15}N) converted to total soil OM and microbial biomass amino acids [19, 20, 37, 38, 40] and fatty acids [19, 20]. As proteins were the most stable fraction, the total hydrolysed amino acids were used for quantifying the total bioNER. At the end of incubation, the contents of bioNER were high in all experiments, and the total NER in soils and sediments were dominated by bioNER [19, 20, 37, 38, 40]. Hence, the majority of the residues in these mass balancing studies was identified as bioNER. These results provide the first direct evidence that nearly all NER from these chemicals constitute natural microbial residues stabilized in OM. In contrast to soil studies, the degradation kinetics of the compounds and bioNER formation in water-sediment microcosms were slower [38, 40]. One important reason for the slower degradation and formation of NER (bioNER or xenoNER) in water-sediment microcosms is the presence of two phases with the resulting mass transfer, when the labelled pesticide was added to the water phase (as recommended in OECD 308) [2]. The degradation (biotic or abiotic) of the compounds, however, takes place mainly in the sediment phase [38, 40]. The results of these studies showed that essentially the formation of bioNER must be considered in environmental fate analyses, particularly when the organic chemical is mineralized quickly. For these compounds NER dominated by bioNER have to be expected.

6 Direct and Indirect Assimilation of Carbon from Chemicals as Two Routes for bioNER Formation

BioNER can be formed either via direct assimilation of carbon from organic chemicals into microbial biomass components as observed for 2,4-D [19], ibuprofen [20], metamitron [37, 38] and glyphosate [40] or indirectly via CO_2 fixation [19]. Heterotrophic CO_2 fixation was observed in a soil biodegradation study with unlabelled 2,4-D performed under a $^{13}CO_2$-enriched atmosphere [19]. This heterotrophic CO_2 fixation will be also relevant for other biodegradable pesticides like glyphosate and metamitron during microbial degradation. Heterotrophic fixation of CO_2 has been reported to be common in soils and was often related to non-growth

metabolism of soil microorganisms [64, 65]. Several studies demonstrated that microorganisms need CO_2 for normal growth, because central anabolic pathways involve CO_2 fixation, e.g. the anaplerotic sequences replenishing the tricarboxylic acid cycle (TCC) [66]. Replenishing the TCC is needed when metabolites are exported from the TCC for the biosynthesis of cell constituents, e.g. aspartate or odd-numbered fatty acids [6, 67]; therefore, a high label in these biomarkers is a strong indication of CO_2 fixation. Hence, whenever isotope-labelled CO_2 is formed in complex environmental samples, the formation of bioNER via CO_2 fixation must be taken into account. Usually it amounts to less than 5% of the overall net mineralization [67]. However, this percentage may be higher if the labelled carbon atom is subject to preferential release as CO_2, for example, if a carboxylic group is labelled in the parent molecule or if labelled CO_2 produced during pesticide degradation is preferentially used by the microbes (before it may leave the cell) instead of using external unlabelled CO_2 for anaplerotic reactions. In general, the position of the labelled atoms in the respective tracer molecule affects the results of NER formation studies and needs to be carefully considered [10, 68]. For instance, a study using 9-[^{14}C]-anthracene, with the label in a position of the molecule which is preferentially released as CO_2, proved that carbon was routed from this chemical via CO_2 and microbial biomass to bioNER [69]. Similar routes of bioNER formation via the direct and indirect assimilation are also relevant for nitrogen turnover.

7 Growth and Starvation Metabolism as Two Routes for bioNER Formation

Microbial metabolism of organic chemicals is also influenced by the metabolic state of the cells which is related to the concentrations of the biocatalysts in comparison to the substrates, for example, $c << k_m$, c around k_m or $c >> k_m$ (see Fig. 3). Thus, the metabolic state affects the extent of bioNER formation. Under given environmental conditions, some microbes are active, some are potentially active and thus able to utilize substrates, whereas others are dormant and do not contribute to turnover processes unless they turn active under more favourable conditions [50, 56]. BioNER can be formed during growth or starvation or even under both conditions, in particular under batch conditions of the OECD tests [1, 2] with initial growth and transition to starvation over time. This may vary depending on compound structure and properties, availability, actual energy content and food requirements of microorganisms, the availability of additional assimilable carbon sources, solid matrix or environmental conditions.

Recent results indicated that $^{13}C_3{}^{15}N$-glyphosate [40] and $^{13}C_6$-metamitron [37, 38] are degraded in two different metabolic routes in soil or sediments, presumably depending on the state of carbon and energy flux in the cells, i.e. growth or starvation metabolism. For instance, bioNER from $^{13}C_3{}^{15}N$-glyphosate were presumably formed

both during growth and under starvation [40]. Initial degradation of glyphosate via the 'sarcosine pathway' (Fig. 4A) seems to be related to microbial growth and for biosynthesis of biomass compounds as shown by the occurrence of co-labelled $^{13}C^{15}N$-glycine in microbial biomass amino acids [40].

The degradation of glyphosate in later degradation phase when the majority of compound in the soil batch cultures was exhausted, proceeds via the aminomethylphosphonic acid (AMPA) pathway and may indicate starvation metabolism. This pathway results in the accumulation of AMPA, which is further metabolized at much slower rates than produced. The single-labelled $^{13}C_2$-glycine was produced from glyoxylate originating from AMPA. The glyoxylate may be used for catabolic pathways and thereby support the starvation metabolism feeding mainly cell maintenance. BioNER from glyphosate were thus formed independent of the degradation pathways. An alternative option for the shift of the metabolic pathway was also investigated by Brock et al. [49] using the Microbial Turnover to Biomass (MTB) modelling approach. The authors found that a potential nitrogen overflow of the cell during glyphosate degradation leads to overstoichiometric nitrogen provision. This, in turn, may cause a shift of the microbial cells to pure carbon metabolism based on glyoxylate and the nitrogen excretion as AMPA.

Another study with $^{13}C_6$-metamitron also highlighted the contribution of different microbial metabolism to the extent of bioNER formation [37, 38]. In contrast to $^{13}C_3{}^{15}N$-glyphosate, bioNER from metamitron were formed only under initial growth conditions, when the compound was degraded via desamino-metamitron as the main metabolite in soil. Later in the experiment, the '*Rhodococcus* pathway' with the formation of 3-methyl-4-amino-6-(2-hydroxymuconate)-1,2,4-triazin-5 (4H)one can be assigned to starvation [37, 38] (see Fig. 4b). The end-products pyruvate and acetaldehyde were used for direct biosynthesis of biomass as proven by the presence of ^{13}C-labelled microbial biomass amino acids and particularly in the dominant ^{13}C-alanine. In contrast to soil, in the water-sediment system, the 'desamino pathway' was detected in the later phase of $^{13}C_6$-metamitron degradation, whereas another pathway (4-dimethylimino-3-methyl-6-phenyl-1,2,4-triazin-5(4H) one) was assigned to growth metabolism [37, 38].

Distinct degradation pathways assigned to either growth or starvation, as observed for glyphosate [40] and metamitron [37, 38], can also be expected for other easily and readily biodegradable organic chemicals. However, also here avoiding nitrogen overflow by the cells may also be an alternative reason since the carbon-nitrogen ratio for this compound is also 3:1. To sum up, degradation of organic chemicals via different pathways during growth or starvation as well as the elemental stoichiometry can also determine the type of NER formation (e.g. xenoNER over the bioNER) and their ultimate contents and composition.

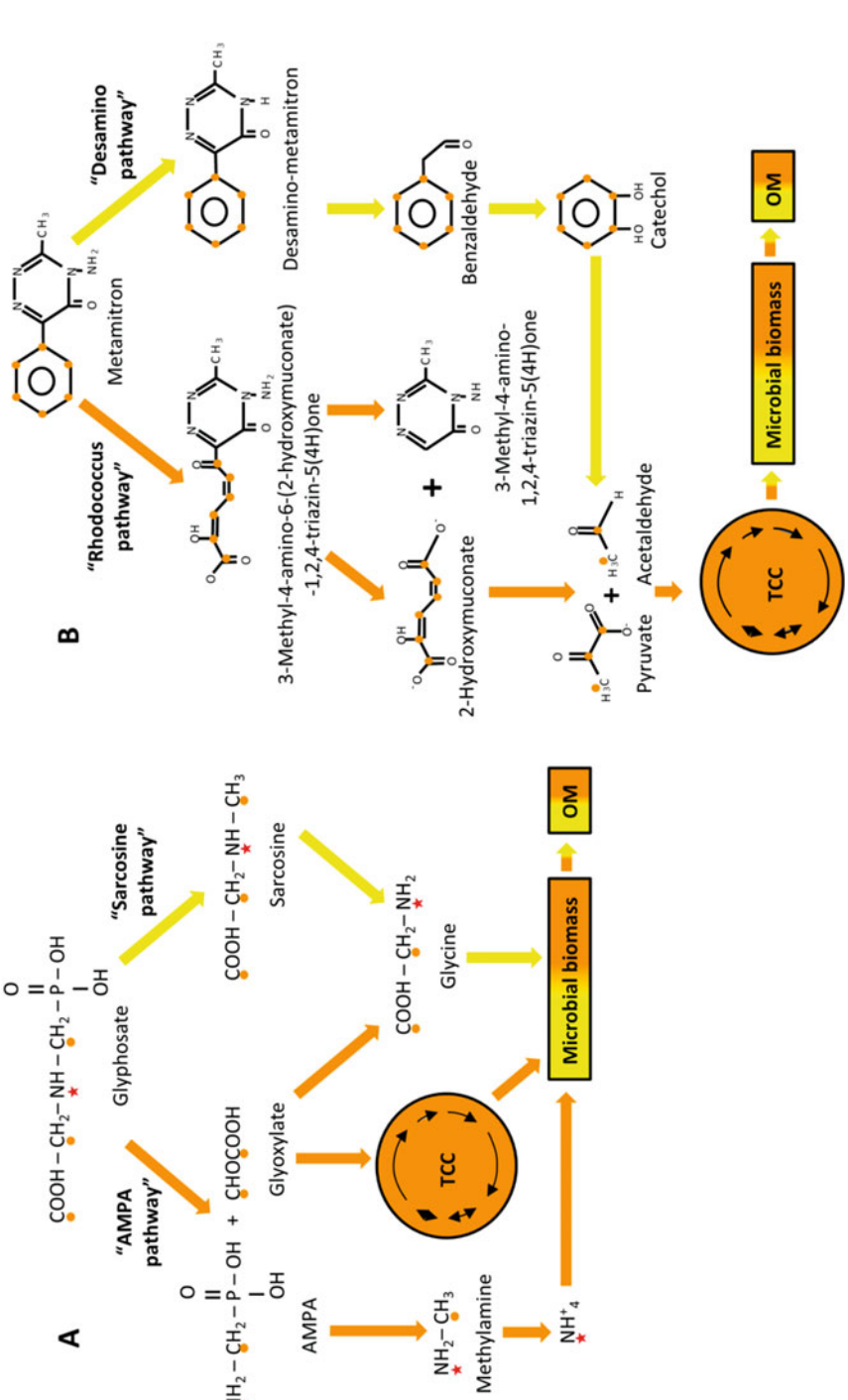

Fig. 4 Growth and starvation in biodegradation of model pesticides. Biodegradation routes of $^{13}C_3\,^{15}N$-glyphosate [40] (**a**) and $^{13}C_6$-metamitron [37] (**b**). Growth or starvation metabolism or even both can contribute to bioNER formation. BioNER from $^{13}C_6$-metamitron are formed during the growth, whereas those from $^{13}C_3\,^{15}N$-glyphosate are formed during both growth and starvation. Yellow arrows, active (growth) metabolism; orange arrows, potential starvation mechanisms. *TCC* tricarboxylic acid cycle, *OM* organic matter, *AMPA* aminomethylphosphonic acid

8 Recent ECHA Suggestions for Differentiation Between the Three Types of NER

Organic chemicals in soils or aquatic environments can undergo different turnover processes as it has been already described in Sect. 1. Two types of xenoNER (types I and II) and bioNER (type III) can be formed during organic chemical turnover in the environment. These three NER types are formed by competing processes, and discriminating analytical methods have been described in the recently published ECHA discussion paper [21, 22] (see Fig. 5). Type I are strongly adsorbed, sequestered or entrapped; type II are covalently bound, both either derived from the parent active compound or its transformation products. Type III are bioNER based on biomolecules after transformation of the labelled carbon (nitrogen) atom to microbial biomass. BioNER can be formed by direct metabolization but may also be formed from released labelled CO_2 via autotrophic and heterotrophic fixation. The remobilization potential and associated potential risk depend on binding strength of organic chemical or its transformation products with solid matrix [6] (see Fig. 5).

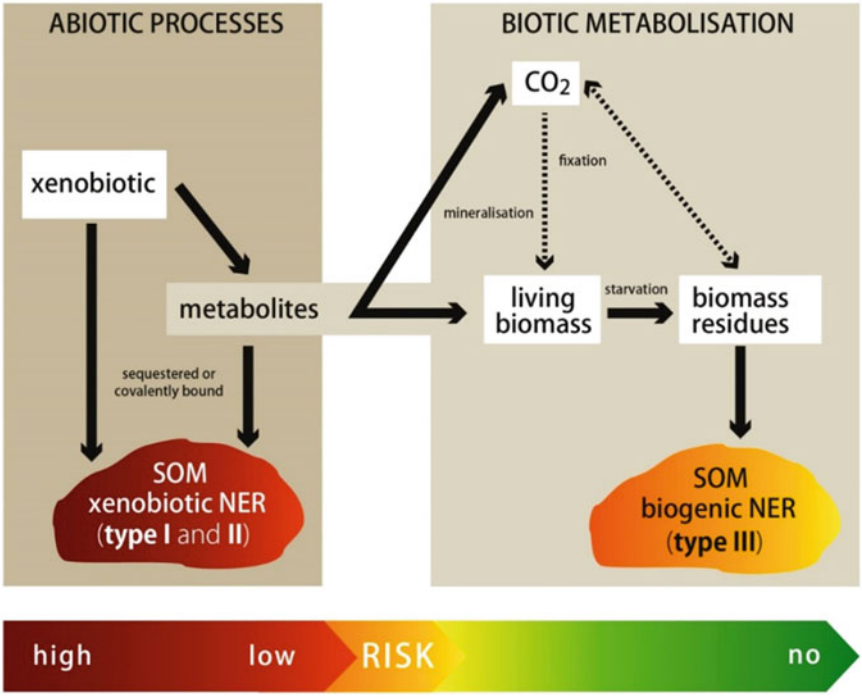

Fig. 5 Biotic and abiotic processes of NER formation and their relevance in risk assessment (modified from Kästner et al. [6])

Chemicals sequestered within solid matrix (as NER type I) can be easily released, whereas chemicals bound to solid matrix via covalent bonding (as NER type II) have a low release potential. However, NER can be also bioNER, a result of carbon or nitrogen assimilation from the chemical into microbial biomass (e.g. lipids or proteins) which is subsequently stabilized in OM after the death. As bioNER contain only biomolecules, they are generally considered to be harmless for the environment.

The differentiation of NER types is planned to be embedded in the general PBT assessment of chemicals under REACH by the ECHA [21, 22]. The ECHA guidance [27, 29] is requesting the analytics to differentiate between three types of NER, in particular for the assessment of the P criterion according to REACH and other regulations. In that case, parent active substance determined as type I NER is considered to increase the degradation half-life (DegT50) of the parent active substance. On the contrary, bioNER are regarded as the product of complete degradation and are expected to decrease the mineralization half-lives. Unknown total NER are considered as remobilizable parent or transformation products, if no additional information is available. Clear indications for bioNER or covalently bound type II NER are considered as 'safe sink' with no or low remobilization potential. In addition, the modelling approach MTB for the potential conversion of chemicals into the bioNER prior to the analytical testing of environmental fate according to OECD has been also proposed. The MTB approach considers the chemical structure, the thermodynamic data of biotransformation and the amount of bioavailable substances [47, 48]. The knowledge about the potential of bioNER formation allows much more efforts for differentiating between the NER type I and type II.

According to the ECHA guidance [27, 29], several liquid-solid extractions and NER remobilizations should be implemented in order to establish a proper risk assessment. After sequential extractions with solvents of increasing strength (starting from mild to harsh), the remaining amount of label in the solid is considered to be total NER. The remaining NER in a solid sample should be then remobilized using a silylating agent [17] to differentiate the NER type I from the NER type II. Extractable parent compound or its transformation products after silylation in the liquid are considered to be NER type I, whereas the label remaining in solid sample is NER type II. Unfortunately, both liquid and solid residues may also contain bioNER; this needs to be corrected for. In this case, another aliquot of the total NER in solid sample should be hydrolysed with 6 M HCL, and the label in amino acids cleaved from proteins should be then quantified and checked for the structural identity. The measured amount of labelled amino acids can be multiplied by a factor of 2 (for incubation times <30 d since biomass consists up to ~50% of proteins; or with a decreasing factor down to 1.1 for long-term experiments >120 day for the relative enrichment of proteins during turnover of microbial biomass in soil) in order to estimate the amount of bioNER.

Although several analytical methods differentiating xenoNER and bioNER have been suggested in the ECHA discussion paper [21], there are still major drawbacks. Analytical methods are often expensive and laborious. Applying those methods,

experienced analysts and highly advanced techniques are required. Furthermore, all regulatory tests are conducted with one active compound, whereas, e.g. pesticides are applied as formulations and often as a mixture of two or three active compounds. The accompanying substances (e.g. surfactants) in the formulation or presence of other active compounds may change the bioavailability and turnover of each active compound. This is only partly considered in regulatory testing of environmental fate. In addition, the MTB modelling approaches predicting the formation of NER are recommended in the ECHA report as a 'screening method' prior to OECD tests [21]. MTB provides only the transformation potential to bioNER but cannot yet correctly reflect how much of this potential is developed under real and complex environmental conditions. For instance, the calculated yield by MTB for some chemicals deviated on average by 49% from obtained experimental data on bioNER, including both over- and underestimations [47]. However, for some pesticides the NER amounts in different soils varied by orders of magnitudes [10].

9 Concluding Remarks and Future Perspectives

Quantitative data on NER and without structural characterization are available in high quantities for ^{14}C-labelled tracer chemicals. Studies identifying and quantifying bioNER formation for several ^{13}C-labelled substances have been already published [19, 20, 37, 38, 40, 70, 71]. In contrast, only one study quantifying bioNER using ^{14}C tracer has been published [72]. However, this study has been validated by an independent investigation in another research lab. Therefore, environmental fate studies of a given active compound should be performed using ^{13}C and ^{14}C tracers for comparison until reliable methods for bioNER analysis using the ^{14}C label and/or more studies with ^{13}C label are available.

A recent study with the slowly degraded antibiotic sulfamethoxazole (SMX) using ^{13}C label showed low mineralization (<0.5%), but sufficient amount of ^{13}C isotope in the microbial DNA and proteins for DNA- and protein-stable isotope probing analysis was detected [73]. However, the concurrent presence of ^{13}C from ^{13}C-SMX bound in DNA and proteins provided evidence of microbial bioNER formation from SMX [73]. SMX is considered to be relatively persistent in the environment with high tendency of NER type I and II formation [74, 75]. The result by Ouyang et al. [73] is thus highly contradictive to the general understanding of productive biodegradation of organic chemicals and formation of bioNER. Based on present knowledge, productive microbial degradation of chemicals requires a certain amount of mineralization accompanied by C transfer into biomass due to thermodynamic reasons. The surprising results obtained by Ouyang et al. [73] indicate that bacteria in complex environments like soil or sediment may utilize organic chemicals as alternative carbon sources without gaining the energy from the

same compound. Therefore, future research should consider the potential influence of multiple uses of carbon substrates on formation of bioNER, in particular those which are hardly biodegradable.

Actual analytical methods differentiating NER types are expensive and laborious. Therefore, new fast and cost-efficient methods for the determination of the NER types should be developed. Only those methods will be easily applied by the chemical industry producing these compounds. A simplified method for the detection of bioNER, which is not based on the determination of more than a dozen of biogenic amino acids separately, could be of big advantage. In addition, better methods for distinguishing type I and type II NER are needed, because silylation [17] or EDTA [63] extraction approaches require high additional efforts for structural as bioNER would have to be assessed in all fractions. In any case, future research should apply the approach on a wider range of compounds particularly pesticides for testing with focus on the slowly degradable compounds.

Using multiple labelled compounds, we can also gain valuable information on the degradation pathways and their shifts according to energy fluxes and stoichiometric imbalances [49] as well as the formation of specific metabolites and transformation products. The determinants of microbial yield formation from a given compound in the presence of other potential substrates (multi-substrate use) are also a research field that is only partly recognized. This outcome could also improve modelling approaches for the environmental fate assessment and particularly NER formation. They are needed, since we cannot test all chemicals. In addition, over-interpretation of bioNER may also lead to a potential 'green-washing' of pesticide residues; therefore these issues should be checked carefully. Furthermore, microbial degradation of chemicals in nature is highly complex and not fully understood yet. Therefore, chemistry (mass balance) should be combined with the biology (e.g. metagenomics, DNA- and protein-stable isotope probing) in order to understand the microbial turnover of organic chemicals and the factors controlling biodegradation. All these future directions could support better testing and risk assessment as well as more sustainable use of chemicals starting from the design of chemicals with high bioNER formation.

Annex

Table 2 Pesticide classification according to mineralization and NER formation: compounds of low (green), intermediate (brown) and high (red) risk of xenobiotic NER formation

ID	Name+label position	CAS number	experimental duration for mineralization (d)	minimum mineralization (% of initial	maximum mineralization (% of initial)	average mineralization (% of initial)	experimental duration for NER (d=	minimum NER (% of initial)	maximum NER (% of initial)	average NER (% of initial)	source
1	Benalaxyl	71626-11-4	100	25	26	25.5	133	18.8	18.8	18.8	Barriuso et al. (2008)
2	Mepanipyrim (Phenyl)	110235-47-7	120	5.4	5.4	5.4	120	26	26	26	Barriuso et al. (2008)
3	Mepanipyrim (Pyrimidin)	110235-47-7	120	2.4	2.4	2.4	120	18.6	18.6	18.6	Barriuso et al. (2008)
4	2,4-D	94-75-7	114	36	36	36	114	27.9	27.9	27.9	Barriuso et al. (2008)
5	2,4-DB	94-82-6	118	42.1	42.1	42.1	118	33.2	33.2	33.2	Barriuso et al. (2008)
6	MCPA-acid	94-74-6	91	54	67	60.5	91	30	34.4	32.2	Barriuso et al. (2008)
7	MCPB	94-81-5	120	58	58	58	120	30	30	30	Barriuso et al. (2008)
8	Mecoprop	7085-19-0	91	25	52	38.5	91	39	51	45	Barriuso et al. (2008)
9	Cyhalofop-butyl	122008-85-9	120	36.1	46.3	41.2	120	33.7	44.2	38.95	Barriuso et al. (2008)
10	Propyzamide/Pronamide (USA)	23950-58-5	90	3.4	3.4	3.4	90	6.8	6.8	6.8	Barriuso et al. (2008)
11	Propyzamide/Pronamide (USA)	23950-58-5	120	33	48	40.5	80	16	27	21.5	Barriuso et al. (2008)
12	Zoxamide	156052-68-5	120	34.4	57.8	46.1	28	25.6	39	32.3	Barriuso et al. (2008)
13	Thiophanate-methyl	23564-05-8	120	7.3	25.7	16.5	120	40	73	56.5	Barriuso et al. (2008)
14	Ethofumesate	26225-79-6		6	13	9.5		16	34	25	Barriuso et al. (2008)
15	Bentazone	25057-89-0	90	2	9	5.5	90	44	80	62	Barriuso et al. (2008)
16	Milbemecin (Milbemectin) A3	51596-10-2	120	14	35	24.5	91	13	40	26.5	Barriuso et al. (2008)
17	Desmedipham (both labels)	13684-56-5	100	7.5	46.4	26.95	100	21.5	67.2	44.35	Barriuso et al. (2008)
18	Desmedipham (AP-labels)	13684-56-5	90	14	19	16.5	90	64	64	64	Barriuso et al. (2008)
19	Phenmedipham (AP)	13684-63-4	120	13.3	16.5	14.9	120	63.6	64.1	63.85	Barriuso et al. (2008)
20	Phenmedipham (Phenoxy)	13684-63-4	120	9.7	11.3	10.5	120	71.3	73.8	72.55	Barriuso et al. (2008)
21	Chlorpropham	101-21-3	200	15	30	22.5		54	78	66	Barriuso et al. (2008)
22	Iprovalicarb	140923-17-7		17.1	59.5	38.3		10.6	27.9	19.25	Barriuso et al. (2008)
23	S-Metolachlor	87392-12-9	90	15.3	15.3	15.3	90	4.6	4.6	4.6	Barriuso et al. (2008)
24	Dimethenamid-P (thienyl)	163515-14-8	120	8	36	22	120	22	44	33	Barriuso et al. (2008)
25	Chlorothalonil	1897-45-6	92	23.8	23.8	23.8	90	63	63	63	Barriuso et al. (2008)

26	Cyazofamid (Phenyl)	120116-88-3	45	14.4	14.4	14.4	59	47.6	47.6	47.6	Barriuso et al. (2008)
27	Cyazofamid (Imidiazole)	120116-88-3	59	11.9	11.9	11.9	45	64	64	64	Barriuso et al. (2008)
28	Tepraloxydim	149979-41-9		66	66	66		25	25	25	Barriuso et al. (2008)
29	Methoxyfenozide (A-Ring)	161050-58-4	120	0.9	3.6	2.25	120	12	27	19.5	Barriuso et al. (2008)
30	Methoxyfenozide (B-Ring)	161050-58-4	120	2.6	2.6	2.6	120	26	26	26	Barriuso et al. (2008)
31	Methoxyfenozide (t-label)	161050-58-4	120	2.7	2.7	2.7	120	24	24	24	Barriuso et al. (2008)
32	Iprodione (phenyl)	36734-19-7		5	5	5		40	75	57.5	Barriuso et al. (2008)
33	Pendimethalin	40487-42-1		1.7	2.4	2.05	90	2	10	6	Barriuso et al. (2008)
34	Etoxazole (t-butylphenyl)	153233-91-1	90	7	15.8	11.4	90	18.6	27.5	23.05	Barriuso et al. (2008)
35	Etoxazole (difluorophenyl)	153233-91-1	90	48	56.4	52.2	90	23	25.5	24.25	Barriuso et al. (2008)
36	Mancozeb	8018-01-7	93	31.5	51.8	41.65	93	46.1	46.1	46.1	Barriuso et al. (2008)
37	Maneb	12427-38-2	32	16	23	19.5	32	62	88	75	Barriuso et al. (2008)
38	Glyphosate	1071-83-6	28-150	32.7	80.1	56.4	28-150	5.1	40.3	22.7	Barriuso et al. (2008)
39	Glyphosate	1071-83-6	112	5.8	9.3	7.55	112	4.6	13.5	9.05	Barriuso et al. (2008)
40	Glyphosate trimesium	81591-81-3	21-150	37	75	56	21-150	20	32	26	Barriuso et al. (2008)
41	Glyphosate trimesium (trimesium)	81591-81-3	9-150	46	74	60	9-150	10	26	18	Barriuso et al. (2008)
42	Bromoxynil	1689-84-5	28	27.3	33.6	30.45	28	72.9	74.2	73.55	Barriuso et al. (2008)
43	Ioxynil (Phenyl)	1689-83-4	48	27.3	27.3	27.3	48	77	77	77	Barriuso et al. (2008)
44	Ioxynil octanoate (Octanoate)	3861-47-0	120	50.2	54.7	52.45	120	38.6	44	41.3	Barriuso et al. (2008)
45	Ioxynil octanoate (Phenyl)?	3861-47-0	128	60.5	66.3	63.4	128	25.2	31.6	28.4	Barriuso et al. (2008)
46	Fenamidone (C-phenyl)	161326-34-7	90	3.6	9.3	6.45	90	24.3	37.4	30.85	Barriuso et al. (2008)
47	Fenamidone (N-phenyl)	161326-34-7	90	5	5	5	90	47.3	47.3	47.3	Barriuso et al. (2008)
48	Imazamox (Pyridine)	114311-32-9	122	0.8	23.6	12.2	122	7.3	17.5	12.4	Barriuso et al. (2008)
49	Isoxaflutole	141112-29-0		1	1	1		6	9	7.5	Barriuso et al. (2008)
50	Spiroxamine	118134-30-8		30.7	44.7	37.7		24.7	26.4	25.55	Barriuso et al. (2008)
51	Acetamiprid	135410-20-7	120	9.6	9.6	9.6	120	32.3	32.3	32.3	Barriuso et al. (2008)
52	Chlorpyrifos	2921-88-2	120	82	82	82	120	4	4	4	Barriuso et al. (2008)
53	Chlorpyrifos	2921-88-2		5	50	27.5		25	25	25	Barriuso et al. (2008)
54	Chlorpyrifos- methyl	5598-13-0		23	69	46		17	26	21.5	Barriuso et al. (2008)
55	Fosthiazate (Thiazolidine)	98886-44-3	84	67	67	67	56	7	7	7	Barriuso et al. (2008)

(continued)

Table 2 (continued)

56	Fosthiazate (Butyl)	98886-44-3	84	27	27	27	56	25	25	25	Barriuso et al. (2008)
57	Indoxacarb (Indanone)	173584-44-6		12.5	29	20.75		39	45	42	Barriuso et al. (2008)
58	Indoxacarb (Trifluromethoxyphenyl)	173584-44-6		1.9	8.4	5.15		5	56	30.5	Barriuso et al. (2008)
59	Oxadiargyl	39807-15-3	92	5.1	10.4	7.75	92	20	24.8	22.4	Barriuso et al. (2008)
60	Flufenacet (Flurophenyl)	142459-58-3	90	10.2	20.8	15.5	90	29.9	56.2	43.05	Barriuso et al. (2008)
61	Flufenacet (Thiadiazole)	142459-58-3	90	31.9	31.9	31.9	90	6	6	6	Barriuso et al. (2008)
62	Metalaxyl-M	70630-17-0	84	22	33	27.5	84	63	73	68	Barriuso et al. (2008)
63	Cinidon-ethyl (Phenyl)	142891-20-1	118	6.1	6.1	6.1	118	79.6	79.6	79.6	Barriuso et al. (2008)
64	Cinidon-ethyl (Indole)	142891-20-1	90	40.7	40.7	40.7	90	49.2	49.2	49.2	Barriuso et al. (2008)
65	Pyraflufen-ethyl	129630-19-9		2.53	2.53	2.53		17	17	17	Barriuso et al. (2008)
66	Pyridate	55512-33-9		19	26	22.5		52	60	56	Barriuso et al. (2008)
67	beta-Cyfluthrin	68359-37-5	84-190	23	36	29.5	84-190	34	42	38	Barriuso et al. (2008)
68	Cypermethrin (cis-isomers)	52315-07-8	168	20	47	33.5	168	21	57	39	Barriuso et al. (2008)
69	Cypermethrin (trans-isomers)	52315-07-8	168	48	61	54.5	168	26	45	35.5	Barriuso et al. (2008)
70	Deltamethrin (Benzyl)	52918-63-5	64	52	65	58.5	64	18	26	22	Barriuso et al. (2008)
71	Deltamethrin (Phenoxy)	52918-63-5	128	52	58	55	128	24	31	27.5	Barriuso et al. (2008)
72	Deltamethrin (Cyano)	52918-63-5	64	62	69	65.5	64	10	20	15	Barriuso et al. (2008)
73	Deltamethrin (Vinyl)	52918-63-5	64	50	70	60	64	14	21	17.5	Barriuso et al. (2008)
74	Deltamethrin (Gem)	52918-63-5	90	36	36	36	90	48	48	48	Barriuso et al. (2008)
75	Lambda-Cyhalothrin (Cyclopropane)	91465-08-6	92	25	59	42	92	12	19	15.5	Barriuso et al. (2008)
76	Flurtamone	96525-23-4	366	24	40	32	366	32	32	32	Barriuso et al. (2008)
77	Pymetrozine	123312-89-0	90	3	15	9	90	21	61	41	Barriuso et al. (2008)
78	Picolinafen (Aniline)	137641-05-5	61	17.4	17.4	17.4	61	43.9	65	54.45	Barriuso et al. (2008)
79	Picolinafen (Pyridine)	137641-05-5	100	22.8	43	32.9	100	21.2	22.7	21.95	Barriuso et al. (2008)
80	Fluroxypyr	69377-81-7		65	65	65		29.7	29.7	29.7	Barriuso et al. (2008)
81	Thiacloprid (Phenyl)	111988-49-9		6.5	34	20.25		22	30	26	Barriuso et al. (2008)
82	Foramsulfuron (Phenyl)	173159-57-4	80	0.3	1.2	0.75	80	74	103	88.5	Barriuso et al. (2008)
83	Foramsulfuron (Pyrimidyl)	173159-57-4	80	2.5	16.3	9.4	80	55	93	74	Barriuso et al. (2008)
84	Quinoxyfen	124495-18-7	200	1.9	1.9	1.9	200	25	25	25	Barriuso et al. (2008)

85	Azoxystrobin	131860-33-8	100-360	2	14	8	100-360	9	24	16.5	Barriuso et al. (2008)
86	Famoxadone (Phenylamino)	131807-57-3	90	11.8	11.8	11.8	90	53.8	53.8	53.8	Barriuso et al. (2008)
87	Famoxadone (Phenoxyphenyl)	131807-57-3	90	13	32.2	22.6	90	29.9	51.4	40.65	Barriuso et al. (2008)
88	Kresoxim-Methyl	143390-89-0	91	17.2	35.2	26.2	91	30.1	47.6	38.85	Barriuso et al. (2008)
89	Picoxystrobin (Pyridinyl)	117428-22-5	119	13.4	32.5	22.95	119	12.4	32.4	22.4	Barriuso et al. (2008)
90	Picoxystrobin (Phenyl)	117428-22-5	113	29.9	54.4	42.15	113	22.4	32.2	27.3	Barriuso et al. (2008)
91	Pyraclostrobin (Tolyl)	175013-18-0	87	4	4	4	87	54.3	54.3	54.3	Barriuso et al. (2008)
92	Pyraclostrobin (Chlorophenyl)	175013-18-0	91	5	5	5	91	56.1	56.1	56.1	Barriuso et al. (2008)
93	Trifloxystrobin (GP)	141517-21-7	105	4	64	34	105	9	27	18	Barriuso et al. (2008)
94	Trifloxystrobin (TP)	141517-21-8	365	57	57	57	365	27	27	27	Barriuso et al. (2008)
95	Ethoxysulfuron	126801-58-9		16.6	16.6	16.6		18.2	18.2	18.2	Barriuso et al. (2008)
96	Flazasulfuron	104040-78-0		2	5	3.5		5	12	8.5	Barriuso et al. (2008)
97	Flupyrsulfuron- methyl (Pyridine)	144740-54-5	90	2	2	2	90	29	29	29	Barriuso et al. (2008)
98	Flupyrsulfuron- methyl (Pyrimidine)	144740-54-5	90	2	2	2	90	39	39	39	Barriuso et al. (2008)
99	Imazosulfuron	122548-33-8	120	3	10	6.5	120	19	67	43	Barriuso et al. (2008)
100	Iodosulfuron	185119-76-0	86	2.1	29.9	16	86	27	39.3	33.15	Barriuso et al. (2008)
101	Mesosulfuron (Phenyl)	400852-66-6	90	6.7	6.7	6.7	90	56.3	56.3	56.3	Barriuso et al. (2008)
102	Mesosulfuron (Pyrimidyl)	400852-66-6	90	6.1	46.8	26.45	90	28	54.8	41.4	Barriuso et al. (2008)
103	Metsulfuron methyl (Phenyl)	74223-64-6	112	32	32	32	98	12	25	18.5	Barriuso et al. (2008)
104	Metsulfuron methyl (Triazine)	74223-64-6	90	11.4	11.4	11.4	90	17.6	17.6	17.6	Barriuso et al. (2008)
105	Metsulfuron methyl (Triazine amine)	74223-64-6		10	10	10		6	6	6	Barriuso et al. (2008)
106	Metsulfuron methyl	74223-64-6	455	38	38	38	455	10	10	10	Barriuso et al. (2008)
107	Oxasulfuron (Phenyl)	144651-06-9	105	36	57	46.5	105	21	27	24	Barriuso et al. (2008)
108	Oxasulfuron (Pyrimidinyl)	144651-06-9	128	21	25	23	128	40	58	49	Barriuso et al. (2008)
109	Oxasulfuron (Oxetanyl)	144651-06-9	79	51	80	65.5	79	5	30	17.5	Barriuso et al. (2008)
110	Prosulfuron (Phenyl+Triazine)	94125-34-5		5	5	5	90				Barriuso et al. (2008)
111	Prosulfuron (Phenyl)	94125-34-5	180	9	9	9	90	12	44	28	Barriuso et al. (2008)
112	Prosulfuron (Triazine)	94125-34-5	180	45	45	45	90	10	10	10	Barriuso et al. (2008)
113	Sulfosulfuron (Imidazo)	141776-32-1		1.6	2.2	1.9		14	41	27.5	Barriuso et al. (2008)

(continued)

Table 2 (continued)

114	Sulfosulfuron (Pyridine)	141776-32-1		8.1	13	10.55		15	33	24	Barriuso et al. (2008)
115	Thifensulfuron-Methyl (Thiophene)	79277-27-3						27	48	37.5	Barriuso et al. (2008)
116	Thifensulfuron-Methyl (Triazine Amine)	79277-27-3						10	38	24	Barriuso et al. (2008)
117	Triasulfuron (Triazine)	82097-50-5	70	2	21	11.5	70	23	42	32.5	Barriuso et al. (2008)
118	Triasulfuron (Phenyl)	82097-50-5	84	2	14	8	84	25	57	41	Barriuso et al. (2008)
119	Molinate	2212-67-1	30	0.96	0.96	0.96	30	2.39	2.39	2.39	Barriuso et al. (2008)
120	Silthiofam	175217-20-6	120	10.62	10.62	10.62	120	44.27	44.27	44.27	Barriuso et al. (2008)
121	Carfentrazone-Ethyl (Phenyl, Carbonyl)	128639-02-1		3	3	3		14.5	15	14.75	Barriuso et al. (2008)
122	Amitrole	61-82-5	7	20	60	40	100	17	19	18	Barriuso et al. (2008)
123	Propiconazole (Triazole)	60207-90-1	84	0.2	2	1.1	84	3.4	47.3	25.35	Barriuso et al. (2008)
124	Propiconazole (Phenyl)	60207-90-1	84	29.3	35.4	32.35	84	23.3	27.3	25.3	Barriuso et al. (2008)
125	Propoxycarbazone (Phenyl)	145026-81-9	88	9.1	49	29.05	88	6.5	29.5	18	Barriuso et al. (2008)
126	Propoxycarbazone (Triazolinon)	145026-81-9	93	1.3	12.6	6.95	93	8.9	65.7	37.3	Barriuso et al. (2008)
127	Florasulam	145701-23-1		4.8	13.5	9.15		29.6	57.1	43.35	Barriuso et al. (2008)
128	Mesotrione	104206-82-8		75	75	75		37	37	37	Barriuso et al. (2008)
129	Acibenzolar-s-methyl (Phenyl)	135158-54-2	90	7.5	44.1	25.8	90	27.7	59.8	43.75	Barriuso et al. (2008)
130	Bifenazate	149877-41-8	119	15.2	23	19.1	119	64	67.3	65.65	Barriuso et al. (2008)
131	Cyclanilide	113136-77-9	120	4.3	4.3	4.3	120	30	30	30	Barriuso et al. (2008)
132	Daminozide	1596-84-5	2	20	59	39.5	2	20	25	22.5	Barriuso et al. (2008)
133	Flumioxazine (Phenyl)	103361-09-7	100	5.6	13.5	9.55	100	62.4	73.6	68	Barriuso et al. (2008)
134	Flumioxazine (THP)	103361-09-7	91	54.9	54.9	54.9	91	29	29	29	Barriuso et al. (2008)

135	Metiram	9006-42-2	90	28	41	34.5	90	38	65	51.5	Barriuso et al. (2008)
136	Forchlorfenuron (Phenyl)	68157-60-8	90	3.07	25.4	14.235	90	16.6	46.4	31.5	Barriuso et al. (2008)
137	Forchlorfenuron (Pyridine)	68157-60-8	120	2.9	5	3.95	120	23.5	25.2	24.35	Barriuso et al. (2008)
138	Maleic hydrazide	123-33-1	90	71.6	71.6	71.6	90	24.5	24.5	24.5	Barriuso et al. (2008)
139	Chlorotoluron	15545-48-9		6.4	13.3	9.85		28.2	62.6	45.4	Barriuso et al. (2008)
140	Isoproturon	34123-59-6		10	22	16		56	68	62	Barriuso et al. (2008)
141	ABAMECTIN	71751-41-2	21 weeks,91	3.2	12.4	7.8	196 d,91	39.1	44.1	41.6	EU approval dossier
142	LINURON	330-55-2	100	<5	<5	5	100	25	30	27.5	EU approval dossier
143	Cyclaniliprole (phenyl)	1031756-98-5	180	0.4	0.4	0.4	180	27.1	27.1	27.1	EU approval dossier
144	Cyclaniliprole (pyrazole)	1031756-98-5	180	0.7	0.7	0.7	180	23.5	23.5	23.5	EU approval dossier
145	8-HYDROXYQUINOLINE	148-24-3	120	9.94	11.6	10.77	120	60.98	79.2	70.09	EU approval dossier
146	Oxathiapiproline (thiazole)	1003318-67-9	120	LOQ	11.81	5.905	120	6.7	38.2	22.45	EU approval dossier
147	Oxathiapiproline (pyrazole)	1003318-67-9	120	LOQ	6.58	3.29	120	9.88	34.2	22.04	EU approval dossier
148	Oxathiapiproline (isoxazoline)	1003318-67-9	120	3.95	3.95	3.95	120	8.24	8.24	8.24	EU approval dossier
149	DIQUAT	2764-72-9	120	85.7	98.3	92	120	1.6	3.5	2.55	EU approval dossier
150	Isofetamid	875915-78-9	100	<5	<5	5	100	70	70	70	EU approval dossier
151	Flumioxazine (Phenyl)	103361-09-7	89;100;120 (Phenyl); 91 (THP)	7.7	13.5	10.6	91;100;120	62.4	73.9	68.15	EU approval dossier
152	Flumioxazine (THP)	103361-09-7	89;100;120 (Phenyl); 91 (THP)	54.9	54.9	54.9	91;100;120	29	29	29	EU approval dossier
153	Fluometuron	2164-17-2	90;100	0.81	1.46	1.135	90;100	5.2	52.3	28.75	EU approval dossier
154	Fluopicolide (Pyridinyl)	239110-15-7	94-98	0	0.2	0.1	94-120	9.2	16.2	12.7	EU approval dossier
155	Fluopicolide (Benzoyl)	239110-15-8	94-99	0	2	1	94-121	4	12	8	EU approval dossier
156	Fluquinconazole (Dichlorophenyl)	136426-54-5	119-365	0.1	2.9	1.5	93-365	4.1	24.5	14.3	EU approval dossier
157	Fluquinconazole (Triazolyl)	136426-54-6	119-366	0.3	10.4	5.35	93-366	7	32.9	19.95	EU approval dossier

(continued)

Table 2 (continued)

158	Glufosinate	126633-48-5, 53369-07-6	120	20	62	41	120	11	38	24.5	EU approval dossier
159	Haloxyfop-P (Phenyl)	96491-05-3	90	32	32	32	90	44	44	44	EU approval dossier
160	Haloxyfop-P (Pyridine)	96491-05-4	90	1	6.3	3.65	90	3.4	38	20.7	EU approval dossier
161	Imazamox	182636-13-1, 114311-32-9	122	0.8	23.6	12.2	90;122	7.3	17.5	12.4	EU approval dossier
162	Imazosulfuron	122548-33-8	120	3	10	6.5	120	19	67	43	EU approval dossier
163	Isoproturon	34123-59-6	100	10	22	16	100	56	68	62	EU approval dossier
164	Isopyrazam (phenyl)	881685-58-1	120	0.2	2.2	1.2	120	2.6	12.8	7.7	EU approval dossier
165	Isopyrazam (pyrazole)	881685-58-2	120	3.2	3.2	3.2	120	25.7	25.7	25.7	EU approval dossier
166	lambda-Cyhalothrin (cyclopropyl)	91465-08-6	120	15	46	30.5	120	13	29	21	EU approval dossier
167	lambda-Cyhalothrin (phenoxy label)	91465-08-7	120	12	30	21	120	12	44	28	EU approval dossier
168	Lenacil	2164-08-1	120	47.6	61.1	54.35	120	19.4	25.8	22.6	EU approval dossier
169	Lufenuron (dichlorophenyl)	103055-07-8	149;360	2	15	8.5	149;360	24.6	74.9	49.75	EU approval dossier
170	Lufenuron (difluorophenyl)	103055-07-9	360	58	58	58	360	28.3	28.3	28.3	EU approval dossier
171	Metalaxyl	57837-19-1	100	23	38	30.5	100	56	56	56	EU approval dossier
172	Metam	137-42-8	21	45.96	86.25	66.105	21	9.88	38.38	24.13	EU approval dossier
173	Metconazole (triazole)	125116-23-6	120	10.3	10.3	10.3	120	39.2	39.2	39.2	EU approval dossier
174	Metconazole (cyclopentanol)	125116-23-7					112	12.5	28.3	20.4	EU approval dossier
175	Methomyl	16752-77-5	92	75	75	75	92	14	14	14	EU approval dossier
176	Metribuzin	21087-64-9	100	15	38.9	26.95	100	36.6	51.8	44.2	EU approval dossier
177	Metsulfuron-methyl (phenyl)	74223-64-6	90	14.5	33	23.75	90	18.2	34.4	26.3	EU approval dossier
178	Metsulfuron-methyl (triazine)	74223-64-7	90	3.2	11.4	7.3	90	10.7	26.1	18.4	EU approval dossier
179	Molinate	2212-67-1	30	0.96	0.96	0.96	30	2.39	2.39	2.39	EU approval dossier
180	Myclobutanil (triazole)	88671-89-0	120	0.2	1.6	0.9	120	4.1	15.9	10	EU approval dossier
181	Myclobutanil (chlorophenyl)	88671-89-1	120	1.7	1.7	1.7	120	8	8	8	EU approval dossier
182	Nicosulfuron (pyridine)	111991-09-4	112	1.3	1.3	1.3	112	35.2	35.2	35.2	EU approval dossier
183	Nicosulfuron (pyrimidine)	111991-09-5	112	16.8	16.8	16.8	112	45.9	45.9	45.9	EU approval dossier
184	Oxadiargyl	39807-15-3	92;125	5.1	10.4	7.75	92;125	20	24.8	22.4	EU approval dossier
185	Oxadiazon	19666-30-9	300	6.41	6.41	6.41	269;365	5.44	35.5	20.47	EU approval dossier
186	Oxamyl	23135-22-0	51;60	45	>70	57.5	51-123	<25	<25	25	EU approval dossier

187	Oxyfluorfen (14C-CPR)	42874-03-3	90-91	0.8	15	7.9	90-91	10.1	43.1	26.6	EU approval dossier
188	Oxyfluorfen (14C-NPR)	42874-03-4	91	0.9	1	0.95	91	12.7	23.8	18.25	EU approval dossier
189	Paclobutrazol (triazole)	76738-62-0	84-120	0.2	11.1	5.65	84-120	4.9	52.2	28.55	EU approval dossier
190	Paclobutrazol (methine)	76738-62-1	120	7.4	7.4	7.4	120	4.4	4.4	4.4	EU approval dossier
191	Pendimethalin	40487-42-1	100	1.7	2.4	2.05	90	2	10	6	EU approval dossier
192	Pirimicarb	23103-98-2	112	1.1	3	2.05	112	9.4	12.3	10.85	EU approval dossier
193	Prochloraz (imidazole)	67747-09-5	119	11	26	18.5	119	15	27	21	EU approval dossier
194	Prochloraz (3H-phenyl)	67747-09-6	364	1	2	1.5	119	15	24	19.5	EU approval dossier
195	Prochloraz (14C-phenyl)	67747-09-7					120;182	23	35.6	29.3	EU approval dossier
196	Prochloraz (trichlorophenyl)	67747-09-8					120	21.3	42.5	31.9	EU approval dossier
197	Profoxydim	139001-49-3	100	32.6	36.8	34.7	100	23	31	27	EU approval dossier
198	Propiconazole (triazole)	60207-90-1	84;105;120	0.2	2	1.1	84;120	14.1	47.3	30.7	EU approval dossier
199	Propiconazole (phenyl)	60207-90-2	84	29.3	35.4	32.35	84	23.3	27.3	25.3	EU approval dossier
200	1,2,4-Triazole	288-88-0	84-168	0.9	48.8	24.85	120	41.8	66.2	54	EU approval dossier
201	CGA 118 245		5	0.1	0.2	0.15	5	9.8	12.3	11.05	EU approval dossier
202	Propoxycarbazone (phenyl)	145026-81-9	88-98;180-361	9.1	49	29.05	88-98	6.5	29.5	18	EU approval dossier
203	Propoxycarbazone (triazolinone)	145026-81-10	93-117;182-365	1.3	12.6	6.95	93-117;182-365	8.9	65.7	37.3	EU approval dossier
204	Prosulfuron (phenyl)	94125-34-5	180	<5	19	12	90	12	44	28	EU approval dossier
205	Prosulfuron (triazine)	94125-34-6	180	<5	45	25	90	10	10	10	EU approval dossier
206	Quinoxyfen	124495-18-7	200	1.9	1.9	1.9	200	25	25	25	EU approval dossier
207	Quizalofop-P (variant quizalofop-P-tefuryl) (phenyl)	119738-06-6	120	22	34	28	120	32	47	39.5	EU approval dossier
208	Quizalofop-P (variant quizalofop-P-tefuryl) (furfuryl)	119738-06-6	30	57	70	63.5	30	15	32	23.5	EU approval dossier
209	Quizalofop-P (variant quizalofop-P-tefuryl) (quinoxaline)	119738-06-6	120-125	2.3	26	14.15	120-125	40	50	45	EU approval dossier
210	Sulcotrione	99105-77-8	120	2.5	73.8	38.15	120	5.9	26.5	16.2	EU approval dossier
211	Tebuconazole (phenyl)	107534-96-3	112	0.4	0.4	0.4	112	16.2	16.2	16.2	EU approval dossier
212	Tebuconazole (triazole)	107534-96-3	58	<0,1	<0,1	0.1	58	14.5	14.5	14.5	EU approval dossier
213	Tebufenpyrad (pyrazole)	119168-77-3	120-122	9.2	43.9	26.55	120-122	2.5	35.5	19	EU approval dossier
214	Tebufenpyrad (benzene ring)	119168-77-3	120-122	16.3	16.3	16.3	120-122	3.5	3.5	3.5	EU approval dossier
215	Tepraloxydim	149979-41-9	100	66	66	66	100	25	25	25	EU approval dossier
216	Thiacloprid	111988-49-9	100	6.5	34	20.25	100	22	30	26	EU approval dossier
217	Tri-allate	2303-17-5	120	19.34	43.98	31.66	120	25.29	35.92	30.605	EU approval dossier
218	Triasulfuron (triazinyl)	82097-50-5	120	12.5	13	12.75	120	19.7	21.5	20.6	EU approval dossier
219	Triasulfuron (phenyl)	82097-50-5	120	8.7	9	8.85	120	25	27.5	26.25	EU approval dossier
220	Triazoxide	72459-58-6	91-120	0.1	0.1	0.1	91-120	18.9	29.8	24.35	EU approval dossier
221	Warfarin	81-81-2	100	10	20	15	100	10	42	26	EU approval dossier
222	Ziram	137-30-4	28	51	57	54	28	31	36	33.5	EU approval dossier

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Impact of Sorption to Dissolved Organic Matter on the Bioavailability of Organic Chemicals

John R. Parsons

Contents

Abstract Dissolved organic matter (DOM) is ubiquitously present in the aquatic environment as well as in soil and sediment pore water. Partitioning-based techniques have generated better insights into the influence of different DOM structural features on sorption for hydrophobic chemicals, but the prediction of pH-dependent sorption for polar and ionisable chemicals remains problematic. Sorption to DOM can reduce the apparent rate of uptake and extent of accumulation of hydrophobic chemicals, although sorption to DOM may in some cases enhance diffusive transport of the contaminants and thereby increase uptake rates during bioaccumulation. Similarly, DOM can act as a surfactant to increase the rate of solubilisation and microbial uptake of soil-sorbed chemicals and therefore their rates of biodegradation. The impact of DOM structure is, however, more complex than can be captured by simple organic carbon-based approaches. In particular, exploration of the influence of condensed aromatic structures in DOM, often referred to as dissolved black carbon, would probably yield better insights into their impact on the bioavailability of high molecular weight PAHs and other hydrophobic compounds. More studies of

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the sorption of polar and ionisable chemicals to DOM would also increase our understanding of its potential impact on the bioavailability of such chemicals.

Keywords Bioaccumulation, Biodegradation, Dissolved organic matter, Passive sampling, Sorption

1 Introduction

Dissolved organic matter (DOM) is operationally defined as the fraction of organic matter in water which can pass through a 0.22–0.7 (often 0.45) μm filter. DOM is often referred to as dissolved organic carbon (DOC) although it also contains important fractions of organic nitrogen and phosphorus. Dissolved organic matter (DOM) is ubiquitously present in the aquatic environment as well as in soil and sediment pore water. The concentration and composition of DOM play a significant role in natural biogeochemical processes but can also have an important impact of the fate and effects of environmental pollutants. Although DOM in the broad sense includes all dissolved organic chemicals, it is often taken to refer to macromolecular, humic substances related components. In general, these humic substances comprise around 50% of the total DOM content of typical river water [1]. The concentration and composition of DOM are highly variable, depending on the source of the organic components, on environmental conditions, such as temperature, pH, ionic strength, and processes such as interactions with solid-phase materials and microbial degradation [1].

Due to its complexity, the analysis and characterisation of DOM is highly challenging, but modern analytical techniques, particularly the application of high-resolution mass spectrometry, enable better insights into the composition and structure of this material. There is continuing debate on the structure of humic materials, with new insights favouring humic substances as dynamic associations of low molecular weight components rather than macromolecules [2]. These associations can form micelle-like structures when dissolved in water. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is a particularly powerful technique to analyse the molecular composition of natural organic matter [3]. Results of analyses using FT-ICR-MS and other advanced NMR and UV-fluorescence techniques indicate that DOM is a complex mixture of low-MW substances and larger-MW biomolecules [4]. Sediment DOM has shown more enrichment of nitrogen- and sulphur-containing compounds in the elemental signature than the overlying DOM [5]. Fluorescent fingerprints indicate that sediment DOM lacks the photo-oxidised and intermediate components which are present in the overlying surface water DOM.

A small fraction of DOM consists of thermally produced highly aromatic structures sometimes referred to as dissolved black carbon. This dissolved black carbon is

widely distributed in the aquatic environment, and even a significant fraction of marine DOM has been thermally altered, either as a result of combustion on land followed by transport to the oceans or by alternation in deep sea sediments [6]. This recalcitrant and potentially highly sorbing fraction appears to comprise around 10% of the dissolved organic carbon that enters the oceans [7].

Depending on the properties of DOM and of the chemicals in question, DOM may have a significant impact on the fate and bioavailability of organic contaminants. In this chapter we first review current knowledge concerning the interactions between freely dissolved organic contaminants and DOM before discussing the impact of this process on the bioavailability of the contaminants.

2 Sorption of Organic Chemicals to DOM

Reversible interactions of dissolved organic matter with freely dissolved specific chemicals are treated as sorption processes that can be quantified with equilibrium partition coefficients (K_{DOM}) calculated from the ratio between the concentration of the chemical sorbed to DOM and that in the freely dissolved phase. These partition coefficients are often normalised to the organic carbon content of DOM and expressed as K_{DOC} values as these values correlate with octanol-water partition coefficients (see below). In the past, measuring K_{DOC} values reliably was challenging as phase separation between the freely dissolved chemical and that sorbed to DOM was not possible. However, newer partitioning-based techniques that do not require phase separation are increasingly being used. These techniques are based on the application of solid sorbents to which the chemical (but not DOM) partitions from the water phase. If the sorbent-water partition constant of the chemical is known, this can be used to calculate the freely dissolved concentration of the chemical from that sorbed to the sorbent. Extraction and analysis of the total concentration of the chemical in solution and subtraction from this of the freely dissolved concentration yield the concentration sorbed to DOM. This is then used together with the freely dissolved concentration and the DOC concentration to calculate K_{DOC}. Solid-phase microextraction (SPME) is a convenient approach to these determinations although other equilibrium sampling approaches are possible [8].

DOM-water partition coefficients for many chemicals can also be estimated from their physical-chemical properties. An extensive survey of published data on K_{DOC} for non-ionic chemicals [9] was used to develop correlations of this parameter with octanol-water partition coefficients of the chemicals. Although satisfactory relationships were derived, they exhibited large uncertainties of up to two orders of magnitude in K_{DOC} values. These were partly attributed to the variability in structure and composition of dissolved organic carbon (DOC) in sediments, soils, and surface waters as well as to the measurement techniques used.

As an alternative to models relating K_{DOC} to the octanol-water partition coefficient, linear solvation energy relationships have been proposed to model the

association of chemicals with dissolved organic matter [10]. Although more chemically diverse K_{DOC} data are needed to produce a more robust model, the linear solvation energy relationship predicts log K_{DOC} for humic acid dissolved organic carbon with a root mean square error of 0.43.

More insight into the influence of different DOM structural features was revealed by using different organic matter fractions extracted from soil to determine sorption isotherms for phenanthrene [11]. Values of K_{DOC} followed the trend lipid > humin (HM) > humic acid (HA) > fulvic acid (FA) > whole soil sample, while the nonlinear exponent values were lipid > FA > HA > whole soil sample > HM.

The composition of DOM can change as a result of biodegradation, resulting in smaller aliphatic and larger hydrophobic neutral fractions compared to the original DOM [12]. The sorption of phenanthrene to DOM before and after biodegradation was analysed using the Freundlich model $C_{OC} = K_{FOC}\ C_i^n$ where C_{OC} is the DOM-bound concentration of phenanthrene (mg kg C^{-1}), C_i is the concentration of freely dissolved phenanthrene (μg L^{-1}) and K_{FOC} (mg kg C^{-1}), and n values (dimensionless) are the Freundlich isotherm model parameters. The microbially induced changes in DOM led to an increase in the isotherm nonlinearity n as well as the extent of sorption of phenanthrene K_{OC} calculated from K_{FOC} at low phenanthrene concentrations (Fig. 1). Specific UV absorbance (a measure of aromaticity) was negatively correlated with the Freundlich n values for both the original and the biodegraded DOM, suggesting that condensed aromatic structures in DOM dominate nonlinear strong sorption.

Dissolved organic carbon and particulate organic carbon partitioning coefficients of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), and benzo[a]pyrene (BaP) were determined along the salinity gradient (0–5.5%) of the Baltic Sea off the coast of Finland (at organic carbon concentrations of 4.93–8.72 and 191–462 mg/L, respectively) and in the laboratory with humic acids and fulvic acids [13]. For all three compounds, sorption was stronger to HAs than to the FAs, and increasing salinity decreased sorption to dissolved humic acids and DOM.

In another study, log K_{DOC} for the binding of pyrene to fulvic acid fractions extracted from soil correlated positively with the aromaticity of the fulvic acid fractions but negatively with the ratio of (O+N)/C and the ratio of UV absorption at 250 and 365 nm [14]. There was no clear trend between log K_{DOC} and the paraffinic carbon content as determined by NMR. The results were interpreted as indicating that sorption was dominated by interactions of π-electrons from the aromatic regions of fulvic acid and pyrene. Similarly, hydrophobicity and aromaticity were the main factors determining the binding affinity of benzo[h]quinoline and benzo[h]quinolinium with humic acids over a range of pH values [15].

Experimental determination of log K_{DOC} values of hydrophobic chemicals is challenging due to the difficulty of working with chemicals with very low solubilities and separating dissolved chemicals from the DOC-bound fractions. Recently, partitioning-based methods that do not require separation, such as passive sampling and passive dosing, have been applied to such determinations. For example, an approach involving passive dosing from a poly(dimethylsiloxane) polymer

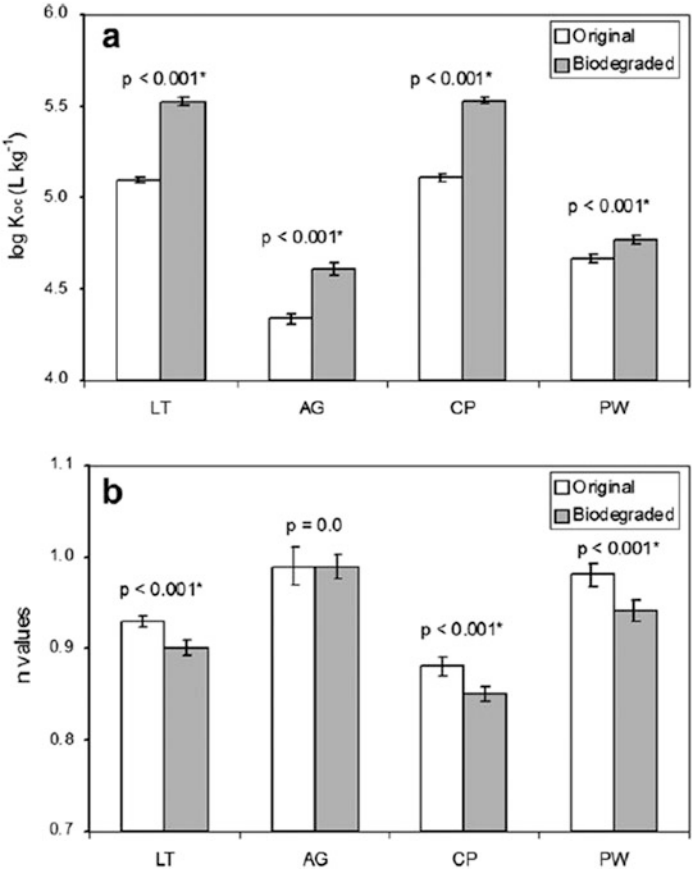

Fig. 1 Comparison of log K_{OC} and n values for the sorption of phenanthrene to DOM samples from *LT* leaf litter, *AG* algae, *CP* compost, and *PW* paddy water before and after DOM biodegradation [12]. (Reprinted with permission from Hur, J., Lee, B.-M., & Shin, H.-S. (2011). Microbial degradation of dissolved organic matter (DOM) and its influence on phenanthrene-DOM interactions. Chemosphere, 85, 1360–1367. Copyright 2011 Elsevier)

preloaded with the chemicals was applied to study the speciation of PAHs in solutions of humic acid, sodium dodecyl sulphate, and other solutes [16]. Derived values of K_{DOC} were consistent with previously published values determined using solid-phase microextraction and fluorescence quenching.

K_{DOC} values for sorption of a range of nonpolar and polar compounds with Suwannee River fulvic acid were determined using headspace and solid-phase microextraction (SPME) methods and used to derive a polyparameter linear free energy relationship (pp-LFER) [17]. A pp-LFER was also calibrated for Aldrich humic acid (HA) using previously published K_{DOC} values. Both experimental and pp-LFER calculated K_{DOC} values for Aldrich HA were around one order of magnitude greater than those for Suwannee River FA. This difference can be explained by

the higher cavity formation energy in Suwannee River FA. The low experimental and calculated K_{DOC} values for halogenated alkanes and alkenes indicate that sorption to DOC is not an important fate process for these chemicals.

Although models to estimate K_{DOC} values for neutral chemicals are available, the prediction of pH-dependent K_{DOC} values (often referred to as D_{DOC}) for ionisable chemicals is problematic [18]. Literature K_{DOC} and D_{DOC} values were used to derive relationships for neutral and ionisable organic chemicals using LSER (linear solvation energy relationship) models for neutral chemicals based on the Abraham solute parameters determining water-solvent partitioning as well as linear regressions for predicting D_{DOC} of organic acids from their octanol/water partition coefficient and dissociation constant (pK_a), to account for the contribution of the neutral and ionic fractions, [19]. The models could predict log K_{DOC} and D_{DOC} values with a root mean square deviation smaller than 0.3 log units.

The sorption of a range of polar and ionic organic contaminants, including pharmaceuticals, industrial chemicals, hormones, and pesticides, to dissolved organic carbon (DOC) was determined using SPME [20]. SPME fibre- and DOC-water partition coefficients of neutral compounds were both linearly related to their octanol-water partition coefficients, whereas those of acidic and basic compounds were pH-dependent and often nonlinear, highlighting the complexity of this process for these chemicals.

The SPME approach was used to conveniently determine partition coefficients for 40 chemicals covering a wide range of physicochemical properties to DOM [21]. Two SPME fibre materials coated with either polydimethylsiloxane or divinylbenzene polydimethylsiloxane were used to cover the range of chemical properties of the chemicals, with polydimethylsiloxane fibres being used for the nonpolar chemicals and divinylbenzene polydimethylsiloxane fibres for the semipolar chemicals. The measured DOC-water partition coefficients correlated well with the octanol-water partition coefficients for the nonpolar chemicals, but the correlation was poor for the full range of chemicals, suggesting that specific binding mechanisms may be involved for the semipolar chemicals. Using this approach, spatial variations in DOM structure and their impact on sorption were studied in the Baltic [22]. The aromatic content of DOM decreased from north to south with concomitant effects on sorption of a diverse set of organic contaminants to DOCs. Clear differences were in the sorption properties of DOM sampled in northern and southern parts of the Baltic Sea, with more contaminants being sorbed to DOM in the Northern Baltic.

DOM from different sources, including terrestrial and aquatic sources, was characterised in terms of their humification index (HIX) [23]. This structural index was used to derive the HIX-based average molecular weight as a parameter to describe the extent of aromatic condensation of DOM and as a parameter that also correlated well with K_{DOC} values of pyrene.

Temperature effects on the sorption to DOM may be significant. An increase of 10°C in temperature resulted in a decreasing sorption of PAHs to DOM, for example, a decrease of K_{DOC} by 0.13 log units for pyrene [24]. The calculated enthalpies of sorption were less exothermic than the (negative) excess enthalpies of

dissolution, and this is consistent with sorption due to non-specific interactions between PAHs and DOM.

Sorption to DOM is in general regarded as always being in equilibrium, and very little attention has been paid to the kinetics of this process. A new approach to studying desorption of organic compounds from dissolved organic matter (DOM) is based on fast solid-phase extraction of the freely dissolved fraction of the chemical when the solution is flushed through a polymer-coated capillary [25]. This technique is referred to as time-resolved in-tube solid-phase microextraction and was applied to two humic acids and a surfactant as sorbents together with pyrene, phenanthrene, and 1,2-dimethylcyclohexane as sorbates. The results indicate that desorption is a two-phase process with a fast desorption step with a half-life of less than 1 s and a slow desorption step with a half-life of more than 1 min. For aliphatic solutes, the fast-desorbing fraction is dominant, whereas the slowly desorbing fraction is important for polycyclic aromatic hydrocarbons.

3 Impact of Sorption to DOM on the Bioavailability of Organic Chemicals

Since sorption to DOM reduces the freely dissolved concentration of chemicals in the aqueous phase, this is expected to reduce the apparent rate of uptake and extent of accumulation in biota. For risk assessment purposes, this effect could be corrected for by basing uptake and accumulation not on the total aqueous phase concentration but on the freely dissolved concentration. Similar effects would be expected on the rates of uptake and transformation by microorganisms, i.e., biodegradation. How significant this impact on bioaccumulation and biodegradation is will depend on both the strength and extent of partitioning to DOM as well as on the concentration of DOM.

3.1 Bioaccumulation

There is a great deal of evidence that the presence of DOM reduces the bioaccumulation of hydrophobic organic chemicals [26]. For example, the accumulation of benzo[a]pyrene, pyrene, and 3,3′,4,4′-tetrachlorobiphenyl, but not of atrazine, by *Daphnia magna* was reduced in the presence of DOM from different sources [27]. The extent of this effect was influenced by DOM properties and water hardness. In a similar study using 13 river waters and 1 humic lake water, reduced bioaccumulation was again observed for benzo[a]pyrene but not for atrazine [28]. Up to 70% of the variation of the effect could be attributed to both the quality and quantity of DOM. There was no significant effect on the bioaccumulation of pyrene, despite the fact that binding of this hydrophobic chemical to DOM was

observed. The effects of lake water DOM on the bioconcentration of benzo[a]pyrene were also much stronger than for 3,3′,4,4′-tetrachlorobiphenyl [29].

In a study to compare the effects on bioaccumulation of DOM from different sources, commercially available humic substances, DOM from a wastewater treatment plant aeration basin, and highly biodegradable DOM from cultures of algae had different effects on the bioaccumulation of fluoranthene, pyrene, and benzo[*a*] pyrene in *Daphnia magna* [30]. The strongest effects were observed for benzo[a] pyrene (up to 80% reduction with humic substances) with pyrene bioaccumulation being decreased by each DOM to a lesser extent. However, fluoranthene bioaccumulation was only reduced by humic substances. In all cases, the solution containing humic substances led to the lowest bioaccumulation.

The accumulation of organochlorine pesticides (OCPs) by Japanese medaka (*Oryzias latipes*) consistently decreased with increasing levels of Aldrich humic acid in the range of 0–15 mg C/L [31]. This trend was closely mimicked by OCP accumulation in triolein-embedded cellulose acetate passive sampling membranes under the same conditions. A similar trend was observed in a field study, suggesting that this passive sampling method can be used as a convenient approach to determine the bioavailability and bioaccumulation potential of hydrophobic organic chemicals in the aquatic environment.

The impact of DOM on bioaccumulation is not limited to hydrophobic PAHs and organochlorine chemicals. The bioaccumulation of two synthetic pyrethroids, bifenthrin and permethrin, by *Daphnia magna* in water samples containing suspended solids from different source sediments consistently decreased with increasing concentrations of suspended solids in the range of 0–200 mg/L [32]. The pesticides adsorbed on either particles or DOM was completely unavailable to *D. magna* for uptake during the 24-h exposure period. The relative contributions of particles and DOM to the reduced bioavailability depended on the organic matter content and texture of the source sediment. The influence of particles was predominant for sandy sediments, but the contribution of DOM became comparable to or even greater than that of particles for sediments containing 1% or more organic matter.

In apparent contrast to the reduced extent of bioaccumulation observed in the studies described above, it has been reported that sorption of hydrophobic organic contaminants to DOM may enhance diffusive transport of the contaminants and thereby increase uptake rates during bioaccumulation. This is consistent with other studies into the effects of DOM on the diffusion of hydrophobic chemicals in water. For example, diffusion of fluoranthene in a system designed to mimic unstirred boundary layers was enhanced in the presence of cyclodextrin, humic acids or sodium dodecyl sulphate micelles [33]. A more extensive study revealed similar enhanced diffusion for most of a set of 12 polycyclic aromatic hydrocarbons by surfactants, humic acid, aqueous soil and horse manure extracts, digestive fluid of a deposit-feeding worm, and root exudates from willow [34]. The extent of enhancement increased with increasing hydrophobicity of the PAHs. In a study of the effects on bioaccumulation, 55 mg/L humic acid enhanced the accumulation rate of benzo [b]fluoranthene by both PDMS-coated fibres and the aquatic worm *Lumbriculus*

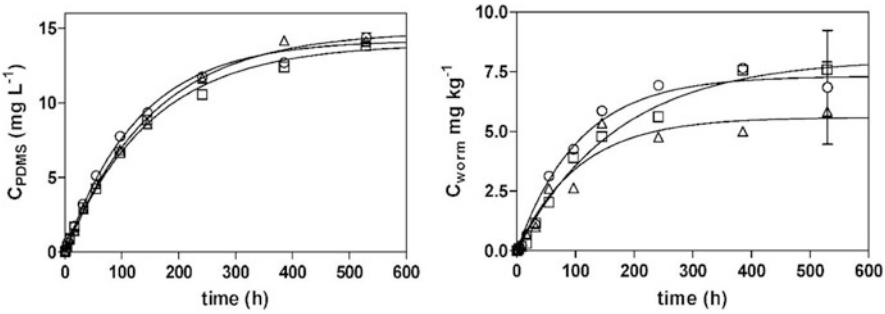

Fig. 2 Enhanced uptake of pyrene by PDMS-coated fibres (left) and *Lumbriculus variegatus* (right) in the absence (squares) and low (triangles) and high concentrations of DOM (circles) [35]. (Reprinted with permission from Ter Laak, T. L., ter Bekke, M. A., & Hermens, J. L. M. (2009). Dissolved organic matter enhances transport of PAHs to aquatic organisms. Environ. Sci. Technol., 43(19), 7212–7217. Copyright 2009 American Chemical Society)

variegatus (Fig. 2) [35]. This effect was not observed for pyrene, and the difference between the two chemicals was explained by the higher affinity of benzo[b] fluoranthene for the dissolved humic material. However, a systematic study of the impact on low concentrations of DOM on the extent and kinetics of bioconcentration of benzo[a]pyrene showed consistently decreased bioconcentration in *Daphnia magna* at all exposure times and no transient enhancement of bioconcentration [36]. Similarly, no enhancement was observed of the bioconcentration of benzo[a] pyrene, tetrachlorobiphenyl, pentachlorophenol, and naphthalene by low concentrations of DOM from a wide range of different aquatic systems.

In another study, the effect of Finnish lake DOM on accumulation of pyrene by *Daphnia magna* was seen in a decreased uptake rate, indicating lower bioavailability of the compound in waters that contain highly aromatic DOM [37]. In apparent contrast, another study indicated that DOM promoted the bioavailability of pyrene for *Daphnia magna* when the freely dissolved concentration of pyrene was kept constant [38]. The bioavailability of pyrene associated with DOM of various molecular weights was ordered as middle molecular weight (5,000−10,000 Da) DOM > lower molecular weight (<1,000, 1,000−3,000, and 3,000−5,000 Da) DOM > higher molecular weight (>10,000 Da) DOM. The results were explained in terms of partitioning of pyrene between DOM and water, the uptake of this DOM and the desorption or release of pyrene from DOM in the gut of *D. magna*.

3.2 Biodegradation

Although dissolved organic matter generally reduces the bioavailability of aqueous hydrophobic chemicals, dissolved humic acids can act as surfactants to increase the rate of solubilisation of soil-sorbed chemicals and may therefore increase their rates of biodegradation. For example, adding 1.5% humic acids to soil slurry microcosms

increased the biodegradation rates of sorbed PCBs [39]. Increasing the concentration of humic acids to 3%, however, resulted in slower biodegradation, indicating that there is a balance between the solubilisation effect and the reduction of freely dissolved concentrations.

Humic acid-sorbed phenanthrene was degraded by bacteria isolated from PAH-contaminated soil, but this was performed by only specific bacterial strains and not by the strains able to degrade dissolved phenanthrene [40]. The rate of biodegradation of humic acid-sorbed phenanthrene was higher than that of dissolved phenanthrene, and this was attributed to bacteria being able to assess the sorbed chemical directly without desorption being required.

In contrast to the results described above, hydrophobic DOM did not enhance the biodegradation of sorbed triflusulfuron methyl in soil [41]. This was attributed to the relatively weak sorption of this pesticide to the hydrophobic DOM, with a K_{OC} of 446.5, being insufficient to enhance desorption. Sorption of triflusulfuron methyl to the DOM was, however, strong enough to reduce the biodegradation rate of the substrate.

Different effects of humic acid at two pH values were observed on the biodegradation of phenanthrene [42]. No surfactant effect was observed at pH 7.8, but this was seen at pH 11.8, with dose-dependent increasing phenanthrene solubilisation and biodegradation rates in soil slurries. However, at the highest humic acid concentrations of up to around 10 mg/g soil, inhibition of biodegradation was observed.

Enhancement of the rates of biodegradation by a representative polycyclic aromatic hydrocarbon-degrading bacterium by DOM was also observed for aqueous phase phenanthrene and pyrene, by a factor of 1.32 and 1.26 at DOM concentrations of 43.14 and 42.15 mg/L, respectively (Fig. 3) [43]. This enhancement was attributed to a combination of faster diffusion of the dissolved compounds in the unstirred boundary layer surrounding microbial cells and direct access of the bacteria to DOM-associated PAHs. No enhancement was observed for fluoranthene in these experiments, suggesting that DOM-sorbed fluoranthene is less accessible for the bacteria.

Enhancement of PAH biodegradation by DOM has also been reported by Smith et al. [44]. Degradation of microcrystalline phenanthrene by *Sphingomonas* sp. LH162 was up to 4.8 times faster in the presence of humic acid. This effect was again attributed to humic acid-mediated faster uptake. Humic acid at up to 1.6% enhanced the solubility and biodegradation of the PAHs phenanthrene, pyrene, and benzo[a]pyrene in liquid media, but this effect was only observed for solubility and not biodegradation in mangrove sediment slurries [45].

In another study humic acids enhanced the biodegradation of pyrene when this was present as solid crystals but did not enhance biodegradation of phenanthrene when this was present in the dissolved phase or provided by partitioning from a polymer [46]. The authors suggest that humic acids can enhance or inhibit biodegradation as a result of a balance between solubilisation of the chemicals on the one hand and inhibition of cell adhesion to the pollutant source on the other.

The degradation rates of two polycyclic aromatic hydrocarbons were proportional to the concentration of biosurfactant in soil-water systems [47] with the

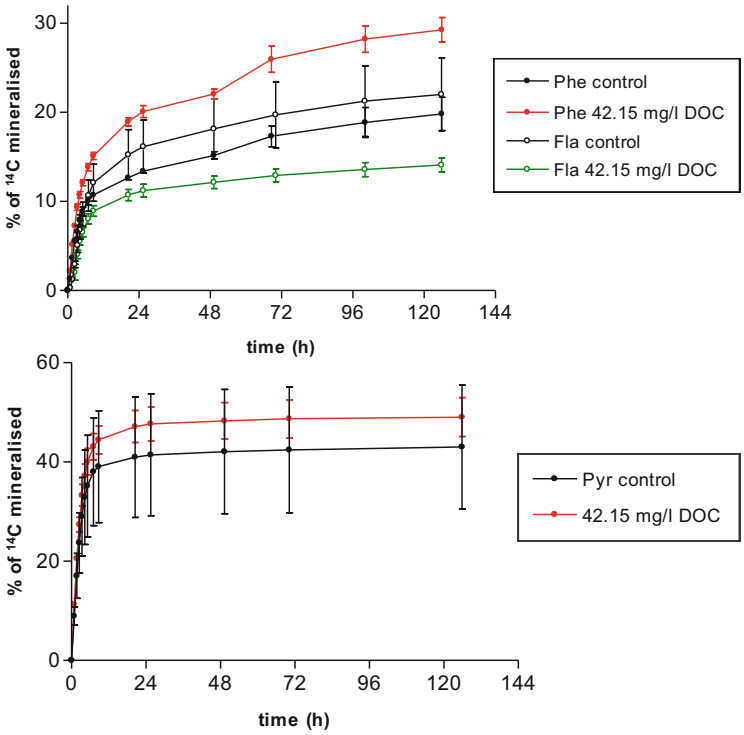

Fig. 3 Effect of DOM on the mineralisation of phenanthrene, pyrene, and fluoranthene [43]

enhancement for phenanthrene being higher than that for pyrene. The addition of soil DOM at an environmentally relevant level inhibited the biodegradation of the PAHs, but adding both DOM and biosurfactant resulted in degradation rates higher than in the presence of only biosurfactant. This effect was attributed to the formation of DOM-biosurfactant complex micelles. Addition of soil DOM produced slightly higher degradation rates than addition of compost DOM, indicating that the chemical structure and composition of DOM also affect the bioavailability of PAHs.

Forest leaf litter was the source of DOM used to study the effect of DOM on the degradation of phenanthrene by the bacterium *Sphingobium* sp. Phe-1 [48]. *P. elliottii* leaf litter decomposed for 12 months used at a concentration of 100 mg/L yielded the highest degradation rate (16.9% in 36 h) and shortened the degradation time from 48 to 24 h. This enhancement of degradation was attributed to the combined effects of proteins and tyrosine in the DOM supplying readily available nutrients that stimulated the biological activity of Phe-1, as well as the effect of fulvic and humic acids in the DOM enhancing phenanthrene bioavailability by increasing the solubility and mass transfer of phenanthrene.

Another example of enhancement of biodegradation of PAHs by DOM is the study reported by Xie et al. [49]. In this case, the extent of removal of phenanthrene increased from 51.1% to almost 100% after 73 h in the presence of humic acids. This

was partly due to enhanced uptake and accumulation of PAHs in lipid inclusion bodies when *Sphingobium* sp. PHE3 was treated with humic acids. The authors conclude that the added humic acid not only acts as a carrier and biosurfactant but can also change bacteria cell wall properties to enhance phenanthrene uptake

Relatively little work has been done on the impact of DOM on the bioavailability of hydrophobic organic chemicals other than PAHs. Triclosan is an example of such a hydrophobic chemical and can be anaerobically degraded by the metal-reducing bacterium, *Shewanella putrefaciens* CN32. Low concentration (0–15 mg C/L) of organic matter (OM) extracted from a peat soil enhanced the degradation rate of triclosan, but this was inhibited by higher concentration (15–100 mg C per L) of OM [50]. It was proposed that the DOM acted as both an electron shuttle and sorbent in regulating the toxicity and degradation of triclosan.

The influence of the interactions between the phenylurea herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) and several humic substances fractions and commercial humic acid (HA) on its biodegradation have been studied [51]. Degradation of diuron in soils inoculated with diuron-degrading bacteria was slower after addition of purified fulvic and humic acids, and the main transformation product formed, 3,4-dichloroaniline, was bound irreversibly to the humic substances.

DOM derived from various sources, including water and sediment of an algal bloom-dominated lake, macrophyte-dominated lake, and humic acid decreased the dissolved concentration of 17a-ethinylestradiol (EE2), but this did not inhibit its biodegradation by an EE2-degrading strain [52]. In fact, DOM enhanced biodegradation with the extent of this effect depending on the source of the DOM. Water-derived DOMs stimulated a more extensive biodegradation of EE2 than the sediment-derived DOMs and HA resulting in the smallest increase in EE2 biodegradation. This effect was attributed to DOM stimulating the growth of the EE2-degrading strain.

4 Concluding Remarks

Sorption or partitioning to dissolved organic matter can have a significant impact on the distribution of hydrophobic chemicals in water as well as in soils and sediments. Sorption to DOM lowers the so-called freely dissolved concentration which is regarded as the bioavailable concentration. This effect has been most extensively studied for polycyclic aromatic compounds but is undoubtedly the case for all hydrophobic compounds. The strength of partitioning depends on the properties of both the chemical in question and the dissolved organic matter. While K_{ow} appears to be a satisfactory predictor of the influence of the chemical's physical chemical properties, things are less clear-cut regarding the influence of DOM properties. Normalising partition coefficients to dissolved organic carbon concentration does not explain all the variability associated with different sources of DOM, nor does normalisation to specific UV absorbance as a measure of aromaticity. Clearly, the

impact of DOM structure is more complex than can be captured by these simple approaches, and more study in this direction would be advantageous. In particular, exploration of the influence of condensed aromatic structures in DOM, often referred to as dissolved black carbon, would probably yield better insights into the partitioning behaviour of highly hydrophobic chemicals such as high molecular weight PAHs to these materials.

The fact that partitioning to DOM can significantly lower the freely dissolved concentration of aqueous phase hydrophobic organic chemicals leads to the expectation that this reduces the bioavailability of such chemicals. This is confirmed by the studies of the bioaccumulation of PAHs and other hydrophobic compounds in the presence of relatively high DOM concentrations outlined above. How significant this effect is in the real world will of course depend on the DOM concentrations under environmental conditions. As well as an impact on the extent of bioaccumulation, DOM may also affect the rates of bioaccumulation. Although sorption to DOM is expected to reduce the rates of bioaccumulation, there is some evidence that partitioning to DOM may under certain conditions lead to faster uptake of hydrophobic chemicals. This may be result of an increased flux of chemical across unstirred boundary layer surrounding biological membranes as a result of transport of DOM-chemical complexes, but other mechanisms may be involved.

Kinetic effects resulting from partitioning of hydrophobic chemicals to DOM are of course important for biodegradation and other non-equilibrium processes. Lowered freely dissolved concentrations will also result in lowered biodegradation rates, but again there is evidence that for certain chemicals and under certain conditions, DOM can enhance the uptake of hydrophobic chemicals by microbes, with faster biodegradation as a result. Perhaps more importantly, DOM can act as either a cosolvent or surfactant to enhance the desorption of soil-sorbed chemicals and thus increase their bioavailability. Since the desorption of aged soil-sorbed chemicals is often the limiting factor in their biodegradation (see Chap. 9 in this volume), this effect may be applicable in the bioremediation of soils. From a societal point of view, this may be the most important impact of sorption to DOM on biodegradation rates, but how important this will be will depend on the chemical properties and the stability of the DOM as well as the mechanisms and strength of sorption of the contaminants to soil and DOM. This is another area where further research is required.

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Part II
Bioavailability and Bioaccumulation

Measuring and Modelling the Plant Uptake and Accumulation of Synthetic Organic Chemicals: With a Focus on Pesticides and Root Uptake

Benjamin M. Jones and Chris D. Collins

Contents

Abstract Plant uptake of synthetic organic chemicals involves the transport of a xenobiotic into the plant cells via the roots or shoots. There is little evidence to demonstrate plants enhance the release of pesticides from recalcitrant fractions in the soil. In the limited studies available, plant uptake is effectively predicted by the bioavailable fraction recovered from passive samplers and mild extractants. These two areas will be fruitful areas for future research. Once the compound enters the plant, there are several potential transport routes prior to translocation to the shoots

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or accumulation within the roots. Modelling the plant uptake of organic chemicals allows for the assessment of risks to human health. There is currently a significant amount of debate within the research community as to the preferred way for quantifying uptake and most appropriate experimental method for measuring uptake. The use of transpiration stream concentration factor (TSCF) predicted by the octanol-water partition coefficient (log K_{OW}) has long been the dominant model; however, recent research has suggested a move away from these predictive relationships. Many studies have been conducted with approximately 200 data points being reported in the peer-reviewed literature; however, statistical analysis has shown that we are no closer to establishing a definitive algorithm to predict plant uptake of organic chemicals. Whilst log K_{OW} could still be an important predictor, other physical-chemical properties, such as molecular weight and hydrogen bond donors, could also play a role. Currently, there is ongoing debate as to whether TSCF is the most suitable measure of plant uptake as it only considers the fraction of the compound that has been translocated to the above-ground plant parts. The plant uptake factor (PUF) considers uptake into the whole plant by measuring the change in concentration of the compound in the uptake solution against the change in volume and may provide a more accurate uptake value as a result. Despite this, both the use of further physical-chemical properties and PUF are relatively new and require rigorous testing by researchers to establish their suitability.

Keywords Environmental fate, Experimental protocols, Root concentration factor, Shoot concentration factor, Transpiration stream concentration factor, Uptake pathways

1 Introduction

Human population growth, increasing industrial production, intensification of agriculture, medical development and chemical advances all lead to the production of synthetic organic compounds, potentially increasing our exposure to toxic chemicals [1–3]. Increased industrial production of naturally occurring and synthetic organic compounds requires regulation specifically in the contamination of crops, soils and groundwater [4]. To protect human and environmental health, it is imperative to be able to predict how compounds will behave to their release into the environment. Plant uptake is an important process when considering the environmental fate or risk assessment of potentially toxic organic compounds [5].

Uptake of organic compounds is a process that involves the transport of a xenobiotic substance into the plant physiological system. It is important to clarify that sorption of a xenobiotic to the surface of plant cells is not considered uptake, the compound must cross the cell wall and into the cell structure. Whilst root uptake is the main pathway transport into plants for most organic compounds, it is also

important to note that this isn't the only pathway for entry the plant's cells, with the soil-air-plant pathway presenting an alternative [6]. Compounds that enter the leaf by this route are susceptible to volatilisation from the soil and are transferred to the air before then being deposited onto the leaf surface. This is likely to be an important pathway for high log K_{OW} compounds (e.g. chlorinated legacy pesticides, e.g. DDT), which are virtually insoluble in water and tightly bound to carbonaceous material in the soil. The low solubility of these compounds and neutral state suggest they are unlikely to be subsequently transported within the plants xylem and phloem and will remain at the point of deposition [7].

Transfer of chemicals post application to soil is one of the dominant routes of environmental contamination. Environmental fate models such as PEARL, PRZM, PELMO and MACRO are used by agrochemical manufacturers and regulators to assess each compound within a range of agricultural scenarios to quantify the potential for groundwater contamination [8–11]. Plant uptake is assumed to be a passive process (although there are a few examples of compounds being taken up actively) within these models with uptake values that are bound between 0 (no uptake) and 1 (complete uptake) [6, 12]. The higher the uptake, the more compound is removed from the profile, therefore reducing leaching and potential aquifer contamination [13]. Another application of modelling plant uptake and groundwater vulnerability from contaminated soil is the risk assessment and exposure from industrially polluted land, which uses alternative models, e.g. CLEA (UK Environment Agency) [14] and CSOIL (Netherlands National Institute for Public Health and the Environment) [15]. Examples of the exposure considered within these models are the consumption of crops, dermal contact with soil or ingestion of soil directly. Unlike to the environmental fate models above which are designed to quantify the potential for reducing transport down the soil profile, the focus of these models is targeted on the edible portions of the crop. For example, if salad leaves are consumed, a compound may partition into the roots only and therefore human exposure is minimal.

This chapter reviews pathways of plant uptake alongside the methods used to quantify and better understand them.

2 Plant Uptake of Xenobiotic Compounds

2.1 Transfer from Soil Solution to the Root Including Bioavailable and Residual Fractions

There is a considerable discussion of the availability of compounds from soils elsewhere in this book that is not repeated here. There was initial interest in cucurbits as potential hyperaccumulators of legacy chlorinated pesticides [16] and the role of root released organic acids in desorbing compounds from the soil matrix [17, 18], whilst more recent research have identified POP transporters in cucurbits which

Table 1 Relationship between passive samplers' crop uptake and other biota

Passive sampler	Pollutant	Crop		Biota		Study
Polyethylene	PCBs[a]	Turnip	0.62[b]	Earthworm	0.85[b]	[24]
Cyclodextrin	∑PAHs[c]	Ryegrass roots	0.29	Earthworms	0.09	[25]
SPME			0.27		0.46	
POM			0.16		0.46	
TECAM	PAH	Wheat roots	0.80–0.93	–		[26]
SPME	∑DDT[d]	Kale leaves	0.99	Earthworm	0.86	[27]
		Cabbage leaves	0.96			

[a]Polychlorinated biphenyls
[b]Reported r^2 value
[c]Sum of polycyclic aromatic hydrocarbons
[d]Sum of dichlorodiphenyltrichloroethane and metabolites

enhance the transfer from root to shoot [19]. With perfluoroalkyl and polyfluoroalkyl substances (PFAS), uptake into wheat was enhanced by combination with earthworms, and uptake into earthworms was enhanced by wheat indicating both were enhancing the release of contaminant from the soil [20]. Unfortunately, these studies were not combined with corresponding measures of available fractions to determine if crops were releasing recalcitrant pollutant fractions in the soil. In one study where available fraction was incorporated, model predictions were improved, but these were spiked soils [21]. In fact, the potential of plants to mobilise residual pollutant fractions in soil is a rarely investigated arena that requires further research.

To date the prediction of plant uptake by passive samplers accounts for an equivalent amount of the variation in accumulation as in other biota, e.g. earthworms using a range of sampling materials (Table 1). The number of available studies is limited; some researchers have found very limited predictive capability with up to two orders of magnitude difference between POM and vegetables accumulating DDT; this variance was postulated to be a consequence of soil heterogeneity [22], whilst others observed no correlation between plant uptake of conazole fungicides and passive samplers as a result of low uptake in SPME fibres or the influence of soil factors (e.g. soil particle size and base saturation) for Empore™ discs and silicone rubber [23].

2.2 Plant Uptake via the Root Pathway

Plant uptake is the transport of organic chemicals dissolved in water into the plant cortex by transpiration. Small compounds (molecular weight $\leq$ 500) can enter the root via the root hairs which vastly increase the surface area [3]. There are three main pathways for the root uptake of solutes: apoplastic, symplastic and transmembrane. Apoplastic is the extracellular transport of the compound, via movement through the cell walls and outside the plasma membrane. Symplastic transport involves

intracellular movement, through the plasmodesmata, gaps in cell walls creating a thread of cytoplasm which allows the transfer of solutes. Transmembrane transport is the intracellular movement of a compound dissolved in solution, passing in and out of the cell through the plasma membrane each time [28].

In order to cross the cell membranes and enter the symplastic pathway, the compound needs to cross the lipid bilayer within the cell membrane. This bilayer is hydrophobic and compounds need to be lipid-soluble to pass through so permeability is very low to polar or ionic compounds [29]. Compounds transported via the symplastic and transmembrane are translocated more readily than the apoplastic transport pathway because they are not blocked by the Casparian strip a collection of suberised cell walls [30]. Therefore, compounds taken up solely by the apoplastic route will not be translocated, unless they at some time cross the plasma membrane of the surrounding cells [3, 31].

Crossing the cell plasma membrane can also lead to a phenomenon called *ion trapping* which results in compound accumulation in the cells. This occurs due to different pH inside, c. pH 7–7.5, and outside cell membrane which can vary substantially. It is possible for a compound to be neutral outside the cell but become ionised in the cytoplasm, thus accumulating inside, as ions are unable to cross the cell membrane [32]. This is dependent on the acid dissociation constant (pKa) of the compound [31]. In practice, acids are increasingly neutral when dissolved in solution where the pH is below the pKa of the compound and bases are increasingly neutral when the solution pH is above their pKa. As the pH gradient between the outside and the inside of the cell increases, the ion trap effect becomes more pronounced. The effect of pH is greatest when pH-pKa is in the range of -1 to 3 for acids and from -3 to 1 for bases [33].

Differential transport within the shoot is often not considered in studies reporting the transport of chemicals to the above-ground parts of plants. In some of the earliest studies, it was noticed that fractionation of compounds occurred up the stem. Briggs et al. in their seminal early work reported this with non-ionic pesticides [34] with high K_{OW} compounds (>2.5) being retarded at the base of the shoot, taking longer to reach the upper sections and doing so in reduced quantities. Compounds of lower K_{OW} concentrated in the upper sections of the shoots as they move more readily in the transpiration stream. This differential transport up the stem was also reported for the PAH fluoranthene [35] and a range of emerging contaminants [36]; the latter noted non-ionic compounds were more readily transported with lower K_{OW} compounds that were more likely to accumulate in fruits.

The picture is further complicated by studies reporting differential transport from root to shoot depending on species and chemical, with preferential transport of neonicotinoids in *Brassica* species and dieldrin in Cucurbitaceae [37]. Significant crop cultivar variation has also been observed with non-pesticide contaminants [38]. Other potential confounding factors are the interaction with nutrition [39] and growth stage dependency [40], and a particular issue is how to quantify the impact of metabolism on transport but also on subsequent toxicological impacts [41–43].

3 Measurement of Plant Uptake

The plant uptake of solute is strongly coupled with the leaching to groundwater [44]. Currently, no standardised methodology for calculating plant uptake values has been agreed, and a lack of consensus within the scientific community has led to further confusion within the literature [45]. The following sections discuss many of the existing equations and derived relationships for measuring the plant uptake of pesticides. This will help to highlight an apparent lack of scientific consensus on the modelling of experimentally derived plant uptake values and help highlight potential avenues for future research.

There are many approaches for measuring plant uptake experimentally and determining where the compound has accumulated in the plant tissue. Although there has been evidence of some active uptake, it is a widely held view that the uptake of pesticides and other synthetic organic compounds is mostly a passive process [6, 12]. Compounds are taken up by plant roots from the soil solution and are taken up during transpiration [6]. Therefore, transport into the plant is closely related to the concentration in the solution, i.e. the bioavailable fraction [46]. In general transfer factors (comparison of the concentration within two compartments within a system) are used to compare accumulation within different compartments within the plant. In the following subsections, these transfer factors, their experimental quantification and model prediction are discussed.

3.1 Root Concentration Factor (RCF)

The roots are the first place that compounds accumulate following their uptake from solution. The RCF is calculated from the ratio of compound concentration in the root to that of the soil solution (Eq. 1) [46].

$$\mathrm{RCF} = \frac{C_{\mathrm{roots}}}{C_{\mathrm{solution}}} \tag{1}$$

3.2 Translocation Factor (TF)

The translocation factor is used to measure the transport of a compound from the roots to shoots. It is calculated from the ratio of compound concentration in the shoot to that of the root (Eq. 2) [47].

$$\mathrm{TF} = \frac{C_{\mathrm{shoots}}}{C_{\mathrm{roots}}} \tag{2}$$

3.3 Transpiration Stream Concentration Factor (TSCF)

The TSCF is the concentration of the compound within the xylem divided by the concentration in solution surrounding the roots (Eq. (3)). This calculation allows for the establishment of a fraction of the translocation to the shoots compared to the amount available to the plant roots. All compounds that are taken up passively have a value between 0–1, providing a simple concept of uptake. A value of 1 means that all compound passively taken up by the roots during transpiration becomes translocated to the shoots. A value of 0.5 means half of the compound taken up by the roots is translocated to the shoots [46, 48]; values greater than 1 are seen if the plant is actively taking up the compound [49].

$$\mathrm{TSCF} = \frac{C_{\mathrm{xylem}}}{C_{\mathrm{solution}}} \tag{3}$$

Another method for deriving the TSCF value is to analyse the concentration of the target compound within the shoots of a plant and normalise this by the amount of water transpired during this period (Eqs. (4)–(6)).

$$\mathrm{TSCF} = \frac{\left[C_{\mathrm{shoots}} * W_{\mathrm{shoots}}\right]}{C_{\mathrm{solution}} * \text{water transpired}} \tag{4}$$

$$\mathrm{TSCF} = \frac{M_{\mathrm{shoots}} / V_{\mathrm{transpiration}}}{C_{\mathrm{solution}}} \tag{5}$$

$$\mathrm{TSCF} = \frac{ln\left(1 - \frac{m}{m_{\mathrm{shoots}} + m_{\mathrm{sol}-t}}\right)}{\ln\left(\frac{V_{\mathrm{sol}-t}}{V0}\right)} \tag{6}$$

Equation (4) measures the concentration of the compound in the shoots and shoot weight to derive the mass in the shoot and divides this by the volume solution transpired multiplied by the concentration in growing solution [50]. Equation (5) uses a similar approach to Eq. (4), except the concentration in the shoots is divided by the transpiration volume. This is then divided by the concentration in solution to compare how much has been translocated [51]. Equation (6) takes the natural log mass of chemical within the shoots over the mass within the shoots and the solution; this is then divided by the natural log of the change in volume during the test period [13]. Unless specifically stated that a correction has been applied for each study, all the equations listed above work on the assumption that the compound is not

phytovolatilised or metabolised after uptake and is therefore present within the plant following sampling [50, 52].

3.4 Plant Uptake Factor (PUF)

Suggested as an alternative to TSCF, the PUF considers uptake into the whole plant rather than the above-ground elements. Originally it was defined as a simple transfer factor between the plant material and the soil solution (Eq. 7) [53]

$$\mathrm{PUF} = \frac{C_{\mathrm{plant}}}{C_{\mathrm{solution}}} \tag{7}$$

$$\mathrm{PUF} = \frac{\ln\left(\frac{m}{m_{\mathrm{sol}-0}}\right)}{\ln\left(\frac{V_{\mathrm{sol}-t}}{V_{\mathrm{sol}-0}}\right)} \tag{8}$$

However, more recently an alternative has been suggested that was derived from a modelling description of plant uptake (Eq. 8) [13]. This new definition of PUF assumes the plant (roots and shoots) is a 'black box' with the roots being surrounded by a solution containing the measured compound [13]. By measuring the change in the mass within the solution and the change in the volume over the same period, the fraction of the mass that is removed by the plant can be determined.

3.5 Laboratory Methods of Measuring Plant Uptake

There are two primary approaches to measuring plant uptake in the laboratory; their strengths and weaknesses are discussed below. If the plant metabolises the test compound during the exposure period, it is not known if parent or metabolite was transported. It is possible to correct the uptake value if the rate of metabolism or volatilisation are known; however, they are difficult to determine and such measurements rarely conducted by researchers [52].

3.5.1 Intact Plant

The first method was devised by Briggs et al. [49] using 10-day-old whole plants that were exposed to test chemical for 24 h then shoots separated from roots for quantification of compound uptake. More recent methods have used this same approach, taking a young plant and measuring the amount of chemical accumulated into the shoots after a fixed exposure period [13, 49, 54]. The majority of the TSCF values within peer-reviewed literature have been conducted using this method [55].

3.5.2 De-topped Plant

The alternative to this method is to 'de-top' the plant and maintain the flow of the xylem using a pressure differential. This method was first reported by Hsu et al. [56], where plants were cut below the node of the first cotyledon and with roots submerged in half-strength Hoagland's solution containing the test chemical. A vacuum is then used to draw the xylem sap up through the transpiration stream [52, 56]. The reported benefits of this method are that the transpiration stream is directly sampled rather than all shoot material with a subsequent estimate of the uptake based on the transpiration [52]. In common with other methodologies, the plants are incubated for a set period.

3.5.3 Future Method Development

Current studies are conducted in hydroponic solution, as a surrogate for the soil system as it is easier to set up in the laboratory. Firstly, it allows for the uptake from a known concentration hydroponic solution for plant exposure [49, 52, 55]. When measuring plant uptake from a soil profile in the laboratory, it is very difficult to transfer the plant from a clean to a contaminated soil profile without significant disturbance. Therefore, the test compound needs to be applied directly to the native profile. In the later situation there are inherent problems with the mixing of the compound within the soil profile, needed for calculations above, although this may be more realistic when compared with applications in the field. Additionally, it has also been suggested that plants grown in hydroponic solution do not always have the same physiology to those grown in soil, with hydroponic roots showing lower rates of root growth and less development of the Casparian strip [3, 57].

TSCF has long been established for measuring the uptake of pesticides and other organic compounds. More recently scientific debate has centred around whether belowground uptake should be also be considered, something that TSCF does not incorporate. When considering environmental fate, plant uptake becomes a sink process that removes the pesticide from the soil pore water and transfers it to the biomass. Hence, TSCF is likely an underestimation of the true uptake from soil [13] and leads to an overestimation of transfer to groundwater.

Most of the research addressing TSCF and PUF have been between the reproducibility of the data and how well each method performs in comparison with previous datasets [13, 49, 52, 54, 56]. Not many tests have been conducted into the consistency of these calculations and how stable their measurements are over time. Adding to this, questions remain about the effect of concentration of the compound and the age of the plant on the uptake. For example, current publications are conducted almost exclusively on young plants [3, 13, 49, 52, 54, 56]. This is likely due to enhance experimental throughput. However, mature regions of roots are known to develop an exodermis that becomes relatively impermeable to water and

solute [3]. It is, therefore, possible that younger plants take up compounds differently to older plants due to the age of the roots [40].

4 Physical-Chemical Relationships Used in the Modelling of Plant Uptake

4.1 Octanol-Water Partition Coefficient (log K_{OW}) and TSCF

Log K_{OW} is defined as the concentration in octanol, divided by the concentration in water within a two-phase system [58]. Log K_{OW} can be considered a measure of the hydrophobicity of a compound and is useful within the application of environmental fate studies due to the observed relationship between a log K_{OW} value and bio-concentration values [3, 13, 49, 52, 54, 56]. Studies of cellular absorption of chemicals across the plasma membrane have shown that compounds with low log K_{OW} values <1.5 can easily pass through the cell wall but may not cross the cell membrane due to its hydrophobic nature. Alternatively, compounds with very high log K_{OW} values >4.5 become trapped within the cell membrane due to their high lipophilicity [31]. The optimum log K_{OW} for cell uptake is between log K_{OW} 2 and 4, where compounds can pass through the cell membrane and are transported [31, 49, 54, 56].

Briggs et al. [49] exposed plant roots to a suite of compounds with a range of log K_{OW} values for a 24 h period when equilibrium was assumed to have been reached. Their relationship derived from their results was Gaussian with an optimal TSCF at approximately 1.5–2 (Fig. 1). The pragmatic approach of Briggs et al. [49] and the simplicity of the relationship allowed for a ready transfer into pesticide leaching models, with the uptake being derived from the log K_{OW} value. This was later removed due to a lack of agreement and reproducibility in later datasets [59]. Further studies into the relationship of TSCF and log K_{OW} have shown similar relationships but exhibit a variation in the point of maximum uptake and slopes of the curve, leading to questions over the use of a universal equation within plant leaching models [52, 54, 56] (Fig. 1.). To address this, Lamshoeft et al. [13] restricted their test compounds to those with log K_{OW} -2 and 2 where models predict potential leaching to the groundwater. The differences between values reported by Dettenmaier [52] and Lamshoeft [13] are considered a result of different test methodologies (de-topped vs intact plant).

Briggs et al. [49]	$\text{TSCF} = 0.784 \exp - [(\log K_{ow} - 1.78)^2/2.44]$	(9)
Burken and Schnoor [54]	$\text{TSCF} = 0.756 \exp - [(\log K_{ow} - 2.50)^2]/2.58$	(10)
Dettenmaier et al. [52]	$\text{TSCF} = \frac{11}{11+2.6^{\log K_{ow}}}$	(11)
Hsu et al. [56]	$\text{TSCF} = 0.7 \exp - [(\log K_{ow} - 3.07)^2]/2.78$	(12)
Lamshoeft et al. [13]	$\text{TSCF} = - 0.0359(\log K_{ow})^2 + 0.1972 \log K_{ow} + 0.5859$	(13)

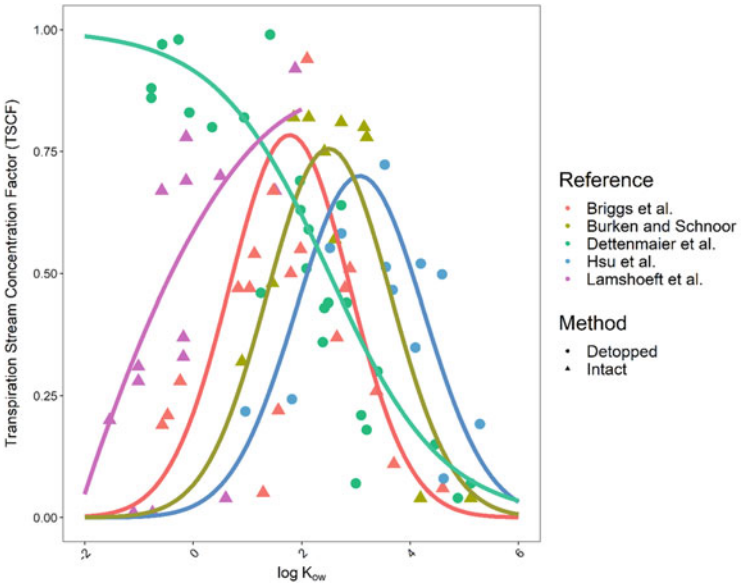

Fig. 1 Modelled relationships for log K_{OW} against TSCF. Briggs et al. [49] (Red); Hsu et al. [56] (Blue); Burken and Schnoor [54] (Gold); Dettenmaier et al. [52] (Green); Lamshoeft et al. [13] (Pink)

Many years of experiments measuring the TSCF has created a large dataset (196 TSCF measurements, 110 unique compounds and 21 plant genera) with no apparent relationship between TSCF and log K_{OW} (Fig. 1) [52]. The variations in relationships proposed could be a function of several variables: different plant species, e.g. barley, poplar, soybean tomato [49, 52, 54, 56]; different measurement methods, e.g. intact method [49, 54], de-topped method [52, 56]; or (more difficult to quantify) operator variation.

5 Modelling Plant Uptake for Environmental Fate Predictions

Recent work has attempted to discover new trends within the dataset using Lipinski's 'rule of five'. The rule of five was developed for the administering of oral medicine to determine absorption by the human intestine if it has five or fewer hydrogen bond donors, ten or fewer hydrogen bond acceptors, molecular weight <500 Da and a log K_{OW} of <5 [60, 61]. Bagheri et al. [62] using a neural network model integrated the 'rule of five' and created a predictive relationship of TSCF ($R = 0.802$), suggesting that log K_{OW} is an important indicator in plant uptake; however, molecular weight, hydrogen bond donors and rotatable bonds should be considered alongside this.

Our focus is pesticides, but many models exist for other organic pollutants which use TSCF and K_{OW} of the chemical and some plant properties [63–66]. More recent work has ranged from simple frameworks where compounds are prioritised based on physicochemical properties, log K_{OW} is <3, its MW is <300, H-bond donors are <3 and H-bond acceptors are <6 [67] or using new statistical techniques, e.g. Bayesian modelling [68] and machine learning [69]. These can enhance our mechanistic understanding and provide guidance on compounds for future study.

One of the major pathways considered during the approvals process for a new chemicals is leaching down the soil profile and into potable water supplies [70]. The environmental fate of pesticides is an important area of study because they are toxic by design and applied deliberately across large areas. It has been shown that approximately 0.1% reaches the target pest [71]. Such data have served to strengthen the drive for regulation of pesticides, and models are a critical tool to assess the leachability of potential plant protection products [72, 73]. Within environmental fate modelling for pesticides, there are currently four main regulatory accepted models adopted: PEARL, PELMO, PRZM and MACRO. All models follow a similar approach for measuring root uptake of plant protection products (Eq. 14); however they take differing approaches to other model elements, such as hydrology. These varying approaches can result in significant differences in the model outputs when sensitivity and uncertainty analyses are conducted [74].

$$M_U = R_L \, F_C \, C_L \tag{14}$$

where M_U is the volumic mass rate of pesticide uptake; R_L is the volume rate of water uptake; F_C is transpiration stream concentration factor or plant uptake factor (TSCF above); and C_L is the concentration of the pesticide in solution.

PEARL (Pesticide Emission Assessment at Regional and Local scales) was developed specifically for use in the pesticide registration process. PEARL is a one-dimensional, dynamic, multilayer model which is coupled with SWAP (Soil Water Atmosphere Plant model) [11]. SWAP uses a finite-difference method to solve Richard's equation, a combination of Darcy's law and the continuity equation for soil water [75]. Within PEARL, the pesticide is assumed to be taken up passively into the roots and subsequently translocated to the shoots.

MACRO is a one-dimensional, non-steady-state model of water, heat and solute transport in a variably saturated layered soil profile. MACRO is a dual-permeability model, whereby soil porosity is classified into micropores and macropores. Micropore water flow is described by Richard's equation and macropore water flow being described using gravity flow [76]. This is a similar approach to PEARL, with both plant uptake equations being comparable.

PRZM (Pesticide Root Zone Model) is a one-dimensional, dynamic compartmental model designed for simulating chemical movement in unsaturated soil systems within and immediately below the plant root zone [8], PRZM-3 added hydrological and chemical processes in the vadose zone [77]. PRZM adds some extra elements compared to PEARL and MACRO. This is the depth and the cross-

sectional area, and whilst this is slightly different to the one given above (Eq. 14), it was not deemed distinct enough to present as a separate equation here.

PELMO (Pesticide Leaching Model) is based on the US-EPA's PRZM model; it is however modified so that it better aligns with the process used by the German authorities for the registration of pesticides. This means that both models are very similar; PELMO also uses Eq. 14, with the addition of cross-sectional area and depth like PRZM, and takes a plant uptake value between 0 and 1 [78].

5.1 Plant Uptake Within Current Environmental Fate Models

Within all models, a plant concentration factor is required, which for passive uptake is constrained between 0 and 1. Previously, if no relevant laboratory data could be found, this value was calculated from the TSCF against log K_{OW} relationship [49, 79]. This relationship has long been contested as discussed above. The current procedure for the setting of a TSCF value is to supply 0 for most pesticides and 0.5 for systemic pesticides which are known to be taken up by the root.

To date, there has been little work published which discusses the effect that plant uptake has on the leaching of compounds [59]. This was conducted using PEARL and centres on the predicted environmental concentration in the groundwater (PEC_{GW}). PEC_{GW} is a measure taken from the model outputs and is defined as the 80th percentile of the mean concentration at 1 m depth. This allows for a quick assessment of the risk of a compound leaching down the profile; compounds are rejected that exceed the regulatory threshold of 0.1 μg/L [59]. Results from this work found that leaching concentration reduced by 24–43% when uptake was set as 1 [80]. This work suggests that there is a significant effect of plant uptake on the leaching behaviour of certain compounds, and it could theoretically reduce the PEC_{GW} to acceptable levels.

6 Conclusion

There is little evidence to demonstrate that plants enhance the release of pesticides from recalcitrant fractions in the soil. There are few studies where plant uptake is effectively predicted by the bioavailable fraction recovered from passive samplers and mild extractants. These two topics should be active subjects for researchers. Plant uptake of xenobiotics has been researched since the 1980s and can be defined in relatively simple terms. However, since the definition of the original relationship (TSCF vs log K_{OW}), there has been little agreement on the multipliers in the equation. This means that progression in this field has been slow, to the point that the current advice remains that plant uptake should be set to 0 for the majority of pesticides and 0.5 for systemic pesticides. Organic compounds are complex, and there are several different factors that affect uptake; recent suggestions point to the

Lipinski ‘rule of five’ being more able to explain the uptake behaviour of organic compounds than just a TSCF against Log K_{OW} relationship [81]. However there remains reasonable doubt and insufficient scientific scrutiny to provide robust values.

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Bioaccumulation and Toxicity of Organic Chemicals in Terrestrial Invertebrates

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Contents

Abstract Terrestrial invertebrates are key components in ecosystems, with crucial roles in soil structure, functioning, and ecosystem services. The present chapter covers how terrestrial invertebrates are impacted by organic chemicals, focusing on up-to-date information regarding bioavailability, exposure routes and general concepts on bioaccumulation, toxicity, and existing models. Terrestrial invertebrates are exposed to organic chemicals through different routes, which are dependent on

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both the organismal traits and nature of exposure, including chemical properties and media characteristics. Bioaccumulation and toxicity data for several groups of organic chemicals are presented and discussed, attempting to cover plant protection products (herbicides, insecticides, fungicides, and molluscicides), veterinary and human pharmaceuticals, polycyclic aromatic compounds, polychlorinated biphenyls, flame retardants, and personal care products. Chemical mixtures are also discussed bearing in mind that chemicals appear simultaneously in the environment. The biomagnification of organic chemicals is considered in light of the consumption of terrestrial invertebrates as novel feed and food sources.

This chapter highlights how science has contributed with data from the last 5 years, providing evidence on bioavailability, bioaccumulation, and toxicity derived from exposure to organic chemicals, including insights into the main challenges and shortcomings to extrapolate results to real exposure scenarios.

Keywords Beneficial arthropods, Bioavailability, Biological traits, Conceptual models, Earthworms, Edible insects, Exposure routes, Pollinators

Terrestrial invertebrates are key components in ecosystems, which play crucial roles in soil structure, functions, and services [1]. Soil structure is characterized by high spatial and composition heterogeneity and is a major driver of soil biodiversity. Invertebrate functions in soils (e.g., carbon transformations, nutrient cycling, structure maintenance, biological populations' regulation) are often related to ecological and morphological traits that include size, morphology and body characteristics, feeding habits, and specific habitat location [2]. The ecology of terrestrial invertebrates, i.e., the interactions among them and their environment, is known to be threatened by many different types of pressures, which can nowadays be included within global changes. These include climate changes, chemical exposure, and biological pressures that will unbalance the ecosystem turning it into an unsustainable environmental compartment. Among the threats, organic chemical compounds are often appearing in agricultural, rural, and urban environments, mainly derived from agricultural practices, industrial activity, wastewater treatment plants (biosolids and/or effluents), or even from groundwater contamination.

1 Exposure Routes and Organismal Traits

Understanding exposure routes of terrestrial invertebrates is paramount in risk assessment, and, therefore, the European Food Safety Authority (EFSA) proposed a new testing strategy, which takes into account the relevant exposure routes for terrestrial organisms and their related effects, specifically for plant protection products [3]. Morphological and feeding traits, along with preferable habitats, discriminate terrestrial invertebrate exposure routes to chemical compounds. This exposure

is also dependent on the time chemical interacts with the target organ/cell and the fate of the substance in soil (Fig. 1). The latter encompasses the bioavailable fraction of the chemical, where the fraction taken up by an organism is related to the dynamic equilibrium from the exchange of the chemical among the soil solid phase, the soil solution, and the biota. Chemicals can be taken up: (1) via direct contact with the surrounding aqueous media, through pore water, by gills or dermally; (2) by ingestion, as particle-bound chemicals, through the ingestion of soil particles or organic matter, by ingesting contaminated prey; or (3) by the respiratory tract, when volatile chemical compounds are present [4]. Within all these processes, we can distinguish between passive dermal absorption of the dissolved chemical fraction in the interstitial water, intestinal uptake of the chemical compound during gut passage (for soil and food items), and exposure through air contamination via the respiratory tract [5]. Besides being species-specific, exposure routes are also dependent on the chemical characteristics that may change the fractions within the different soil compartments (soil particle-pore water-air). For chemicals with log $K_{ow} > 5$ (K_{ow}: octanol-water partitioning coefficient), uptake from soil particles may become more relevant than from pore water, especially in high organic soils, as hydrophobic chemicals tend to adsorb more efficiently to soil organic matter [6].

Soil exposure comprises the duality of exposures through pore water (dermal and/or "drinkable") and soil particle ingestion. Dermal uptake is usually measured in terrestrial invertebrates (e.g., earthworms) by the filter paper method, where organisms are in direct contact with the study chemical. The Organisation for Economic Co-operation and Development (OECD) Guideline 207 [7] advises this initial screening test to further identify potentially toxic chemicals in soil, although it has to be noted that results obtained with this test (values in μg cm^{-2}) cannot directly be translated to soil concentrations. The second step advised already includes a soil exposure test. This allows discrimination between main exposure routes, depending on the chemicals. In addition, as an effective exposure route, this test is also used to infer on chemical modes of action [8]. Collembolans are known to use the ventral tube to ingest water as a way to balance their fluid and electrolyte content [9]. In this sense, they have often been used to approach porewater exposure in soils, although in recent years few studies have been published on organic contaminants toxicity to collembolans specifically accounting for porewater concentration [10].

Litter exposure route is also key for some macrofauna decomposers, like isopods [3]. As major litter transformers, evaluating effects from litter contamination is crucial in rural and urban ecosystems, but there are not so many recent studies available on this exposure route [11]. Besides litter, exposure through feeding has shown recently to be important regarding, for example, plastics and fibers [12–14].

Volatile chemical compounds can potentially provide an extra route of exposure through the respiratory system [15]. When looking at the respiratory system of insects, as an example, a diffusion gradient is generated, and O_2 is dissolved in a small fraction of water in order to be exchanged by diffusion into the cells [16]. A similar pattern occurs with isopods, where air dissolves in the surrounding moisture comprising their pleopods (pseudolungs) and allowing O_2 to diffuse [17]. Considering that volatile compounds can also be trapped in this water and diffused into cells,

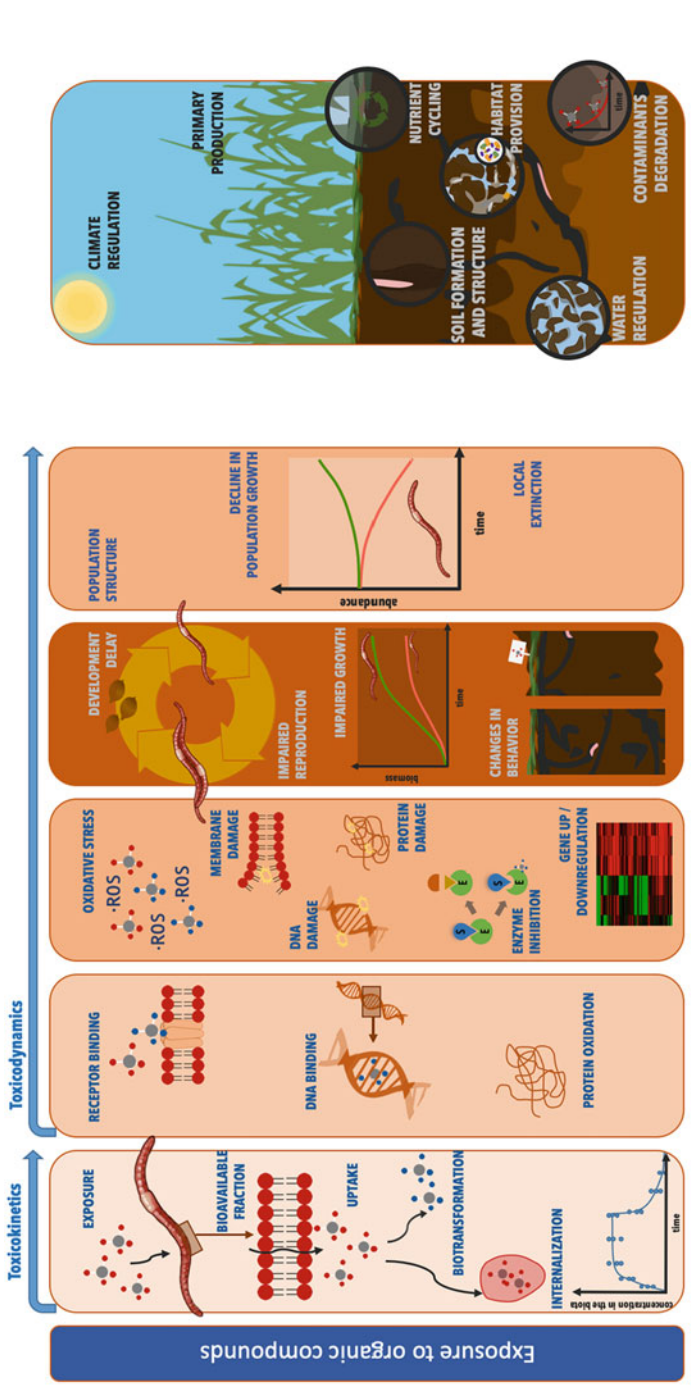

Fig. 1 Bioavailability, toxicokinetic, and toxicodynamic endpoints that have been reported as targets, at different levels of biological organization, from a potential exposure to organic chemicals, leading at a final stage to changes in ecosystem functions and services

this may be an important route to explore as it enters directly into the circulatory system of soil invertebrates. So far, no studies on this are available.

2 Bioaccumulation and Toxicity

Chemical partitioning in the soil is dependent on the soil properties, and it is widely known that different soil types provide, in a general sense, different bioavailable fractions. But, the bioavailable fraction cannot be disconnected from the exposure route involved nor the organism's physiology. If this were just a question of chemistry, one would suggest that the toxicity of a chemical to plants would be similar to that for collembolans, as their exposure is mainly through pore water, which is not the case. Toxicity is surely dependent on chemical uptake (related to the bioavailable fraction), but the organism's physiology is key regarding toxicity (toxicodynamics; presence of specific receptors; metabolic capacity) (Fig. 1). In animals, the distribution of chemical compounds from their gut system to the cells is then again fractionated (bioaccessible fraction), and only a percentage reaches the target organ/cell (bioactive fraction) [18]. The mode or mechanism of action of a chemical will trigger the effects induced, according to the concentration that reaches the target.

The bioaccumulation concept is paramount to understand toxicity, by looking at the amount taken up by the organism, the loss by several processes including egestion, metabolism, transfer to offspring, and growth (e.g., molting), and how chemicals are internalized by the organism. Other factors like feeding traits, habitat use, reproduction, age, biotransformation ability, or energy demand are also crucial in determining bioaccumulation patterns [19]. Therefore, both concepts and data (bioaccumulation and toxicity) are used for the risk assessment of chemical compounds.

For organic chemical compounds, chemical persistence, expressed as their half-life, is key to understand toxicity and bioaccumulation, considering that exposure concentration may vary in time. In addition, their biotransformation through gut passage is also important regarding the observed effects, the gut microbiome being of great importance. Persistent organic pollutants (POPs) are important compounds to study as they tend to stay longer in the environment and their properties potentiate bioaccumulation and toxicity to terrestrial organisms. They are considered hydrophobic and lipophilic, having high affinity with cell membranes, tending to accumulate into lipids rather than entering the aqueous fraction in cells. Toxicity is known to be exerted through a disturbance of membrane integrity by itself and also as a path for the partitioning of pollutants into biological membranes. Also, these substances are accumulated in the lipid fraction to reduce the amount circulating in the animal's plasma, but this may potentially lead to biomagnification in trophic chains.

Bioaccumulation factors are, therefore, calculated as a ratio between the concentration of the substance in the biota, corrected for the lipid content, and the

concentration in the soil [20]. This, therefore, enables comparison across species but also when dealing with different stages of animal development. For example, in the ontogeny of insects, different lipid concentrations are present throughout development [21], and, therefore, this should also be taken into account. In this way, the variation due to variable lipid content is eliminated.

In the case of nonpolar organic compounds (e.g., polycyclic aromatic compounds (PACs)), some are nonreactive or with a nonspecific mode of action (also known as baseline toxicity), which is exerted through narcosis due to the nonspecific interaction between lipophilic chemical molecules and the phospholipids in biological membranes [22, 23]. Narcosis-type effects are reversible, and toxicity thresholds can be extrapolated from organism to organism, with a correction for lipid content [22]. For reactive compounds, where specific modes of action are present (e.g., neurotoxicity, endocrine disruption, genotoxicity), mortality and sublethal effects can occur in specific taxa at low doses, with organisms eliciting lower body burdens when compared to those from nonreactive compounds.

3 Models

In the last years (or even decade), few studies are available reporting the use of models to predict bioaccumulation or toxicity of organic chemical compounds to terrestrial invertebrates. Models and tools like the equilibrium partitioning theory (EqP theory), QSARs, or DEBtox tool (Fig. 2) that require complex and expensive datasets are nowadays less frequently applied than when they were first described.

The EqP theory, described in the later 1980s and early 1990s for aquatic organisms, is a tool that enables the estimation of the internal concentration of hydrophobic chemicals in biota [24]. In soils, this relationship is determined by the porewater concentration and relates to soil properties and several constants like desorption and adsorption rate constants. The partitioning coefficient (K_p) defines the dynamic process of chemical sorption to the soil particles and desorption, which leads to the presence of contaminants in pore water. This process is mainly driven by soil properties (e.g., organic carbon content) and by the chemical K_{ow} (K_p increasing with increasing K_{ow}). Bioconcentration factors (BCF) for chemicals accumulating in organisms whose accumulation is mainly driven by porewater, therefore, depend on the chemical K_{ow} (and lipid content of the organism), with higher BCFs for chemicals with higher K_{ow}s. When relating bioaccumulation patterns in organisms to the chemical concentrations in soil, a bioaccumulation factor (BAF) is calculated. BAF is the ratio of BCF and K_p as it captures both the uptake from porewater and the sorption of the chemical from porewater to the soil solid phase. Since for nonpolar organic chemicals both the BCF and K_p are related to the K_{ow}, the BAF is independent on the K_{ow} but dependent on the organic carbon content of the soil and the lipid content of the organism. In addition, as it relates to other constants dependent on the organism's physiology and behavior, for a more accurate prediction, the EqP theory can be adapted regarding the organism tested. Using earthworms as an example, the

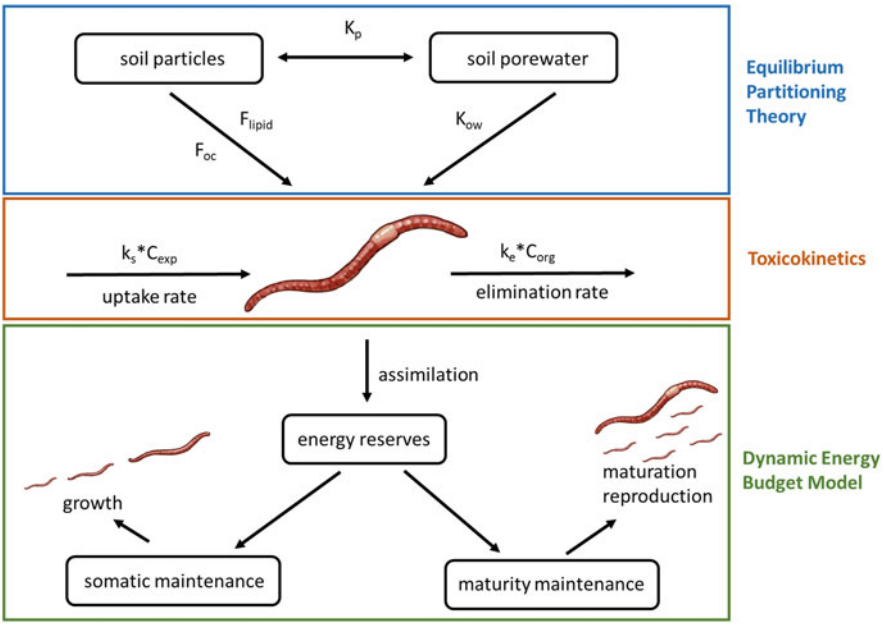

Fig. 2 Mathematical models and tools to estimate biological responses in terrestrial invertebrates, considering exposure routes (soil particles and soil porewater), uptake and elimination kinetics, and how assimilated organic chemicals change somatic and maturity maintenance. K_p partitioning coefficient, K_{ow} octanol-water partitioning coefficient, F_{lipid} lipid fraction, F_{oc} organic carbon fraction, k_s uptake rate constant, C_{exp} concentration of exposure, k_e elimination rate constant, C_{org} concentration in the organism

EqP theory can, therefore, include a dietary uptake rate constant for soil ingestion and an uptake rate constant for pore water [25], for cases where no equilibrium is reached. In addition, the metabolism, reproduction effort, and growth of organisms can also be included as rate constants.

Nowadays, to ensure all requests from the European Union regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), several approaches are being used based on intelligent test systems to decrease animal testing and use existing datasets. Software and statistical tools (e.g., in silico methods) enable the extrapolation from one chemical to similar chemical compounds, using read-across, or from quantitative structure-activity relationships (QSARs), where toxicity is related to chemical properties. In the latter case, QSARs for soil organisms can be developed as linear regression relationships between LC_x/EC_x (lethal/effective concentrations at x %) based on the dissolved fraction, bioavailable in the pore water (in mol L^{-1}), and chemical lipophilicity expressed as log K_{ow} (negative regression) [26]. For this, soil-specific coefficients like the sorption coefficient, based on the carbon-water partitioning and the organic matter fraction present, are determined in a specific soil. Solid-phase microextraction (SPME) is an effective tool to assess interstitial concentrations of organic chemicals

in soils reliably. As an example, for the collembolan *Folsomia candida*, whose chemical exposure route is known to be exclusively through porewater, a QSAR was developed by Giesen and van Gestel [26] for six chloroanilines. EC_{10} and EC_{50} values for effects on reproduction were used, based on porewater concentrations measured by SPME and estimated from nominal soil concentrations and soil-water partitioning coefficients. Measured and estimated porewater concentrations were comparable only for tetra- and pentachloroaniline, with a decreasing degree of chlorination inducing a higher disparity between modeled and measured concentrations. Therefore, some extra optimizations were needed regarding the bioavailable fraction. Several QSARs for organic carbon normalized partitioning coefficient (K_{oc}) or Freundlich soil-water partition coefficient (K_{foc}) use as the independent variable the octanol-water partitioning coefficient (K_{ow}), the molecular connectivity index (MCI), or water solubility. Both K_{oc} and K_{foc} reflect the adsorption of chemicals to soil particles (affinity), where higher values indicate higher sorption. These two coefficients are derived through linear and nonlinear distribution of the coefficient, respectively [27]. In practice, K_{foc} is more appropriate for chemicals for which sorption ability depends on their concentration. More recently, Eckel [28] derived a novel calculator to estimate K_{foc} for soils. In this study, K_{foc} for 41 pesticides in 18 agricultural soils was predicted from subcooled liquid solubility, with robust estimates when compared to the existing literature. This estimation accounts for ionization of the compound and determines its solubility as a liquid at room temperature, with the final aim of achieving robust estimates for both solids and liquids and neutral and anionic compounds.

Toxicokinetic/toxicodynamic (TKTD) models simulate effects in time during an exposure scenario, accounting for the interaction between the bioavailable fraction and the uptake and elimination of the chemical in a defined organism (toxicokinetics) but also how the chemical interacts with the cellular/organ target, traducing that into effects (toxicodynamics). The General Unified Threshold model of Survival (GUTS) is a unifying TKTD framework for predicting the time course of survival, which has different assumptions, data requirements, and complexity [29]. All GUTS versions use the external concentration to estimate an individual damage dynamic and which further translates into an individual hazard state variable, resulting in simulated mortality when an internal damage threshold is exceeded [30]. The toxicodynamic component of GUTS deals with death mechanisms in different ways, assuming that (a) death rate is identical for all individuals in a population, and the threshold parameter for lethal effects is fixed – stochastic death (SD) model –, and (b) effects are distributed among individuals, and once an individual tolerance is exceeded, the organism dies immediately – individual tolerance (IT) model [30].

The Dynamic Energy Budget model (DEBtox) is another TKTD tool that has been used for mechanistic models to infer on stressor effects on the life-history traits

of individual organisms. This enables the extrapolation to higher and lower levels of biological organization. The advantage of this tool is the integration of a time course of effect data within one consistent framework. These data gather time point series for several endpoints like survival, reproduction, and growth (Fig. 2). For example, Jager et al. [31] infer on the modes of action of chlorpyrifos in *F. candida*, where reproduction, growth, and mortality are modeled and modes of action predicted based on the results obtained from multiple endpoints in life-cycle toxicity tests. In this specific case study, chlorpyrifos affected the process of egg production but also aging through oxidative stress. More recently, bee species responses to chemical mixtures have been compared using conceptual pharmacological models (concentration addition and independent action) and the DEBtox model [32]. The use of time series is indeed advised as changes in time may occur and can, therefore, be predicted. This is also highlighted by Hesketh et al. [33], who reported the benefits of evaluating chronic exposure instead of acute (short-term) effects for toxicity tests with the honey bee *Apis mellifera*. In this case study, DEBtox was used to infer on the potential survival up to 30 days and 90 days of summer and winter worker lifespans. Despite the advantages that this kind of modeling brings to regulation, the effort to gather data is high, and therefore not many studies have been carried out with terrestrial invertebrates.

4 Organic Chemicals and Interactions with Biota

This section includes a summary of the scientific literature of the last 5 years on the bioavailability and effects of organic chemicals on terrestrial invertebrates following the biological organization represented in Fig. 1. The information is focused on:

(a) Plant protection products (herbicides, insecticides, fungicides, and molluscicides), pharmaceuticals (veterinary and human), PACs, polychlorinated biphenyls (PCBs), flame retardants, and personal care products;
(b) Key terrestrial invertebrates for ecosystem functioning including annelids, arthropods, and mollusks;
(c) Bioaccumulation data;
(d) Effects at sub-organism level (genotoxicity and biochemical, morphological, and histological alterations);
(e) Effects at individual and population levels (life-history traits and behavior).

Soil annelids, especially earthworms, are by far the most studied group, with information covering many different organic chemicals and, in some cases, some of their main metabolites. Nevertheless, it is also referred at bioaccumulation and toxicity information on other invertebrate groups.

4.1 Plant Protection Products

4.1.1 Herbicides

Bioaccumulation of Herbicides

Bioaccumulation studies of herbicides in terrestrial invertebrates are scarce due to difficulties in their chemical determination; most of the studies are focused on earthworms. For example, greater bioaccumulation of atrazine has been found in *Metaphire guillelmi* (BAF 0.42) than in *Eisenia fetida* (BAF 0.08) [34]. The authors attributed this to the fact that *E. fetida* uptake is mainly through dermal absorption, whereas that of *M. guillelmi* is largely affected by gut processing in which physical grinding and surfactant-like materials could facilitate atrazine desorption from the soil. Tejada et al. [35] reported greater bioaccumulation of oxyfluorfen in *Allolobophora molleri* (BAF 4.0–4.5) than in *E. fetida* (BAF 3.0) and *Lumbricus terrestris* (BAF 1.0–1.5). Goto and Sudo [36] found higher bioaccumulation risk of trifluralin and pendimethalin in *Eisenia* spp. (BAF 9.1 and BAF 5.8, respectively) than in *Pheretima* spp. (BAF 0.93 and BAF 0.27, respectively) (BAFs calculated from kinetic parameters). Jing et al. [37] reported enantioselective bioaccumulation of fenoxaprop-ethyl in *E. fetida*, with a preferential accumulation of the R-enantiomer (BAF 1.4) over the S-enantiomer (BAF 0.17). For the majority of the previously referred studies, the lack of BAF standardization for earthworm lipid content and soil organic carbon makes it difficult to compare different species and herbicides.

Effect of Herbicides at Sub-Organism Level

Herbicides can cause DNA damage in terrestrial invertebrates. This has been shown, for example, for the pure active substances fomesafen and mesotrione in *E. fetida* [38] and glyphosate-based herbicides in the land snail *Cantareus aspersus* [39]. Herbicides can also alter gene expression. For example, the pure active substance 2,4-dichlorophenoxyacetic acid (2,4-D) may upregulate superoxide dismutase, glutathione S-transferases, and catalase genes expression in *Eisenia andrei* [40], while siduron-based herbicides may induce downregulation of metallothionein and the expression of heat shock protein genes in *E. fetida* [41]. In the honey bee *A. mellifera*, paraquat may downregulate glutathione S-transferase, superoxide dismutase, and peroxiredoxin gene expression levels, but not those of catalase, cytochrome P450s, and vitellogenin genes [42].

Herbicides favor the production of reactive oxygen species (ROS) [38], which can overcome the antioxidant defenses of terrestrial invertebrates, causing lipid peroxidation [40, 43, 44]. Invertebrates can counteract this through the activation of certain antioxidant enzymes (e.g., catalase, superoxide dismutase, peroxidase, glutathione peroxidase, glutathione reductase) [38, 40]. However, some studies also

found decreasing activity of antioxidant enzymes or no effects after herbicide exposure [43, 45]. Herbicides can also induce changes in the activity of enzymes involved in xenobiotic detoxification (e.g., glutathione S-transferases and carboxylesterases) [43, 46] and in hydrolysis of acetylcholine neurotransmitter (acetylcholinesterase) [43, 44].

Little information exists on the possible morphological and histological alterations induced by herbicides in terrestrial invertebrates. This is the case of glyphosate-based herbicides in the earthworm *Eudrilus eugeniae* (e.g., setal anomalies, epidermal lesions, clitellar swelling) [44], as well as in the cellular ultrastructure of the hypopharyngeal glands of *A. mellifera* [47]. On the contrary, Druart et al. [48] found no effects of glyphosate-based herbicides on the male genital apparatus of the land snail *C. aspersus*.

Effect of Herbicides at Individual and Population Levels

Life-History Traits Herbicides can induce earthworm mortality, either through dermal contact in filter paper tests of short duration or through medium-/long-term exposure to soil conditions. In the case of filter paper tests, greater toxicity to *Eisenia* spp. has been found, for example, for diquat and tembotrione ($LC_{50} < 10$ μg a.i.[1] cm^{-2}), compared to glyphosate and siduron (LC_{50} ~10–100 μg a.i. cm^{-2}) or imazamox ($LC_{50} > 100$ μg a.i. cm^{-2}) [41, 49]. Herbicide metabolites can sometimes be more toxic than parent compounds (e.g., fenoxaprop-ethyl and quizalofop-ethyl metabolites for *E. fetida*) [37, 50]. In the case of earthworms exposed to herbicide-spiked soils, several species are commonly used, although most of the information refers to *E. fetida* (e.g., $LC_{50} < 10$ mg a.i. kg^{-1} d.w.[2] for terbuthylazine, ~100–500 mg a.i. kg^{-1} d.w. for acetochlor, and >1,000 mg a.i. kg^{-1} d.w. for butachlor) [51–53]. Plenty of information exists on earthworm survival in glyphosate-spiked soils. As pure active substance, glyphosate only causes adverse effects on earthworm survival (e.g., no observed effect concentration, NOEC >50,000 mg kg^{-1} d.w. for *E. fetida* in field soil; NOEC 478 mg kg^{-1} d.w. for *E. fetida* in OECD artificial soil) at levels well above the predicted environmental concentration (PEC 5.7–6.6 mg kg^{-1} d.w.) [54–56]. This trend has also been shown for its main metabolite in soil (aminomethylphosphonic acid, AMPA), with field-relevant concentrations having no effects on earthworm survival (e.g., NOEC 1,000 mg kg^{-1} d.w. vs. PEC 2.0–6.2 mg kg^{-1} d.w.) [55–57]. However, glyphosate-based herbicides may induce earthworm mortality at field-recommended application rates [58, 59]. Negative effects on survival of the enchytraeid *Enchytraeus crypticus* have been found upon exposure to atrazine from cocoon stage (LC_{10} 125 and 378 mg a.i. kg^{-1} d.w. for pure active substance and commercial formulation, respectively), while no effects have been reported upon exposure of

[1] a.i. – active ingredient.

[2] d.w. – dry weight.

adults (NOEC >200 and >400 mg a.i. kg^{-1} d.w. for pure active substance and commercial formulation, respectively) [60]. Compared to annelids, the effect of herbicides on the survival of other terrestrial invertebrates is less studied. Several studies found negative effects of herbicides, generally at field-realistic concentrations, on the survival of ants [61], bees [62], beetles [63], collembolans [64], isopods [65], ladybugs [66], predatory mites [67], snails [58], and spiders [68]. However, there are also studies reporting no effects of realistic field concentrations of herbicides on the survival of terrestrial arthropods and mollusks [55, 69–72].

Similar to survival, most of the studies evaluating herbicides' effect on terrestrial invertebrate reproduction are focused on annelids. Glyphosate pure active substance causes no effects on earthworm reproduction at field-realistic concentrations (e.g., NOEC ≥470 mg a.i. kg^{-1} d.w. for *E. fetida* in artificial and field soils) [54, 55]. Its main metabolite AMPA shows a variable effect by being able to stimulate or not affect earthworm reproduction at concentrations similar to PEC values [55–57]. On the contrary, glyphosate-based herbicides have been found to negatively affect earthworm reproduction at field application rates [45, 59]. In the case of other herbicides, it is described, for example, that nicosulfuron can stimulate earthworm reproduction, oxyfluorfen can reduce it, depending on the study species, and tembotrione has no effects when applied at recommended field rates [35, 43]. The effect of herbicides on enchytraeid reproduction is also highly variable. Adverse effects are described for atrazine, especially when comparing the pure active substance (EC_{50} 161 and 236 mg a.i. kg^{-1} d.w. when exposed from adult and cocoon stages, respectively) with commercial formulations (EC_{50} > 400 mg a.i. kg^{-1} d.w.) [60]. Negative effects on enchytraeid reproduction have been also reported for commercial formulations of phenmedipham (especially in acidic soils and/or with low organic matter content) [73]. On the contrary, no effects have been found for commercial formulations of metsulfuron-methyl [74]. For other terrestrial invertebrate groups, some studies have reported negative effects of realistic field concentrations of herbicides on the reproduction of beetles [63], collembolans [64], isopods [65], and snails [48]. However, most of the studies evaluating herbicide effects on arthropod and mollusk reproduction found no toxic effects [55].

Herbicides can affect terrestrial invertebrate growth. Several studies reported lower growth of earthworms in the presence of herbicides (e.g., 2,4-D, glyphosate, terbuthylazine) but at concentrations generally exceeding field-realistic levels [40, 55, 75]. Some herbicide metabolites can also affect earthworm growth (e.g., AMPA at concentrations <2.5 mg a.i. kg^{-1} d.w.) [57]. Few studies have assessed the effects of herbicides on the growth of other terrestrial invertebrates. Gomes et al. [60] found effects of atrazine on *E. crypticus* growth (variable response depending on whether it is applied as a pure active substance or commercial formulation). Druart et al. [48] and Ogeleka et al. [58] found effects of glyphosate-based herbicides on the land snails *C. aspersus* (growth stimulation) and *Archachatina marginata* (growth inhibition), respectively. Herbicides can also affect invertebrate development, in this case, most of the studies being focused on arthropods. Exposure to recommended field application rates of commercial formulations of several

herbicides (e.g., 2,4-D, atrazine, glyphosate) has been reported to affect the development of the beetle *Zygogramma bicolorata* [63]. Freydier and Lundgren [66] found negative effects of commercial formulations of 2,4-D and dicamba on the development of the ladybug *Coleomegilla maculata*, while no effects of glyphosate-based herbicides have been reported on the ladybug *Harmonia axyridis* [71]. Molting can also be affected by herbicide exposure in bees [69], collembolans [64], isopods [65], and spiders [76].

Behavior Some terrestrial invertebrates can avoid herbicides. This is the case, for instance, of *E. andrei* against metsulfuron-methyl [74], *E. crypticus* against atrazine [60], the collembolan *F. candida* against glyphosate [77], and the spider *Neoscona theisi* against glyphosate [46]. On the contrary, other studies revealed non-avoidance response of terrestrial invertebrates when exposed to herbicides [45, 74, 77]. The avoidance behavior of terrestrial invertebrates against herbicides may depend on specific soil properties. Chelinho et al. [73] assessed the avoidance response of *E. crypticus* against a phenmedipham-based herbicide in soils with different pH, organic matter, and texture and found higher toxicity in sandy soils with low pH.

The effect of herbicides on terrestrial invertebrate mobility is highly variable. Decreasing adult mobility is reported for *C. maculata* after exposure to 2,4-D and dicamba pure active substances [66]. Sanogo et al. [78] found immobility effects of commercial formulations of atrazine and diuron on beetles of the genus *Crenitis*. Higher activity has been reported for the spider *Pardosa milvina* when exposed to recommended field application rates of glyphosate [79]. In the case of *A. mellifera*, higher mobility has been found upon exposure to recommended field application rates of commercial formulations of bentazone but not for metamitron [80].

The effect of herbicides on terrestrial invertebrate fodder or prey consumption is also highly variable most of the studies being focused on some arthropod groups. Field-realistic concentrations of glyphosate did not affect the consumption of sugar-spiked solutions by *A. mellifera* [81], while decreasing fodder consumption has been reported upon exposure to bentazone and metamitron [80]. Recommended field application rates of 2,4-D and dicamba did not affect the prey consumption of *C. maculata* [66]. On the contrary, recommended field application rates of glyphosate increased the consumption of prey by *P. milvina* [79].

4.1.2 Insecticides

Bioaccumulation of Insecticides

Few authors have studied bioaccumulation of insecticides in nontarget terrestrial invertebrates in recent years. Qu et al. [82] reported similar bioaccumulation of two fipronil enantiomers (R and S) in *E. fetida*, although their degradation inside the body was enantioselective with a preference toward S-fipronil. Also, bifenthrin and lambda-cyhalothrin presented different bioavailability and enantioselective bioaccumulation in *E. fetida*, where the less toxic enantiomer was preferably

bioaccumulated [83]. This is in line with the study of Ye et al. [84] on fenvalerate and esfenvalerate, with the latter characterized by higher toxicity and lower BAF (BAF 1.4–1.6 for fenvalerate and 0.8–0.9 for esfenvalerate). Liu et al. [85] reported concentration- and exposure time-dependent bioaccumulation of two dinotefuran metabolites in *E. fetida*. Besides chlorpyrifos hydrophobicity, Svobodová et al. [86] emphasized the role of soil organic matter and clay content in the bioavailability and bioaccumulation of this insecticide in *E. andrei*.

Effect of Insecticides at Sub-Organism Level

Insecticides can induce genetic alterations in terrestrial invertebrates. Several studies reported DNA damage in earthworms upon exposure to sublethal concentrations of cypermethrin (*Pheretima peguana*) [87], the neonicotinoids imidacloprid and dinotefuran (*E. fetida*) [88, 89], and the keto-enol insecticide spirotetramat (*E. fetida*) [90]. This is not in accordance with Wang et al. [91] who found no DNA damage in *E. fetida* exposed to the neonicotinoid guadipyr at concentrations up to 100 mg a.i. kg^{-1} d.w. Cardoso et al. [92] found DNA damage in *F. candida* at field-recommended concentrations of dimethoate (0.4 mg a.i. kg^{-1} d.w.). Proteins related to glycolysis can be affected by low doses (e.g., <NOEC for reproduction of 43.8 mg a.i. kg^{-1} d.w.) of tebufenozide in the collembolan *Yuukianura szeptyckii* [93]. Neonicotinoids can induce gene downregulation in the brain of honey bee workers, namely, those encoding the enzymes related to glycolysis and lipids. The authors argued that such effects could further impair honey bee physiology, behavior, and survival [94].

Insecticides can induce ROS production in terrestrial invertebrates, leading to alterations in the activity of antioxidant enzymes (e.g., *E. fetida* exposed to imidacloprid) (e.g., [88, 95]). Velki et al. [96] reported species-specific biomarker responses to organophosphate and pyrethroid insecticides in soil microcosms for several earthworm species (*E. andrei*, *L. terrestris*, *Lumbricus rubellus*, and *Octolasion lacteum*). The authors indicated higher responses to the organophosphates dimethoate and pirimiphos-methyl, especially for the activity of acetylcholinesterase, carboxylesterase, catalase, and glutathione S-transferases. Although lack of correlation between biomarker responses in *E. fetida* and the presence of organochlorine insecticides in agricultural soils was observed [97], neurotoxicity of dimethoate in the isopod *Porcellionides pruinosus* was documented, alongside with oxidative stress and lipid peroxidation [98]. Oxidative damage was also described for the land snail *Cantareus apertus* upon exposure to carbaryl-contaminated food [99]. Balieira et al. [100] reported activity of the antioxidant enzymes glutathione peroxidase and catalase in *A. mellifera* exposed to imidacloprid. However, Zhu et al. [101] found no effects of imidacloprid on the activity of esterase, acetylcholinesterase, glutathione S-transferases, and invertase in honey bees surviving a spray tower experiment.

Insecticides can affect the structure and integrity of terrestrial invertebrate cells. For instance, cypermethrin negatively affected cell viability in coelomocytes of

P. peguana, alongside the micronucleus frequency and pinocytic adherence activity [87]. Leomanni et al. [99] documented alterations of hemocyte lysosomal membrane stability in *C. apertus* as a consequence of carbaryl-induced oxidative stress.

Insecticide exposure can also lead to morphological and histological alterations in terrestrial invertebrates. Saxena et al. [102] reported that carbamide (carbaryl and carbofuran) and synthetic pyrethroid (cypermethrin and fenvalerate) insecticides led to cuticular membrane damage and disintegration of circular and longitudinal muscles in the earthworms *E. andrei* and *Metaphire posthuma*. Neonicotinoids can induce disruption of the epidermis and midgut tissue in *E. fetida* [95]. The organophosphate insecticide monocrotophos can induce morphological and histological changes in *E. eugeniae* (e.g., clitellum swelling, epithelial cells damage) [44].

Effects of Insecticides at Individual and Population Levels

Life-History Traits Terrestrial invertebrate survival has been largely assessed in scientific studies on insecticides (e.g., [75, 103–106]). The survival of the isopod *Porcellio scaber* can be affected by imidacloprid and thiacloprid (LC_{50} 7.6 and 32 mg a.i. kg^{-1} d.w., respectively), but not by chlorantraniliprole [104]. Salvio et al. [107] reported no mortality of the slug *Milax gagates* upon exposure to chlorpyrifos and cypermethrin. These insecticides, however, had a lethal effect on the isopod *Armadillidium vulgare* [107]. Insecticide metabolites can be more toxic than the corresponding parent compounds (e.g., pyriproxyfen metabolites in *E. fetida*) [108]. Zhu et al. [101] evaluated the survival of *A. mellifera* workers exposed to imidacloprid in a spray tower experiment. While the concentrations >80 mg a.i. L^{-1} caused mortality, the bees continued dying even after 48 h of posttreatment time. This is one example that illustrates the situation in which short-term toxicity tests may not show possible long-term consequences of chemicals exposure. The same study underlines the importance to address different exposure duration for insecticides with different physicochemical characteristics.

Diverse effects of insecticides on terrestrial invertebrate reproduction are documented. According to Leitão et al. [106], the organophosphate ethoprophos induced lower reproductive output in laboratory bioassays with *F. candida* (EC_{50} 0.03 mg a.i. kg^{-1} d.w.), compared with *E. andrei* and *E. crypticus* (EC_{50} 8.3 and 68.5 mg a.i. kg^{-1} d.w., respectively). These authors reported negative effects of ethoprophos on *F. candida* and *E. andrei* reproduction upon exposure to soils collected from a greenhouse experiment [109]. *F. candida* reproduction was also affected by chlorantraniliprole, with lower toxicity in soils with higher organic matter content. Toxicity was not observed in the case of *E. crypticus* and the mite *Oppia nitens* reproduction [104]. de Lima e Silva et al. [105] found higher sensitivity of *F. candida* and *E. andrei* reproduction to neonicotinoids than for *O. nitens*. Both imidacloprid and fipronil affected the reproduction of *F. candida* at doses comparable to PEC values (0.230 and 0.096 mg a.i. kg^{-1} d.w., respectively) [103]. A consistent reproduction response of *F. candida* to imidacloprid was found over

three generations, while for thiacloprid recovery was obtained from the second generation [110]. Such responses were explained by the persistence of imidacloprid versus fast degradation of thiacloprid. Multigenerational and transgenerational exposures to the pyrethroid etofenprox induced significant changes in egg size of *F. candida*, which can imply severe consequences at the population level [111]. Bori et al. [112] evaluated commercial formulations of imidacloprid covering from the manufacturer recommended doses to the worst-case scenario representing an excessive application (0.13–2 mg a.i. kg^{-1} d.w.). The authors found effects on *E. fetida* reproduction (EC_{50} 1.4 mg a.i. kg^{-1} d.w.; NOEC 1 mg a.i. kg^{-1} d.w.), but not on *F. candida*, and argued that adjuvants and solvents present in the formulation might have contributed to toxicity. The need for more scientific studies on ecotoxicity and risk assessment of adjuvants was also highlighted in the review by Mesnage and Antoniou [113].

Insecticides may impair the growth and development of terrestrial invertebrates. Body weight reduction of *E. fetida* was reported upon exposure to commercial formulations of both organophosphates (field-recommended dose of 47 mg a.i. kg^{-1} d.w. and above it) and imidacloprid (0.13–2 mg a.i. kg^{-1} d.w.) [75, 112]. Body weight of *P. scaber* was not affected by chlorantraniliprole [104], but it was significantly lower in the presence of thiacloprid [105]. The molting frequency of *Y. szeptyckii* was affected by tebufenozide [93]. Yu et al. [114] reported no effects of imidacloprid on the development time, pupation, and adult emergence of the ladybug *Coccinella septempunctata*, while egg production and hatching were compromised.

Behavior Avoidance is a commonly reported endpoint in laboratory insecticide exposure studies. Bori et al. [112] found avoidance behavior of *E. fetida* against soils spiked with an imidacloprid-based formulation, while this was not the case of *F. candida*. Avoidance of the predatory mite *Hypoaspis aculeifer* was a sensitive endpoint in exposure to deltamethrin, dimethoate, and chlorpyrifos [115]. However, avoidance/preference behavior can provide false-positive results. For instance, the ryanoid insecticide chlorantraniliprole impacted *F. candida* locomotion, preventing them from avoiding the spiked soils [104].

Flight behavior of honey bees has been used as an endpoint upon insecticide exposure. Williams et al. [116] found no effects of field concentrations of the neonicotinoids thiamethoxam and clothianidin (4 and 1 μg a.i. kg^{-1} d.s., respectively) measured in pollen on the mating flight behavior of honey bee queens. These authors, however, emphasized that their observations were not aligned with other studies regarding honey bee workers (references cited by Williams et al. [116]).

The impact of insecticides on terrestrial invertebrate prey or fodder consumption may vary. Maple leaf consumption by *P. scaber* was reduced in imidacloprid-spiked soils (EC_{50} 6.7 mg a.i. kg^{-1} d.s.) [105]. Byrne et al. [117] found no effect of imidacloprid on the consumption of honey bees foraging on citrus flowers. Overmyer et al. [118] documented negative effects on *A. mellifera* feeding of thiamethoxam at concentrations >100 mg a.i. L^{-1} at the individual level and >50 mg a.i. L^{-1} at the colony level, both in the range of concentrations reported

in other field studies. Wang et al. [119] reported increased food consumption and stimulated digging and foraging in invasive ants exposed to low doses of imidacloprid (0.01 μg a.i. L^{-1}) but suppression above 0.25 μg a.i. L^{-1}. These authors raised the concern that such complex behavioral changes in invasive ants might occur with other beneficial ant species upon exposure to neonicotinoids. Low levels of imidacloprid affected ladybugs by reducing their consumption of aphids, ultimately reducing adult body weight and inducing slower development, underlining the relevance of looking into effects on predatory species [120]. This agrees with Bredeson et al. [121] who reported altered quality of *Rhopalosiphum padi* aphids for the predatory species *C. maculata* upon exposure to thiamethoxam.

4.1.3 Fungicides

Bioaccumulation of Fungicides

Most of the current studies do not consider the bioaccumulation of organic fungicides in terrestrial invertebrates, probably because of their complex analysis. The available studies are only focused on earthworms, showing, for example, bioaccumulation of tebuconazole, furalaxyl, pentachloronitrobenzene, and tolclofos-methyl in *E. fetida* [86, 122–124]. Moreover, fungicide bioaccumulation can be related to specific soil properties, such as organic matter and clay content, which can increase sorption and decrease fungicide bioavailability in soils [86].

Effect of Fungicides at Sub-Organism Level

Few studies evaluated genotoxicity of fungicides on terrestrial invertebrates. Certain fungicides, like carbendazim, induced DNA damage in coelomocytes of *E. fetida* at concentrations above 0.4 mg a.i. kg^{-1} d.w. after 7 days of exposure [125]. Chlorothalonil can impact *F. candida* by altering several pathways, including detoxification and excretion, immune response, cellular respiration, protein metabolism, and oxidative stress defense [126]. In the same species, Qiao et al. [127] revealed a general downregulation of the expression levels of multiple genes when exposed to 87 mg a.i. kg^{-1} d.w. of pentachlorophenol. Fungicides can also induce transcriptional alterations in genes encoding enzymes related to oxidative phosphorylation and metabolism in bees [128].

Fungicides can increase ROS production and induce oxidative damage. For example, both pentachloronitrobenzene (0.1 mg a.i. kg^{-1} d.w.) and tolclofos-methyl (0.01 mg a.i. kg^{-1} d.w.) induced ROS production and increased lipid peroxidation in *E. fetida* despite the higher activity of the enzyme superoxide dismutase [122]. Wang et al. [129] also found alterations in the antioxidant defense system of *E. fetida* exposed to dimethomorph but only above the recommended application rates (>100 mg a.i. kg^{-1} d.w.). Beyond oxidative damage, other biochemical responses can be sensitive to fungicides as shown by Rico et al. [130] in *E. fetida* exposed to

carbendazim, tebuconazole, and prochloraz (alterations on cholinesterase, dehydrogenase, and alkaline phosphatase enzyme activities). Morgado et al. [131] reported higher metabolic costs (energy reserves and consumption) of mancozeb exposure to early life stages of *P. pruinosus*.

In the last years, very few studies described the possible effects of fungicides on morphological and histological alterations in terrestrial invertebrates. One example is the finding that carbendazim (4 mg a.i. kg^{-1} d.w.) and prochloraz (286 mg a.i. kg^{-1} d.w.) can induce morphological changes in the body wall and gastrointestinal tract of *E. fetida* [132].

Effects of Fungicides at Individual and Population Levels

Fungicides can affect the survival, growth, and reproduction of terrestrial invertebrates. The majority of the studies used earthworms as model species, exposing them to azoxystrobin, carbendazim, chlorothalonil, dimethomorph, furalaxyl, mancozeb, pentachloronitrobenzene, prochloraz, tebuconazole, and tolclofos-methyl [106, 122, 123, 129, 130, 132, 133]. Few other soil invertebrates have been used to study fungicide effects: the enchytraeids *Enchytraeus albidus* and *E. crypticus* [106, 134, 135], *F. candida* [107, 136, 137], *P. pruinosus* [131], and *H. aculeifer* [134]. For most of these studies, a negative impact on at least one life-history parameter is described when invertebrates are exposed to fungicide-spiked soils. Fungicides can also induce effects on bees such as the timing of pupation and metamorphosis into adult bees [138], decrease in larval survival and malformations during development [139], or even negatively impact colony health [140].

Schnug et al. [141] used a soil-multi-species test system with four different collembolan species and one earthworm species exposed to picoxystrobin for 8 weeks. The authors found a lower sensitivity of *F. fimetaria* compared to the other collembolan species and that earthworm performance was correlated to both collembolan abundance and bait-lamina consumption.

4.1.4 Molluscicides

There is a lack of information, in the past 5 years, on the bioaccumulation and effects at the sub-organism level of molluscicides in terrestrial invertebrates, so only effects at individual and population levels are presented.

Effect of Molluscicides at Individual and Population Levels

Life-History Traits Several studies described the adverse effects of molluscicides on the survival of target organisms, such as slugs and snails. McDonnell et al. [142] evaluated the potential molluscicidal action to the land snail *Cornu aspersum* of several essential oils (bitter orange, cedarwood, cinnamon, clove bud, eucalyptus,

garlic, lemongrass, peppermint, pine, rosemary, and spearmint) and the terpene d-limonene. The clove bud oil was the most effective (LC_{50} 0.03%), followed by pine (LC_{50} 0.08%) and spearmint (LC_{50} 0.10%) oils, while d-limonene showed the lowest toxicity. The high efficacy of the clove bud oil can be related to its high content of eugenol which has known insecticidal and herbicidal effects [142].

Among the nontarget organisms, earthworms are one of the most affected groups by molluscicidal baits [143]. However, recent studies have suggested that recommended agricultural doses of metaldehyde-based molluscicides have no deleterious effect on the survival and growth of *E. fetida* and *L. terrestris* [75, 144]. For other terrestrial invertebrates, Cardoso et al. [145] evaluated the effects of metaldehyde and methiocarb bait products to *F. candida* by exposing organisms to single and pulse (recommended application mode by manufactures) doses. The authors showed higher toxicity of metaldehyde to collembolan survival (LC_{50} 102.4 and 69.6 mg a.i. kg^{-1} d.w. for single and pulse exposure to metaldehyde, respectively; no effects of methiocarb), while methiocarb affected reproduction more (EC_{50} 58.4 and 19.8 mg a.i. kg^{-1} d.w. for single and pulse exposure to metaldehyde, respectively; EC_{50} 39.1 and 12.5 mg a.i. kg^{-1} d.w. for single and pulse exposure to methiocarb, respectively).

Behavior Molluscicides exposure may alter the feeding behavior of target organisms. This is, for example, the case of the slug *Arion vulgaris* exposed to metaldehyde, especially in less irrigated systems, as slug recovery is affected in drier environments and also because watering reduction diminishes molluscicide losses by leaching [144]. Cardoso et al. [145] found no effects of metaldehyde baits on the avoidance behavior of *F. candida.* They also found a preference response for methiocarb baits, which may indicate no adverse effects of this molluscicide or even the presence of some attractants in their composition.

4.2 Pharmaceuticals: Veterinary and Human

4.2.1 Bioaccumulation of Pharmaceuticals

Bioaccumulation studies of pharmaceuticals in terrestrial invertebrates are scarce in the recent literature, and only a few reports using earthworm species are available. Carter et al. [146] evaluated the fate and uptake of different human pharmaceuticals including the antiepileptic carbamazepine (39 μg kg^{-1} d.w.), the anti-inflammatory diclofenac (49 μg kg^{-1} d.w.), the antidepressant fluoxetine (80 μg kg^{-1} d.w.), and the lipase inhibitor orlistat (65 μg kg^{-1} d.w.) using *E. fetida*. These pharmaceuticals accumulated in the earthworms, with BAF values ranging from 2.3 for carbamazepine to more than 22 for orlistat. Soil properties (mainly pH) are also essential factors that would change the uptake and accumulation of pharmaceuticals by earthworms [147].

4.2.2 Effects of Pharmaceuticals at Sub-Organism Level

Pharmaceuticals can affect terrestrial invertebrates by inducing genotoxicity. Gao et al. [148] described alterations in the expression levels of two target genes in different segments of *E. fetida* exposed to the veterinary pharmaceutical albendazole for 14 days. Regarding human pharmaceuticals, Chen et al. [149] reported effects of diclofenac on neural metabolic processes in *F. candida* at 200 mg kg^{-1} d.w., as well on the upregulation of immunity-related genes.

Pharmaceuticals can also induce biochemical alterations in terrestrial invertebrates. For human pharmaceuticals, Oliveira et al. [150] described increasing lipid peroxidation levels and inhibition of the enzyme acetylcholinesterase in *F. candida* exposed for 96 h to the antiepileptic carbamazepine (4 mg kg^{-1} d.w.) and the antidepressant fluoxetine (0.4 mg kg^{-1} d.w.), respectively. Using the same compounds, but on a multigeneration approach, Oliveira et al. [151] also found increasing oxidative stress and impaired neurotransmission in *F. candida*, especially following carbamazepine exposure at field-realistic concentrations. For veterinary pharmaceuticals, Guimarães et al. [136] observed that the antioxidant mechanisms of *F. candida* were dynamically activated along with generations when exposed to 1 mg kg^{-1} d.w. of ivermectin.

4.2.3 Effects of Pharmaceuticals at Individual and Population Levels

Life-History Traits Human pharmaceuticals can affect terrestrial invertebrates with adverse effects on survival, growth, and reproduction. For example, this was the case of *F. candida* exposed to fluoxetine, carbamazepine, and diclofenac [149–151]. Pino et al. [152] evaluated the lethal toxicity of a battery of 18 human pharmaceuticals such as nonsteroidal anti-inflammatory drugs, blood lipid-lowering agents, β-blockers, and antibiotics to *E. fetida*. From all the tested compounds, ibuprofen (LC_{50} 64.8 mg kg^{-1} d.w.) showed the highest acute toxicity to earthworms, followed by diclofenac (LC_{50} 90.5 mg kg^{-1} d.w.) and simvastatin (LC_{50} 92.7 mg kg^{-1} d.w.).

Veterinary pharmaceuticals are also the focus of different studies using terrestrial invertebrates. A battery of pharmaceuticals (ivermectin, fipronil, fluazuron, and closantel) has been evaluated using *F. candida* in tropical Brazilian soils [153]. The results confirmed higher chronic toxicity of fipronil (EC_{50} 0.19 mg kg^{-1} d.w.) and ivermectin (EC_{50} 0.43 mg kg^{-1} d.w.), followed by fluazuron (EC_{50} 3.07 mg kg^{-1} d.w.). Closantel did not show severe effects on *F. candida*. Alves et al. [154] reported adverse effects of fluazuron on the reproduction of *E. andrei* and *F. candida* (EC_{50} 20.8 mg kg^{-1} d.w. and 4.48 mg kg^{-1} d.w., respectively). The same species have been used to assess the effects of nicarbazin and monensin used in the poultry industry [155]. Monensin showed the highest toxicity, especially in terms of collembolans reproduction (EC_{50} 101 mg kg^{-1} d.w.) [155].

Behavior The few available studies in this field indicate that some terrestrial invertebrates can avoid pharmaceutical-spiked soils. This is, for example, the case of *F. candida* against carbamazepine, using a light avoidance innovative test at very low concentrations (AC_{50} 0.04 mg kg^{-1} d.w.) [151]. Alves et al. [154] found avoidance response of *F. candida* and *E. andrei* against fluazuron (AC_{50} 1.73 and 4.78 mg kg^{-1} d.w., respectively), highlighting the higher sensitivity of this behavioral response compared to reproduction (EC_{50} 20.8 mg kg^{-1} d.w.).

4.3 Polycyclic Aromatic Compounds

4.3.1 Bioaccumulation of Polycyclic Aromatic Compounds

Bioaccumulation of PACs has long been regarded as an environmental concern. Early toxicokinetic studies confirmed the bioaccumulative potential and identified main uptake routes (e.g., [156, 157]). Soil properties and aging time were found crucial for PAC bioaccumulation in earthworms, leading to marked differences in BAF and toxicokinetic parameters [158]. A peak-shaped accumulation curve was reported for phenanthrene and pyrene, resulting from the degradation and desorption, with consequent reduction of PAC bioavailability [158]. BAFs were significantly higher for soils with high total organic carbon, ranging between 2.1–37.2 for phenanthrene and 2.0–26.1 for pyrene. The distribution of accumulated PACs within soil organisms is another topic explored in recent years. A hierarchical method for extending whole-organism toxicokinetic studies was described, by addressing sub-organism, tissue, and subcellular fractionation of phenanthrene in *E. fetida* [159]. Phenanthrene partition varied dynamically with exposure concentration and through time, probably distributed by the earthworm circulatory system [159]. Heterogeneous distribution at organ level may reflect not only the main routes of exposure but also the ability of earthworms to transport PACs toward less susceptible body locations or where detoxification takes place [160]. These processes are species-specific and valuable for explaining general or endpoint-specific differences in sensitivity to PACs [160]. Ecophysiology traits might mediate PAC exposure, leading to different BAFs, as shown by Zhang et al. [160] for *E. fetida* (BAF 8.64), *Pheretima guillelmi* (BAF 107), and *M. guillelmi* (BAF 350). No differences were, however, found between *E. fetida* and another endogeic earthworm species (*Aporrectodea caliginosa*), which highlights the complex and sometimes conflicting results of PAC bioaccumulation within the soil compartment. Bioaccumulation of field-relevant PAC mixtures has also been assessed, including field-contaminated soils, soil amendments, or relevant mixtures/formulations containing multiple PACs (i.e., lubricants, oils). Rorat et al. [161] assessed PAC bioaccumulation in *E. andrei* exposed to sewage sludge in vermicomposting experiments for 5 weeks. Body concentrations in earthworms depended on the vermicomposting mixture used, leading to distinct accumulation patterns of individual PACs, even though total PAC mixtures did not show evidence of bioaccumulation (BAF 0.07–0.74)

[161]. Recent studies reported increased bioavailability of PACs from biochar-amended soils. For instance, Malev et al. [162] reported PAC bioaccumulation in *E. andrei* after exposure to a biochar-soil matrix. Prodana et al. [163] found increased levels of naphthalene-type metabolites in earthworm tissue upon exposure to soil amended with woodchip biochar particles.

4.3.2 Effect of Polycyclic Aromatic Compounds at Sub-Organism Level

Some PACs can be genotoxic to terrestrial invertebrates. Benzo[a]pyrene induced DNA damage to coelomocytes of *E. fetida* at 1 mg kg^{-1} d.w. [164]. A similar result was reported for *E. andrei* in Sforzini et al. [165]. The genotoxicity caused by some PACs (including benzo[a]pyrene) has been attributed to a biotransformation product by microsomal monooxygenases cytochromes P450 [166]. PACs were also linked to genotoxic effects arising from exposures to environmentally relevant complex mixtures (e.g., oil-contaminated soil [167]). Benzo[a]pyrene decreased lysosomal membrane stability in coelomocytes and chloragogenous tissue of *E. andrei* and increased neutral lipid accumulation and lysosomal/cytoplasmic volume ratios [165]. Alterations in ROS-scavenging enzymes and oxidative stress levels have also been reported. Duan et al. [164] found changes in ROS-scavenging enzymes (superoxide dismutase and catalase) in *E. fetida* after 14 days of exposure to benzo-[a]pyrene, but not lipid peroxidation at concentrations below 500 mg kg^{-1} d.w. For the same species and chemical, Ye et al. [168] denoted an increase in superoxide dismutase and peroxidase activities and failure to reach a new homeostasis status after 56 days at 10 mg kg^{-1} d.w. Glutathione S-transferases alterations were reported for phenanthrene and fluorene in *E. fetida* [169]. Recent OMICS have highlighted important differences in toxicity pathways elicited by PACs to soil organisms, as shown by Roelofs et al. [170] for *F. candida* and *E. crypticus* after exposure to phenanthrene. Whereas no strong induction of biotransformation pathways was observed in *E. crypticus*, upregulation of genes encoding all phases of biotransformation/detoxification (I/II/III) was found in *F. candida*. Similarly, Holmstrup et al. [171] found upregulation of genes related to biotransformation/detoxification and general stress handling proteins (i.e., Hsp70) in *F. candida* exposed to phenanthrene.

4.3.3 Effect of Polycyclic Aromatic Compounds at Individual and Population Levels

Most of the recent work on the toxicity of PACs to terrestrial invertebrates has been conducted with earthworms (e.g., [172], collembolans [173], and, to a lesser extent, mites [115] and isopods [174]). Overall, collembolans are the most sensitive group, particularly when considering survival (e.g., LC_{50} values generally one order of magnitude lower than those for enchytraeids) (see [175, 176] and references therein). Earthworms have generally proved lower sensitivity than collembolans but higher than enchytraeids. However, Gainer et al. [177] showed greater sensitivity of

earthworms to lubricating mixtures including PACs and aliphatic compounds probably related to a higher uptake due to their bigger size. Deviations from nonpolar narcosis might occur for sublethal endpoints, indicating that more specific responses might be present [178]. This makes it difficult to predict species-specific sublethal responses and compels a case-by-case analysis of their ecotoxicological importance. For instance, earthworm growth inhibition was a sensitive endpoint for some PACs [137], and so was biomass variation in terrestrial isopods [174]. Phenanthrene-contaminated soils triggered avoidance responses of *E. fetida* [173] and *H. aculeifer* [115], but not of *E. crypticus* and *F. candida* [173]. Again, slightly different results can be obtained for mixtures containing PACs, such as lubricating oils, which caused strong avoidance responses of *E. fetida*, *F. candida*, *O. nitens*, and *H. aculeifer* with only *E. crypticus* showing no response [179].

4.4 Polychlorinated Biphenyls

4.4.1 Bioaccumulation of Polychlorinated Biphenyls

Understanding the bioaccumulation patterns has long been a priority for PCBs due to their high stability and hydrophobicity. However, bioaccumulation studies with PCBs in terrestrial invertebrates were almost exclusively conducted with earthworms. In recent years, the main focus is on understanding PCB bioaccumulation patterns under a wide range of exposure conditions. Differences in the toxicokinetics of PCBs were found for natural soils with markedly distinct properties, including different uptake and elimination rate constants and time to reach internal steady-state concentrations [158]. Moreover, earthworm density and, mostly, feeding activity can also mediate bioaccumulation of PCB 153, with non-fed earthworms showing twofold higher BAFs than fed individuals [180]. Assessing stereoselective bioaccumulation of chiral PCBs in earthworms has been a recent line of research. For example, significant stereoselectivity for PCBs 91, 95, and 149 during uptake and elimination phases has been shown in *E. fetida*, leading to variable enantiomer fractions over time [181, 182]. This indicates that toxicokinetics is partly driven by biological processes. An additional line of bioaccumulation-related research has focused on assessing the efficiency of soil amendments in the remediation of PCB-contaminated soils. Although promising as a remediation tool for PCBs, variable biota bioaccumulation patterns highlight the complexity related to product properties, application doses, protocols, and time, among others (e.g., [183, 184]).

4.4.2 Effect of Polychlorinated Biphenyls at Sub-Organism Level

Ecotoxicity studies evaluating sub-organism level effects of single PCBs in terrestrial invertebrates are scarce and most date back to the 1990s. Most of these studies

focused on earthworm coelomocyte immunoassays as surrogates for mammalian toxicology and reported, among others, decreased immunocompetence and macrophage-related functions (e.g., [185]). There is a paucity of new approaches on PCB toxicity to terrestrial invertebrates. Recent studies denoted the induction of DNA damage to coelomocytes of *E. fetida* exposed to soil spiked with a standard PCB mixture at 0.25 mg kg^{-1} d.w. [186]. Dose-dependent increases of the ROS-scavenging enzymes (catalase, superoxide dismutase, and peroxidase) were also found in PCB-spiked soils, without signs of lipid peroxidation [186]. Similarly, Shen et al. [187] also found increased activity of antioxidant enzymes in earthworms exposed to field soils contaminated with PCBs.

4.4.3 Effect of Polychlorinated Biphenyls at Individual and Population Levels

As for sub-organism approaches, few ecotoxicity studies at the individual level have been conducted in recent years. Duan et al. [186] found growth inhibition in *E. fetida* exposed to a standard PCB mixture, with significant effects registered at lower concentrations than for effects on oxidative stress enzymes [186].

4.5 Flame Retardants

4.5.1 Bioaccumulation of Flame Retardants

There is a lack of recent information on the bioaccumulation of flame retardants in terrestrial invertebrates. A higher bioaccumulation potential was found for perfluoroalkyl substances, compared to halogenated flame retardants, in *E. andrei* exposed to an agricultural soil amended with anaerobically digested municipal waste and composted sludge [188]. Huang et al. [189] reported bioaccumulation of decabromodiphenyl ether (DecaBDE) in *P. guillelmi*. Using ^{14}C labeled-DecaBDE, these authors found that DecaBDE extractable fraction may lead to underestimating the total bioaccumulated DecaBDE. Low bioaccumulation potential of tri-n-butyl phosphate (TBP) in the earthworm *Perionyx excavatus* was reported by Wang et al. [190]. These authors also detected TBP biotransformation products, revealing specific detoxification mechanisms in *P. excavatus* for this xenobiotic.

4.5.2 Effects of Flame Retardants at Sub-Organism Level

Liang et al. [191] reported that 2,2′,4,4-tetrabromodiphenyl ether (BDE-47) and decabromodiphenyl ether (BDE-209) altered energy- and amino acid-related metabolism and the nerve activity in *E. fetida*. Shi et al. [192] reported the upregulation of superoxide dismutase and heat shock protein Hsp70 gene expression upon exposure

of *E. fetida* to hexabromocyclododecane and tetrabromobisphenol A (TBBPA), with the latter inducing higher effects. Dechlorane plus, a polychlorinated flame retardant, induced oxidative stress and genotoxicity in *E. fetida* [193].

4.5.3 Effects of Flame Retardants at Individual and Population Levels

TBBPA induced higher mortality for *M. guillelmi* than for *E. fetida* [194]. The authors argue that this difference could be related to the distinct exposure routes of both earthworm species, as *M. guillelmi* is more exposed to TBBPA through soil particle ingestion while *E. fetida* mainly through dermal uptake. Shi et al. [192] reported increased *E. fetida* body mass upon exposure to TBBPA. As reviewed by Rothenbacher et al. [195], the most sensitive endpoint for TBBPA was *E. andrei* reproduction (EC_{50} 0.12 mg kg^{-1} d.w.) and has been used to derive the predicted no effect concentration of 0.012 mg kg^{-1} d.w. Since the early 2000s, there are no updates regarding the endpoints of interest for the risk assessment of TBBPA.

4.6 Personal Care Products

4.6.1 Bioaccumulation of Personal Care Products

Similar to the majority of previously referred compounds, only a few studies cover the bioaccumulation of personal care products in terrestrial invertebrates. Most of the recent studies focused on the antimicrobial agent triclosan and its main soil metabolite (methyl-triclosan), with special attention to earthworm bioaccumulation. For instance, Chevillot et al. [196] assessed the bioaccumulation of these compounds in *E. andrei* exposed to both a triclosan-spiked soil (BAF 2.6 and 0.5 for triclosan in juveniles and adults, respectively; no detection of methyl-triclosan) and a soil amended with biosolids from a wastewater treatment plant containing triclosan (BAF 2.0–2.5 for triclosan and methyl-triclosan). Macherius et al. [197] also evaluated the bioaccumulation of triclosan and methyl-triclosan in different earthworm species of a soil amended with biosolids. The parent compound showed higher BAFs compared to the metabolite (4.2–13.9 for triclosan and 1.2–5.1 for methyl-triclosan). Both studies concluded that the presence of methyl-triclosan in earthworm tissues is also related to triclosan methylation inside the organism. Havranek et al. [198] evaluated the bioaccumulation of triclosan, galaxolide, and tonalide in the earthworm *Dendrobaena veneta* exposed to a soil amended with contaminated sludge. The authors found the higher transfer of triclosan from the sludge to the earthworms (transfer factor 0.8) than those of galaxolide (transfer factor 0.1) and tonalide (transfer factor 0.02). These results could be explained from the possible excretion and/or metabolization of galaxolide and tonalide in earthworms compared to triclosan. Rivier et al. [199] described greater bioaccumulation of triclosan,

compared to galaxolide and tonalide, in *A. caliginosa* exposed to a soil amended with contaminated sludge.

4.6.2 Effect of Personal Care Products at the Sub-Organism Level

Personal care products can induce genotoxicity in terrestrial invertebrates. Some authors indicate that triclosan can induce DNA damage to earthworm coelomocytes (e.g., *E. fetida*; EC_{50} 8.9 mg kg^{-1} d.w.) [200], while others describe no effects [196]. Triclosan can also alter the transcriptional expression levels of some genes as described by Lin et al. [200] for the heat shock protein Hsp70 gene in *E. fetida* (upregulation after triclosan exposure; EC_{50} 1.8 mg kg^{-1} d.w.). Novo et al. [8] evaluated the effect of an organic UV filter (4-hydroxibenzophenone, 4-OHBP) on the transcriptional expression levels of endocrine, stress, and energy-related genes in *E. fetida*. Exposure to 4-OHBP induced an increase of the ecdysone receptor gene (endocrine-related gene), while it decreased the genes CuZn superoxide dismutase (oxidative stress-related gene) and glyceraldehyde-3-phosphate dehydrogenase (energy metabolism-related gene).

Personal care products can also induce alterations at the biochemical level. Ma et al. [201] indicated that triclosan could stimulate the antioxidant defense machinery of *E. fetida* (e.g., enzymes superoxide dismutase, catalase, and peroxidase). Despite the induced antioxidant activity, it may not be enough to protect organisms from oxidative damage as indicated by the increased lipid peroxidation. Wang et al. [202] evaluated the effects of triclosan on the activity of the enzymes superoxide dismutase, catalase, and peroxidase of the land snail *Achatina fulica*. Increasing enzyme activity levels were found upon exposure to low concentrations. However, catalase and peroxidase activity inhibition occurred at high concentrations leading to increased lipid peroxidation.

4.6.3 Effect of Personal Care Products at Individual and Population Levels

Personal care products can negatively affect terrestrial invertebrate survival. This is, for example, the case of *F. fimetaria* and *A. fulica* exposed to triclosan [202, 203]. However, there are also studies indicating no effects of triclosan on earthworm survival [196, 198]. Besides the variable effects reported on survival, triclosan generally alters reproduction. Lin et al. [200] described reduced reproduction in *E. fetida* exposed to triclosan. Chevillot et al. [196], however, found positive effects of triclosan on *E. andrei* reproduction. Personal care products can induce both increased (e.g., *D. veneta* exposed to triclosan, galaxolide, and tonalide; *E. andrei* exposed to triclosan) and decreased (e.g., *E. fetida* and *A. fulica* exposed to triclosan) invertebrates' growth [196, 198].

4.7 Mixtures

Agricultural practices are a good example of complex exposures that vary in their composition in time and concentration, where pesticides are applied in pulses, in a sequence, or simultaneously. This leads to a complexity of effects due to TKTD processes that vary depending on the mode of action of the substances, the organisms' physiology, and sensitivity to the substances. In addition, there are several processes and interactions that may occur leading to differences in responses: (1) chemical and physicochemical interactions, affecting exposure and bioavailability; (2) physiological interactions at uptake sites, interfering with the quantity taken up by organisms; (3) physiological and biochemical interactions during internal processing leading to a certain amount of substance available at the molecular target site; and (4) interactions at the target site(s), leading to different processes on intoxication.

Several models have been used to predict mixture toxicity, some based on old pharmacological models: the concentration addition and independent action models, which differ regarding the concept of the similarity or dissimilarity of chemical modes of action, respectively. These two models assume that there is no chemical interaction inside the organism and that chemicals may act as dilutions of each other (concentration addition) or are response additive, measuring the joint probability of effect from all chemicals in the mixture (independent action) [204].

In the work of Morgado et al. [205], a multiple biomarker approach was used to infer on possible time-dependent mechanisms of chlorpyrifos and mancozeb mixtures in the terrestrial isopod *P. pruinosus*. At recommended doses for agriculture practices, isopods revealed impaired detoxification and oxidative stress-related enzymes, although with some ability to recover and with juveniles showing higher stress upon exposure than adults. This difference regarding age or state was seen especially for energy-related parameters, showing associated metabolic costs.

The ladybug *C. maculata*, a beneficial insect in cropland, is prone to be exposed to pesticide mixtures. In the study of Freydier and Lundgren [66], second instars of ladybugs were exposed to nonlethal effects of 2,4-D and dicamba applied as pure active ingredients and in commercial formulations. The commercial formulations were more toxic than the active ingredients, showing adjuvants increase the efficacy of these compounds in nontarget species. Effects were observed at the survival level of organisms, growth, and the proportion of males produced. Although the authors conclude that dicamba did not increase the lethality of 2,4-D to ladybug larvae, no clear conclusion was derived regarding the interaction pattern occurring when these two formulations were mixed. This highlights the need for complex experimental designs, in order to cover a high range of exposure doses, which enables the prediction of toxicity using the already mentioned conceptual models and deriving interaction patterns like synergism or antagonism.

In the study of de Santo et al. [206], three soil invertebrates, *E. andrei*, *E. crypticus*, and *P. minuta*, were exposed in a laboratory trial to the herbicide metsulfuron-methyl and also to its mixture with mineral oil (as adjuvant).

The herbicide at the recommended dose did not represent any harm to the test species, but when used along with the mineral oil, effects on reproduction were observed for the three species. The combination of the herbicide and the mineral oil did not affect the feeding activity of soil fauna, in a field trial.

Besides mixtures of two, three, or four organic compounds, studies with more complex mixtures are scarcer. One example is the long-term study of Chevillot et al. [207] where *E. fetida* was exposed to complex mixtures of 7 neonicotinoids, 54 pesticides (including the previous 7 neonicotinoids), and 69 organic compounds (54 pesticides and 15 pharmaceuticals), using artificial soil at relevant field measured concentrations. Bioaccumulation of neonicotinoids under a joint exposure to low concentrations of multiple organic compounds was related to other individual (e.g., decrease in reproduction) and molecular (e.g., DNA damage) adverse effects.

Considering the predictions from the IPCC-Intergovernmental Panel on Climate Change, deviation of mixture toxicity from the expected patterns has also been highlighted due to changes in exposure conditions (e.g., soil moisture, temperature). In the study of Morgado et al. [131], the isopod *P. pruinosus* was exposed to chlorpyrifos and mancozeb at different soil moisture contents (mimicking drought and flood scenarios). Moisture did not affect the mixture toxicity, where additivity was the more parsimonious pattern observed. However, soil moisture content did influence the effects of individual pesticides and, as a consequence, of the pesticide mixture itself, with the major contribution for toxicity arising from the interaction of each pesticide with in the soil mixture.

In the study of Bednarska et al. [208], the earthworm *E. fetida* was exposed to chlorpyrifos, copper, and different temperatures (10 and 20°C). Chlorpyrifos significantly affected acetylcholinesterase activity, while Cu induced low levels of effect with no potentiation in joint exposures. The assimilation rate constant for chlorpyrifos was higher at 20°C for the single chlorpyrifos exposure, but also under co-exposure with Cu, the elimination rate constant behaved similarly, being only significant for chlorpyrifos single exposure.

5 Bioaccumulation in Edible Terrestrial Invertebrates: Link to Human Exposure

One of the major concerns for the next 30 years is how to feed the 9 billion people that the world is expected to have in 2050 [209]. Oceans are overfished, the land is overexploited, and climate change and water scarcity may lead to the search for innovative food production solutions [210]. The farming of edible insects has been presented as one of the best sustainable solutions, challenging the reuse of sub-products and other wasted feedstocks, reintroducing these components into the food value chain [209, 210]. Insects have a high content of nutrients and proteins, and their use as food has valuable environmental advantages over conventional meat, producing nutritional food sources with low environmental impact.

The data available on the transfer of chemical contaminants from different substrates to the insects is minimal, and there is a need to comply with the applicable food safety regulations, especially for residues of pesticides, veterinary pharmaceuticals, and PACs in insects, that could be taken up and accumulated by terrestrial invertebrates [211–213]. The majority of the studies evaluating the potential accumulation are on the black soldier fly (*Hermetia illucens*), one of the most used insects for food and feed for animals and humans.

The accumulation of veterinary pharmaceuticals may occur, as reported by Charlton et al. [211], who detected nicarbazin in *Musca domestica* growing on poultry manure. However, other studies report the opposite, with no accumulation of different antibiotics and one antiepileptic in *H. illucens* larvae grown in a composting system to produce organic fertilizer [213]. In order to combat infections and diseases in the rearing systems, antimicrobial agents should be used for prevention. Consequently, there is a need to find the right equilibrium between avoiding the toxic effects of the drugs for rearing insects and the need to control possible insect infections [214]. Insects used for food and feed are also prone to pesticide accumulation. Results indicate that pesticides with the higher log K_{ow} tend to bioaccumulate in edible insects, while those with a lower log K_{ow} tend to be readily excreted by the insects [212]. Fungicides were efficiently metabolized and degraded by *Tenebrio molitor* after exposure to substrate contaminated with metalaxyl, epoxiconazole, benalaxyl, and myclobutanil [215, 216]. Different PACs (benzo[a]pyrene, benzo [a]anthracene, benzo[b]fluoranthene, and chrysene) were also found in the fly larvae [211], but no maximum limits for PACs in animal feed are set.

Nowadays, this line of investigation is crucial, and more studies are needed for a better comprehension of how insects that serve for food and feed accumulate toxic compounds that could be biomagnified at higher levels in the trophic chain and, eventually, negatively impact humans. Because of that, joint efforts are needed to update the legislation for these types of food sources, as already is in place for other "traditional" food sources.

6 Final Remarks

Soil risk assessment of organic chemicals remains a challenge for the years to come. From the scientific literature addressing the bioaccumulation and toxicity of these compounds to terrestrial invertebrates, in the last 5 year period, the main gaps and research needs identified are related to:

– Biodiversity beyond standardized species. The majority of the studies available focused on groups of organisms used in the standardized laboratory tests (i.e., earthworms, collembolans, predatory mites, and honey bees), but little information exists on other terrestrial invertebrates with crucial roles in soil structure and functioning such as ants, beetles, ladybugs, snails, and spiders.

- Ecological relevance of dermal contact bioassays. A large number of studies evaluated the toxicity of organic chemicals through filter paper contact tests and/or topical applications. As soil exposure conditions are not considered, the outcome of these studies cannot be used by regulators for soil risk assessment or for specific chemical risk assessment (e.g., plant protection products).
- Ecotoxicological endpoints required. For soil risk assessment, LC_x and EC_x values are critical endpoints to derive insight into the hazard and risk of organic chemicals. Still, nowadays, risk assessors prefer to be informed on NOEC and LOEC as valuable endpoints, which are scientifically unprecise and biased and that could be replaced by EC_{10} or EC_{20}s. The majority of the studies do not report either of these ecotoxicological endpoints, which are of particular importance for new emergent organic chemical compounds.
- Inconsistency in units' reporting. The consensus is missing among researchers in reporting the units of ecotoxicological endpoints, which hampers their use in soil risk assessment. Moreover, reporting details on compound application methods, soil properties including bulk density and thickness of the soil layer to which a compound is applied, would allow for the conversion of units.
- Broader concentration ranges for low levels of biological organization. The growing number of studies covering effects at the sub-organism level represents a step further in understanding the modes of action of organic chemicals. However, the complexity of this type of study often hinders the inclusion of several test concentrations, not allowing regulators to consider them for soil risk assessment since no ecotoxicological endpoints can be derived.
- Scarcity of bioaccumulation studies for terrestrial invertebrates. The existing models and tools on bioaccumulation and toxicity of organic chemicals to terrestrial invertebrates are requested under the REACH regulation. The general lack of scientific literature on the toxicokinetics of organic chemicals in terrestrial invertebrates is primarily associated with the relatively high costs of chemical analysis and the absence of well-established and/or standardized analytical chemical methods and protocols for specific organic compounds and residues in the soil matrix and animal tissues. BAFs reported in the current literature are very often not standardized for organism lipid content and soil organic carbon content, being one of the limitations when comparing the results of different studies and for different test species. Beyond soil risk assessment, the knowledge of the bioaccumulation of organic chemicals, alongside the necessary optimization and development of quantification methods, could directly contribute to food safety regulations regarding the use of edible terrestrial invertebrates.
- Information on mixture toxicity. Most of the currently available studies on the mixture toxicity of organic chemicals consider the approach based on concentration addition and/or independent action recommended by ECHA. Albeit the advances, most of the studies focus on earthworms and binary and/or ternary mixtures, emphasizing the need to address the effects on other terrestrial invertebrates and for more complex mixtures. The latter should also cover commercial formulation components (e.g., adjuvants). Further complexity arises from climate change predictions, whose effects might potentially interact with the toxicity of

mixtures of organic chemicals, but such research is up to now scarce. Mixture toxicity studies in terrestrial invertebrates have generally been focused on individual-level endpoints. Additional research at both lower and higher levels of biological organization would improve one's ability to predict potential deviations from additivity by, respectively, improving the mechanistic knowledge on mixture toxicity and assessing the ecological significance of such deviations at the community or ecosystem level.

– Higher-tier studies. Although their long-known importance, not enough effort has been put on developing integrated approaches that account for species interactions and soil ecosystem functioning (e.g., microcosm and mesocosm studies) in the context of organic chemical exposure. Likewise, the soil compartment is still behind aquatic counterparts in terms of the development and application of modeling approaches to extrapolate the results of laboratory toxicity experiments to the field for organic chemicals. Such higher-tier studies are critical for improving the ecological realism of soil toxicity assessments and extrapolating the effects from laboratory to field conditions.

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Assessment of the Oral Bioavailability of Organic Contaminants in Humans

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Contents

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Abstract Bioavailability estimates the actual internal uptake or absorption of contaminants that enter the body (internal dose) and helps in providing a more accurate estimation of the human risks than the usage of total concentration. This is important for exposure assessment for children in relation to their hand-to-mouth activities. For example significant reductions of the bioavailability of long-term contaminated soils have been demonstrated using various animal models. The measurement for bioavailability involves various uncertainties for organic contaminants. It is crucial to determine the parameters that influence the results of bioavailability. This chapter provides a summary of the current state of knowledge for the determination of bioavailability for a range of organic contaminants. The information provided will be useful in facilitating further research efforts for the investigation of bioavailability of contaminants in conducting exposure assessments.

Keywords Bioaccumulation, Bioavailability, Diffusion, Dissolution, Electrophilic, Perfusion rate, Permeability rate

1 Introduction

Human exposure to various environmental contaminants has been of worldwide concern for decades. Babies, children and pregnant women are a particular concern in exposure assessment as the ingestion of environmental contaminants may have deleterious effects on children birth weights, cell function and lung functions [1]. Human health risk assessment (HHRA) is used for assessing the potential impact of a hazard, such as contaminants, on the health of a person, a group of people or a community. Some key steps in HHRA, such as the results from the exposure assessments and the information obtained from the hazard assessment helps us to address the question 'What is the magnitude of the exposure and the related adverse effects of the contaminants?' When this information is used in the risk characterisation step of HHRA, scientists can answer the question 'How far away is the remediation or the clean-up goal?'. Bioavailability is defined as the rate and the extent of the compound reaching the bloodstream [2]. Bioavailability, exposure dose and exposure frequency are three main factors in exposure assessment. Unlike exposure dose and frequency, the bioavailability assessment has more uncertainties in its determination due to the following factors: (1) the influences from properties of tested soils; (2) the fate and speciation of environmental contaminants that control their transport; (3) the sources of the environmental contaminants and (4) the model applied to determine bioavailability. Therefore, minimising the uncertainties in bioavailability assessment will significantly contribute to a more accurate exposure assessment.

Chemical speciation, bioavailability, bioaccumulation and toxicity are key issues in assessing the risk of contaminants to the environmental ecosystem and to human

health. The fate of organic contaminants in soil influences bioavailability as well as in windborne dust and sediments [3]. Bioavailability is a key parameter that determines the adverse effects resulting from the exposure to that substance. Therefore, the toxicity of compounds depends on their bioavailable fraction.

The estimation of bioavailability for organic contaminants is thus critical for conducting a human health risk assessment [4]. The latest National Environmental Protection Measure of Australia encourages the use of site-specific oral bioavailability data of contaminants when available [5]. However, there is considerable uncertainty regarding the estimation of bioavailability for organic contaminants, e.g. the selection of animal model, dosing matrix, selection of reference materials, exposure period, selection of biomarkers and identification of any metabolites. For example, compared to rodents and mice, swine are preferred for human health risk assessment as they share many similar traits to humans, such as body weight, anatomy, genetics and physiology [6, 7]. The greater cost for conducting a swine study limits the use of swine in the investigation of bioavailability. In addition to the cost constraint, there has been limited investigation of the interspecies correlation for different organic contaminants. Various dosing approaches have been investigated for the swine studies which have been conducted, e.g. using oil, food components, soil, artificial soil, sands. Both short- and long-term exposure periods have been reported as well, for a limited range of organic contaminants. Given the limitations, only limited animal studies have been conducted for organic contaminants: PAHs [3, 8–11], PCBs [12], DDT [13], and PFAS [14]. Thus the investigation of bioavailability for single and mixed compound organic contaminants requires ongoing efforts.

The bioavailability of a contaminant can be affected by sorption, cation exchange capacity and pH, as well as the presence of other interfering organic contaminants [8, 15–17]. For example, the reduction of the bioavailability of organic compounds in some contaminated soils due to the effect of various parameters has been reported, e.g. PAHs [18, 19].

We devote this chapter to the concepts of bioavailability, the measurements of in vivo bioavailability, sample collection, treatment and analysis of organic substances during bioavailability testing and the validation of in vitro studies with the use of animal models which mimic human physiology, biochemistry and anatomy. Furthermore, various factors and parameters which influence the assessment of the bioavailability of organic contaminants, including the comparison of both the in vivo and in vitro models, the selection and design of models for bioavailability assessment and how to minimise uncertainties from modelling, will be discussed in this chapter.

2 Concepts of In Vivo Bioavailability

2.1 *Concepts of Absolute Bioavailability and Relative Bioavailability*

Bioavailability in terms of contaminants reaching the human body by inadvertent ingestion through the hand-to-mouth behaviour of a child can be explained in a simple approach. It is basically the fraction of ingested contaminant that reaches the systemic circulation (blood) [20, 21]. However, in reality this involves both the rate and the extent to which a chemical moiety is absorbed from the gastrointestinal tract and becomes available at the site of action. Measuring the chemical at the site of action (at cellular or tissue level where the chemical exerts its adverse/toxic effects) is not always possible to achieve. Therefore, measuring the chemical in the general circulation is performed instead. This bioavailability value can range between 0% meaning no ingested contaminant reached the blood to 100% meaning all the contaminant that was ingested reached the bloodstream. The oral bioavailability of a chemical is usually <100% due to different factors including degradation or metabolism of the chemical prior to the absorption, incomplete absorption, and/or first-pass metabolism [22].

There are two terms, 'absolute bioavailability' and 'relative bioavailability' that are used as a measure of the systemic exposure of a chemical in both animal and human studies [21, 23]. In the following diagram, the authors explain the absolute bioavailability (AB) and relative bioavailability (RB) with respect to animal studies, conducted using soil for testing chemical bioavailability in soil. Figure 1 shows how a soil particle contaminated with B[a]P (Benzo(a)pyrene) enters a child's gastrointestinal system via the oral route and how it becomes bioavailable to the child who ingested the soil particle due to their hand-to-mouth behaviour.

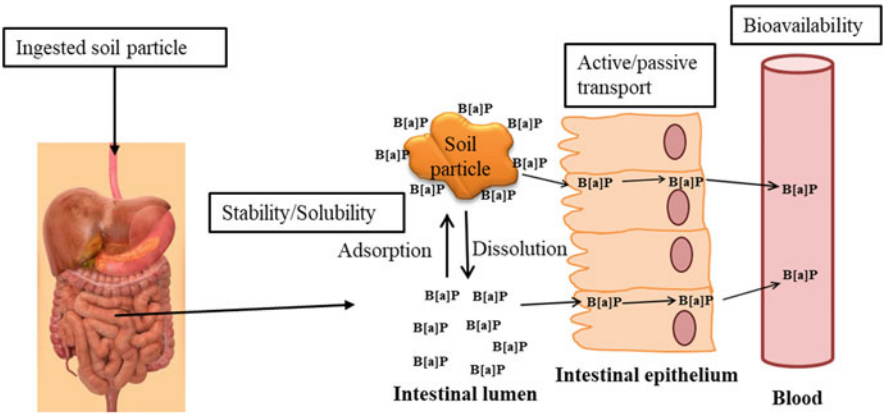

Fig. 1 Simplified diagram illustrating human intestinal absorption and bioavailability of B[a]P from an ingested soil particle

Absolute bioavailability is the amount of contaminant from the soil that reaches systemic circulation relative to an intravenous (IV) dose. An IV dose is assumed to have 100% bioavailability as the chemical is injected directly to the systemic circulation (blood). RB is the amount of chemical from the soil that reaches the systemic circulation relative to different formulations (nonintravenous) such as via an oral solution [6, 21].

Bioavailability of an organic contaminant like B[a]P can be derived from the area under the blood B[a]P concentration-time curves (AUC) corrected for the dose [3], using the results from B[a]P administered as an IV (intravenous) dose, oral solution or as spiked soils. Absolute bioavailability (AB) and relative bioavailability (RB) can be calculated using Eqs. (1) and (2), respectively. Absolute bioavailability (AB) can be calculated by comparing the AUC for the oral treatment with the AUC for the intravenous (IV) treatment [3, 23].

$$\mathrm{AB}\ (\%) = \frac{\frac{\mathrm{AUC_{oral}}}{\mathrm{Dose}}\,(\mathrm{oral})}{\frac{\mathrm{AUC_{IV}}}{\mathrm{Dose}}\,(\mathrm{IV})} \times 100\% \tag{1}$$

In Eq. (1), $\mathrm{AUC_{oral}}$ is the area under the blood chemical concentration-time curve for an oral chemical solution dose. $\mathrm{AUC_{IV}}$ is the area under the blood chemical concentration-time curve for an IV dose of the same chemical. $\mathrm{Dose_{oral}}$ is the dose of orally administered chemical (units: μg/kg). Dose $_{\mathrm{IV}}$ is the dose of intravenously administered chemical (units: μg/kg).

$$\mathrm{RB}\ (\%) = \frac{\mathrm{AUC_{soil}}/\mathrm{Dose_{soil}}}{\mathrm{AUC_{solution}}/\mathrm{Dose_{solution}}} \times 100\% \tag{2}$$

In Eq. (2) $\mathrm{AUC_{soil}}$ is the area under the chemical concentration in blood-time curve for an orally administered contaminated soil. $\mathrm{AUC_{solution}}$ is the area under the chemical concentration in blood-time curve for an orally administered chemical. $\mathrm{Dose_{Soil}}$ is the dose of chemical (units: μg/kg) in the orally administered soil, while $\mathrm{Dose_{solution}}$ is the dose of chemical (units: μg/kg) in the orally administered solution.

Bioavailability of an ingested compound has been described as consisting of three processes:

1. Release from the dose matrix
2. Transport across the intestinal epithelium
3. Reaching systemic circulation without being metabolised

$$\boldsymbol{F} = \boldsymbol{F}_b \times \boldsymbol{F}_a \times \boldsymbol{F}_h$$

where *F* is the bioavailable fraction of the oral dose; *Fb* is the fraction of an external dose that could be released from soil (bioaccessibility); *Fa* is the fraction of *Fb* that

could be transported across the intestinal epithelium; and *Fh* is the unmetabolised fraction of *Fa* that finally reaches systemic circulation.

2.2 Bioavailability Process and Limitations

There are a few limitations to the bioavailability concept. Researchers have access to only a limited number of body fluids including blood and urine. Even after years of bioavailability research, they are not able to access or mimic the fluids surrounding the target tissues/cells which play a major role in target stimulation and adverse effects.

When an organic contaminant such as PAHs (polycyclic aromatic hydrocarbons) reaches the gastrointestinal tract, it has to overcome several barriers to reach the target molecules at the cellular level and to cause adverse effects at cellular, tissue or organ level. One of the first barriers that contaminants entering through the oral route come across is the enzymatic activity and acidity of the gastric solutions. The food content at any given time affects the binding of these PAHs and hence the bioavailability [8]. These are all physio-chemical barriers that the contaminant encounters. Then once the contaminant reaches the intestinal epithelium, it meets the first biological barrier. This barrier utilises both active and passive transport of substances to facilitate the movement of chemicals through it. Most of these contaminants, which have the same characteristics such as valency will be utilising the same transport pathway to reach the systemic circulation. During the intestinal absorption, the molecules navigate using two mechanisms:

- Passive diffusion
- Carrier-mediated pathways

2.2.1 Passive Diffusion

During the diffusion process, solute molecules are transported from one place to another place through a physical barrier following a random molecular motion. If this diffusion is passive diffusion, it means that the molecules can cross the lipid bilayer of the cell membranes without having to spend energy. Instead the molecules move from a high to low electrochemical gradient across the membrane.

The positively charged smaller molecules can cross the cell membranes and move across the cell better as both sides of the lipid bilayer are negatively charged [22].

2.2.2 Carrier-Mediated Transport

Carrier-mediated transport is the transport mediated by membrane transport proteins. Chemicals entering the gastrointestinal tract may be hydrophobic, electrophilic,

contain a positive or negative charge or have a combination of these properties. Cell membranes are equipped with proteins embedded across them. These transport proteins allow larger positively charged molecules to travel in and out of the cells. This is called carrier-mediated transport [24].

2.3 Rate Limiting Steps in the Gastrointestinal Bioavailability

There are several steps that hinder the absorption of chemicals through the gastrointestinal tract. These factors affecting absorption can be listed as:

- Dissolution rate
- Perfusion/permeability rate
- Gastric emptying rate

These factors are included in Fick's first law (Eq. (3)) which provides a quantitative description of diffusion through a given surface area [25].

$$J = Aj = -AD\frac{dC}{dX} \tag{3}$$

where, J = rate of passive diffusion for nonionized molecules (unit: amount/time); A = the area through which diffusion occurs (unit: length squared); j = total flux per unit area (unit: amount/[time*area]); D = diffusion coefficient (diffusivity) (unit: area/time); C = concentration (unit: amount/volume); X = distance (unit: length); $\frac{dC}{dX}$ = change in concentration (chemical) gradient (unit: amount/volume).

2.3.1 Dissolution Rate

Dissolution is one factor that determines the absorption of chemicals in the gastrointestinal tract. When accidental ingestion of a soil particle by a child has occurred, once that soil particle is in the gastrointestinal tract, the contaminants carried by the particle can be released and some is reabsorbed. This release of contaminants from the soil particle is called the dissolution process. Therefore, the rate of dissolution is one of the rate limiting processes in the absorption of chemicals. Furthermore, intestinal absorption will be dissolution rate limited for those chemicals that are tightly bound to solid particles or in forms which do not favour dissolution.

2.3.2 Perfusion Rate

The rate at which the contaminant reaches from the site of absorption to the active site, where it acts to cause an adverse effect, will determine the concentration of this chemical in the blood. The gastrointestinal tract has a rich supply of blood vessels

and lymphatic vessels. Therefore, the rate of fluid flow in blood or in the lymphatic system, which is the perfusion rate, will affect the chemical concentration in the blood. For chemicals with a high dissolution rate and which can easily diffuse through membranes, the perfusion or the amount of blood or lymph supply to the intestinal epithelium will be the rate limiting step for the absorption.

2.3.3 Diffusion/Permeability Rate

When the soil is ingested by hand-to-mouth behaviour of a child, the chemical of concern needs to dissociate, disintegrate and dissolve in the gastrointestinal (GI) solutions, allowing them to be freed into the GI solutions for them to diffuse across the enterocytes (the cells of the intestinal lining). Once in the free dissolved form, the contaminants will cross the intestinal barriers and easily reach the body fluids such as the blood or lymphatic flow. When the concentration gradient across the intestinal membrane is high, the diffusion rate is high [22].

Diffusion coefficient or diffusivity (D) is an indication of the mobility of a chemical across a given diffusional barrier. It is affected by temperature, physico-chemical properties of the chemical entering the gastrointestinal tract and the diffusional barrier itself (Eq. 4). The Stokes-Einstein equation represents the relationship between the above-mentioned factors to the diffusivity (D);

$$D = \frac{kT}{6\pi\eta r} \tag{4}$$

where, D = diffusion coefficient in a given diffusional barrier (unit: area/time); k = Boltzman constant ($1.3806503 \times 10^{-23}$ $m^2kg/s^2/K$); T = absolute temperature (unit: degree); π = pi (no unit); η = viscosity (unit: amount/[time×length]); r = radius of the diffusant (unit: length).

As shown in the above Stokes-Einstein equation, diffusivity is inversely proportional to the radius of the diffusant. Therefore, in a constant temperature in a given diffusional barrier, as the size of the chemical that needs to cross the barrier increases, the diffusivity decreases. Hence the size of the chemical molecule limits passive diffusion. In addition to this, lipophilicity of the molecule affects the absorption process across the intestinal epithelium. Since the cell membranes are made of phospholipids, lipophilic chemicals such as organic contaminants will have a higher rate of passive diffusion compared to hydrophilic chemicals.

2.3.4 Gastric Emptying Rate

From Fick's first law, we can see that passive diffusion is directly proportional to the surface area of the gastrointestinal epithelium. Since the small intestine is equipped with villi and micro villi which increase its absorption surface immensely, while at the same time having a thinner membrane compared to the stomach, its absorption

process is efficient [22]. Having these physiological characteristics makes the small intestine an ideal site for chemical absorption. Therefore, the rate limiting factors that affect the time taken for the ingested food to reach the small intestine will determine the amount of contaminant absorbed. Some of the rate limiting factors for gastric emptying can be food intake or intense exercise [22].

2.4 Computational Modelling and Pharmacoinformatic Approaches to the Prediction of Oral Bioavailability

HHRA is a way of assessing the potential impact of a hazard on the health of a person, a group of people or a community [26]. HHRA has a few important steps:

1. Issue identification – Identify the problem or situation that is affecting human health, e.g. B[a]P contamination.
2. Hazard assessment: Assess the possible toxic or adverse health effects associated with this hazard (e.g. skin, lung and/or bladder cancer due to B[a]P exposure).
3. Dose response assessment: Understand the dose response relationship(s) with regard to the chemical/s of concern (skin rash or eye irritation with redness and/or a burning sensation with lower levels of B[a]P exposure and development of carcinogenic effects with increased level and duration of exposure in humans).
4. Exposure assessment: Develop a site or conceptual model(s) including pathways connecting the chemical source to the receptors. In other words, the model shows how the hazardous substance reaches the humans who are exposed to it. Here data is collected for analysis, e.g. analyse the soil, water and/or air concentration of the B[a]P in the affected area, identify who are exposed to this hazard and how they may have been exposed.
5. Characterise the risk: This step involves the data collected in the above-mentioned processes and uses the data to calculate and predict the magnitude and the nature of the health risk and the hazard posed in the past, present or future.

This HHRA process is an important step in the risk management process. Using these HHRA results, advice or recommendations are made by authorities to ensure that human health is protected. This involves a risk communication process [27]. Bioavailability is incorporated in the exposure assessment step in the HHRA process. For example in this step, the chronic daily intake of B[a]P derived from exposure to soil via hand-to-mouth behaviour can be calculated using Eq. (5) [28];

$$\mathrm{DI} = \frac{(\mathrm{Cs})(\mathrm{IR})(\mathrm{CF})(\mathrm{EF})(\mathrm{BA})(\mathrm{ED})}{(\mathrm{BW})(\mathrm{AT})} \tag{5}$$

where, DI = daily intake (mg/kg/day) (either mean daily intake or chronic daily intake); Cs = chemical concentration in soil (mg/kg); IR = ingestion rate (mg soil/day); BA = bioavailability (%); CF= conversion factor (10^{-6} kg/mg), EF =

exposure frequency (days/year), ED = exposure duration (years), BW = body weight (kg), AT= averaging time (days – period over which exposure is averaged) = EF × ED.

Bioavailability testing using suitable animal models that mimic human physiological and anatomical functions is a long, labour-intensive, ethically challenging and an extremely costly process [3, 17, 29]. A number of general pharmacokinetic principles and properties apply to all chemicals; these include absorption, distribution, metabolism, elimination (ADME), half-life and steady-state concentration, and linear versus nonlinear pharmacokinetics, which collectively contribute to the bioavailability of a chemical. When evaluating chemicals for potential health effects, scientists tend to use both chemical toxicity as well as exposure information [26, 30]. Chemical exposure shows how much of a chemical one may be exposed to via particular pathway(s) such as eating, breathing, drinking and/or skin contact.

Computational models that are used for predicting oral bioavailability and the human health risk from chemical(s) are very important in protecting the human health effects, especially the health of the most vulnerable people in a society such as children, pregnant mothers and the elderly. Suitable application of these predictive models will bring a rational and efficient way of using in vivo and in vitro as well as epidemiological data for the betterment of HHRA [17, 26, 31]. One such scientific approach used to understand the health risks of chemicals is physiologically based pharmacokinetic (PBPK) modelling. PBPK models are computational mathematical models which link and process how a chemical enters the body through various routes such as through eating, drinking, breathing and skin contact, the amount of chemical that reaches the bloodstream and how the chemical is carried into various tissues via blood, and how the body changes these chemicals (metabolism) and then eliminates the chemical. These models incorporate the human physiological, anatomical and biochemical processes [31, 32]. For example, these PBPK models are developed using mathematical values called "parameters" and equations that describe characteristics and processes of the body such as gender, body weight, blood flow rate and metabolism rate. These PBPK models provide a critical link between exposure information, bioavailability and chemical toxicity as well as being an important tool for using animal, in vitro, and computer-based experiments to inform chemical evaluations. The PBPK model relates the amount of chemical exposure to the receptors (humans) to the amount of chemicals found in the blood and tissues of the receptors at different points of time. For example if you are a child and you are exposed to a certain level of B[a]P from soil through hand-to-mouth behaviour, the PBPK models such as the one developed by the Agency for Toxic Substances and Disease Registry (ATSDR) from USA, has a generic, seven-compartment PBPK model for six priority volatile organic compounds (VOCs): benzene (BEN), carbon tetrachloride (CCl(4)), dichloromethane (DCM), perchloroethylene (PCE), trichloroethylene (TCE) and vinyl chloride (VC) [31]. If a well-designed PBPK model is used, depending on the blood/tissue organic chemical concentrations predicted, the risk of the adverse effect can be predicted using these models. The same process can be done for many other chemicals, thereby linking possible chemical concentrations in the environment to blood or tissue chemical

concentrations. This helps the researchers to prioritise which chemicals may have the highest likelihood of leading to adverse health effects.

Some authorities such as the US Environmental Protection Agency (EPA) use PBPK models to understand what animal bioavailability and toxicity data means for humans. This process is called 'extrapolation'. A PBPK model that describes a chemical in a laboratory animal can be utilised for humans by changing the parameters used in the model. Using human physiological data helps to predict more human relevant effects [33]. In other instances the PBPK models are used for 'route-to-route extrapolations' meaning the use of data from one exposure route to predict the risks of a chemical via another route of exposure [33]. For example if the scientists have laboratory data obtained for the exposure of B[a]P through the oral route, it may be used to develop a PBPK model, with necessary adjustments, in order to estimate the disposition of B[a]P following inhalation of air containing B[a]P in the workplace that is more relevant to a population in a certain exposure scenario such as working in coking, coal-tar and asphalt production plants, or in smokehouses or where local trash is burned [34]. PBPK models can be modified to better predict the adverse health outcomes by chemicals in a specific group of people like populations with certain diseases such as diabetes, heart conditions, obesity, or elderly people or children. These different groups have different physiologies, and their biochemical processes and chemical bioavailability can be affected as a result of it. Therefore, estimates of blood or any other tissue concentrations will be different among them.

Another such sophisticated risk assessment model was recently developed by Australian scientists, called raCARE™. It has been developed by scientists from CRC CARE (The Cooperative Research Centre for Contamination Assessment and Remediation of the Environment) and University of Newcastle, NSW, Australia. The model incorporates a chemical database, toxicity assessment values, exposure assessment parameters and calculations, and risk characterisations for various chemicals. This model also incorporates the RB values determined from lab studies to demonstrate the influence of bioavailability on the risk calculations. It estimates exposure at a population level and also relates external exposure and internal exposure using toxicokinetic models. It comprises a chemical database of about 60 chemicals and their physical, chemical and toxicological/reference dose data. The model guides users in carrying out human health risk assessments across several software modules, including chemicals, exposure, toxicity, risk, demography and RB prediction input/output, and Integrated Exposure Uptake Biokinetic and Benchmark Doses. Web-based pharmacokinetic (PK) models are freely available online (hhra.net) for a range of applications in data analysis, data simulation and parameter fit. In 2018/2019, the model was extended to include rankCARE™, software initially developed in collaboration with BHP to rank contaminated sites based on risk. Coupling the compliance model with rankCARE™ allows realistic prediction of risk from exposure to contaminants.

The ability of these models to help in predicting the approximate human health risks from the above-mentioned data, as well as physicochemical properties and the chemical structures of the chemical compounds, has a great practical benefit for

saving human lives. One of the crucial aspects of developing any computational model is the availability of relevant and accurate data sets [35].

3 Measurement of Relative Bioavailability (In Vivo Studies)

In the last two decades, in vivo animal studies were widely applied as biological systems to measure the RB of organics, and these results were extrapolated to humans to assess the risk of human exposure to contaminated soils [3, 8]. The basic approach used to obtain the RB of organic contaminations is to administer both organic contaminated soils and references to animals and measure the concentration of organic contamination in animal biomarkers (liver, kidney, urine, blood, etc.), using Eq. 2 to calculate the RB. Several animals have been used in in vivo studies, such as minipigs, swine, mice and rats, to measure the RB based on oral exposure of B[a]P and other organic contaminants [3, 36, 37]. Given the high cost and long duration of these experiments, and the trend of the ethical approval processes becoming more and more stringent, the in vivo studies have decreased in Europe and the USA. The number of in vivo studies has increased in China in the past few years due to suitable funding support and the high demand of research.

The factors such as animal species, body weight (BW), exposure period, exposure type and biomarkers of collection should be meticulously considered when designing in vivo animal studies. Each of these factors may influence the results of the measured RB. Here we will use rodents (rats and mice) and swine to discuss these factors since there are more data available from these species.

3.1 Animal Model Selection

Among the different animal species being tested, swine are the optimal models to measure organic RB if the researchers have sufficient funding and facilities. This is because swine provide a better simulation of the process of an infant's and child's growth, metabolism and development [3, 38, 39]. This is the reason that both the US EPA and Europe have employed the swine model to measure the RB of chemicals and validate the in vitro methods [39, 40]. The rats and mice are alternative choices to the swine model and are more affordable. Few studies has been carried out using mice and rats to measure B[a]P RB in soil [3, 41]. The only research that has been carried out to compare the interspecies difference was a comparison of RB between swine and mice/rats using the same spiked soils [3]. A significant correlation between the rat and swine models was identified, and the less than 1 slope factor between the RBrat and RBswine indicated that the RB in the rat would be 0.26 of that in swine. This is primarily due to a higher plasma concentration of B[a]P in rats in comparison to that in swine according to the dose responses of freshly spiked silica sand as reference material (Fig. 2). However, the potential risk for adverse

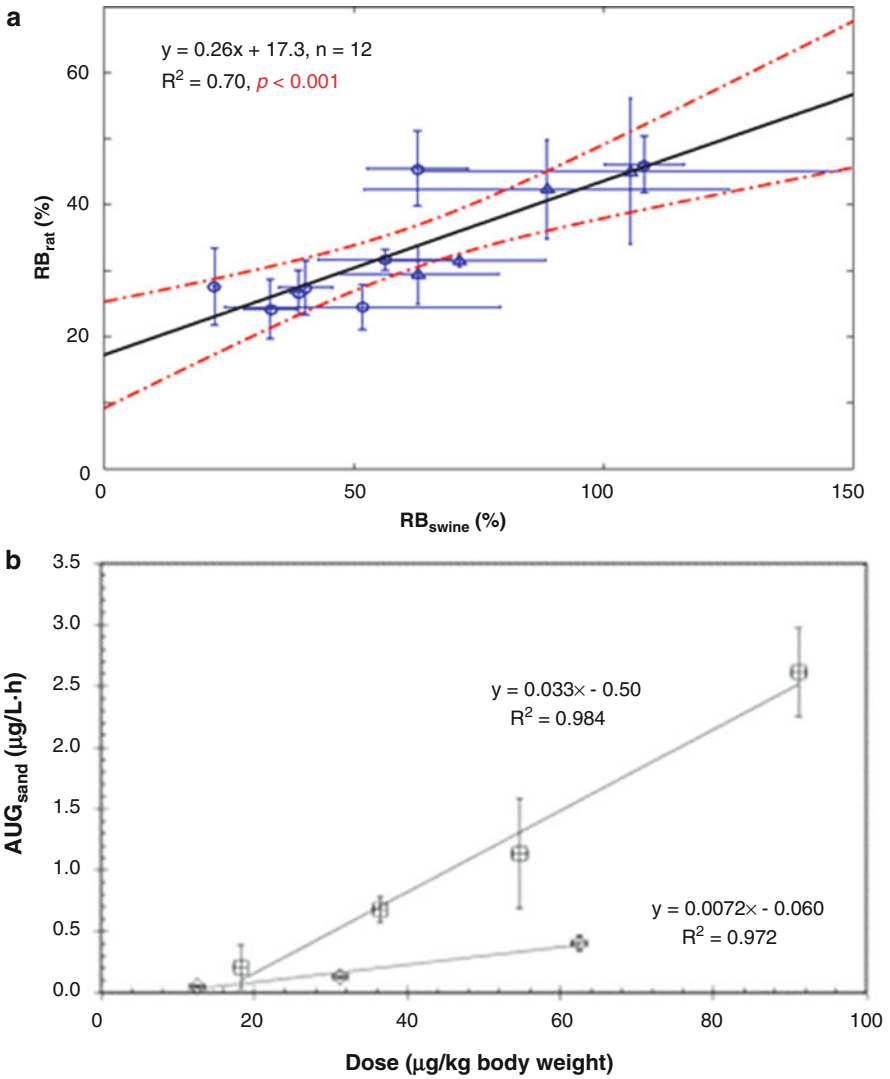

Fig. 2 Comparison of bioavailability results from rat and swine model (**a**) results from spiked soils ($n = 12$, triangle indicate data from four soils at Day 50 and circle indicate data from eight soils at Day 90); (**b**) dose-response relationship (square indicates rat and diamond indicates swine) (adapted from [3])

health effects was not clear, as for B[a]P, it is the active metabolites which induce the carcinogenic effect in cells. In future, more detailed studies focusing on both the uptake and metabolism of the organic compound should be carried out. Moreover, most animal studies were carried out at a much higher concentration that humans will likely be exposed to and so the bioavailability results from these studies may

not be valid at environmentally relevant concentrations for human exposure. The modern mass spectrum technologies, with careful sample processes, enable measurement of trace concentrations of substances in biological samples for calculation of the relative bioavailability. Investigation of soils from different sources and different mixed organic contaminant combinations to account for the natural or anthropogenic mixed organic contaminations is needed to provide more confidence in extrapolating results obtained from animal studies to the human scenario.

3.2 Body Weight

Dose-response assessment for human health using toxicological effect information from laboratory animals often requires extrapolation to humans. The US EPA endorses use of a body weight scaling to the ¾ power (i.e. $BW^{3/4}$) as a general default procedure to extrapolate toxicologically equivalent doses of orally administered agents from animal study results to humans, based on the overarching assumption that measurable characteristics of anatomy and physiology scale as a function of BW.

3.3 Exposure Period

Different from most toxicity studies, animal studies focusing on the measurement of contaminant bioavailability are usually carried out for a relatively short period of time. Many studies measure a substance's concentration over time, following a single dose. This approach is good enough to allow calculation of relative bioavailability of the contaminant compared to a reference material. Occasionally, multiple doses are required to enable a measurable concentration of the substances to accumulate in the biological samples. Hardly any bioavailability studies apply a repeated dose to reach a steady state.

3.4 Exposure Mode

Both fasting and fed states have been employed in previous studies, and the fasting state is more popular because this is equivalent to the situation where children and babies are prone to ingest soils when they feel hungry [39]. However, food constituents such as oil and protein are likely to increase the oral bioavailability of hydrophobic organic contaminants such as PAHs and PFAS. The lipid phase inside food could enhance the solubility of these compounds. Moreover, excretion of bile acid could form emulsions that facilitate transfer of these compounds to the

gastrointestinal epithelial cell. However, too much of the oil phase may retain PAHs in the food matrix and result in a lower absolute bioavailability. Large amounts of oil also result in lower oral bioavailability of PFOA, due to competitive uptake with lipids to the gastrointestinal system. Soil samples should be ideally ground to <250 μm to allow formation of a more uniform suspension for oral dosing. Often, a metal lubricated gavage tube/needle is used. The selection of the size of needle and gavage tube should be considered to minimise pain to the animal. The volume of solution administered to the animal should be decided based on the total volume of the animal's stomach. Normally, a volume of no more than 3 mL per time can be administrated to a rat with a BW of 300–350 g, and no more than 0.2 mL per time can be administrated to a mouse with a BW of 20–25 g. In the fed state, organic contaminated soils can be administrated into animal food according to the concentration of organic contaminants as well as the daily uptake dose of the animal. The input of contaminated soils should not influence the taste of animal food to avoid the changes in ingestion of organics during the exposure period.

3.5 Biomarkers

Biomarkers of animals such as liver, kidney, urine and blood are widely used to measure RB of organic contaminants in soils. Blood plasma is an important biomarker as data can be obtained at different time points during the whole experiment to monitor the trend of RB through determination of the AUC curve. When planning the time points for blood collection, the metabolisation half-time of the particular contaminant and the total blood volume for the selected animal should be considered. The total volume of collected blood samples should not exceed 10% of the total blood volume of the animal [42]. If repeat blood samples are required, then a maximum of 0.6 mL/kg BW/day or 1% of total blood volume of animal will be applied within 24 h [42]. The total blood volume of an animal can be estimated as 55–70% of the animal's BW. Generally, swine offer more choices of biomarkers such as repeated blood samples, liver, kidney, and urine, followed by rats, whereas mice are the least useful due to their small body mass [3, 8, 43].

3.6 Exposure Dose

It is critical to select the appropriate dose for a contaminant bioavailability study. Ideally environmentally relevant concentrations should be tested, provided a measurable concentration in the biological samples can be achieved. The latter is highly reliant on the detection limit of sample analysis. Bioavailability measured at an extremely high dose is likely to underestimate the risk for human exposure. Where the metabolism rate for elimination of the organic contaminant changes, the dose-response curve of the substance will no longer be linear. Valid comparison among

different treatments should ensure that the metabolic clearance is constant, in other words, the linearity of the dose-response curve should be checked beforehand. The range of the linear dose-response curve may vary when using different animal models due to the pharmacokinetics, e.g. AUC changes with the body weight scaling. The toxicity and metabolism rate of specific administered contaminants should also be considered; these data, however, are often not available.

Case studies of PAH RB for swine, rats and mice can be found as per below:

1. Swine [39]
2. Rats [3]
3. Mice [41]

4 Sample Collection, Treatment and Analysis for Organic Contaminants

Measurement of RB for organic contaminants normally involves usage of AUC for blood samples or different biomarkers, e.g. kidney, liver or urine, depending on the chemical or the metabolite of interest. The biological samples need to be treated to eliminate the matrix effects prior to being analysed by analytical instruments, e.g. GC-MS-MS (gas chromatography-tandem mass spectrometry), and/or LC-MS-MS (liquid chromatography with tandem mass spectrometry). This section summarises information on the sample collection, sample pre-treatment and analysis for the biological samples concerning organic contaminants.

How samples are collected, handled and stored is of the utmost importance in ensuring good-quality data from the study being conducted. In addition to conventional plasma, serum or whole blood, other matrices including tissues (virtually any tissue/organ in the body that the contaminant may get distributed to, e.g. kidney, liver), faeces, urine, etc. can be collected for the investigation of RB. The number of samples collected will depend on the experimental plan. Generally, blood/serum samples will be collected at different time points to derive the area under curve (AUC). Other biomarkers including liver, kidney, urine and bone will be collected at the end of the experiments. Special attention should be given to sampling containers and additives to avoid cross contamination and matrix effects. For example, PTFE tubes are not suitable for collection of PFAS contaminated serum/blood.

Various anticoagulants can be considered for maintaining the blood and subsequent plasma produced from the whole blood to ensure it remains fluid and relatively free from large clots, including ethylenediaminetetraacetic acid (EDTA), heparin, oxalate, citrate and fluoride. Additives such as antioxidants can be added in the sampling tubes for stabilisation of analytes of interest. A typical protocol for preparation of plasma includes selecting a tube with an appropriate anticoagulant, draw and gently mix blood and anticoagulant, centrifuge for 10–20 min (at 1,500–2,500 g), transfer plasma supernatant, and store at a specific temperature (e.g. −20°C or −80°). The major difference between plasma and serum is that no

anticoagulants are used in the collection of serum and all the fibrinogen and associated proteins are removed through the clotting process. Therefore, serum always contains less protein material than plasma, leading to a cleaner sample extract [44].

Duan et al. [3] investigated the bioavailability of B[a]P in contaminated soils through collection of serial blood samples using rat. The blood samples (~ 0.25 mL) were collected from tail veins of the rat in heparinized tubes over a time series (0.25, 0.5, 1, 1.5, 2, 4, 6, 8 and 24 h) following oral administration of spiked soil or sand. Plasma was separated immediately by centrifugation at 1,037 g for 15 min and about 0.12 mL aliquot of a sample was taken and stored in an amber glass vial (4 mL) with a PTFE-lined cap at −20°C until extraction. The extraction of B[a]P from plasma was carried out using a similar approach as Duan et al. [8]. Briefly, 1.5 mL hexane was added to each vial and subjected to sonication (40 kHz, 5 min) twice. The faeces samples were collected for each individual rat in the first 12 h post-oral dosing or IV injection and then every 24 h until 72 h. Before extraction faeces samples were stored at −20°C. The faeces samples were homogenised with anhydrous sodium sulphate (about three times the volume of the faeces) in a blender after thawing the faeces from −20°C to room temperature. The faeces samples were then extracted using an aggressive solvent mixture of dichloromethane and acetone (1:1) following ultrasonic extraction indicated in US EPA 3550, to estimate the total B[a]P in rat faeces. Briefly, each extract was sonicated, shaken and centrifuged prior to being combined for evaporation. An aliquot of the sample was then filtered prior to analysis using HPLC.

Juvenile swine were used for bioavailability testing for PAHs [8, 45]. The swine used [8] were 8–10 weeks of age at approximately 30 to 35 kg live weight. The animals were housed individually and carefully cared for according to the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (National Health and Medical Research Council: Canberra, seventh Edition, 2004). The animals were grouped randomly ($n = 3$) and dosed at 1.25 g soil on a dry weight basis per kg of pig body weight. Time-course blood samples (10 mL) were taken from the jugular vein catheters prior to feeding (0 h) and at 0.25 h, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after dosing. The blood samples (10 mL) were centrifuged immediately at 3,452 g for 20 min at 4°C and the plasma supernatants were stored at −20°C prior to extraction and analysis. Plasma aliquots (4 mL) were extracted by solvent using hexane by vigorous shaking and facilitated by sonication twice at 40 kHz for 5 min. The extraction tubes were further conditioned by shaking at 300 rpm for 40 min prior to being centrifuged for 30 min (4,000 g, 4°C) to separate the solvent phase. The solvent supernatant was combined and evaporated prior to analysis using HPLC vial.

In another study [45], swine were divided into four groups ($n = 6$) and exposed orally to PAHs in artificial soil, food (dough ball), corn oil, and a certified reference material (CRM soil), daily, for 7 days. The CRM soil is a natural clay soil collected from a contaminated site in the USA and contained 15 individual PAHs. Artificial soil was made according to Environment Canada guidelines [46] and was spiked with benzo[a]pyrene (BaP) and anthracene according to Reid et al. [47]. The food and corn oil were also spiked with BaP and anthracene. The swine were dosed with

5 mg kg-bw^{-1} daily of both anthracene and BaP in the artificial soil and food exposure groups. Swine exposed to PAHs in corn oil were given 2.5 mg kg-bw^{-1} daily of both BaP and anthracene. Swine exposed to PAHs in the CRM were given 0.17 kg-bw^{-1} daily of BaP equivalents. Blood samples were collected from the jugular vein of the swine on days 1 and 7 of exposure at 1, 2, 3, 4, 6, 8, 12 and 24 h post-exposure. Serum was separated by centrifugation and stored at 4°C pending analysis of PAHs. The tissue samples, including samples of stomach, jejunum, ileum, proximal colon and liver were collected after the swine were euthanised following dosing at 1, 2, 3, 4, 6, 8 and 12 h post-exposure.

Mice were used for investigation of PCB bioavailability in soils [12]. The female Balb/c mice weighing 20–25 g was used. Silica sand was used as a reference material for RBA calculation. The acid-washed silica sand (<250 μm, 0.2 g) was spiked with PCBs at 200 mg kg^{-1} for each congener and incorporated into mouse chow (Qinglong Mountain Company, Nanjing, China). Mice were exposed with chow containing 0.2 g of PCB-spiked sand for 4 d. After 4 d, adipose tissue, liver and kidneys were collected after the mice were sacrificed. Freeze-dried tissues were ground and extracted three times using n-hexane: acetone (v/v 1:1) in an ultrasonic bath. Lipid debris in extracts were removed by digestion with concentrated H_2SO_4 (3 mL) prior to being further purified using a column containing 2 g of anhydrous sodium sulphate, 2 g of florisil and 3 g of silica gel. Elutes were filtered prior to analysis.

The bioavailability for PFAS in food components [48] were also investigated using female Balb/c mice weighing 22–25 g. All mice were raised under standard animal house conditions (12 h light/dark cycle, 22 ± 2°C, and 50 ± 5% humidity) and were acclimated for 1 week before bioavailability assays. The food samples were spiked with PFOA and fed to the mice which were then fasting for another 5.5 h prior to the supply of standard mouse chow. Food samples containing 1 mg/kg PFOA were administered to mice at 0.3 g/d or 0.3 mL/d for 7 d. The liver samples were collected for PFOA quantification after the mice were sacrificed. The concentration of PFOA in liver was used for calculation of RB. The extraction methods for blood samples and liver samples for PFAS analysis are demonstrated in Fig. 3. Briefly, the blood samples (0.2 mL) were added to 50 μL internal standards (20 μg/L) followed by the addition of 1 mL 0.5 M TBAS and 2 mL 0.25 M Na_2CO_3. Methyl tert-butyl ether (MTBE) was added for extraction under shaking (300 rpm for 10 min) and sonication for 10 min. and the samples were then centrifuged (3,000 rpm for 10 min). The supernatants from three extractions were combined prior to evaporation under nitrogen gas. The whole livers were placed in 15-mL polypropylene (PP) tubes containing 10 mL of Milli-Q water and homogenised by blending at 10,000 rpm (PRO200, PRO Scientific, USA). An aliquot of the sample (1 mL of homogenised sample) was transferred to another PP tube, to which 1 mL of 0.5 M tetrabutylammonium hydrogen sulphate (TBAHS) solution and 2 mL of sodium carbonate buffer (0.25 M, pH 10) were added. 13C4 − PFOA (>99%, Wellington Laboratory, Canada) were added as a surrogate. After mixing, the slurries were extracted with 5 mL of MTBE by shaking for 20 min. After centrifugation at 4,000 rpm for 10 min, the MTBE layer was transferred into a clean PP tube,

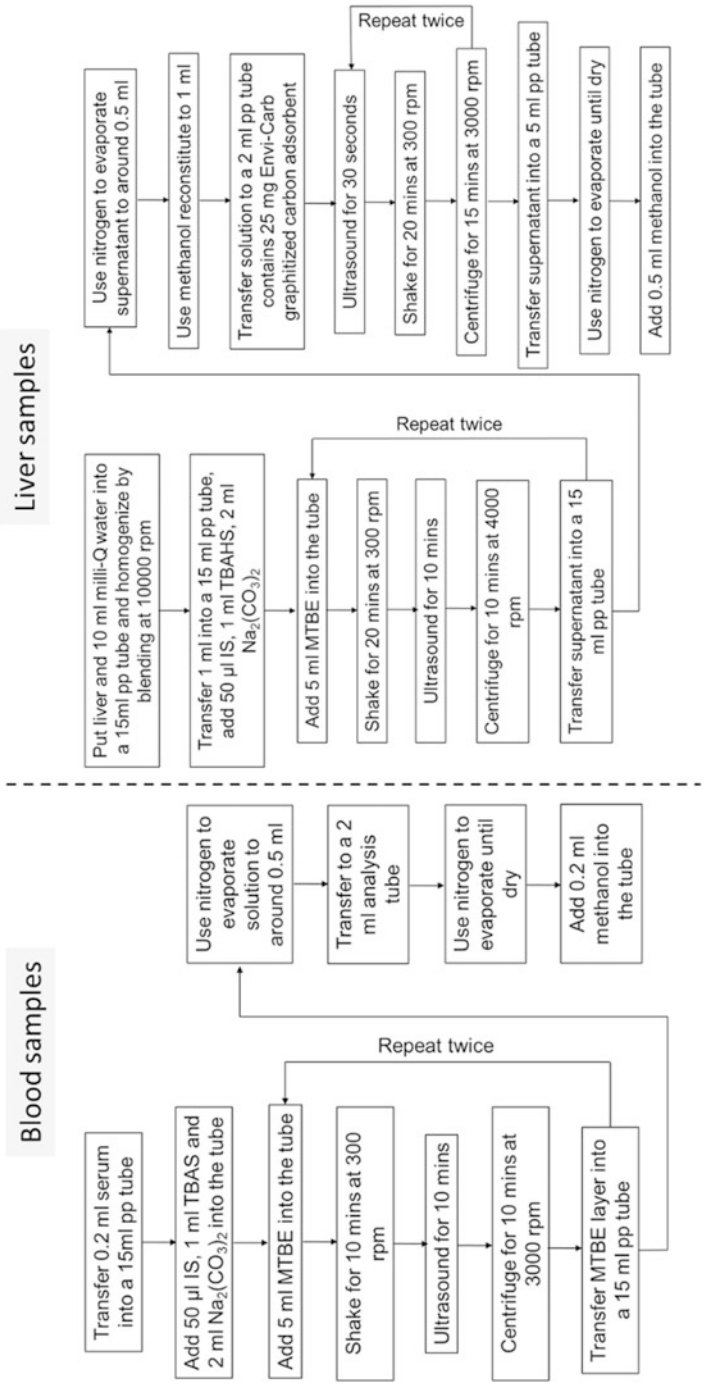

Fig. 3 Extraction methods for blood and liver samples for PFAS analysis

and the extraction procedure was repeated twice. The collected extract was reduced in volume to ~0.5 mL under nitrogen and reconstituted to 1 mL through the addition of methanol. The methanol extract was then purified by transferring to a 2 mL PP tube containing 25 mg of Envi-Carb graphitised carbon adsorbent. The extract was sonicated for 30 s, shaken for 20 min at 250 rpm, and then centrifuged at 3,000 rpm for 15 min. The purification procedure was repeated twice, and then all supernatants were combined in a 5 mL PP tube. The collected supernatants were then evaporated to near dryness under nitrogen and reconstituted in 0.5 mL of methanol for PFOA analysis [14]. A similar approach was reported by Joudan et al. [49].

5 Validation of In Vitro Studies Using In Vivo Studies

Animal-based experiments are required to study the bioavailability of hydrophobic organic contaminants (HOCs) in soil. However, there are critical issues associated with animal-based testing including associated cost and time implications, challenges with reproducibility, technical requirements, and ethical considerations. Hence, animal-based testing is being de-emphasised [50, 51]. Over the past few decades, the focus is shifting to the development of simple in vitro bioaccessibility measurements to predict bioavailability in an uncomplicated manner. In vitro or oral bioaccessibility refers to the dissolved contaminant concentration in simulated gastrointestinal fluid [52, 53] and is usually regarded as a conservative estimate of in vivo or oral bioavailability [54]. The use of in vitro bioaccessibility measurements may help overcome the critical issues associated with in vivo bioavailability measurements. However, in vitro bioaccessibility measurements must be validated against in vivo bioavailability and then standardised for realistic applications in risk assessments, as well as regulatory acceptance.

5.1 Correlations Between In Vivo and In Vitro Methods (IVIVC)

Much effort is increasingly being devoted to the development of in vitro bioaccessibility tests, but much less effort appears to be devoted to the validation of these tests. Although a number of studies have reported in vitro bioaccessibility to in vivo bioavailability correlations or relationships (IVIVC) for inorganic contaminants such as lead and arsenic [40, 55–57], published information on IVIVCs for organic contaminants is conspicuously inadequate in the literature. Figure 4 describes the IVIVC obtained for different HOCs (hydrophobic organic contaminants) from different studies and shows only four data points for HOCs, such as benzo[a]pyrene, dibenz[a,h]anthracene, PCB (polychlorinated biphenyls) #52 and PCB #118. Whereas in vitro bioaccessibility overestimated in vivo bioavailability

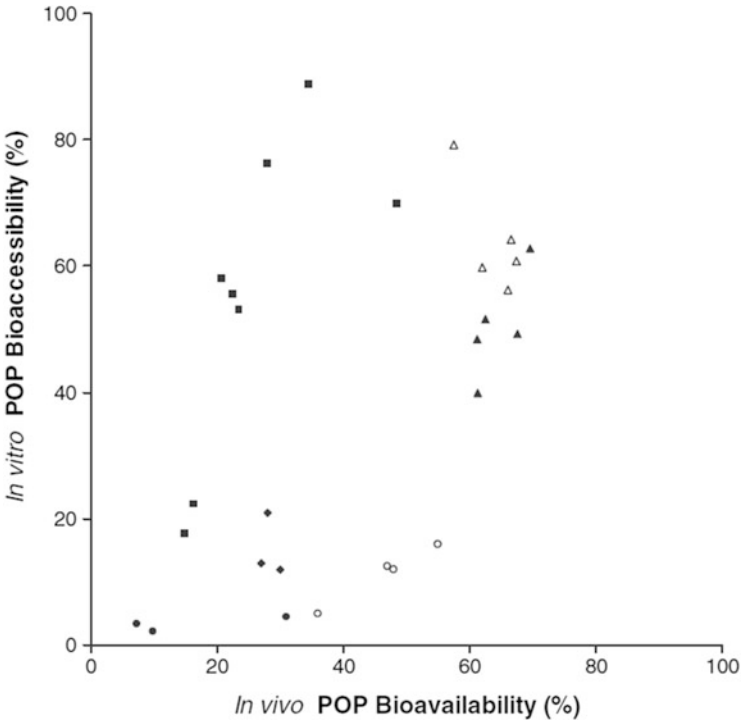

Fig. 4 Correlation between in vivo persistent organic pollutant (POP) bioavailability and in vitro POP bioaccessibility. Data are presented for phenanthrene (■ = in vivo method: rat [blood]; in vitro method: [58]) from [59], benzo[a]pyrene (● = in vivo method: mouse [urine]; in vitro method [60]; (○ = in vivo method: swine [urine, faeces]; in vitro method: [60]) from [61], dibenz[a,h]anthracene (◆ = in vivo method: swine [urine, faeces]; in vitro method: [60]) from [61] and PCBs (▲ = PCB #118, Δ = PCB #52: in vivo method: rat [blood]; in vitro method: [58]) from [62]. Adapted from [63]

for benzo[a]pyrene and dibenz[a,h]anthracene, in vivo bioavailability for PCB #118 may be underestimated [63].

An IVIVC ($r = 0.73$, $p < 0.05$) was reported for phenanthrene in a study that utilised four soils spiked at two different phenanthrene concentrations [59]; however, a relative oral bioavailability (RB) of over 100% was reported for three of the soils. Another study reported an IVIVC with an R2 of 0.81 for benzo[a]pyrene using seven soils; however, the study combined three soils dosed to mice and four soils dosed to juvenile swine, and combined observations that were based on different measured end points in the animals were utilised towards the determination of the reported IVIVC [61]. In a different investigation, the RBA of B[a]P in juvenile swine fed with spiked soils ($n = 8$) was significantly correlated with simple solvent shake extractions, such as dichloromethane/acetone (R2 = 0.67, $p < 0.05$) and butanol (R2 = 0.75, $p < 0.01$), but not with hydroxypropyl-β-cyclodextrin or Milli-Q water extractions [8]. Similar findings were also reported in a different investigation

recently [64]. However, bioavailability surrogates will need to mimic the gastrointestinal physiology of relevant animal models (such as swine or mouse), an attribute which is missing in simple solvent extractions. The few studies that reported IVIVCs for organic contaminants either utilised a small number of soils or mostly spiked soils. The fate and behaviour of HOCs in spiked soils are expected to be different from HOCs in well-aged field-contaminated soils [65], and hence oral bioaccessibility and bioavailability will be different as well. The credibility and regulatory significance of such reported IVIVCs will need to be established, particularly using a wide range of field-contaminated soils, prior to their use in routine risk assessments.

Often, in vitro bioaccessibility measurements of HOCs tend to underestimate in vivo bioavailability measurements [13]. In vivo bioavailability has been reported to be underestimated by up to 2,000 times using bioaccessibility values derived from a 'Fed ORganic Estimation human Simulation Test' (FOREhST) and fugacity modelling [41]. Such observations have been explained to result from the absence of a sink in in vitro assays that simulate the activities of the intestinal epithelium in the gut of test animals [66–69]. Recent method developments for in vitro bioaccessibility testing include an absorptive sink to counter the observed underestimation of in vivo bioavailability [53, 70]. Silicone-based materials, C-18 discs and Tenax are some of the absorptive sinks that have been utilised in this regard [10, 52, 54, 65, 68]. A positive relationship ($R2 = 0.53$, $p = 0.04$) was recently reported between in vitro bioaccessibility (as measured by a physiologically based extraction test that incorporated silicone rods) of benzo[a]pyrene in 8 soils (spiked at 50 mg/kg and aged for 500 days) and the RBA reported in a different study that dosed the same soils (spiked at 50 mg/kg and aged for 50 days and 90 days) to juvenile swine [65]. While the relationship reported was interesting, it was also noted that the soils compared were aged at different times [65]. It would be interesting to validate such observations using soils subjected to similar ageing periods and preferably contaminated soils. Still, in one study where an absorptive sink was included to measure the oral bioaccessibility of polycyclic aromatic hydrocarbons in field-contaminated soils ($n = 8$), a poor relationship was reported with in vivo bioavailability ($R2 = 0.45$, $p < 0.07$) [10].

Overall, considering the usefulness of in vitro bioaccessibility measurements in risk assessments, it is clear that there is a critical need for more studies that lead to the development of IVIVC for organic contaminants in soils and a need to develop standard operating procedures for bioaccessibility measurements.

6 Challenges and Expectations of Bioavailability Studies

Despite many decades of research on bioaccessibility and bioavailability, it is still challenging to accurately estimate contaminant bioavailability due to the uncertainties related to animal models, modelling uncertainties, different soil properties affecting contaminant bioavailability, speciation and source differences among

contaminants. More research efforts are expected to minimise uncertainties in measuring bioavailability and address the connection between contaminant speciation, soil properties and bioavailability.

Future remedial action must be taken on contaminated lands both in Australia and overseas based on prediction models or in vivo studies based on more accurate and reliable data obtained from studies on a high number of soils from varying contaminated sites, as well as with the use of properly validated bioavailability and risk predictive models. This section addresses some areas needing improvements with regard to bioavailability assessment.

6.1 *Improvement of Available In Vitro Models and Statistical Prediction Models*

The small intestine is the main place for contaminant absorption. The absorption in the small intestine is a dynamic process and involves exposing the ingested contaminants to the stomach as well as intestinal pH values. A bioaccessibility test has been proposed for organic contaminants from the BARGE group (the Bioaccessibility Research Group of Europe) called 'Fed ORganic Estimation human Simulation Test' (FOREhST)[71]. These models should be improved to address the mixed contaminant bioavailability. Future studies should be directed towards formulating bioavailability prediction models based on the contaminated sites of concern, such as depending on the contamination source.

Further studies should be performed in order to revise the NEPM (National Environment Protection Measures), EIL (Ecological Investigation Levels) and HIL (Health Investigation Levels) values with the use of a broad set of data on single and mixed bioavailability of organic compounds aimed at protecting human and ecological health.

6.2 *Prediction of Soil Properties to Compound Bioavailability*

Studies have demonstrated that soil properties may potentially predict bioavailability of compounds such as benzo(a)pyrene [8]. However, given that soil is a complex and heterogeneous system with varying physicochemical properties, the limited number of soils and sources of contamination studies make the prediction of bioaccessibility based on soil properties a challenging objective. More detailed studies could narrow the uncertainties concerning correlations between soil properties and bioaccessibility of compounds for different mixed compound contaminated soils.

6.3 Contaminant Forms and States of Speciation Relate to their Bioavailability

There are many limitations when samples are being examined with sophisticated techniques such as SEM, XRD and XANES, and sometimes the results cannot show all the chemical forms and binding statuses.

7 Conclusions

A risk-based approach incorporating bioavailability concepts is a smart and attractive evidence-based option in terms of both cost and in situ management of contaminated sites. The bioavailability measurements can be successfully incorporated into tools to reduce risks in contaminated sites. Therefore, more research should be carried out to reduce the uncertainties related to bioavailability assessment of contaminants. Steps should be taken to properly validate in vitro study results on organic contaminants with reliable in vivo studies with regard to single as well as mixed contaminated soils. Furthermore, standard operating procedures should be prepared detailing such validated in vitro methods. The researchers, environmental authorities and other stakeholders should reach agreement on the selection of proper bioavailability methods and suitable human health risk assessment models to be used for the site assessment and remediation processes. Priority should be given to provide proof on how a successful bioavailability concept can be used in the contaminated site management process but a similar emphasis should also be given to communicating the effectiveness of this process to the general public as well as the authorities and all other stakeholders in this process.

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Part III
Impact of Sorption Processes on Toxicity, Persistence and Remediation

Carbon Amendments and Remediation of Contaminated Sediments

Sebastian Abel, Inna Nybom, and Jarkko Akkanen

Contents

Abstract Sediments are archives of human activities and other environmental changes in the aquatic environment. In many cases they reflect past and present activities in their catchment. Therefore, elevated concentrations of many types of micropollutants, including hydrophobic organic compounds, are found in sediments. However, sediment is not necessarily the final sink for contaminants, as they can pose a threat to local biota as well as to human health. In cases where sediments are toxic or the contaminants bioaccumulate in organisms and/or biomagnify in food chains, remedial actions are considered. This chapter introduces briefly the most common sediment remediation methods including monitored natural recovery and environmental dredging and capping, but the focus is on more recently introduced activated carbon-based sediment amendment technology. All methods come with advantages but also with problems, and these may be contaminant and site specific.

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Overall, carbon amendment technology is shown to be a worthy alternative to the traditional methods. Further development is ongoing to broaden the applicability, for example, and promote biodegradation of the contaminants and environmental conditions under which it can be applied.

Keywords Activated carbon, Bioavailability, Contamination, Remediation, Sediment

1 Introduction

Sediments are an important part of the aquatic environments, offering habitats for a wide variety of organisms and playing an important role in the control of biogeochemical cycles. Sediments consist of material eroded from the watershed and materials produced within the water body itself. In many cases materials from land dominate. These reflect both natural and anthropogenic processes in the watershed. The solid matter in sediments is a mixture of organic and mineral particles of varying type and size. The quality of sediment varies both among different aquatic systems and within a water body. Heavier and bigger particles are more likely to settle in high-energy environments, whereas lighter and finer materials are deposited in low-energy environments. Seasonal and episodical variations between low- and high-energy conditions (e.g., high flow vs. low flow, storm events) create dynamic sediment conditions at certain sites.

In aquatic systems, sediments form archives of human activities and other environmental changes. They offer a sink for many types of micropollutants but especially for hydrophobic organic chemicals (HOCs) characterized by low water solubility and in most cases also persistence. Once in the sediment, these compounds are well preserved due to low temperature, darkness, and often anoxic conditions, where bio-, photo-, and chemical degradation are negligible. Since the sediments collect pollutants, they may also pose risks to local organisms. Problems can be manifested in two primary ways: (1) the sediment is toxic to benthic fauna, and/or (2) the contaminants bioaccumulate in organisms and can biomagnify in food webs, leading to toxic effects at the top of the food web. Thus, high chemical concentrations can be found, for example, in predatory fish, which can eventually lead to human health risks if the fish from the area are used as a human food source.

Often the total concentration of a chemical in sediment does not directly correlate with the bioaccumulated concentration in organisms or with the observed toxicity. The fraction of chemical that is responsible for the uptake and effects in biota is called the bioavailable fraction. Sorption to sediment (natural) organic matter was noted to play a major role in controlling bioavailability of many organic compounds [1]. However, this could not conclusively explain all variations in sorption [2, 3]. HOCs were shown to have orders of magnitude higher sorption to

sedimentary carbonaceous materials (e.g., black carbon) than to amorphous organic matter. In addition, sorption to carbonaceous materials was found to be stronger than to the amorphous organic matter, emphasizing the importance of black carbon-type materials as a sorbent phase in sediments [4].

These observations led to an investigation of different carbonaceous materials and their influence on bioavailability of HOCs in sediments [5, 6]. Activated carbon (AC), a manufactured carbonaceous material, was found to have a strong sorptive ability [7], and thereafter its effects in sediments were tested [8]. It was quickly deduced that this strong sorption of HOCs to black carbon and AC could be utilized for active remediation of contaminated sites. As summarized in the benchmark paper by Ghosh et al. [9], and discussed further in the latter parts of this chapter, contaminated sediments can be artificially enriched with these strong sorbents to lower the bioavailability and therefore the risks of HOCs.

In the water phase of aquatic systems, remediation of HOC contamination is "easy." Stopping the discharges will result in the contaminants being degraded, diluted, and/or transported elsewhere. With sediments, things are much more complicated. Due to the ability of sediments to accumulate and store contaminants, high concentrations can be found decades after the termination of the discharge. As the problem rarely solves itself, sediment remediation often needs to be considered. There's no universal remedy that would fit all sites, contaminants, and situations. Therefore, we need to continue developing new approaches and refine the old ones so that we have methodologies for all kinds of contamination situations.

This chapter will briefly introduce commonly used sediment remediation methods, with special focus on the use of AC amendments as a stabilizing method for contaminants in sediments. The chapter reviews recent scientific literature on the topic, including possible ecological risks and remediation potential, i.e., sorption capacity of AC amendments, and outcomes of field applications.

2 Sediment Remediation

2.1 Need for Remediation

The need for sediment remediation can arise for several reasons. The fundamental issues are the protection of ecological integrity and human health. The concentration of one or several contaminants exceeding the limits set in national legislation (e.g., sediment quality guidelines) can trigger further evaluation and lead to remedial actions. However, in many countries such guidelines do not exist [10], and, without binding legislation, decisions on whether to take actions are made case by case. Common reasons for initiating remediation include toxicity of the contaminated sediment to benthic organisms, bioaccumulation of the contaminants in the aquatic food chain, and associated human health risks through organisms used as food. The question as to whether remediation is required needs proper risk assessment and expert analysis of weight of evidence. In addition, the financial costs of the

remediation are always an issue. When the decision to remediate has been made, the most suitable remediation method is chosen after weighing the risks and benefits of the alternatives.

2.2 Traditional Methods

There are three common sediment remediation methods used for sediments contaminated with HOCs: (1) monitored natural recovery (also referred to as monitored natural attenuation), which entails closely following natural processes (physical, chemical, biological) that transform, degrade, immobilize, and/or isolate contaminants in the sediment; (2) dredging or excavation, whereby contaminated sediment is removed, treated, and landfilled; and (3) in situ capping, in which clean material is applied to cover the contaminated sediment on site, physically isolating it from the surrounding environment and reducing exposure of aquatic organisms as well as contaminant fluxes from sediment to water. Common to all three methods is that continuous monitoring is required to ensure that the remediation effort is effective in short and in long term. These methods do not work for new or ongoing contamination, and therefore the first step of all remedial actions should be to eliminate the active sources of the contamination.

All three approaches have their advantages, but none is trouble-free [11]. Dredging is the only method that removes the contaminants from the site. Monitored natural recovery causes the least disturbance, whereas capping and dredging disturb the habitat. Monitored natural recovery may entail restrictions in the use of the area, which could have economic impacts. Despite the possible disadvantages, for several years there has been a clear emphasis on in situ methods, especially capping and monitored natural recovery, for example, in the USA [12].

2.2.1 Monitored Natural Recovery

Monitored natural recovery is the least invasive remediation method, and it is based on natural processes [13]. In monitored natural recovery, the site is actively monitored, and the progress is assessed to ensure that the risks are reduced over time. Physical, chemical, and biological processes affect either the contaminants themselves or the surrounding environment. Physical processes include burial through ongoing sedimentation of clean material, which forms a natural cap. Concentrations at the site can also decrease as a result of downstream transport of particle-associated contaminants. Chemical attenuation processes can be divided into two types. Contaminants can be sorbed by sediment particulate matter, which reduces their bioavailability. Other chemical processes (e.g., hydrolysis) can lead to degradation or transformation of the chemicals to less harmful breakdown products. Microbial biodegradation can be an important biological process that transforms the

contaminants into more water-soluble and less toxic compounds, in some cases leading to full mineralization.

This method comes with some concerns [14]. Depending on the site characteristics and the remediation goals, recovery can take decades. Thus, this approach will affect and restrict the use of the area for a long period of time, during which the local organisms and humans are still at risk of being exposed to the contaminants. Also, environmental changes or extreme weather events may cause movement of the deposited sediment beds, re-exposure of the highly contaminated layers, and transport of the contaminated sediment into new areas. In addition, a method, which does not contain active remediation, can also encounter resistance among the public. To speed up the natural remedial processes, enhanced monitored natural recovery approaches can be applied, combining environmental engineering with monitoring. These enhancements include the locally restricted, active treatment of highly contaminated hotspots, for example, by removal (dredging) or isolating (capping) of contaminants. This can help in reducing the direct toxic effects of the contaminant to the local micro- and macrofauna, allowing contaminant-degrading organisms to thrive [15].

2.2.2 Dredging

Environmental dredging refers to removal of contaminated sediments from predetermined areas and relocation and treatment of the dredged material. Typical equipment for dredging includes grabs, buckets, or different types of suction dredgers. In theory, environmental dredging is straightforward, but practice has shown that it is not always successful. In addition, the method disturbs the local ecosystems [16, 17]. Increased turbidity of water due to suspended sediments and their deposition to different sites throughout the water body can result in habitat changes. Dredging can also re-expose contaminants buried deep in the sediment, and resuspended contaminated sediment particles can increase the concentrations of contaminants in water. This can lead to an increase in exposure and thus increased tissue concentrations in organisms. Longer-lasting problems can be caused by dredging residuals, i.e., contaminated material that is not dredged but is instead left on site or material that escapes from the dredge [17, 18]. Despite the disadvantages, environmental dredging is still an actively used remediation method, especially when persistent chemicals need to be removed from the aquatic environment.

2.2.3 Capping

Traditional capping is a remediation method where contaminated sediment is covered with clean materials on site. The capping materials are inert, such as easily obtainable rock, gravel, and clean sediment, which are used to create up to 50 cm thick layers on top of the contaminated sediment. Along with the natural materials, synthetic materials such as geotextiles can be used. The cap physically separates the

contaminated sediment from the surrounding environment, preventing resuspension and transport of the contaminated sediment. At the same time, clean habitats are created for a new succession of benthic communities and other organisms.

The limitations of the traditional in situ caps are, for example, that the thick caps are not suitable for shallow sites. Also, the geotextiles can contain persistent and toxic compounds [19]. On the one hand, capping has been shown to be efficient in reducing the diffusion of contaminants through the caps due to the increased diffusion distance [20]. However, the caps do not stop the migration of the contaminants through the cap in all cases due to advection with groundwater seepage [21]. To overcome some of these challenges, active capping and thin layer capping have been developed [22], where the capping materials contain sorbents such as AC with a high affinity for the contaminants (see Sect. 3.1).

3 Sediment Remediation with Carbon Amendments

Remediation with sorbent amendments is a relatively new in situ remediation option that has been extensively studied since the early 2000s. In this remediation approach, sorbent material is added on top of or mixed into the sediment. The aim is to stabilize the contaminants in the sediment by reducing their freely dissolved concentrations. The contamination is not physically removed, but rather the treatment targets to the reduction of the bioavailable and bioaccessible fractions. In general, the term bioavailability includes internal processes in the organism in addition to processes in the environment [23, 24]. However, here the bioavailable fraction is defined as the fraction of a chemical in the environment that is free to be taken up by organisms and pass biological membranes. In the aquatic environment, it is equal to the freely dissolved fraction. The bioaccessible fraction can be defined as the fraction of a contaminant in sediment that can desorb and thus become bioavailable.

There is a general consensus within the scientific and regulatory community that the bioavailable and bioaccessible fractions of a sediment-associated contaminant are the key drivers for the observed effects of a substance in the environment [25]. Bulk sediment concentrations, which are measured by total, exhaustive extraction of the contaminated sediment with solvents, are far less indicative of the actual risk they pose. Measuring the bioaccessible fraction relies on non-exhaustive extraction of the sediment, using adsorbents instead of organic solvents. The adsorbent is mixed with a sediment-water slurry for different durations to assess fast (<24–48 h mixing) and slower desorbing fractions of a contaminant. The total capacity of the applied sorbent must exceed the fraction of interest, since the saturation of the adsorbent will lead to underestimation of chemical concentrations. The most widely used sorptive phase in this application are Tenax® beads, which provide a high capacity for organic contaminants and are easily separable from the sediment slurry. Numerous studies have demonstrated that Tenax extraction can be used reliably to assess the bioaccessible contaminant fraction in sediments (as reviewed by Lydy et al. [26]).

Bioavailability can be approximated by focusing the measurement on porewater concentrations or chemical activity of a contaminant. Several approaches have been proposed and validated over the recent decades. Solid-phase microextraction (SPME) and other passive sampling methods, such as silicone-coated jars [27], have been widely used due to their simplicity and comparably low costs. The key difference to desorption methods, e.g., Tenax extraction, is the non-depletive nature of the passive sampling [28]. Instead, a thermodynamically stable equilibrium is formed between the chemical activities of a compound between the sampler medium (SPME fibers, silicone, or other polymers) and the aqueous phase. Distribution coefficients, specific to the used sampling material, are then used to calculate the porewater concentration of the contaminant. An overview on the underlying concepts of different approaches to passive sampling has been given by Mayer et al. [29].

Several materials have been tested to constrain contaminant fluxes from sediment to water, for example, apatite, chitosan, and thiols to sequester metals [30–32] and organoclays and AC for organic contaminants [17, 33]. Likely due to its established use in water and wastewater treatment, AC amendments have received most attention as a new in situ remediation technique for contaminated sediments [17, 34–36].

In the years since AC-based sediment remediation has been proposed, the remediation efficiency of the method has been evaluated in numerous laboratory and field studies using passive sampling approaches and a range of organisms. The applicability and suitability of AC treatments have been demonstrated for sediments containing polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), pesticides, and other similar chemicals [37–43]. In addition, organo-metal-contaminated sediments have been successfully remediated with AC amendments [44, 45]. In many of these studies, the application of AC reduced the contaminant bioavailability by up to 95%.

3.1 Activated Carbon as a Sorbent

AC is produced by activating a carbonaceous base material, such as charcoal (biogenic ACs or activated biochars) or black coal (petrogenic ACs). Most commonly, this activation is achieved by applying high temperature (>500°C) reinforced with a controlled amount of oxidizing gas (e.g., steam, oxygen, or carbon dioxide). Alternatively, activation may be carried out chemically, using corrosive reagents [46]. Both activation methods aim at opening the pore space in the carbonaceous base material, leading to an end product with highly porosity and a high specific surface area that can easily exceed 1000 m^2/g [37].

The increase in surface area increases the number of potential sorption sites for chemicals. Therefore, the highly porous AC, with high specific surface area, has a strong affinity for HOCs. Before remediation the contaminants are presumably at an equilibrium between (organic) sediment particles and the sediment porewater. Once AC is brought into the system, the contaminants begin to redistribute from their

previous compartments to the introduced sorbent particles. This redistribution is characterized by an initial, fast phase, in which the fast-desorbing fraction of the contaminant load is sequestered by the AC. This leads to quickly decreasing aqueous (porewater) concentrations of the contaminants within a few days to a month [47, 48]. The slower desorbing fraction of HOCs, however, may require several months before it is transferred to the applied AC [8]. In general, the share of this slowly desorbing fraction rises with increasing hydrophobicity of the contaminant [49].

For an accurate assessment of AC remediation efficiency, it can be important to consider this two-stage mass transfer of contaminants to the AC particles. When only the quickly reduced aqueous concentrations are measured, the actual success of the remediation might be overestimated. The remaining, slow-desorbing faction that is still bound to sediment particles might not be reflected in the aqueous concentrations. Nonetheless, it can still become bioavailable to organisms that ingest the contaminated sediment particles due to an increased solubility of the contaminant in the presence of digestive fluids [50, 51]. This is reflected to some extent by the – on average – higher measured remediation efficiencies of AC amendments in studies, which focus on passive sampling methods (Fig. 3). McLeod et al. [52, 53] found that the physical mass transfer of contaminants from sediment particles to sorbent particles is a more important mechanism behind the beneficial impact of AC amendments, compared to the reduction of aqueous concentrations. Therefore, the AC amendment's efficiency in reducing concentrations in biota exposed primarily through sediment ingestion may be lower than expected based on the reduction in the freely dissolved concentration. Hence, measuring the freely dissolved porewater concentrations (passive sampling) or freely dissolved and bioaccessible fractions (e.g., different extraction methods) can be suitable methods to approximate bioaccumulation reductions after sorbent remediation. However, directly measuring the uptake into benthic organisms will yield a much more accurate evaluation.

3.2 Applicability

The high porosity and light texture of AC poses technical challenges to its application in situ. Two different application methods for AC amendments have been used: (1) mixing AC into the sediment on site or applying it premixed with inert material, such as sediment or sand, and (2) direct application as a thin-layer cap. Mixing AC into the sediment during application helps to stabilize the amendment and provides homogenous distribution of AC particles in the biologically active upper sediment layers. Active mixing can significantly accelerate the mass transfer of contaminants, thus yielding fast control of HOC fluxes. On the other hand, mixing technologies may increase the operational costs of the amendment, especially in deeper water [36]. In suitable areas, such as tidal zones [39] or relatively shallow water bodies [64], mixing AC into the sediment can provide quick and efficient contaminant sequestration.

In thin-layer capping, AC is applied to the sediment surface, which can reduce the need for heavy or specialized equipment and thus make the remediation more cost-effective. To enhance the settling, and to improve the placement accuracy, inert binders (e.g., clay) or weighting agents can be added. With direct application, the addition of foreign material to the site is minimized. Therefore, thin layer AC capping may be especially suitable at sites where retaining the water depth is vital, as AC caps generally are only few centimeters thick [43]. Thin-layer capping with AC can create a closed barrier preventing contaminant fluxes from the sediment to the overlying water [65], which can be a strong advantage, especially for heavily contaminated sites where an immediate improvement is desired.

Long-term monitoring of in situ field studies has shown that AC can be well retained in the amended sites. No losses of AC were observed in the lower Grasse River (NY, USA), from plots amended by mixing or by thin-layer capping, during 3-year monitoring [40, 64]. Burial of the amendment due to newly deposited sediment was observed. In addition, downward fluxes of PCBs from water to sediment were reported during the postplacement monitoring period, suggesting that AC can reduce PCB fluxes from sediment in the long term. High stability of a thin-layer powdered AC cap was observed also on active harbor in Bremerton (Washington, USA), after 33 months of deployment [43]. Slightly increasing performance of the amendment over time was reported with increasing reductions of PCB availability to a clam *Macoma nasuta* and polychaete worm *Nephtys aecoides*. Similarly, post-amendment monitoring in Greenland fjords (Norway), 5 years after thin-layer capping, showed increased effectiveness of powdered AC containing caps in reducing polychlorinated dibenzodioxin and dibenzofuran (PCDD/F) fluxes from sediment to water [65]. However, thin layer caps are susceptible to disturbance by environmental factors such as currents or winds, and losses of AC from the amended sites have been reported. The AC levels can be reduced by lateral mixing with untreated surrounding sediment carried into or the AC spread outside the remediated areas [54, 66]. In addition, ongoing sources of contamination, such as newly settling contaminated sediment particles, may hinder the remediation efficiency [54, 67]. On sites with low sediment stability, deep water, or slopes, additional measures such as covering the AC amendment with a layer of sand, clay, or gravel may be required [36].

In addition to the amendment stability, it is important that applied AC gets mixed into the sediment, as this reduces the diffusion distances for the sediment-associated contaminants and facilitates a more complete mass transfer to the AC particles. In thin-layer capping, incorporation of AC into the sediment relies on natural process such as bioturbation, sediment deposition, or other physical processes. For example, in tidal estuarine wetland in upper Canal Creek in Maryland (USA), close to 100% of AC was retained after 10 months of deployment, but the vertical mixing of AC into the wetland sediment proved to be slow [66]. The burrowing activity of the local benthic fauna plays a key role in incorporating AC into the sediment [54, 66, 68, 69]. Thus, AC amendment-induced effects to bioturbating benthic fauna are an important factor in thin-layer cap stability.

In general, AC amendments are considered less costly compared to traditional dredging and capping. The main costs of the AC amendments are the material and the operational costs. The materials costs are relatively low, approximately 2–3 €/kg AC [36]. Operational costs, however, are highly dependent on the amendment technique and site characteristics. The selection of the most suitable approach is always determined by site-specific characteristics, as well as the ecological and health risks posed by the contaminated site [36, 70].

3.3 Remediation Efficiency

The raw material used to produce the AC seems to be of only minor importance for the remediation efficiency. Experimental studies have shown both equal [55] and lower performances of biogenic ACs compared to petrogenic ACs in HOC bioavailability reduction [71, 72]. The difference in performance is likely explained by the pore structure of the ACs in synergy with the molecular size of the target contaminant. Certain biogenic ACs may exhibit smaller pores, which can lead to fast pore blockage by large molecules and thus reduced adsorption capacities [71, 72].

The most dominant factor determining the contaminant binding potential is the particle size of the ACs. It is evident that the remediation efficiency markedly increases with decreasing sorbent particle size. As reviewed by Rakowska et al. [34], finer powdered ACs (PACs) have two major advantages. Firstly, the distribution within the sediment is denser for PAC, given that the same doses are applied. This reduces the required contaminant diffusion pathways between sediment and AC particles. Secondly, the external surface area becomes larger in proportion to the quantity of applied sorbent. The accessibility of pores within the AC particles can be obstructed and, thus, especially for larger molecules, hamper their adsorption [71]. This issue can be further exacerbated when the outer pores of larger AC particles (granular AC; GAC) are clogged by sediment particles, reducing the available pore space for contaminant adsorption significantly [56]. In the most severe published cases, this has caused GAC to bind insufficient or even no measurable amounts of contaminants [37, 52, 56].

The remediation efficiencies of AC amendments show a clear dose-response relationship (Fig. 1). In laboratory studies it has been shown that even small PAC doses below 0.5% of the amended sediments' dry weight (dw) can lead to a measurable reduction in the bioaccumulation of organic contaminants [55, 78]. Increasing the dose is initially followed by a fast increase in remediation efficiency, as reviewed, for example, by Janssen and Beckingham [35]. The maximum measured remediation efficiency reported in the literature varies and seems to depend on the utilized test setups. The highest efficiencies with the least variation are measured when contaminant bioavailability is determined with passive sampling methods (Fig. 1). When actual bioaccumulation is measured, the observed variation in the results depends largely on the test species used. In most laboratory studies, the achieved reduction of freely dissolved contaminant concentrations and

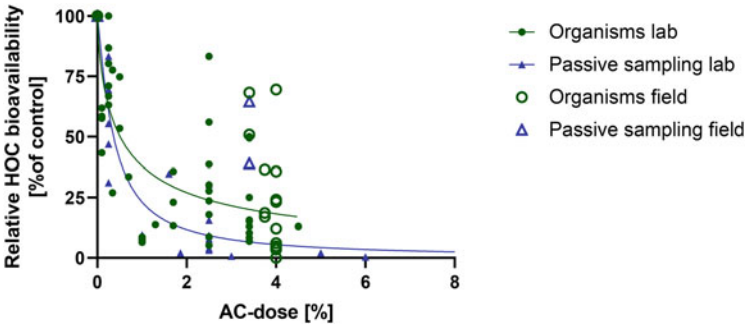

Fig. 1 Published remediation efficiencies vs. applied activated carbon doses (powdered activated carbon particle sizes <300 μm; mixed into the sediment; doses as sediment dry weight %). The datasets were grouped by sampling methods. Studies using organisms measure the actual bioaccumulation from the amended sediment into test organisms, while passive sampling approaches measure freely dissolved porewater concentrations of a contaminant. Closed symbols show results from laboratory (lab) studies, while field results are shown as open symbols. Due to the short span of applied AC doses in field trials, no dose-response curve was fitted. R^2-values for the remediation efficiencies obtained from laboratory studies are 0.46 (organisms) and 0.84 (passive sampling). Data from [37, 40, 52, 53, 55, 57–61, 73–77]

bioaccumulation in organisms reaches up to 95%. The AC doses needed to reach these drastic reductions in contaminant bioavailability often lie below 2.5%. Only few laboratory studies have found significant benefits from further increasing the AC concentrations within the contaminated sediment. Under field conditions, however, higher doses are often required to reach similar remediation efficiencies. In part, this is due to the risk of loss of applied AC under environmental conditions. In such cases, the effective AC dose can be significantly lower than the applied target dose [54, 68]. This is especially critical for field applications, as the time required for AC amendment to develop its full remediation potential can be much longer (up to several years) than in a confined laboratory microcosm [40, 41, 65]. Nonetheless, if a sufficient amount of sorbent is retained over longer time periods, AC amendments have proven to yield similar effectiveness in field trials as shown in laboratory studies [70].

One of the fundamental principles of the remediation method is that the reduction of contaminant bioavailability and bioaccumulation in benthic organisms will lead to reduced HOC transport in the aquatic food chain [9, 70]. Based on laboratory studies, it has even been suggested that AC treatment could reduce HOC transport from aquatic to terrestrial food chains if the concentrations in benthic invertebrates going through metamorphosis (developing from sediment dwelling larvae stage to flying adults) decrease as a consequence of reduced HOC bioavailability [58]. However, experimental field-scale evidence to support the reduction of HOC food chain transfer is scarce. Kupryianchyk et al. [74] demonstrated reduction in bioaccumulation of PCBs in a food chain (macrophytes, zooplankton, macroinvertebrates, and fish) with 4% sediment dw PAC treatment, and an order of magnitude decrease in concentration was observed in fish. However, the

experiment was executed in artificial 15-m long ditches, with benthic invertebrate communities and fish established at the beginning of the experiment. The influence of the interrelated surrounding areas is a much more important factor in the field than in (semi-) closed experimental systems. Thus, the effects of AC amendment on higher trophic levels may be less apparent in the field or require longer time to be established.

3.4 Secondary Ecological Effects

Organisms that exhibit severe adverse reactions to an untreated contaminated sediment will generally only see benefit from AC amendments. This can be the case for sites with either highly sensitive benthic fauna or high contaminant levels. Although there may be hidden adverse effects caused by the AC itself, the benefit of a reduced contaminant toxicity outweighs the risk in these cases. Tomaszewski et al. [75], for example, found a notably increased survival rate of the mussel *Mytilus edulis* after contaminated sediment was amended with AC. Kupryianchyk et al. [73] found that highly toxic sediment (PAH content >1,100 mg/kg) became habitable again for the benthic macroinvertebrates *Gammarus pulex* and *Asellus aquaticus*; there was no survival of the test organisms in the untreated sediment.

Nevertheless, a large share of sites that are remediated with AC does not show these extremely high levels of contamination. For such sites, there are indications that AC treatments may cause direct adverse effects. Undesired, negative side effects of the AC amendments are most strongly seen in deposit-feeding benthic species.

Analogous to the remediation efficiency, the particle size and dose of AC are the major factors determining the magnitude of adverse effects [35]. Finer PAC particles can cause severe effects to organisms, while the adverse effects of the coarse GAC particles only manifest at unrealistically high doses or not at all (Fig. 2a). Sediment-dwelling benthic worm *Lumbriculus variegatus* has been observed to avoid ingesting PAC-amended sediment [57, 78], and AC exposure which induced internal damages on *L. variegatus* and *Chironomus riparius* gut wall microvilli layer has been reported [57, 79]. Co-sequestration of nutrients by the AC is another suspected cause of the observed adverse effects on benthic organisms, although it is not considered to be the main contributor [80]. Despite several studies reporting adverse effects of PAC amendments, the mechanism causing the negative effects is not fully understood.

The sensitivity of benthic organisms to PAC exposure varies considerably between different species. Figure 2b shows an overview of 15 studies that measured biological effects of PAC amendments on 9 species. Clear adverse effects were reported in all available studies for *L. variegatus*, *C. riparius*, and *G. pulex.* Millward et al. [59] found *Neanthes arenaceodentata* to be similarly sensitive. However, the published results for this organism are not as consistent and might be influenced by experimental factors such as external feeding of the test organism during laboratory bioassays [80]. On the other hand, organisms such as *A. aquaticus* and *Leptocheirus*

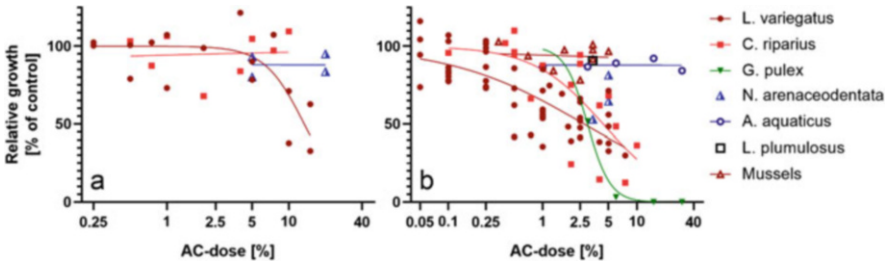

Fig 2 Adverse effects of (**a**) granular activated carbon (GAC; particle size >300 μm) and (**b**) powdered activated carbon (PAC; <300 μm) in relation to the applied doses (mixed into the sediment, based on sediment dry weight %). The graphs show that (1) GAC produces significantly lower adverse effects in comparable doses and (2) adverse effects are dependent on species. While some species show strong adverse reactions to already small PAC doses (closed symbols), others are significantly less sensitive (open symbols). The fitted curves show a clear dose-response reaction to PAC (**b**) for the sensitive species *Lumbriculus variegatus* ($R^2 = 0.60$), *Chironomus riparius* ($R^2 = 0.56$), and *Gammarus pulex* ($R^2 = 0.98$). Adverse reaction to GAC was observed with *L. variegatus* only ($R^2 = 0.56$), with dosages much higher than generally applied for sediment remediation. No significant increase of adverse effects with increasing AC dose was found for *Leptocheirus plumulosus*, *Neanthes arenaceodentata*, *Asellus aquaticus*, and any of the examined mussel species. Data from [52, 53, 55–61, 73, 75, 78–81]

plumulosus and different mussel species show limited or no adverse effects, even at high doses of PAC (Fig. 2).

For AC amendments on a field scale, observed adverse effects depend, to a large extent, on the composition of the benthic fauna at the treated site. Given that the benthic fauna varies widely between different aquatic ecosystems and even within a single lake [54], the observed effects of AC amendments can vary accordingly. The results reported for field studies range from significant disturbances following the application of AC [82] to no measurable adverse effects [39]. The magnitude of the adverse effect can further depend on the general habitat or sediment quality prior to the AC application, where organisms that are already living on the margin of their habitable conditions are more negatively affected [78, 80].

The strong dependency of both adverse effects and remediation potential on the AC particle size can complicate the material selection. This is especially the case when benthic communities at a potential treatment site are dominated by sensitive species. When minimizing the risk for adverse effects at such sites is prioritized and coarser GACs are used, the success of the remediation might be compromised due to the low contaminant binding potential of these materials. The direct comparison of remediation potential and adverse effects of different ACs illustrates this trade-off (Fig. 3).

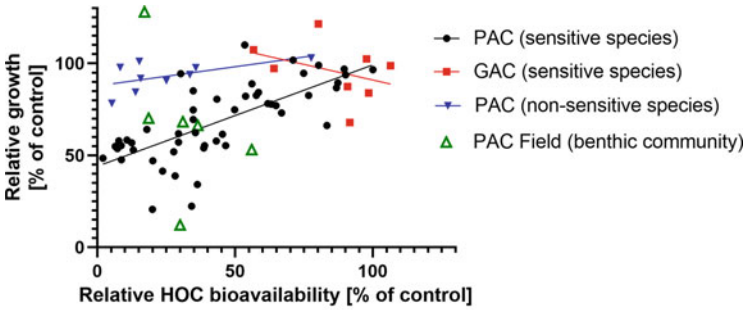

Fig. 3 AC-induced adverse effects correlate strongly with the beneficial effect of reduced contaminant bioavailability for sensitive species (*Lumbriculus variegatus*, *Gammarus pulex*, *Chironomus riparius*). The reduced bioavailability of hydrophobic organic contaminants with powdered activated carbon (PAC; particle size <300 μm) treatment is high, with also strongest secondary effects to sensitive organisms ($R^2 = 0.51$, $p < 0.01$). The opposite holds true for granular activated carbon (GAC; >300 μm), to which even sensitive species rarely show adverse effects, but also the remediation efficiency is low (insignificant correlation). Significant correlation of adverse effects and remediation potential is not found for nonsensitive species. Field studies focus on benthic communities, which can be dominated by either sensitive or resistant species. Results vary and can be positioned in either groups on the graph. All data points represent the adverse effects and remediation efficiency measured for a single AC treatment. The data for the graph was collected from studies that report both values [37, 40, 41, 52–63]

4 Concluding Remarks and Future Prospects

Considering the extent of anthropogenic chemical pollution and the complexity and diversity of contaminated sites, AC amendment methods are a valuable supplement to traditional sediment remediation approaches. Results from both laboratory and field studies have laid a foundation for AC amendment-based sediment remediation. AC amendments have been proven to efficiently reduce freely dissolved concentrations, bioaccumulation, and fluxes of HOCs, and it is currently the most widely used in situ stabilization amendment worldwide. In addition, the carbon produced under controlled temperature conditions and absence of oxygen (slow-pyrolysis biochars) have shown to be extremely persistent and have higher carbon sequestration potentials compared to the naturally produced pyrogenic carbon (wildfire charcoal) [83]. However, to choose the optimal site-specific remediation approach, balanced consideration of the potential benefits, ecological effects, and costs is required.

Several field-scale studies have confirmed the remediation potential of AC amendments, and in long-term monitoring, AC has proven to be a stable sorbent, retaining its ability to reduce bioavailable HOC concentrations over several years. In addition, due to ongoing mass transfer, the effectiveness of AC application can even increase over time, attaining its full treatment capacity years after application. Despite the many examples of successful remediation with AC amendments, site-specific factors such as high organic matter content or dynamic sedimentation or sediment transport processes can lower the remediation efficiency.

The direct (i.e., secondary ecological) effects of AC amendments on benthic organisms have been extensively studied. The results have varied from no observed effects to significant effects on abundance, biomass, and number of species. In general, the observed effects on benthic organisms have been less severe in the field compared to laboratory experiments, and often (but not in all cases) adverse effects of AC amendment in the field have diminished within 1 to 2 years after the amendment. Nevertheless, the effects on benthic fauna should not be overlooked when planning remediation project. When multiple remediation approaches are viable at a given site, their potential adverse effects should be evaluated and weighed against each other. Adverse effects to benthic fauna can endanger the stability of the AC amendment, because if the benthic fauna disappear, then the incorporation of the AC into the sediment will slow. Depending on the areal extent of the AC treatment, the areas surrounding the AC placement can serve as reservoirs for recolonization. AC treatments are often limited to a small area within a water body [9], thus limiting the adverse effects to a relatively low fraction of the total ecosystem fauna. The beneficial effects, however, could extend throughout the food chain and over time lower the contaminant burden in the whole ecosystem.

Balancing the AC dose and particle size of the amendments is a key factor in seeking to reduce negative effects to benthic fauna. Where higher dose and smaller particle size may provide more efficient or quicker sequestration of HOCs, they are also more likely to induce greater stress to organisms. New innovations can provide future solutions to reduce the adverse effects of the sorbent material by altering the AC properties so that it is less disruptive to benthic organisms [56]. Developing AC remediation products further could allow this remediation method to move from a purely passive, bioavailability-reducing role towards active contaminant removal. Magnetized AC, which can be retrieved from the sediment [60, 61], allows for the extraction of contaminants. AC can function as a substrate for microbial growth, and biofilms formed on the particle surfaces can enhance degradation of chemicals [84]. AC has been shown to stimulate the diversity of PAH-degrading microbes, and under anaerobic conditions, AC amendment was shown to significantly induce naphthalene degradation [85]. The novel approach of inoculating AC particles with contaminant-degrading bacteria combines enhanced biodegradation with sorption [86, 87]. The advantage of such bioamended AC is that, in addition to reduction of the bioavailable fraction, it can also reduce the total concentrations of the contaminants in the sediment. Development of AC amendments in this direction could promote the wider adoption of the method, as in many countries, the regulations on the reuse and management of contaminated sediment are based on total concentration [9, 36]. Although bioavailability is being increasingly incorporated into risk assessment [88], legislation in many cases still lags behind. In addition, the acceptance of the remediation approach among the general public could improve if the method also provides contaminant removal.

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Why Biodegradable Chemicals Persist in the Environment? A Look at Bioavailability

Jose J. Ortega-Calvo, Felix Stibany, Kirk T. Semple, Andreas Schaeffer, John R. Parsons, and Kilian E. C. Smith

Contents

Abstract Biodegradable chemicals may become persistent due to reductions in their bioavailability thereby impacting on the rate and extent of biodegradation in soils and sediments. This chapter examines this – commonly neglected –

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contradictory face of persistence assessments from the light of the latest advancements in bioavailability science. They include the microbial influences on bioavailability, the different sorption capacities of carbonaceous components of soils and sediments, and the dissimilar bioavailability shown by chemicals when they are present as non-extractable residues. We also discuss possible pathways to improve the realism in persistence assessments from standardized biodegradation tests by incorporating new bioavailability-based approaches. Innovations of the standard tests are possible through the modified chemical application of enhanced dispersion and passive dosing. In addition, we offer a proposal for integrating bioavailability measurements into standard simulation tests with soils and sediments, by using desorption extraction and passive sampling methods to assess the removal of the bioavailable fractions, in addition to the total extractable concentration of the chemical.

Keywords Bioavailability, Biodegradation, Microorganisms, Non-extractable residues, Persistence, Sorption, Standardized tests

1 Introduction

In this chapter, we will consider biodegradable chemicals as a broad group of toxic organic substances of anthropogenic origin which can be broken down biologically, mainly by microorganisms. In the case of complete biodegradation, an organic chemical is transformed into innocuous, simple inorganic molecules, such as carbon dioxide, oxygen, and water, i.e., the chemical is mineralized. However, biodegradation can also be incomplete resulting in transformation products (i.e., metabolites), whose toxicity may differ substantially from the parent molecule. A biodegradable chemical will remain in a given environment if (1) microorganisms with the required metabolic abilities are absent or present in insufficient numbers; (2) the environmental conditions are not favorable for biodegradation to occur, and/or (3) the organic contaminant is present in such a form that it cannot be taken up by the microbial cells, i.e., it is not bioavailable. Any of these individually or together leads to persistence as biodegradation does not occur or only occurs very slowly, and this has strong implications for the assessment and management of the risks caused by chemical pollution [1].

The chapter begins by explaining the different aspects of persistence and biodegradability. These two concepts are often confused, and their differentiation is essential to understand the key position of bioavailability in the environmental fate of biodegradable chemicals. The main processes involved in bioavailability will be then discussed in connection with biodegradation, focusing on the latest advances in understanding the microbial influences on these processes; the different sorption capacities of carbonaceous components of soils and sediments; and the dissimilar

bioavailability shown by chemicals when they are present as non-extractable residues (NER). The chapter will conclude with the prospective introduction of bioavailability within current standardized and non-standardized procedures to determine biodegradability. We hope that the reader finds useful the inclusion within this chapter's contents of our research and teaching experience in biodegradation and bioavailability from the last 5 years at the Spanish National Research Council (CSIC), RWTH Aachen University, the University of Amsterdam, and the Lancaster University.

2 Persistence Versus Biodegradability

Chemicals are classified as persistent if they are not subject to biological, chemical, or physical degradation processes. On the one hand, in product development, persistence is often technologically desired (by aiming at a higher product quality through durability). On the other hand, it is ecologically undesirable if persistent chemicals are emitted into the environment, as this might lead to an accumulation of the chemical in environmental media with potentially negative effects on organisms [2]. Thus, in an environmental regulatory context, persistence is a major hazard criterion, often assessed together with bioaccumulation and toxicity (e.g., in the PBT criteria). Persistence in this context is almost always assessed in terms of biodegradation. Biodegradation may be carried out by many organisms, but most important from an environmental point of view is microbiological biodegradation. The persistence of a substance can be described quantitatively by the time required to remove a certain fraction of the initial concentration, such as its half-life, in specific environmental compartments. The range of biodegradation rates is very wide. For instance, relatively simple contaminants such as benzene, toluene, or phenolic compounds are often readily biodegradable, whereas known problematic chemicals such as dichlorodiphenyltrichloroethane (DDT) and dioxins are quite refractory and often have biodegradation half-lives of several years [3]. However, environmental biodegradation rates of the same compound can vary by orders of magnitude among different aquatic and terrestrial ecosystems.

The persistence of a substance is essentially determined by its molecular structure. For example, natural and artificial polymers (such as plastics), chlorinated, nitrated or sulfonated aliphatic and aromatic hydrocarbons, as well as hydrocarbons with branched side chains often exhibit persistence. Within these chemical groups, many organochlorine compounds, which are often used as pesticides (e.g., DDT) show a high persistence in the environment. However, persistence depends to a large extent on ambient conditions. Not only the presence of specific degrading populations of microorganisms controls biodegradability but also the environmental conditions at the respective location, such as temperature, pH, concentrations of electron acceptors, nutrients and co-substrates, humidity, and light. In addition, the physicochemical properties of the chemicals that are being degraded play an important role – here not only the intrinsic degradability based on the chemical structure,

but also factors influencing their behavior in the environment such as volatility, hydrophobicity, and lipophilicity are important in determining their bioavailability to microorganisms.

The environmental dimension of bioavailability can be best understood at the intersection between sorption and biodegradability. In principle, a chemical needs to exist in the dissolved phase before it can be taken up via passive diffusion across the microorganism membrane and be accessed by the degrading enzymes [4]. However, large numbers of chemicals sorb significantly to particulate organic and mineral matter in soils and sediments. These sorbed chemicals may undergo biodegradation after desorption. In some cases, however, the biodegradation does not rely on desorption into the aqueous phase before it can proceed but occurs through direct contact with possible transfer of the chemical across the lipid membrane. When the contaminant has to first desorb into the water phase before it can be degraded, and this desorption is slow, this can result in a biodegradation rate that is so slow that the chemical is considered to be persistent due to limited bioavailability rather than due the intrinsic lack of biodegradability of the compound [4]. This has important practical implications since degradation half-lives measured with experimental setups containing any kind of natural or artificial sorbing matrices form the basis of both regulatory persistence assessments and exposure modeling. Therefore, a compound might be classified as being persistent due to slow desorption, despite it in fact being readily degradable by microorganisms. Therefore, it is important to clearly distinguish between such desorption effects and the actual biodegradation process.

3 Bioavailability Processes

In the broadest terms, bioavailability addresses the issue as to whether an organic contaminant exists in a physical state such that it can be taken up and degraded by the relevant microorganisms [5]. As discussed above, a wide range of processes can affect the bioavailability of organic contaminants. These include sorption/desorption kinetics and equilibria, spatial mismatches between the degrading microorganisms and contaminant molecules, as well as specific microbial growth strategies aimed at ensuring maximum access to the contaminant substrate [6–9].

Considerable effort has been invested into understanding how bioavailability influences biodegradation, together with the chemical and environmental properties that control this. Here, different analytical approaches are commonly applied to differentiate between the total and bioavailable fractions of the organic contaminant. Such methods can be roughly separated into those that target the microbially available fraction of contaminant in the matrix (e.g., mild extractions or desorption-type methods) [10] and partitioning approaches that specifically target the freely dissolved fraction (e.g., passive sampling) [1, 11].

Especially in water-saturated systems, like sediments, the freely dissolved concentrations (C_{free}) are relevant since diffusive uptake of the dissolved contaminant molecules into the microbial cells is a major uptake pathway [4]. An elevated

dissolved concentration external to the degrading microbial cells leads to a steep diffusive gradient and thus higher uptake rates [4, 12]. However, whether this uptake actually controls the overall biodegradation rate further depends on the subsequent steps in the process (e.g., whether the relevant enzymes become saturated or repressed at higher concentrations). Furthermore, simply determining these dissolved concentrations using partitioning-based methods cannot provide a complete picture of contaminant bioavailability. For example, when the external pool of dissolved contaminant molecules is depleted due to microbial uptake and biodegradation, then the desorptive resupply from the surrounding matrix-sorbed contaminant pool influences bioavailability [8]. This can lead to a situation where dissolved concentrations are low, but biodegradation is maintained by an efficient resupply from the sorbed pool. In this case, simply measuring the dissolved concentrations might lead to incorrect conclusions about the contaminant having a low bioavailability. This is particularly relevant for hydrophobic organic compounds (HOCs), where a significant mass fraction is associated with the surrounding soil or sediment matrix (see above) [13].

Research performed during the last 5 years has improved our current understanding on how bioavailability affects biodegradation. The advances include (1) microbial influences on bioavailability processes, (2) differing phase exchange mechanisms among carbonaceous components of soils and sediments, and (3) evidence showing the biodegradability of chemicals present in non-extractable residues. It is conceivable that, if properly integrated, this knowledge will allow a more realistic assessment of organic chemicals, identifying those situations where biodegradability, bioavailability, and intrinsic persistence can be distinguished from one another.

4 The Microbial Component of Bioavailability: Solubilization and Cell Positioning

Microorganisms can operate on the bioavailability processes at two different levels (Fig. 1). On the one hand, the distribution of the chemicals between the soil or sediment particles and the aqueous phase can be modified by microbial substances (strategy A). On the other hand, the bioavailability of chemicals can be increased by the positioning of the microbial cells in relation to the pollution source, for example, by an enhanced dispersal of microorganisms throughout the polluted matrix (process B) or by a direct contact with the contaminant, thereby enabling biodegradation to proceed more rapidly (process C). These pathways have been investigated from the point of view of microbial actions on recalcitrant contaminants subject to bioremediation [14]. Perhaps because the experimental tests currently applied to determine persistence in prospective risk assessment of chemicals were not designed for looking at these phenomena, their relevance in those regulatory scenarios remains completely unknown.

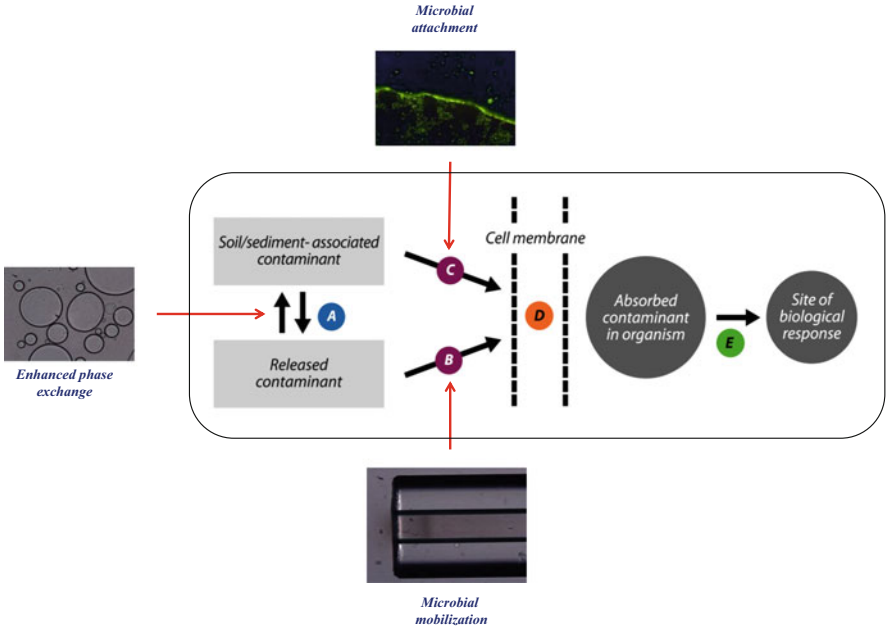

Fig. 1 Microbial modes of action on bioavailability processes, in relation to the biodegradation of organic contaminants. Biodegradation can be enhanced through solubilization (represented by the surfactant action on phase exchange; process **A**), tactically driven microbial mobilization (represented in the figure by chemotactic bacteria; process **B**), and attachment to interfaces, which allows the direct acquisition of the sorbed contaminant (process **C**). (**D**) represents the contaminant uptake by the microbial cells, necessary for biodegradation, eventually leading to mineralization or co-metabolism (**E**). Figure adapted from [15]

Microorganisms can influence the phase exchange of chemicals through the production of surfactants and extracellular enzymes, the ionic displacement of charged compounds, or changes in the pH of sorbent surfaces [16]. Solubilization by biosurfactants is probably the most common way by which microorganisms influence the bioavailability of HOCs. The capacity for producing surface-active compounds by microorganisms was recognized as early as 50 years ago, and, since then, significant advancements have been made in understanding why and when some microorganisms excrete biosurfactants, as well as their chemical nature, environmental biodegradability, and physicochemical properties [17, 18].

Obviously, the extent to which biosurfactants can influence the in situ biodegradation of HOCs in a given environment will strongly depend on the occurrence, numbers, and activity of biosurfactant producer cells in the existing microbial community. These cells should be able to deliver sufficient biosurfactant to cause the micelle-driven solubilization of the contaminants, and the biosurfactant should not negatively affect the overall activity of the contaminant degraders. In addition, provided that the primary mechanism of association of the biosurfactants to the soil and sediment is an adsorption process at the solid-water interface, the biosurfactant

efficiency may be different between the various sorbed pools present in the contaminated soil or sediment. The solubilization of fast-desorbing contaminants by biosurfactants may enhance biodegradation, provided the toxic effects and metabolic competition caused by multiple contaminants present in the mobilized mixtures, or the nutrient demands associated with this mobilization, do not limit the activity of contaminant-degrading populations [19]. The efficiency of biosurfactants in mobilizing slowly desorbing chemicals may decrease, as compared with the fast-desorbing pool, as a result of the limitations imposed by the intra-aggregate diffusion of the aged contaminants and biosurfactants [20]. However, biosurfactants may still be more efficient in enhancing the biodegradation in soils and sediments that have reached a slow desorption profile after conventional bioremediation [19] and phytoremediation [21], possibly by avoiding the limitations mentioned above with the enhanced phase exchange of the fast-desorbing fractions. Furthermore, recent findings show that the rhizosphere can enhance the biosurfactant efficiency in mobilizing the residual, slowly desorbing contaminants, through biochemical influences on the sorbed biosurfactants [22]. As a result, the effects of biosurfactant-aided phase exchange on the biodegradation of HOCs in natural and engineered environments are often site-specific and difficult to predict.

Recent research has also shown that the flow of the contaminants to the contaminant-degrading microbial communities can be significantly affected by the microbial positioning along the contaminant paths, which may result in an enhanced or diminished bioavailability. These spatial influences may operate on the contaminants associated with the aggregates within the soil or sediment and on those already released into the aqueous phase and being transported at macroscopic scales (Fig. 1). The movement of contaminant-degrading microorganisms in water-saturated porous media is usually limited by their high deposition rates and adhesion to surfaces, as well as by the extremely restrictive fluid dynamics operating at the aqueous microenvironments surrounding the microbial cells. These limitations can be overcome by flagellated microorganisms through behavioral responses to a variety of stimuli, including chemical gradients, leading to a tactic mobilization to distant contaminant sources and, subsequently, to an increased rate of contaminant acquisition. The positive impact on contaminant biodegradation of such tactic mobilization has recently been examined using a model, passive dosing column system, and a representative soil bacterium, *Pseudomonas putida* [23–25]. By inducing different cellular motility patterns in response to a variety of chemical effectors (including different of sources of dissolved organic matter and nanoparticles), bacterial transport was enhanced, which promoted the mineralization rate of naphthalene desorbing from a passive dosing device located a few centimeters away. This bacterium was also used as an experimental model for examining the potential role of bacterial motility in the co-metabolism and biosorption of pyrene in a porous medium [26]. The study indicated that motile bacteria may even increase, through these two processes, the risk associated to contaminant mobilization in soils.

Another set of microbial phenomena, recently linked to bioavailability and biodegradation, is caused by eukaryotic zoospores and mycelia, by acting as biological effectors on the positioning of contaminant-degrading bacteria. The studies

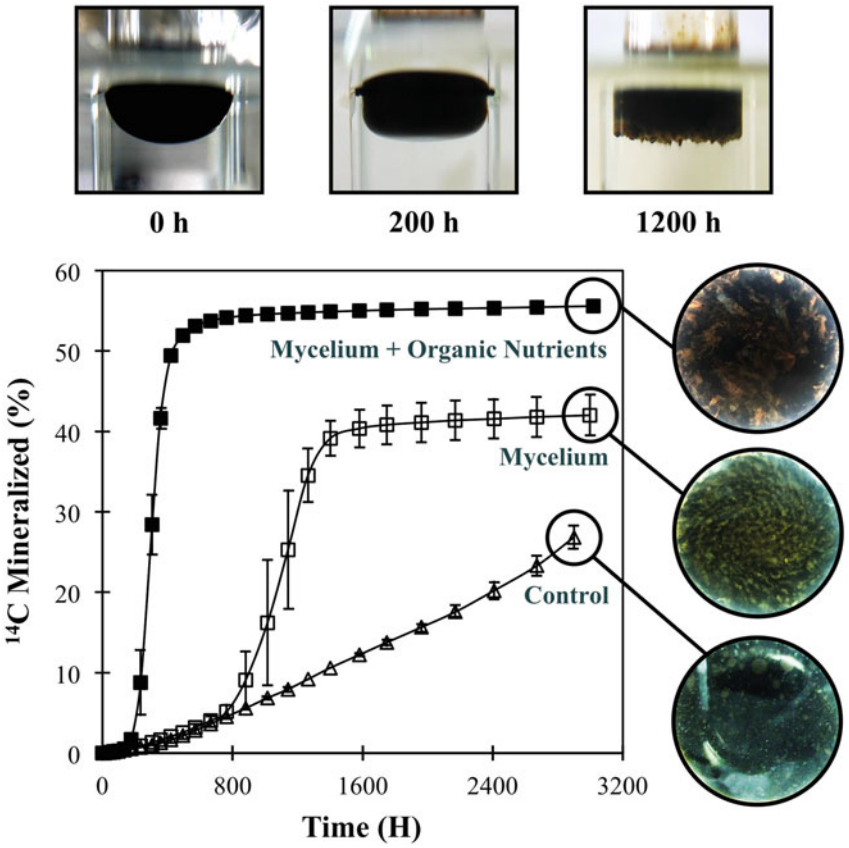

Fig. 2 The mycelium of the oomycete *Pythium oligrandrum* enhanced mineralization of phenanthrene by cells of the bacterium *Mycobacterium gilvum* attached at the interface between a nonaqueous phase liquid (NAPL), composed by heavy fuel, and water. Without mycelium (control), the bacterium did not develop biofilms at the interface and degraded linearly the compound, following the rate of partitioning into the aqueous phase. Reproduced with permission [29]

revealed that the motile zoospores, produced from oomycete mycelial networks, mobilized both non- and self-propelled PAH-degrading bacteria and formed microbial consortia at NAPL/water interfaces. Such microbial consortia are initiated by the settlement of zoospores at the interfaces between NAPLs and water, which is followed by germination and the formation of mycelial networks by zoospore cysts onto and into the NAPLs. This sequential phenomenon is likely the initiation of complex biofilms that can enhance PAH bioavailability and sustain the growth of PAH-degrading bacteria attached at the NAPL/water interface, where substrate acquisition occurs directly from the NAPL, at a higher rate than in the surrounding bulk aqueous phase (Fig. 2) [27–29]. These results extend the concept of "mycelial pathways" for the dispersal of contaminant-degrading bacteria, explained elsewhere in this series [30].

The opposite effect to these biological effectors was observed with humic acids [31] and biosurfactants [32], which inhibited the attachment of contaminant-degrading bacterial cells to the surface of silicone acting as a passive doser for pyrene. This effect compensated for the enhancement in phase exchange of the chemical and thus resulted in either an inhibition or no effect on contaminant biodegradation by these solubilizers. Recent research has also shown that bacteria attached to hydrocarbon/water interfaces can penetrate sub-micrometer pores through physical forces imposed by growth, colony extension, and biosurfactant production [33, 34]. The resulting cell plasticity facilitates the bacterial translocation and passage through theoretically inaccessible, sub-micrometer pores, thus allowing the biodegradation of occluded contaminants to occur.

5 The Geochemical Component of Bioavailability: Organic Matter and Black Carbon

Organic matter (OM) and black carbon (BC) are ubiquitous in soils and sediments. These have been shown to have a major role in controlling bioavailability and hence biodegradation [35]. During recent years, the traditional, one-phase organic carbon (OC) partitioning model has been expanded for hydrophobic contaminants to include both the uptake into OM and onto BC. The new model has been useful in understanding field observations of the solid-water distribution coefficient for many contaminants, which have evidenced a higher sorption capacity than would have been expected on the basis of OM content only. A detailed examination of the sorption mechanisms to these two materials in connection with bioavailability of NOCs in soils is provided elsewhere in this series [36]. When they become dissolved in the porewater of soils and sediments, OM and BC may also contribute in parallel to the sorption processes, with an impact in bioavailability that may be different to the solid-phase OM and BC [37].

Mainly as a result of the recently identified implications of the strong sorption to BC and similar materials (i.e., activated carbon and carbon nanomaterials) for risk assessment and management of nonionic organic contaminants, the biodegradation and persistence of contaminants associated to these sorbents have been addressed in several recent studies. Zhang et al. examined the biodegradation in suspensions of the bacterium *Mycobacterium vanbaalenii* PYR-1 of phenanthrene and nitrobenzene sorbed to different carbonaceous materials, including graphite, activated carbon, and carbon nanotubes [38]. The results showed that the strong sorption to these materials, as evidenced by decreases in C_{free}, limited the biodegradation of these contaminants. However, the transformation was not solely controlled by C_{free} but also by the attachment of the bacterial cells to the surface of the sorbents. This physical association changed with the surface characteristics of the materials, in accordance with their capability to establish polymeric interactions with the bacterial cells, and

facilitated the direct uptake of the sorbed chemicals. This indicated that the strong sorption to these materials was a reversible process.

A new approach based on the strong sorption capacity of activated carbon has recently been proposed to allow the bioremediation of soils highly contaminated with crude oil (up to 15%), what theoretically would not result in a successful contaminant removal due to toxicity [39]. A mixed sorbent, composed of activated carbon and diatomite, allowed significant reductions in the content of total petroleum hydrocarbons and minimized the production of oxygenated petroleum by-products and the associated soil phytotoxicity. These positive results were explained on the basis of the high porosity and the reversible sorption capacity of the amendment.

6 Bioavailability of Biodegradable Chemicals Present as Non-extractable Residues

Biodegradable xenobiotic chemicals in solid matrices like soil or sediment undergo transport, binding, and degradation processes that depend on the physicochemical properties of the substance and the soil and on environmental conditions. When using isotope-labelled compounds, often radioactive ^{14}C-labels, it is possible to establish a mass balance of their fate in solid matrices like soil. If the thoroughly extracted soil is combusted, a third fraction beside extractable and volatile and mineralized residues can be quantified due to the labelling. This fraction comprises so called non-extractable residues (NERs), i.e., residues that cannot be extracted without changing the structure of the matrix.

NERs vary in the amount that is formed and in the type of binding: residues may be entrapped or sequestered in the pores and voids of the soil matrix (type 1 NERs, nomenclature see Schaffer, et al. [40]), or they may be covalently bound to humic matter (type 2 NERs). A third type of NER is formed by microorganisms which use structural elements from the contaminants by direct catabolism, by co-metabolism in presence of other readily degradable substrates, or indirectly by fixation of carbon dioxide released from the isotope-labelled xenobiotic. By these processes, parts of the isotope-labelled carbon atoms can be incorporated into microbial biomass, forming biogenic NERs (bio-NERs or type 3 NERs) [41, 42].

All chemicals form NERs to different extents in soil and sediments, some in very high amounts, but in most cases nothing is known on their chemical structures and the potential for long-term release and biodegradation. There has been many years of debate about whether NERs have to be considered as “safe” sink, i.e., as dead-end metabolite or as long-term source due to stabilization and (very) slow remobilization of the parent compound and/or primary, xenobiotic metabolites. A thorough review on bio-NERs is included elsewhere in this volume, where the methodology of stable isotope labelling, such as with ^{13}C, as an alternative for analysis of bio-NERs is described [43]. Here, we will focus on xenobiotic NERs, i.e., type 1 and 2 NERs, respectively. However, we would still like to give a short note on bio-NER, which

can be extracted by hydrolyzing the NER containing matrix under drastic acidic conditions. This treatment will release amino acids from the matrix which subsequently are purified by ionic exchange chromatography. Bio-NERs of ^{14}C-labelled chemicals will also contain the label and can thus be quantified, for instance, by ^{14}C-thin-layer chromatography [44].

Recently, methods for differentiation of the three NER types have been developed. An extraction sequence was proposed [40] to obtain a solid matrix that only contains NERs. This sequence comprises as a first step the use of aqueous solutions to determine the amount of residues being easily desorbed and second the use of organic solvents or solvent mixtures to extract thoroughly the matrix and finally exhaustive extraction methods like Soxhlet or pressurized liquid extraction. Then, the matrix is considered not to contain any more extractable residues, i.e., only NERs. The resulting matrix has subsequently to be treated with agents which disaggregate the humic matter in order to differentiate NER containing entrapped residues – such type 1 NERs will be released after this treatment – and covalently bound residues (type 2 NERs), which will remain in the matrix. For humic matter disaggregation, treatment with a silylation agent like trimethylchlorosilane is performed, which will replace exchangeable protons and thus break hydrogen bridges of humic matter. Alternatively, strong chelating agents like EDTA will remove bridging metal ions from humic matter [45]. Upon disaggregation, sequestered, entrapped residues will be released and can be analyzed by chromatographic and spectroscopic methods.

Entrapped NERs have recently been suggested to be considered in the persistence assessment of chemicals because they are slowly released from the matrix under natural conditions [40]. However, evidence for the presence of biodegradable organic contaminants and/or their primary, xenobiotic metabolites in NERs so far is limited. NERs of the fungicide cyprodinil have been analyzed by ^{13}C-NMR spectroscopy: primary metabolites and the parent compound have been detected [46]. Berns et al. [47] applied ^{15}N solid-state NMR spectroscopy in combination with Density Functional Theory (DFT) calculations and characterized the binding mode of simazine NERs in soil which consisted of metabolites rather than the parent compound [47]. Nonylphenol, a degradation product of widely used nonylphenolethoxylate tensides, forms high amounts of NERs in soil, which according to ^{13}C-NMR spectroscopy and sequential chemical degradation studies is predominantly bound by ester bonds to humic matter [48]. Also antibiotics from pig manure form NER in soil when added as fertilizer: sulfadiazine rapidly binds irreversibly to soil, and ^{13}C-NMR revealed that the parent compound is contained in fulvic acids in low concentrations [49, 50]. Recently, the influence of a chemical charge in ionizable organic chemicals with respect to the formation of NERs has been investigated. There are three structurally similar compounds on the basis of dodecylbenzene derivatives, one neutral at pH 7, i.e., a phenol derivative, one negatively charged containing a sulfonic acid functional group at the benzene ring, and the third positively charged with a trimethylammonium group at the ring. Silylation of the extracted, only NER containing soil revealed that the positively

charged derivative formed mainly type I NER, whereas the others formed covalently bound and biogenic NERs [51].

Analyses of NERs with the above methods still have some uncertainties. This pertains primarily to the extraction procedures for removing the extractable residues to obtain the matrix containing only NERs. Extraction agents have to be chosen to obtain sufficient extraction efficiency. In addition, the solvents for optimal extraction efficiency have to be changed during the incubation of a xenobiotic in an environmental matrix because the properties of the extractable residues will change. Another methodological uncertainty is the extraction procedures for investigating NERs (silylation for type 1 and type 2 NER differentiation and acidic hydrolysis for bio-NER quantification): it is likely that silylation extracts other residues besides xenobiotic residues, and they also may contain bio-NERs. Therefore, type 1 non-extractable residues must be analyzed to address this uncertainty. However, a method for analytical differentiation needs to be developed. Chemical analysis of type 2 NER, which is strongly bound and not releasable under natural conditions and not even with organic solvents, is however not possible with classical chromatographic methods. Analysis like solid-state NMR is possible but limited by insufficient sensitivity. This means there remains some uncertainty in the determination of xenobiotic and biogenic residues in these fractions.

As further uncertainty, neither the silylation method to distinguish type 1 and type 2 NER nor the method to identify bio-NERs has yet been standardized which will be the next step, for instance, by round robin testing. Structural identification of type 1 and type 2 residues is a technical challenge. As a pragmatic approach, the released amount of NERs after silylation can be taken as type 1 NER and that remaining in the matrix as type 2 NER. Assuming that the relative amount of type III NERs, which is determined independently by the described acidic hydrolysis method, is also the same in the type 1 and type 2 NER fractions, it is possible to estimate the absolute amounts of types 1 and 2.

From a regulatory point of view, the differentiation of the NER types should be embedded in the general persistence assessment of chemicals. Type 1 NERs do have remobilization potential, whereas type 2 is considered to be irreversibly bound (unless there are indications for the opposite). Therefore, type 1 NERs have to be considered in the persistence assessment. It is accepted that bio-NERs are of no environmental concern.

7 Persistence and Chemical Management

Ultimately a major motivation for studying the environmental fate of an organic contaminant is to better understand its concentrations in a particular habitat, given these determine organism exposure, uptake, and toxicity [52]. These exposure concentrations reflect the net balance between the contaminant inputs and losses over time. Although various loss processes impact an organic contaminant's persistence in a particular environment, in the majority of cases, microbial degradation

remains a major removal mechanism [53]. However, understanding and quantifying the role microbial degradation has in persistence remain challenging given the large number of interacting factors. These include the intrinsic properties of the organic contaminant (e.g., toxicity, recalcitrant (sub)structures), microbial factors (e.g., microbial diversity, abundance, and growth strategies), and environmental conditions (e.g., presence of co-substrates, temperature, oxygen availability) [5, 7, 54].

A robust risk assessment and management strategy for organic contaminants thus requires quantitative measurements of their persistence that are both reliable and relevant [1]. Given the central role of biodegradation has here, this involves determining microbial degradation in laboratory tests that are highly standardized (e.g., the OECD series [55]). Here, one major challenge is ensuring that the results from these artificial tests can be used to make reliable judgments about their likely persistence in the environment [56]. In other words, can the quantitative result coming from standardized tests be applied to the situation in the field? Answering this is not trivial, given the complex set of interacting factors that determine how fast and to what extent rate microbial degradation occurs. As discussed above, this requires careful consideration of whether the microbial aspects and physical state of the organic contaminant within the tests are (1) environmentally relevant and (2) do not to limit biodegradation.

Although many factors can potentially influence microbial degradation in such tests, the following discussion is confined to the impact the bioavailability of organic contaminants on the results. Nevertheless, it is emphasized again here that the bacterial diversity and numbers as well as the test conditions are equally relevant in this context. As an example, several studies and reports have all highlighted the importance of ensuring that microbial diversity and abundance are adequately reflected in biodegradation tests used for regulatory processes [57, 58].

8 Bioavailability in the OECD Test Series

Several chemical regulatory frameworks exist, within which standardized tests for biodegradability are applied to assess the likely persistence of organic contaminants in the environment. In the following, the OECD suite of biodegradation tests is used as an example to highlight the importance of considering bioavailability [55]. These OECD biodegradation tests can be separated into three groups arranged in a tiered system of increasing complexity (Table 1):

- Ready biodegradability (or screening) tests
- Inherent biodegradability tests
- Simulation tests

In general, the OECD series of biodegradation tests aim to provide some quantitative measure of a compound's biodegradability as the basis for classifying whether it is likely to persist in the environment or not. Particularly for the lower two tiers, it is not the intention to reproduce environmentally relevant microbial and

Table 1 OECD biodegradation tests [55]

OECD method	Description	Year of publication/ latest update
Ready biodegradability		
301A	DOC die-away test	1981/1992
301B	CO_2 evolution test	
301C	Modified MITI test (I)	
301D	Closed bottle test	
301E	Modified OCDE screening test	
301F	Manometric respirometry test	
310	CO_2 in sealed vessels (headspace test)	2006/2014
Inherent biodegradability		
302A	Modified SCAS test (semi-continuous activated sludge)	1981
302B	Zahn-Wellens/EMPA test	1981/1992
302C	Modified MITI test (II)	1981/2009
Simulation		
303A	Aerobic sewage treatment: activated sludge units	1981/2001
303B	Aerobic sewage treatment: biofilms	
304	Inherent biodegradability in soil	1981
305	Bioconcentration: flow-through fish test	1981/1996
306	Biodegradability in sea water	1992
307	Aerobic and anaerobic transformation in soil	2002
308	Aerobic and anaerobic transformation in aquatic sediment systems	2002
309	Aerobic mineralization in surface water	2004
311	Anaerobic biodegradability of organic compounds in digested sludge: measurement of gas production	2006
312	Leaching in soil columns	2004
314	Biodegradability of chemicals discharged in wastewater	2008

environmental conditions within the test setup. Rather these tests are intentionally stringent in order to identify readily biodegradable compounds to assess whether a compound can be degraded or not. In contrast, the more involved simulation tests do attempt to reproduce environmental conditions and also provide data that can be used to describe the biodegradation kinetics.

Given the central role bioavailability has in determining both the rate and extent of biodegradation, the following sections focus how it can impact the results coming from different types of regulatory biodegradation tests used for assessing persistence. This is relevant since a more detailed consideration of test bioavailability can help reduce incorrect classifications as to a compound's persistence. For example, limited biodegradation of a certain contaminant observed in a test might be attributed to either an inherent recalcitrance due to its structure or to a low bioavailability limiting its uptake and biodegradation. The first would indicate a likely persistence in the environment, whereas the second not necessarily so. Should bioavailability be

different in the field, then the persistence observed in the test would no longer be relevant.

Ready Biodegradability Tests These provide a stringent first testing tier and indicate whether a chemical is rapidly degradable or not over a period of 28 days [55]. Biodegradability is determined by measuring one of several nonspecific endpoints (e.g., reduction in dissolved organic carbon, production of carbon dioxide, biochemical oxygen demand, production of inorganic carbon). All these tests take place in aqueous suspensions with varying but low solid densities, require application of above mg/L test compound concentrations, and use microbial inocula with different sizes and that are obtained from a range of sources (e.g., sludge or effluent from wastewater treatment plants, surface water, soil, a mixture thereof) [55]. These tests provide a simple yes/no answer as to the likelihood of a compound's biodegradation and are not intended to result in a biodegradation rate. Nevertheless, biodegradation kinetics are implicitly included since the pass/fail criterion is partly based on a certain degree of microbial removal within a specified time period (which is the definition of a rate). Two extremes of compound bioavailability might be envisaged in these ready biodegradability tests.

At one extreme are the HOCs. Although such compounds will preferentially sorb to any solid phases such as organic matter present in the tests, given the rather low concentration of solids (<30 mg/L), any limited bioavailability due to sorption is hardly relevant. More challenging is when the starting concentrations of test compound are well above a few milligrams per liter. These high concentrations are needed to ensure sufficient substrate for a reliable measurement of the different test endpoints. However, these initial concentrations exceed the aqueous solubilities of many HOCs by orders of magnitude which unavoidably results in an additional solid HOC phase. Bioavailability during the test is then mainly determined by dissolution of these solid HOCs. Several studies have shown this dissolution-controlled biodegradation of organic compounds, highlighting the limited biodegradation when the dissolution process is slow as is often the case for HOCs [59, 60].

Such bioavailability-limited biodegradation has been recognized for HOCs in these tests, and a number of modifications, already standardized, have been suggested to increase their bioavailability. These include physical treatments such as sonication to better bring HOC into solution, sorption to inert supporting materials such as silica or dispersing them via surfactants, emulsifiers or dissolving in inert silicone oils [61, 62]. Some of these treatments increase biodegradability in such tests, most likely via modifying the test compound bioavailability. However, exactly which treatment leads to an increased biodegradation depends on the compound and whether it exists in solid or liquid form [61]. Also, some of these preparation methods might not be accepted by regulators for making conclusions on the ready biodegradability of tested compounds [62], although they are considered in the REACH regulation [63]. Therefore, unless bioavailability is determined in parallel, it remains unclear under what conditions and by how much these modifications can alter bioavailability.

Perhaps one solution to avoid these excessively high starting concentrations of poorly soluble organics is the use of radiolabeled ^{14}C compounds. Here, the significant increase in analytical sensitivity would permit much lower starting concentrations, while still allowing $^{14}CO_2$ evolution to be followed. This approach would also be useful for testing toxic compounds (see below). However, this is approach has higher costs and safety concerns and furthermore is restricted to those compounds for which the radiolabeled form is available.

At the other extreme, there are those highly soluble organic compounds. These remain dissolved at the mg/L concentrations introduced at the test start and are thus readily bioavailable. These high dissolved levels facilitate the biodegradation process but for certain compounds could lead to inhibition or even toxicity [9]. This is particularly relevant for test compounds with high toxicities toward bacteria, such as the antibiotics. In fact, several studies clearly indicate that elevated concentrations of such microbially active compounds can lead to either reduced biodegradation and/or shifts in the microbial community composition. Therefore, too high a bioavailability of especially microbially active organic compounds can also limit biodegradation in this type of test.

Inherent Biodegradability Tests These are designed to highlight those organic contaminants which possess an inherent potential for biodegradation. They are similar in design to the above ready biodegradation tests in that they take place in the aqueous phase, consider similar endpoints, and require above mg/L starting concentrations [55]. The major differences are that the inocula are more environmentally representative, are introduced in larger amounts, and thus have a higher chance of containing sufficient numbers of competent degraders and that the criteria for a compound being flagged as being biodegradable are not as stringent [57]. In terms of test compound bioavailability though, the same considerations apply as above. Too high a bioavailability can lead to inhibition or toxicity, and conversely too low a bioavailability can limit biodegradation.

Simulation Tests These are the most complex biodegradation test systems and aim to assess both the rate and extent of biodegradation in laboratory setups that mimic either the aerobic stage of a wastewater treatment plant or an environmental compartment such as soil, sediment, or surface water [55]. In contrast to the above tests, the starting concentrations of 1 to 100 μg/L are lower and closer to environmental levels. Nevertheless, it should be noted that for very hydrophobic compounds, even these reduced starting concentrations are still well in excess of their aqueous solubilities, leading to the same issues with bioavailability being limited by dissolution as described above. Furthermore, the significantly high amounts of solid materials in such simulation tests can significantly impact particularly HOC bioavailability via sorption [56]. Since the time between test compound addition and the test start is minimal, it is unlikely that typical aging processes will play a significant role in reducing bioavailability [64]. However, given that the substance is freshly spiked, it could be interesting to study the competing processes of desorption into the aqueous phase versus diffusion deeper into the organic material due to an internal

non-equilibrium. Such diffusion into the inner regions of the organic material would lead to a reduction in bioavailable contaminant for the degrading microorganisms.

Given the complexity of the processes occurring within the solid phase and how these impact bioavailability, it is probably simpler to apply some of the methods discussed above that provide a measure of bioavailability during the test. This possibility will be discussed in the next section. This would have the advantage that it becomes easier to explain those factors that are controlling biodegradation in such simulation tests and furthermore simplifies comparison between different tests since the organic contaminant levels are expressed in the same "currency." As an example, when the bioavailabilities are similar but the biodegradation rates are different, this would indicate that other factors play the determining role (e.g., different microbial communities due to different inocula sources). A major disadvantage with this approach is that as yet there is neither a universally accepted definition of bioavailability within the context of microbial degradation nor a single method to determine this.

9 Non-standardized Approaches for Assessing Biodegradation

Challenges in reliably measuring the microbial degradation of HOCs have led to the development of new methods for improving their handling in such biodegradation tests. These requires introducing the HOCs into the test setup at the low levels which are at or below their respective aqueous solubilities and then buffering these over a prolonged period to permit measurement of the biodegradation endpoint. One such approach is partitioning-based dosing (or passive dosing), where test HOCs (or mixtures of HOCs) are first loaded into an inert polymer which is then brought into contact with the medium containing the degrading microorganism. The HOC then partitions from the loaded polymer into the aqueous medium where it is taken up and degraded by the bacteria [65]. The freely dissolved and bioavailable concentrations of HOC in the medium are defined by equilibrium partitioning concentrations. Therefore, bioavailability in the test setup is well-defined and can also be controlled via loading the polymer to different levels. Furthermore, the decrease in these freely dissolved concentrations due to the biodegradation is compensated by further partitioning from the reservoir of HOC in the polymer. This is important, since, despite the low freely dissolved concentrations inherent to HOCs, a sufficient turnover of the test HOC is ensured for robust measurement of the relevant endpoint. Another advantage is that co-solvents are not required to introduce the HOCs, which avoids issue of toxicity, enzyme inhibition, co-metabolism, etc. Partitioning-based approaches have been successfully applied to measure the biodegradation kinetics of HOCs and their mixtures [65–67]. However, they are not yet standardized, and work remains before they will be considered as part of the suite of biodegradation tests for regulatory purposes.

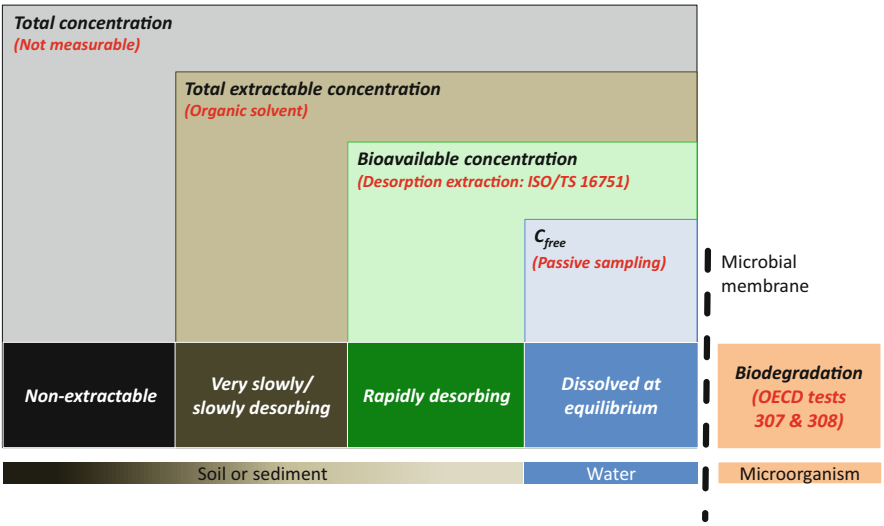

Fig. 3 Proposal for integrating state-of-the-art bioavailability science into current OECD simulation tests, by incorporating desorption ISO methods and passive sampling determinations into the standard simulation tests for soils (OECD 307) and sediments (OECD 308). C_{free} freely dissolved concentration at equilibrium. Figure adapted from [1]

Although bioavailability is still not part of OECD standardized schemes, it is possible to incorporate it into the current OECD simulation tests taking into account the recent developments (Fig. 3). In our proposal, it would be possible to assess the removal of the bioavailable fraction (however it is measured) instead of or as well as of the total amount of chemical. For example, desorption extraction methods, recently standardized as ISO/TS 16751 [68] and described in detail in another chapter of this series [10], can be used in simulation tests with soils (OECD 307, see Table 1). This is similar to previous attempts to use standardized desorption extraction as a tool to evaluate the performance of bioremediation using bioavailability assessments in a wide variety of PAH-contaminated soils [21, 22]. The importance of assessing bioavailability during a bioremediation approach was highlighted in these studies, because this measurement provides a more realistic risk-based information than that provided by total contaminant concentrations only. For example, such assessment allows an estimate of the likelihood of success or failure of the bioremediation method by offering the amount of a chemical that may be removed through biodegradation in relation to the limits set by a regulator. The single time-point Tenax extraction ISO method resulted a reliable and robust way to determine bioavailability of contaminants in a wide set of samples from different treatments (phytoremediation, biostimulation, and bioaugmentation). With sediments (OECD 308), the use of passive sampling [11] could also be very useful to determine the evolution of bioavailability, opening possibilities for capturing, in prospective risk assessment of chemicals, the knowledge already applied in retrospective assessment regulations with remediation of polluted sediments [69].

10 Concluding Remarks

Referencing the results of biodegradation tests to the bioavailable rather than total concentrations would be useful for a better understanding of the often conflicting results that are produced. Direct measurement of compound bioavailability in the simpler screening types tests is likely impractical for routine inclusion. However, targeted measurements might play a useful role. When a compound is observed to be poorly degraded in such a screening test, experimental confirmation of a high test bioavailability would indicate that the observed persistence is either due to its recalcitrant nature or perhaps even toxicity. In contrast, when this limited biodegradation is accompanied by a measurement indicative of a reduced bioavailability, then consideration should be given to modifying the test such that this is no longer limiting. In contrast, routine inclusion of bioavailability measurements in the simulation tests would greatly assist in explaining the observed biodegradation rates but also facilitate comparison between different test media and chemicals. The prerequisite here though is the acceptance of a universal definition of microbial bioavailability and a set of methods to measure this.

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Bioavailability as a Microbial System Property: Lessons Learned from Biodegradation in the Mycosphere

Lukas Y. Wick

Contents

Abstract Bioavailability for contaminant degradation requires a deep understanding of the ecology of degrader microbial systems. It hence should be perceived as a microbial systems property. In this chapter we summarize recent research on microbial ecology of contaminant biodegradation in the mycosphere (i.e., the microhabitat

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surrounding and affected by mycelia). By forming unique transport networks, mycelial fungi are highly adapted to cope with complex heterogeneous habitats and to grow under conditions of uneven availability of their vital resources. Combining concepts from bioavailability, ecophysiology, and microbial ecology, our chapter discusses the impact of fungal networks on chemical and bacterial transport and their effects on contaminant bioavailability and degradation. It thereby provides generic information on key factors, processes, and ecological principles that drive contaminant biotransformation in the mycosphere.

Keywords Biodegradation, Ecology, Fungal-bacterial interactions, Microbial systems, Mycosphere

1 Bioavailability and Contaminant Degrading Microbial Systems

1.1 Introduction

Being main drivers of biogeochemical cycles, microbial systems [1] are also key to the degradation of environmental contaminants. Rate and extent of the degradation of chemicals, however, depend on the molecular property, the availability to degrading organisms [2–4], and the environmental conditions that sustain the activity and abundance of degrader biomass. After anthropogenic release, contaminants typically end up in terrestrial systems, i.e., important ecosystems for biogeochemical nutrient cycling by fungi and bacteria [5]. The fungal kingdom comprises a vast diversity of taxa expressing various morphologies that range from single-celled yeasts to large multicellular organisms with complex interconnected networks (mycelia) of minute, protoplasm-filled tubes called hyphae [6]. Mycelia efficiently spread in heterogeneous habitats such as soil, where they promote microbial contaminant degradation by their own catabolic potential and multifarious interactions with degrader bacteria [7, 8]. Whereas other reviews focus on fungal ecology [5, 9], the biochemical versatility of fungi [10–13], or the 'untapped potential' for remediation [7], this chapter addresses the question how interactions between fungi, bacteria, and their habitats influence the bioavailability and biodegradation of organic chemicals. It focuses on mycelial fungi and biodegradation in their mycosphere (i.e., the microhabitats surrounding and affected by hyphae and mycelia [14]). Please note that the terms "biodegradation" and "biotransformation" are not consistently used in the literature: while some associate "biodegradation" to the ultimate breakdown into CO_2, NH_4^+, or H_2O (also referred to as "mineralization"), others refer to "biodegradation" as the disappearance of the contaminants and do not distinguish between incomplete transformation to metabolites ("biotransformation") and "mineralization." Here, we mostly use "biodegradation" in a sense encompassing both "biotransformation" and "mineralization."

1.2 Bioavailability as a Driver of Biodegradation

The term "bioavailability" is used to denote the *degree of interaction of chemicals with living organisms* [15], and several biological and chemical methods for assessing bioavailability have been described [4]. For contaminant biodegradation we adopt the approach by Bosma et al. [16] defining bioavailability as the *rate of a chemical's mass transfer to microbial cells relative to their intrinsic catabolic degradation potential*. This perspective points at the relevance of mass fluxes for "degradation processes" and discriminates bioavailability for degradation from bioavailability for "non-degradation" processes that lead to poisoning or inhibition of the receptor organism [17].

Low bioavailability for biodegradation may arise if the environmental concentration of a chemical is small (e.g., as for organic micropollutants) or if the flux of a chemical to the degrading cells is minimal (e.g., as for poorly water-soluble or sorbed chemicals [18]). The flux may also become nearly zero for compounds such as plastic polymers [19]. The mode of emission of chemicals also has significant effects on their spatial distribution, and bioavailability. While accidental spills of industrial chemicals lead to massive localized contaminations and potentially high chemical fluxes to cells, waterborne transport of, e.g., micropollutants may result in diffuse low-level contamination that may not sustain sufficient degrader biomass. Rein et al., for example, found that concentrations of <5–10 nM of polycyclic aromatic hydrocarbons (PAH) did not meet the maintenance requirements of the degrader population [20]. According to Bosma et al., several processes (Fig. 1) determine the bioavailability of a degrader cell: the release and transport of the chemical from the source to the cell ("availability to degrader") and the cell's uptake and rate of biodegradation and the respective changes of cell activity ("activity of degrader"). Productive biodegradation (i.e., biotransformation that promotes the build-up and maintenance of biomass) only takes place if chemicals can be transported across the microbial cell membranes into the cytosol where they are metabolized and used for cell maintenance and growth, a process that may in particular influence the bioavailability of chemicals present at low concentrations [21, 22]. Large molecules need to be depolymerized by extracellular enzymes prior to uptake (e.g., as often performed by fungi), whereas uptake of ions, polar molecules, or molecules with very low saturation concentrations (e.g., high molecular weight PAHs) may rely on energy-dependent cellular uptake systems. Such uptake, however, can only take place if the microbes draw advantage from degradation of such chemicals. Co-metabolic contaminant biodegradation, by contrast, generally is not growth-linked and depends on the use of non-specific enzymes to degrade environmental compounds. Co-metabolism is an often underappreciated facet of microbial contaminant biodegradation, which may increase contaminant bioavailability and produce more available metabolites (cf. the metabolites in Fig. 1) in spite of little benefit for the degrader. Co-metabolism can occur under various aerobic and anaerobic environmental conditions and for a wide variety of contaminants and co-substrates [23]. Having the potential to degrade chemicals even at minute

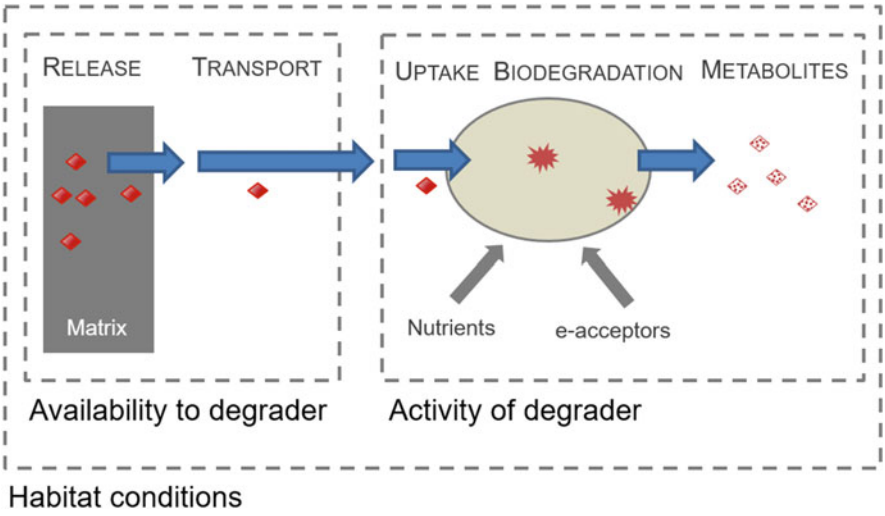

Fig. 1 Schematic overview of the processes of the main processes driving the bioavailability and biodegradation of an inherently biodegradable chemical. Bioavailability is a dynamic feature that is determined by the release, transport, uptake, and transformation of the chemicals at the cellular site of response. It depends on the presence of the chemical, the abundance and catabolic activity of degrader cells, and the prevalent habitat conditions

concentrations ("micropollutants") co-metabolic degradation may allow for cleanup endpoints in the parts per trillion range [23]. As for metabolic degradation, however, the environmental conditions have to allow high abundance and activity of the degraders.

1.3 Microbial System Properties as Drivers of Bioavailability

The heterogeneous distribution of nutrients, pH, temperature, water or terminal electron acceptors is thought to be a key driver for the high diversity and the spatial variations of the activity and abundance of terrestrial microorganisms [24–26]. One gram of surface soil contains up to 10^9–10^{10} prokaryotic cells, hundreds of meters of fungal hyphae, and 10^8–10^9 viruses [26]. Such values convert to >5 tons of prokaryotic and 1–15 tons of fungal biomass per hectare [26]. Despite such high biomass, only a small fraction (0.17%–0.02%) of the specific surface area of soil is considered to be covered with microorganisms [27, 28]. Contaminant biodegradation in such microbial systems hence relies on appropriate fluxes of matter and energy to and between degrader organisms to ensure sufficient microbial activity [29]. Thereby, contaminant transport to and uptake into a cell also depend on morphological, physiological, and behavioral characteristics (functional traits) of the microbes [18]. These traits may include possibilities to adjust the uptake of

chemicals, for instance, by excretion of surface active molecules (biosurfactants) or expression of high affinity uptake systems. Dispersal and chemotaxis, are further self-locomotive traits that allow microbes to control their exposure to chemicals [30]. For unicellular organisms such as bacteria the effectiveness of dispersal depends on the presence of water as major factor controlling bacterial movement in soil. Finally, the activity of a particular organism is always affected by interactions with other organisms in the same habitat. Major microbial interactions during chemical biodegradation are the competition for substrates and nutrients, but also predation or cooperation by syntrophy or by protection against pathogens or predators. Spatiotemporal variations of terrestrial habitat conditions may also cause stress and disturbances [31–33] to the degraders. Such harsh conditions may request high cellular adaptive capacity and a high degree of intercellular interactions [34, 35]. Moreover, the presence of contaminants also exerts a selective pressure on microbial communities [36]. Serving as carbon and energy sources they may favor degrader over non-degrader communities or trigger the evolution of microbial communities toward the degradation of new chemical structures. Differential metabolic potential and sensitivity of organisms to toxic effects of chemicals will hence contribute to microbial biodiversity of a contaminated microbial system, by favoring organisms that take profit from contaminant input and simultaneously distressing organisms that may suffer, e.g., from potential limitation of nutrients and electron acceptors utilized by contaminant degraders.

2 Traits of Mycelial Fungi Relevant for Contaminant Biodegradation

Fungi colonize nearly all habitats of our planet and shape many terrestrial ecosystem functions. Comprising estimated 12 Gt of carbon, fungi form the third most abundant biomass on our planet after plants (450 Gt C) and bacteria (70 Gt C) [37]. In the following, we outline three major characteristics of mycelial fungi that make the mycosphere a hotspot for high contaminant bioavailability and biodegradation.

2.1 Fungi Are Ubiquitous and Also Present in Contaminated Habitats

With more than 144,000 known species [6] and up to 3.8 million undiscovered species, fungi significantly contribute to the taxonomic diversity of microbial systems. In moist, aerobic terrestrial habitats containing complex natural organic matter [38], up to 300 taxa in 0.25 g of soil have been described [38], thereby accounting for up to 50–75% of the microbial biomass. Such abundance is triggered by the capacity

of saprotrophic fungi to depolymerize constituents of animals, wood, and other plant material [6, 39]. Some fungi are highly adapted to extreme environmental conditions. They may grow at low oxygen partial pressures, temperatures ranging from −5 to +60°C, pH values of 1 to 9, or at water activities of as low as 0.6533. The transport of water in fungal hyphae [40] thereby promotes their tolerance to drought and contributes to the maintenance of relevant ecosystem functions during drought stress [41]. Melanized fungi have even been found to use radioactivity for growth [42] or to survive simulated Martian environmental conditions [43]. Fungi are also often found in contaminated environments [7] although still poor knowledge exists on fungal community responses to contaminant mixtures and remediation approaches [8, 44].

2.2 *Fungi Have a Broad Catabolic Potential and Decouple Contaminant Transformation from Biomass Formation*

The species richness and abundance of fungi often goes along with a substantial diversity in biochemical functionality. Saprophytic fungi play a significant role in the decomposition, sequestration, and production of polymeric organic matter (e.g., lignin, lipids, carbohydrates, and proteins [39]). They normally attack high molecular weight compounds with extracellular oxidoreductases. The low specificity of these enzymes also enables the co-metabolic transformation of structurally diverse pollutant classes [7]. Metabolites produced may either be subject to intracellular catabolism (and used for biomass production), be secreted in the form of conjugates, or form bound residues of soil constituents [7]. Despite commonly being considered as aerobic organisms, reports on the presence of fungi in anoxic habitats [45, 46] and anaerobic fungal transformations of contaminants exist (e.g., [47–49]). Although saprophytic bacteria and fungi often share similar biogeochemical functions, they express different suitability to degrade contaminants. The overall catabolic potential of fungi for degrading organic contaminants is broad. Bacteria and archaea, by contrast, often use contaminants as sole sources of carbon and energy by a series of highly specific biochemical pathways requiring corresponding terminal electron acceptors [7]. The availability of a contaminant to specialized degrader organisms thus becomes central for biomass production, i.e., is key to the feedback loop of contaminant uptake and biomass formation [18]. For poorly available chemicals (and unlike for fungal enzymes that remain expressed even at low contaminant concentrations [13]), specific degradation pathways may not be expressed in bacteria. At such conditions bacteria and archaea may enter dormancy, undergo sporulation, or start using more available substrates (while at best co-metabolizing the contaminants). Given the often oligotrophic nature of soil, plant root-derived exudates are a major driver of co-metabolic fungal degradation. Phytoremediation (i.e. the degradation of soil contaminants in presence of plants) has to be regarded as a

result of the complete root zone including bacteria, fungi, and plants [50]. Plant-associated microbial communities can be seen as "a sunlight driven hotspot for the turnover of organic chemicals" [51]. Mycorrhizal symbioses rely on the effective mycelial transfer of mineral nutrients and water to the plant symbiont in exchange for photosynthates that account for up to 30% of the host plant's net carbon fixation. The "mycorrhizosphere" (i.e., the habitat around and affected by mycorrhizal fungi) hence is a prominent hotspot of microbial activity and the biodegradation of contaminants such as chloroaromatics, polycyclic aromatic hydrocarbons (PAH), and explosives.

2.3 *Fungi Adapt Well to Habitat Heterogeneity and Create Suitable Niches for Contaminant Biodegradation*

The highly fractal structure of interconnected mycelial networks enables fungi to exploit the three-dimensional space of their habitats. Fungi are also less sensitive to environmental heterogeneities (e.g., in water and nutrient availability) than bacteria, because they are able to decouple activity from local habitat conditions [52]. Driven by a turgor pressure of up to 600 kPa (i.e., ca. the pressure inside a bike tire) [53], hyphae may grow at speeds of >20 μm min^{-1} [53] and even extend into submicron pores of soil matrices [54]. Fungi thereby may exhibit mycelial lengths of $\approx 10^2$ m g^{-1} in arable and up to 10^4 m g^{-1} in forest topsoil [7]. Expressing hydrophobic cell wall proteins (hydrophobins), many hyphae are also able to overcome air-water interfaces, bridge air-filled soil pores, and access heterogeneously distributed soil nutrient and carbon sources. The concurrence of an adaptive mycelial morphology in response to environmental conditions and a bi-directional cytoplasmic streaming promotes an effective mycelial foraging strategy that combines growth of feeder hyphae in favorable (nutrient-rich) environments with expansion of exploratory hyphae into new areas. Although the diameter of their hyphae measures 2–10 μm, mycelial networks can extend up to an area of hectares [55]. Fungi thereby also act as engineers for bacterial life [56] by creating habitats for efficient contaminant biodegradation in several ways: (1) Intrahyphal translocation and release provides C-metabolites and N and P nutrients that can be used for bacterial activity and co-metabolic degradation [57] (Fig. 2b). (2) Production of large quantities of hydrophobins shape water infiltration properties and, thus, the availability of water to bacteria. (3) Transport of water from areas of high to areas of low water potential [40] enables microbial activity [41, 58, 59] (Fig. 2b). (4) Hyphae of filamentous fungi also mobilize a wide range of hydrophobic contaminants by vesicle-bound cytoplasmic transport ("fungal pipelines" [60] or "nutrient mobile links" [9]), and transported compounds become available to distant bacterial degraders [51, 61]. As a consequence of these multiple interactions with bacteria,

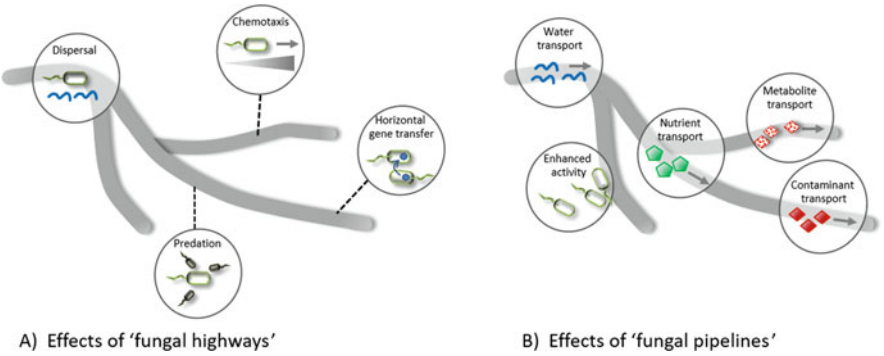

Fig. 2 (**a**) Liquid films along hyphae ("fungal highways") facilitate various ecological processes relevant for contaminant biodegradation including random or chemotactic bacterial dispersal toward contaminant hotspots, horizontal gene transfer, and predation by bacterivores. (**b**) Matter transport by hyphae ("fungal pipelines") comprises the translocation and release of water, nutrients, metabolites, but also contaminants. Both transport processes increase the bioavailability of resources and contaminants for bacteria, their activity, and hence contaminant biodegradation in the mycosphere

fungal networks can also be considered as key players in microbe-driven chemical ecology [62].

Beside active degradation of PAHs, fungi also exert a selective force on the bacteria in the mycosphere [63] due to their release of nutrients and signaling molecules [64]. The bacterial diversity in the mycosphere can range from a few to several hundreds of species and depends on the fungus [65, 66]. Bacterial activity may also be beneficial for fungi, e.g., when fungiphilic bacteria provide specific nutrients or degrade antifungal toxins in exchange for fungal products. Taking into account such multifaceted interactions, the concept of bipartite bacterial-fungal interactions is shifting toward complex interaction networks (sometimes also conceptually named as "meta-organisms" [67]). The scaffold of the mycelia also serves as efficient dispersal vectors ("fungal highways" [68] or "genetic mobile links" [9, 69]) for bacteria, thereby promoting their (random or taxis-driven) access to soil habitats [70]. Contaminated soil habitats often remain poorly accessible to bacteria as their active dispersal is restricted by the poor connectivity of surfaces and discontinuity of water phases in the absence of episodes of water flow or bioturbation. Such restrictions can be overcome by "fungal highways." Liquid films forming along hyphae further enable transport and close cell-to-cell contact of initially spatially separate bacterial conjugation partners along the network structures. Hyphae thereby form a hotspot for horizontal gene transfer between bacteria (HGT) and evolution endowing bacteria with new genetic traits for contaminant degradation [71].

3 Linking Mycosphere Traits and Processes to Bottlenecks of Contaminant Bioavailability

Three conceptual bottlenecks limit the bioavailability and biodegradation of contaminants in soil: (1) insufficient concentration of contaminants for cellular uptake (bottleneck 1: "availability to degrader"), (2) insufficient activity and abundance of microbial communities carrying the necessary catabolic potential (bottleneck 2: "activity and abundance of degrader"), and (3) poor temporal stability of the degraders' performance (bottleneck 3: "functional stability of degrader"). In the following, we link the above-outlined fungal traits and mycosphere processes to these mutually interwoven bottlenecks (cf. also Table 1 and Fig. 3).

3.1 Bottleneck 1: Availability to Degraders

Insufficient uptake arises if contaminant fluxes toward degrader cells are too low to address their full catabolic potential [18] (Fig. 1). In microbial systems, such restriction typically occurs if contaminants are present at minute concentrations or if they are matrix-bound (sorbed), poorly water soluble, or present as solid chemicals. The high surface area of mycelia in conjunction with good sorption properties of the chitin and chitosan in fungal cell walls increases the availability of poorly concentrated chemicals with octanol-water partition coefficients of log D_{ow} of $\leq$3.0 [72]. Assuming a hyphal diameter of 10^{-5} m and mycelial length of 100 to 10,000 m g^{-1}, the surfaces of fungi may amount up to $\approx$0.03–0.3 m^2 g^{-1}_{soil} and comprise up to 0.01–1% of the specific surface area of soil [73]. By physical [74] and chemical [7] weathering of surfaces, mycelia further promote the release of matrix-associated [53, 75] or polymer-bound chemicals [76]. Fungi thereby produce significant amounts of amphiphilic surface active compounds that mediate the release of contaminants [77] and metals [78] and promote chemical transport and uptake to organisms [79]. Typical structures of fungal biosurfactants comprise sophorolipids, protein-lipid/polysaccharide complexes, glycolipids, or glycolipoproteins. Fungi also often decrease the pH in the mycosphere [80, 81] and modulate the speciation, release, and availability of pH-sensitive and/or matrix-bound chemicals. Acidic habitat conditions also enhance the dissolution of unless insoluble mineral elements and – in conjunction to fungal metabolites serving as bacterial carbon and energy sources – lead to improved nutrient availability and activity of surface weathering bacteria [72, 77, 78, 81]. The ubiquity and the widespread networks of mycelia in soils are further prone to transport sorbed chemicals over distances up to the cm range [60] and, hence, to increase the contaminant availability to degrader cells distant from the sources. This is a particularly important process in heterogeneous vadose environments [61], where air-filled pores restrict the transfer of water-bound chemicals and bacteria.

Table 1 Overview of mycelial traits and processes on habitat and bacteriome conditions being presumed to be relevant for microbial (bacterial) activity and the bioavailability and biodegradation of organic chemicals

Key traits of mycelial fungi	Main effects on bioavailability	Availability to degrader (bottleneck 1)		Activity and abundance of degrader (bottleneck 2)			Diversity and stability of degrader (bottleneck 3)	
		Release	Transport	Uptake	Activity	Abundance	Diversity	Stability
Release of primary and secondary metabolites	Mycelial release leads to enhanced availability of nutrients and carbon compounds and cross-feeding [106].	Metabolites are resource for matrix weathering organisms that promote release of chemicals. Organic acids change pH and affect release of pH-sensitive chemicals.	Organic acids change pH and thus solubility and transport of pH-sensitive chemicals.	Metabolites are resource for energy-driven or co-metabolic chemical uptake.	Metabolites are nutrient source for (co-metabolic) degradation [41], Khan et al. (2020) unpublished. Bacteria and fungi mutually exchange nutrients [107] and metabolites (cross-feeding) [57, 69, 107, 108]. Release of secondary metabolites alters the availability of nutrients in soil [109].	Metabolites enable biofilm formation on hyphae [110] and fuel bacterial dispersal along hyphae Metabolites are source for tactic colonization [111, 112]. Synergetic cross kingdom interactions under oligotrophic conditions [85] and increase of habitat carrying capacity of bacteria [20, 89].	Metabolites select for fungiphilic bacteria [56, 63, 64, 113]. Secondary metabolites modulate community composition (by acting as antibiotics [114] or signals to other organisms) [62, 115].	Metabolites form carbon and energy sources to fulfill maintenance requirements for degrader organisms [16, 20]. Fungal-bacterial synergetic cross kingdom interactions stabilize ecosystem functions [116].
Production of biosurfactants and exopolymeric substances	Biosurfactant and EPS release promotes solubilization of hydrophobic chemicals [117, 118] and hyphal growth.	Surfactants and EPS mediate chemical release from matrix [119].	Micelles promote transport of dissolved chemicals and mobilize liquid chemicals by emulsification [79, 120].	Micelles promote cellular chemical uptake [79].	EPS and micelles promote degradation by bacteria and fungi [79, 121].			Hydrophobins enable hyphal and bacterial spreading in air-filled matrices [7].
Saprotrophic growth using unspecific extracellular enzymes or radical-mediated transformations	Saprophytic growth decouples fungal biomass abundance from contaminant availability. Unspecific attack of complex and polymeric molecules increases release of chemicals and metabolites [7].	Unspecific attack promotes biochemical weathering of matrix and release of entrapped chemicals and nutrients [122].	Enzymes modulate compound mobilization [123].	Degradation of complex polymers leads to molecules available for bacterial uptake [124]	Transformation of complex polymers [125, 126], hydrophobic, recalcitrant chemicals, and micropollutants [124, 127] produce metabolites modulating bacterial activity.		Metabolites shape mycosphere bacterial community composition [56, 63].	Metabolites form carbon and energy sources to fulfill maintenance requirements for degrader organisms [16, 20].

Network-based growth: effect on matter transport	Mycelia decouple resource location from location of growth and help to overcome heterogeneous distribution of carbon sources and/or nutrients [128, 129].	Mycelial enmeshment of soil matrix [130, 131] promotes close contact and direct chemical uptake by hyphae and bacteria.	Mycelia form scaffold for matter transport in air-filled and heterogeneous matrices. Hyphae promote matter and contaminant transport [128]. Mycelia enrich hydrophobic compounds by biosorption and increase accessible concentration to mycosphere bacteria [132].	Hyphae transport chemicals to immediate vicinity of bacterial surfaces [61].	Mycelia increase bacterial degradation activity [61, 133]. Mycelia bridge NAPL-water interfaces [133] and increase degradation.	Hyphae take up and transport chemicals/nutrients to communities distant from source [60, 134]. This increases habitat carrying capacity of degrader bacteria [89]. Mycelial matter transport provides carbon and energy source for spore germination and bacterial growth in oligotrophic habitats [58].	Hyphae form networks for signaling molecules and modulate cell-to-cell interactions [62]. Mycelia retain bacteriophages [101] infecting bacteria.	Networks allow for spatial decoupling of resource and bacterial/fungal growth location [135]. Hyphae fuel bacterial dispersal to zones of heterogeneously distributed chemicals (increased accessibility) [83] or out of zones of disturbance.
Network-based growth: effect on bacteria transport	Mycelia are a scaffold for bacterial dispersal and promote transport through air-filled pores [14].	Mycelia enable transport of bacteria to sorbing matrices promoting mixed bacterial-fungal biofilm formation, increased biodegradation [77], and bacteria-induced weathering of matrix.	Bacteria are transported to contaminant source [136].		Mycelia enable chemotactic movement along [70] and to mycelia. Dispersal networks promote bacterial transport and degradation at low matric and osmotic potentials [99]. Dispersal of predators along hyphae affects degradation [91, 136, 137].	Mycelia form scaffolds for tactic movement to chemoattractor sources [70]. Dispersal of predators decreases bacterial biomass [91, 136, 137]. Dispersal networks promote bacterial transport and abundance at low matric and osmotic potential [99].	Mycelia form scaffold for transport of predators [91, 137]. Mycelia promote horizontal gene transfer among bacteria and evolution [71, 100]. Mycelia transport chemical signals and shape cell-to-cell interactions [62]. Mycelia increase co-migration-induced invasion effects [138].	Mycelia allow for recolonization of disturbed areas [98]. Mycelia enable dispersal and habitat colonization at low matric potentials [68].

(continued)

Table 1 (continued)

Key traits of mycelial fungi	Main effects on bioavailability	Availability to degrader (bottleneck 1)		Activity and abundance of degrader (bottleneck 2)			Diversity and stability of degrader (bottleneck 3)	
		Release	Transport	Uptake	Activity	Abundance	Diversity	Stability
Modulation of water regime, pH, and salinity	Mycelia induce change of hydraulic flow regime and water availability. Hyphae modulate pH and salinity at the microscale [139].	Mycelia modulate surface wettability [140] and chemical release due to changed surface wetting.	Mycelia increase waterborne solute transport. Mycelia decrease water intrusion by hydrophobins [141]. Mycelia change pH and affect transport of pH-sensitive compounds.	Mycelia potentially change uptake of pH-sensitive chemicals [139].	Mycelia increase microbial activity due to water transport [58].	Mycelia increase bacterial abundance in areas of low water availability due to water transport [58].	Mycelial influence presence and diversity of fungiphilic degrader bacteria [56, 63, 142].	Mycelia alleviate drought stress and salt stress for bacterial communities [99].
Modulation of matrix integrity	Turgor pressure apical growth promotes physical effects on matrix integrity and subsequent release of entrapped chemicals [53, 75] [75].	Mycelia promote bio-weathering of matrices and surfaces [74, 75, 143] and availability of entrapped chemicals and nutrients.	Mycelia influence soil aggregate structure [80] and change hydraulic conductivity and solute transport [144].					Mycelial bioturbation allows for colonization of new matrices, nutrient sources, and habitats.

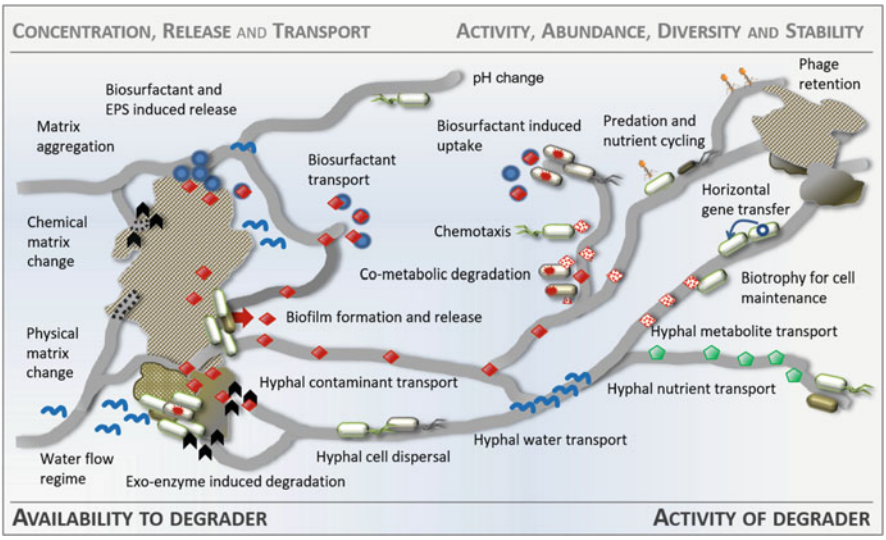

Fig. 3 Schematic overview of mycosphere processes promoting the bioavailability and biodegradation of contaminants. These processes (i) stimulate the release and transport of matrix-bound chemicals, (ii) increase the contact probability between contaminants and degrader cells, (iii) fuel synergetic bacterial-fungal exchange of nutrients, (iv) promote co-metabolic degradation of contaminants, (v) endorse evolution in bacterial communities, and (vi) modulate physical and chemical habitat conditions. For detailed descriptions, please refer also to Table 1

3.2 Bottleneck 2: Activity and Abundance of Degraders

Efficient contaminant transfer to cells occurs if cells are able to efficiently take up and degrade the chemicals. This is the case if they draw advantage from degradation or if co-substrates promote their uptake and degradation. Next to high contaminant fluxes, microbial habitats hence also must provide sufficient nutrient and energy fluxes to degrader cells to sustain their activity and abundance. Low nutrient transfer to cells not only limits the functional performance of cells but also evokes inadequate intracellular regulation and expression of metabolic contaminant degradation pathways and, thus, impairs the survival, abundance, and evolution of bacterial degrader communities. The impact of fungi on the physiology, regulation, and expression of metabolic pathways in bacteria is still poorly studied. Mycelia, however, have been described to serve as networks for random and chemotactic dispersal of bacteria ("fungal highways," Fig. 2a), thereby endorsing the contact probability between degrader cells and contaminant sources and, hence, the activity and abundance of degrader organisms [82, 83]. Mycelia further enable the transport of contaminants and fungal metabolites (e.g., low molecular weight peptides, organic acids, sugars or sugar alcohols, metal-mobilizing or antimicrobial compounds [84]) to distant bacteria and sustain bacterial-fungal cross-feeding [85] and bacterial activity and abundance, resp. [82, 86, 87]. Such cross-feeding is

particularly important [88] under oligotrophic conditions [85] where fungi increase the habitat carrying capacity of bacteria [20, 89]. It also allows for carbon flows within the mycorrhizosphere where bacteria have been split into "plant-feeders" and "fungus-feeders" [88]. Independent of the habitat, bacterial-fungal interactions play an important role in biogeochemical nutrient and carbon cycling [86, 87] and thus also for the turnover of contaminants. Another trait of (mainly saprotrophic) fungi comprises the use of unspecific extracellular enzymes and/or radical-mediated transformations that also allow for the co-metabolic breakdown of complex polymeric compounds, recalcitrant chemicals, or organic micropollutants. Subsequent transformation products act as carbon and energy sources for energy-driven bacterial contaminant uptake and/or co-metabolic degradation as detailed above. Mycelial cytoplasmic streaming also goes along with "active" transport of water by the "fungal pipelines" enabling bacterial activity in otherwise dry areas [58]. A recent study, for instance, has shown that hyphal release of water and nutrients induced the germination of *Bacillus subtilis* spores [58]. By enmeshing soil aggregates [80], some fungi also modulate the hydraulic conductivity [90] and water flow regimes and thereby promote waterborne transport and mutual contact of chemicals, nutrients, and bacteria. An often overseen aspect is the effect of mycelia on predation and subsequent cycling of nutrients within contaminant degrading communities. Recent studies have analyzed the joint effect of predation and dispersal networks on contaminant degradation by linking spatial abundances of degrader and predator bacteria to the degradation of the major soil contaminant phenanthrene [91]. The data found suggested that predation facilitated by (mycelial) dispersal networks support the build-up of an effective bacterial biomass and, henceforth, contaminant biodegradation in heterogeneous systems such as soil [91, 92].

3.3 *Bottleneck 3: Functional Stability and Diversity of Degraders*

Adopting this chapter's concept of bioavailability, it becomes clear that contaminant bioavailability comprises both a spatial [93] and a temporal dimension. Even though sufficient microbial degradation capacity may exist at a given point of time, environmental conditions (e.g., temperature, water, or nutrient availability) may fluctuate, and alter the composition, functional diversity and stability of degrading microbial communities [36, 94]. This may also include the loss of the genotypic and phenotypic diversity of degrader organisms and/or their expression of contaminant-specific functional traits (Fig. 4). The presence of contaminants and their varying fluxes to degrader cells, may further impact the fitness and performance of competing degrader organisms. For instance, bacteria and fungi may strive for the same nutrient and electron acceptor pools. Depending on the situation, bacterial-fungal interactions may be

competitive (e.g., induced by antimicrobial compounds [95]) or range from apparently random physical interactions to specific commensal or symbiotic associations [95, 96]. Bacterial biotrophy of extracellular fungal products (e.g., organic acids, sugars, or polyols [84]) may be a commensal interaction that has been discussed as an effective strategy to fuel bacterial dispersal along hyphae. Such dispersal promotes bacterial access to new contaminant sources [83] or help bacteria to efficiently drop out of unsuitable habitats. Although many studies exist on the use of bacteria and fungi in bioremediation approaches (cf. [97]), still limited information exists on their [62] physical and metabolic interactions during contaminant degradation in complex environments. Experiments in synthetic and in silico microbial habitats however revealed that hyphae promote the stability of contaminant biodegradation by (1) translocating bacteria and nutrients for enhanced recolonization and recovery of degrader communities after disturbances [98], (2) distributing water from wet to dry habitats and thereby shaping suitable local matric potentials for improved bacterial degradation [58] and fungal compound mineralization [41], and (3) sustaining bacterial dispersal and compound degradation capacity at low osmotic and matric potentials [99]. Mycelia also serve as novel ecological routes for enrichment and dissemination of antibiotic resistance genes [100] and as a focal point [71, 100] for horizontal gene transfer (HGT) between genetically distinct bacteria. HGT is an important evolutionary mechanism that endows bacteria with new genetic traits in favor of contaminant degradation [71]. Recent work has also discussed the mycosphere as arena for bacteriophage retention [101] and phage-induced exchange of genetic elements among microbial communities [102, 103]. Phage predation [104] in the mycosphere may form an important evolutionary force for microbial degrader communities and their adaptation to changing environmental and contaminant conditions, respectively.

4 Lessons Learned: Contaminant Bioavailability Stretches over Various Organizational Levels and Requires Deep Understanding of the Ecology of Degrader Microbial Systems

Bioavailability for contaminant degradation in soil requires a deep understanding of the ecology of degrader microbial systems. The drivers of bioavailability in the mycosphere thereby stretch over different organizational levels and scales including the molecular, cellular, community, and system level (Fig. 4). At the molecular level, the structure and the physicochemical properties of the chemical will determine the abiotic interactions and potential intrinsic recalcitrance toward existing biochemical degradation pathways (and the evolution of new pathways, respectively). Such chemicals are unlikely to be quickly degraded without the production of harmful or persistent degradation products. At the cellular level, mass transfer into the cell (i.e., by active or diffusion-driven cellular uptake) and biochemical transformation

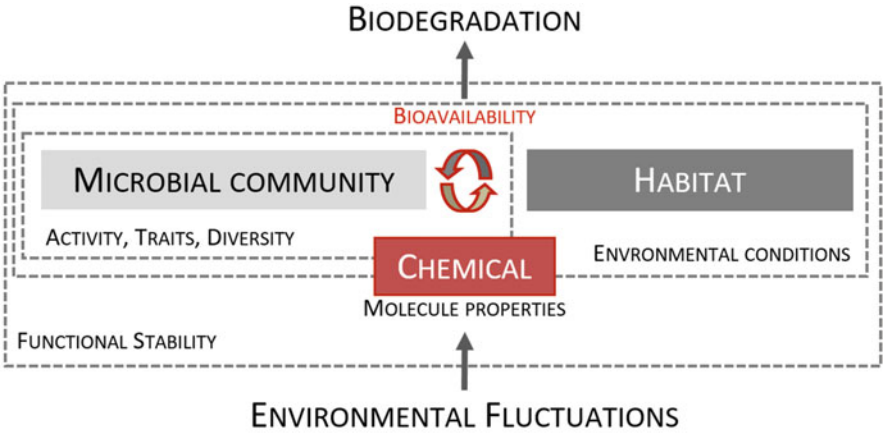

Fig. 4 Bioavailability for contaminant degradation requires a deep understanding of the ecology of contaminant degrading microbial systems in the mycosphere. The drivers of bioavailability of inherently degradable chemicals thereby stretch over different organizational levels and scales including the molecular, cellular, community, and microbial system level. The biodegradation of a chemical depends on its physicochemical properties and intrinsic structural stability toward (bio-) chemical reactions (molecule properties), its presence and distribution in a given habitat, and also the functional potential and effectiveness of microbial communities (traits). All these aspects are subject to and result from ever-changing environmental fluctuations

capacity by the cell will determine the biodegradation (cf. Fig. 1). These processes have been shown to be modulated by the mycosphere in several ways. At the community level, the presence of contaminants also exerts selective pressure on the abundance and diversity of microbial communities [36]. Increased exchange of genetic elements in the mycosphere also triggers the evolution of their genetic potential to degrade new chemical structures or biodiversity changes. Finally, at the system level, the prevailing habitat conditions must provide sufficient proliferation to allow for ongoing contaminant bioavailability and degradation ("functional stability"). This may also require microbial interactions with plants (e.g., in the rhizosphere [51]) or other higher organisms such as soil-dwelling animals. Last but not least, even though we here focus on biodegradation, microbial system considerations on compound bioavailability also need to account for competing abiotic degradation processes (e.g., photo-degradation, heterogeneous catalysis at matrix surfaces, or hydrolysis) that may affect available concentration of chemicals and their epi-metabolome [105] in any microbial system.

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Part IV
Methods for Measuring Bioavailability

Bioavailability and Bioaccessibility of Hydrophobic Organic Contaminants in Soil and Associated Desorption-Based Measurements

Anthony C. Umeh, Ravi Naidu, Olugbenga J. Owojori, and Kirk T. Semple

Contents

Abstract Many publications on contaminant bioavailability in soils often state that the use of total contaminant concentrations in risk assessment is an overly conservative approach. Such conservatism makes traditional risk assessment approaches and contaminated land decision-making expensive. The risk-based approach to

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contaminated land management strives to identify and manage the potential risks of significant harm being caused to humans and ecological receptors, following exposure to contaminated land. Risk-based approaches are more cost-effective than the traditional approaches from the perspective of contaminated land management. Contaminant bioavailability or bioaccessibility is one of the critical concepts that underpins risk-based approaches to contaminated land management. Bioavailability describes the fraction of the total contaminant concentration that desorbs from soil and is immediately available to cause harm to a living organism, after passing through the organism's membrane. Bioaccessibility describes what is available and potentially available under natural environmental conditions and during realistic timeframes. The reliable measurements of either contaminant bioavailability or bioaccessibility is therefore critical; in this regard, a thorough understanding of contaminant sequestration and desorption behaviour is required. This chapter discusses the fate of HOCs in soils, bioavailability and bioaccessibility of organic contaminants and their associated desorption-based measurements.

Keywords Bioaccessibility, Bioaccumulate, Bioavailability, Desorption, Hydrophobicity, Sequestration

1 Introduction

Chemical contamination is a global problem. Over the years, thousands of organic and inorganic chemicals have been released into the environment, particularly through anthropogenic sources. In many contaminated sites, hydrophobic organic contaminants (HOCs) are present and of concern as they are persistent in the environment and can bioaccumulate in living organisms and display toxic and carcinogenic behaviour. Examples include polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), dioxins and furans, polychlorinated biphenyls (PCBs) and dichloro-diphenyltrichloroethane (DDT) and other chlorinated pesticides. Emerging contaminants, e.g. per- and polyfluoroalkyl substances (PFAS) and pharmaceuticals, are also gaining increasing global attention. These chemicals are transported through the environment where they may end up in matrices, such as soils, sediments, water and air, in a wide range of concentrations. These emerging contaminants may also pose risks to living organisms following exposure to contaminated matrices.

The health risks posed by HOCs to humans and other ecological receptors and the potential for (significant) harm to be caused following exposure are traditionally thought to be a function of the total concentrations in the contaminated matrix. It is assumed that the total HOC concentrations in soils are 100% bioavailable to humans, plants and animals and can cause harm following exposure. The total concentrations of HOCs in soils and sediments are routinely determined in laboratories for risk

assessment purposes. For this determination, soils are sampled and subjected to an exhaustive solvent extraction, at high temperature and pressure with or without high-energy input agitation. The determined total concentration, with supporting regulation, is then considered for contaminated site management to protect potential human and ecological exposures. Each of the components of the source-pathway-receptor relationship is managed.

Over the last 20 years, there are mounting evidence showing that the total concentration of contaminants in soils are not 100% bioavailable, particularly for long-term contaminated soils [1]. The presence of HOCs in soils, or the determination that HOCs are present, may not equate to significant harm being caused or the significant possibility of such harm being caused following exposure. Different factors cause the reduction of HOC concentrations that humans and ecological receptors may be exposed to. One such factor is the time from which an organic contaminant first enters the soil and the time to soil sampling and analytical measurement [2]. The longer the time HOCs spends in soils after first entry, the more likely that its concentrations will be reduced. Also, the physicochemical properties of soils and contaminants of interest, including environmental factors, such as temperature, may influence the contaminant concentrations that humans and other ecological receptors may be exposed to. The hydrophobicity of organic contaminants means that their phase distribution will be influenced not only by the surrounding matrix (soil or sediment) and biological properties but also by physicochemical sequestration processes with increasing soil-HOC contact time, including diffusion into the 3-D structure of the soil and sorption to soil surfaces [3–5].

It is now accepted that the traditional approach to contaminated site assessment and management certainly overestimates the fraction or concentration of contaminants in soil that may be readily available or bioavailable to cause harm to living organisms following exposure [6, 7]. Therefore, it is meant to be conservative but in some cases can be overly protective. Moreover, the emerging risk-based approach to contaminated land risk assessment and management questions whether the total contaminant concentration is really needed to be completely cleaned up in the first place [8, 9], especially when considering that the stringent clean-up levels are difficult to achieve economically using current technologies. In the risk-based approach, identifying and managing the potential risks of significant harm are important [8, 9]. Risk-based approaches help to prioritise the effective management of contaminated sites that have potential to cause harm and as such be more cost-effective, compared to the traditional approach. Reliable measurements of either contaminant bioavailability or bioaccessibility are therefore critical for risk-based approaches to contaminated land management. The aims of this chapter are to briefly discuss the fate of HOCs in soils and bioavailability and bioaccessibility of organic contaminants and to discuss the desorption-based extraction methods for associated measurements in detail.

2 Conceptual Fate of HOCs in Soils

After release into soil, HOCs are subjected to different physicochemical and biological fate (Fig. 1). They can be volatilised, leached, photo- (chemically) or photochemically oxidised, biodegraded and taken up by living organisms [1].

With increasing soil-HOC contact time, the amount of HOC that can be extracted from soil tends to decrease [11], as a result of increased intra-soil processing [3]. In addition, the mobility, bioavailability, bioaccessibility and toxicity of the HOC tend to decrease as well [12–14]. This is known as 'ageing' (Fig. 2).

Key processes that occur during ageing include sorption and diffusion (or sequestration) and have been extensively reviewed elsewhere [2, 3, 5]. Sequestration is a number of physical processes which cause chemicals to persist in soils and involve the molecular diffusion of contaminants into micro- and nanopores, through a pore-filling mechanism, where they may become entrapped or occluded. Sorption is a combination of physical adsorption, partitioning into organic matter, and chemical binding, of HOCs to soil matrices. Overall, HOCs can interact with mineral and organic matter fractions and other sorbent matrices in soils, either through physical sorption, chemical sorption (van der Waals forces, hydrophobic interactions, electrostatic bonding, and hydrogen bonding), entrapment or occlusion in micropores [3, 15, 16].

The nature and extent of sorption of HOCs to soil is simply described by the soil-to-water partition (K_d) coefficient at equilibrium [17, 18]. Classically, it is assumed that the organic matter (rubbery and glassy) is the major partitioning phase for HOCs in soils [17, 19, 20]; hence, the K_d is often normalised by the fraction of organic carbon in soils to determine the organic carbon-water partitioning coefficient (K_{oc}). The K_{oc} describes how mobile HOCs are in soil. At high K_{oc} values, HOCs are likely to be retained in soil than in the dissolved pore water phase and vice versa. However, soils and sediments are very complex and heterogeneous matrices with a wide range of sorbent phases. Sorbent phases, such as black carbon, other carbonaceous materials and clay minerals, can also contribute to entrapping HOCs [3, 4, 11, 21–25]. Hence, the sole use of K_{oc} to predict or explain the nature of sorption, HOC sorption, is rather too simplistic [26, 27]. The quality and quantity of the different sorbent phases should be considered to understand the real nature and extent of HOCs sequestration in soils.

The effect of ageing and resulting sequestration processes is that the contaminant concentration in soils will not be 100% bioavailable [14, 28]. A fraction is freely dissolved in soil pore water and freely available; another may be weakly sorbed but potentially available, and another is strongly sorbed and may not be readily available. Also, there may be a fraction that is very strongly sorbed to the soil matrix (the so called non-extractable residues), and thereby recalcitrant and no longer considered available under natural environmental conditions. How much of each fraction is present in soils at a particular time is highly dependent on soil properties, such as organic matter and clay (in terms of quantity and type), cation exchange capacity, soil texture and structure, surface areas and pore size characteristics [11, 13, 14, 29],

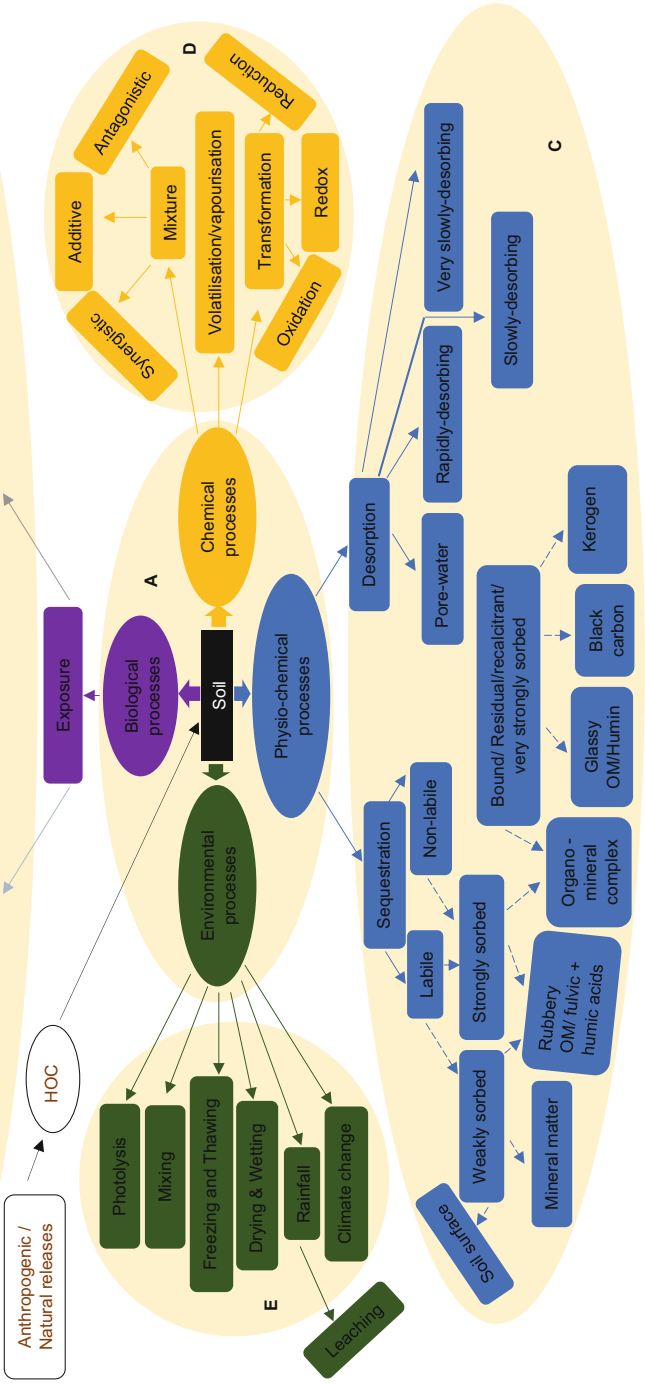

Fig. 1 Schematic fate and behaviour of hydrophobic organic contaminants in soil: Implications of biological, chemical and physicochemical processes. (**a**) Ageing effects. (**b**) Bioavailability influences. (**c**) Sorption-desorption processes. (**d**) Chemical reactions. (**e**) Environmental influences. The processes described in (**a**) may influence risks from exposure (**b**). No connotation to arrow lengths, sizes of shapes and dotted lines and outlines. However, dash arrow lines signify that there are uncertainties associated with these processes. HHRA is human health risk assessment; SGV is soil guideline value; EHRA is environmental health risk assessment; HOC is hydrophobic organic contaminant; OM is organic matter (Modified from [10])

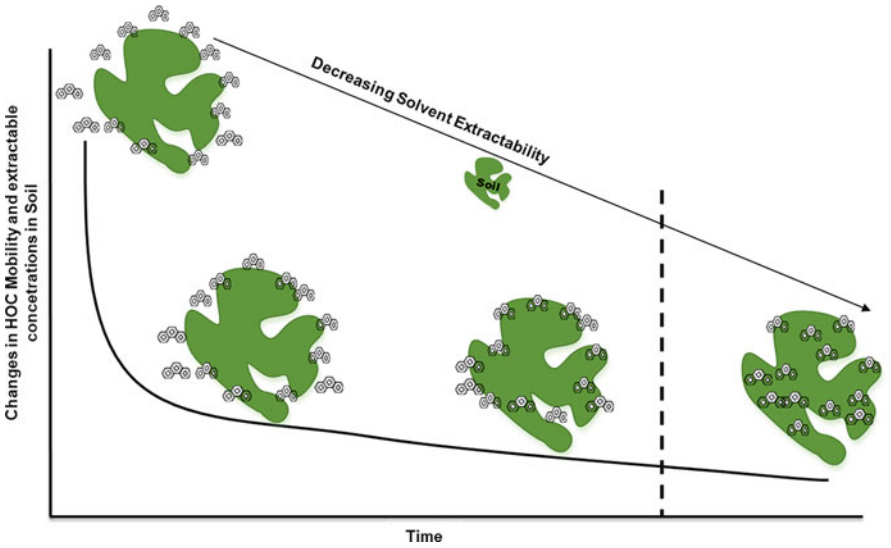

Fig. 2 Impact of soil contact time on HOC mobility and extractability

as well as dependent on the contaminant's physicochemical properties, and prevailing biological (e.g. biodegradation) and environmental conditions (e.g. temperature) [5, 30–33].

2.1 Temporal Fractionation of HOCs in Soil

2.1.1 Sorption

As ageing progresses, sequestration and sorption processes become increasingly dominant [2, 4, 5]. The total contaminant fraction in soils is delineated into different fractions of contaminants according to their interactions in soils (Fig. 3). The dissolved fraction which is present in the soil aqueous phase may be freely dissolved, associated with dissolved organic carbon as colloids or complexes, or with other constituents, or sorbed to surfaces of fine mineral particles [34]. The freely dissolved fractions are available for easy uptake by soil organisms [35–37]. The fraction of contaminants that are sorbed to soil surfaces, such as the surfaces of clay minerals, or that partition into soft (or rubbery or amorphous) organic matter surfaces in soils can be weakly sorbed [3]. Overall, the weakly sorbed are associated with characteristic weak chemical interactions such as van der Waals, dipole-dipole, dipole-induced dipole and hydrogen bonding [3, 38, 39].

The strongly sorbed contaminant fractions are associated with nanosized pore structures; carbonaceous sorbents, such as black carbon; and glassy organic matter (e.g. humin) and clay complexes [3, 39]. The strongly sorbed HOCs may become

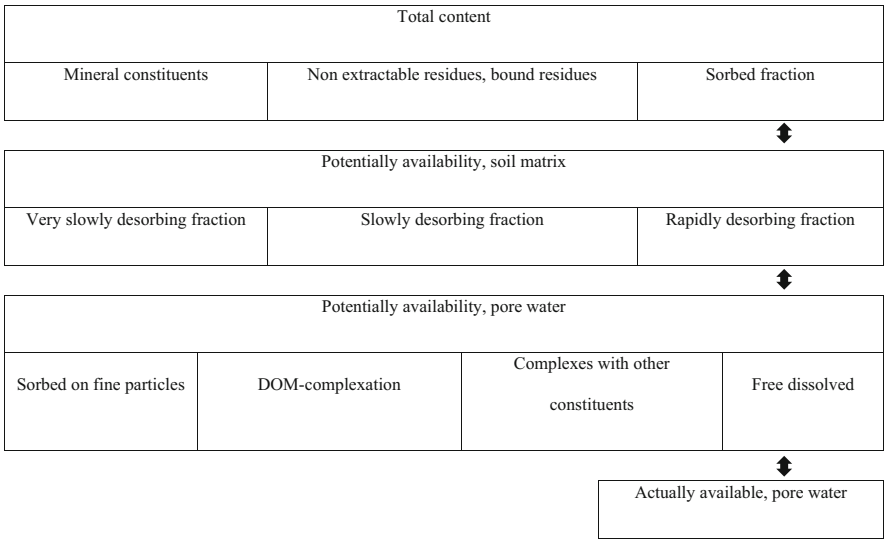

Fig. 3 Availability of contaminants in soil as a result of complex interactions in the soil matrix and pore water (adapted from [34])

very resistant, persistent and irreversibly sorbed in soils, particularly when covalently bound within soil matrices. The strongly sorbed HOC residues have also been classified to include at least two types, from the perspective of environmental relevance [39, 40]. Type I residues involve residues that exhibit low to high stability and reversibility since they are adsorbed to surfaces or occluded in pores and are principally involved in physical interactions with the soil matrix [39]. Type II residues are covalently bound residues with high stability and low reversibility potential [39]. Understanding such classifications may be important in the risk assessment of soils, where potential reversibility of HOC residues in soils are of interest.

Non-linear sorption of HOCs at increasing concentrations in soils has been reported in sorption isotherm studies [15]. The observed non-linearity is associated with the heterogeneity of sorption sites within the soil [41]. For instance, the soil organic matter simply consists of soft (or rubbery) and hard (or glassy) phases. Adsorption of HOCs to the glassy phase is known to be stronger than the adsorption to the rubbery phase, and this forms the basis of the dual phase sorption theory [15, 42]. Multiphase sorption of HOCs in soils is reasonable, considering the heterogeneity within soil matrices [3]. Overall, the microscale locations of HOCs in soils and the strength of interactions (sequestration and sorption) between HOCs and the soil matrix will influence the extent of sorption.

For the sake of simplicity, the different HOC fractions above were described as if each fraction were distinct; in reality, these fractions are a complex continuum. The key information is that the potential for organic contaminants to be released in soils, at such amounts or concentrations that can pose significant harm or significant

possibility of significant harm, reduces with time. Hence, it is not the total concentration of HOCs in soils that should be relevant in risk assessment, but the fraction of the total HOC concentration that is available to cause harm to exposed organisms.

2.1.2 Desorption

Prior to an HOC becoming toxic to an exposed organism, the HOC must be released and taken up from soils in considerable amounts under suitable physical and environmental conditions. Hence, the rates and extents of release and uptake of HOCs from soils are important. In historically contaminated soils, desorption is one of the key processes that influences HOC bioavailability and bioaccessibility [43–45]. For instance, it has been shown that microbial mineralisation of phenanthrene in soil terminated because the amounts of readily available or desorbable phenathrene plateaued, regardless of the presence of catabolically active microbes and enough nutrients [46]. To understand contaminant release and bioavailability (and bioaccessibility), a clear understanding of how much of the sorbed contaminant can be desorbed from soils now or in the future [11, 47, 48], including associated rates and extents [24, 33].

Non-exhaustive extractions (explained in detail later) of aged soils containing sorbed HOC residues and subsequent modelling of associated desorption behaviour have shown that desorption occurs in two or three phases [24, 49, 50], as depicted in Fig. 4. The first phase is rapid and describes the fast desorption of dissolved or weakly sorbed HOCs into surrounding soil pore water. Because the weakly sorbed or rapidly desorbing fractions are readily bioavailable, they are likely to be considerably depleted, particularly in long-term contaminated soils, due to biotic and/or abiotic losses and extensive sequestration. The second and third phases are slow and describe the slow and very slow desorption of more strongly sorbed HOC fractions, due to the tortuous diffusion from remote sites. Recent studies have

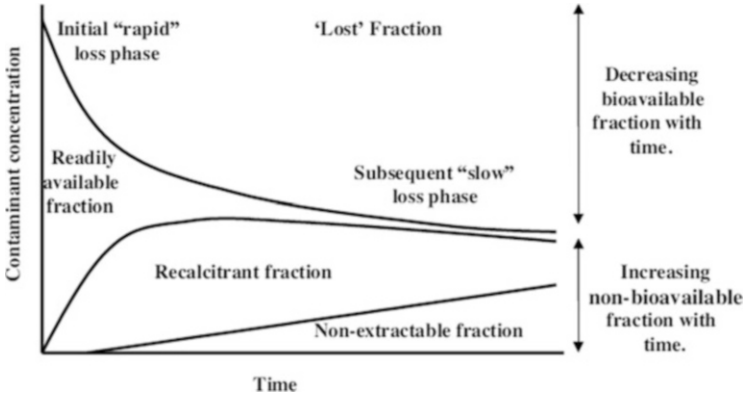

Fig. 4 Biphasic loss curve of hydrophobic organic contaminants highlighting the impact of sorption and ageing on contaminant bioavailability (adapted from [51])

confirmed that while these strongly sorbed fractions may be released slowly with time, the amounts that are released are extremely small and unlikely to pose risks to biota [11–13, 45, 47]. Where contaminant fractions are covalently bound or physically entrapped and immobilised, they may become recalcitrant or non-desorbable [39].

Desorption Kinetics

Two and three compartments, first-order equations are often used to describe the desorption behaviour of HOCs in contaminated soils (Eqs. 1 and 2), and associated mechanisms have been described in detail elsewhere [17, 23, 52, 53].

$$\frac{S_t}{S_0} = F_{\text{rap}} * e^{-k_{\text{rap}}*t} + F_{\text{slow}} * e^{-k_{\text{slow}}*t} \tag{1}$$

$$\frac{S_t}{S_0} = F_{\text{rap}} * e^{-k_{\text{rap}}*t} + F_{\text{slow}} * e^{-k_{\text{slow}}*t} + F_{\text{very slow}} * e^{-k_{\text{very slow}}*t} \tag{2}$$

where S_0 and S_t are HOC concentrations in the soil or sediment sample at time 0 and time t, respectively. F_{rap}, F_{slow} and $F_{\text{very slow}}$ are the rapidly, slowly and very slowly desorbing fractions, respectively. k_{rap}, k_{slow} and $k_{\text{very slow}}$ are the associated rate constants with each desorption fraction, respectively.

The rapidly desorbing fractions are often described in terms of fast extents (F_{rapid}) and rates (k_{rapid}). The slowly desorbable fractions are described by slow or very slow extents (F_{slow} or $F_{\text{very slow}}$) and rates (k_{slow} or $k_{\text{very slow}}$). Studies have shown that k_{slow} can range from 10^{-2} to 10^{-4} for different soils and HOCs, and generally up to two orders of magnitude lower than k_{rapid} [50], particularly in soils with carbonaceous materials with strong capacity to sequester HOCs [24].

3 Contaminant Bioavailability and Bioaccessibility

Contaminant bioavailability and bioaccessibility are critical considerations during risk-based contaminated land assessment and management (Fig. 5). Bioavailability is defined as 'that fraction of a chemical which is freely available to cross an organism's cellular membrane from the medium the organism inhabits at a given time' [6]. By freely available, there is a perception of immediacy, i.e. the contaminant is available now to cross an organismal membrane [6]. Only after crossing the membrane can the HOC be subjected to other processes such as uptake, transformation, toxicity, storage, degradation and elimination [54].

Specific to human health risk assessments, a bioavailable compound, often determined in vivo [14, 55–58], is that fraction that crosses the intestinal epithelium from the gut fluid into the blood to exert toxicity to cells, tissues, organs and systems

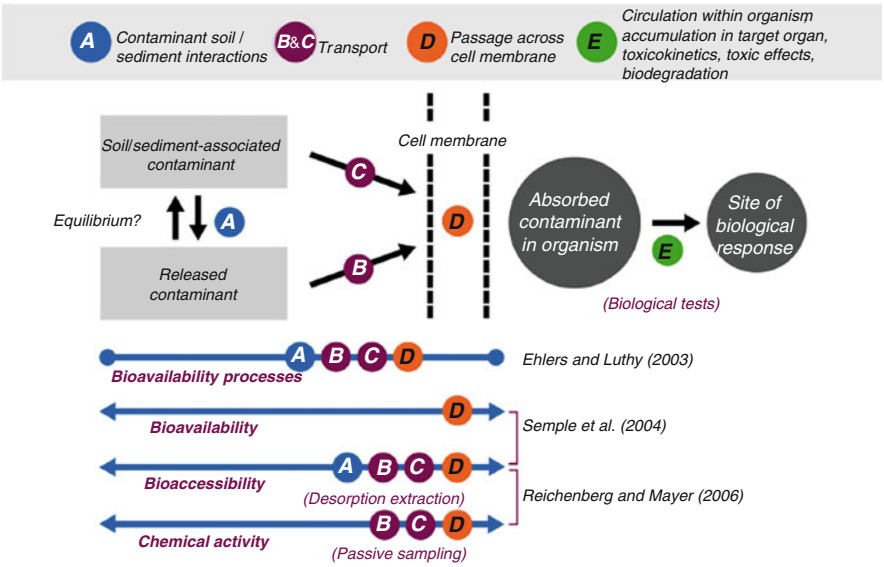

Fig. 5 Overview of scientific concepts of the bioavailability of organic chemicals (adapted from [7])

(Fig. 6). Bioaccessibility refers to the fraction of a chemical that is available now plus what may become available [6, 54]; in other words, there are spatial and temporal constraints delaying the organism's access to the chemical (Fig. 5). According to the National Research Council report [59], bioavailability processes describe the 'individual physical, chemical, and biological interactions that determine the exposure of organisms to chemicals associated with soils and sediments'. In Reichenberg and Mayer's paper [54], bioaccessibility and chemical activity were considered as two important and complementary constituents of bioavailability. A summary of the different bioavailability definitions can be found elsewhere [5]. Specific to human health risk assessments, the bioaccessible compound is the fraction of the total concentration of contaminants that is dissolved in the gut fluid with a potential to cross through the intestinal epithelium to become bioavailable and is often determined in vitro [13, 55, 57, 60, 61]). For soil and mammalian systems, the bioaccessible fraction is a more conservative estimate, being often greater than the bioavailable fraction, but less conservative than that determined by total extraction.

It is immediately apparent from these definitions that bioavailability and bioaccessibility concepts are discipline-, site-, chemical-, environmental matrix- and condition-specific. For instance, bioavailability may be related to how much of a contaminant in soils can be biodegraded towards assessing the success of a bioremediation strategy for a site [46]. Bioavailability may also relate to the fraction of a contaminant that could pass through the gut membrane of earthworms and cause genotoxicity [12, 37, 62, 63] or the amount that can be dissolved in the human gut membrane and cause toxicity [13, 55, 64]. While bioavailability and bioaccessibility

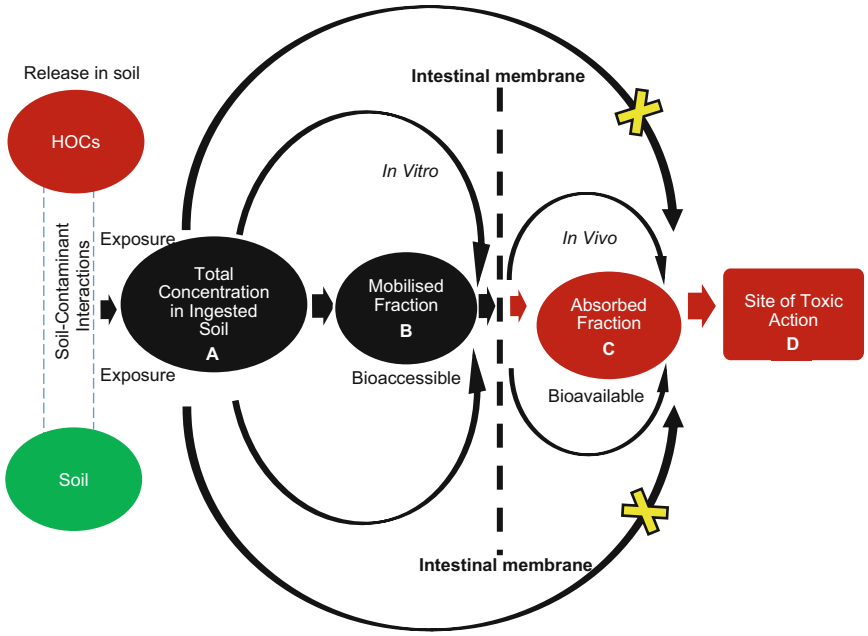

Fig. 6 Contaminant mobilisation via a simulated gastrointestinal fluid. (**a**) Contaminated soil with a known total extractable concentration is introduced into an in vitro bioaccessibility model. (**b**) Fraction of A which is mobilised. (**c**) Fraction of (**b**) which crosses intestinal epithelial membrane, absorbed and may adversely impact cells, organs, tissues and/or whole organismal systems and associated functions (**d**). Yellow 'X' symbol = total concentration does not imply absorbable concentration, hence, the use of total concentration to predict potential for harm following exposure to HOCs-contaminated soil has been reported to be overly conservative (adapted from [10])

considerations are important in risk assessment, the goal and endpoints of the risk assessment needs to be considered carefully in site-specific risk assessments [10, 65].

3.1 Measurement of HOC Bioaccessibility

To achieve a more forensic approach to the risk assessment of contaminated land, a reliable measure of bioavailability or bioaccessibility must be achieved to support the decision-making process. Ideally, bioavailability of target contaminants to the actual receptor would be the best approach; however, studies involving invertebrates and larger animals are expensive, time-consuming and technically demanding. The other important consideration is that bioavailability is organism- and even species-specific [28], adding a degree of uncertainty into the risk assessment process. An alternative approach is to use an in vitro extraction method, which can be used to

assess contaminant bioaccessibility, which is more conservative, and used as a surrogate for bioavailability in risk assessment.

Contaminated soils have been extracted with 'harsh' solvents, 'mild' organic solvent and organic solvent-water mixtures, surfactant solutions, aqueous solutions consisting of complexing agents such as cyclodextrins or adsorbent resins such as Tenax and XAD and supercritical fluid (CO_2), as well as persulphate oxidation of contaminated soils [51, 66]. Of these methods, HPCD and Tenax extractions are widely used for bioaccessibility testing particularly for ecological health risk assessments [38, 67, 68], considering the relative ease of the extraction procedure, strong correlations with endpoints such as biodegradation, and the reproducibility of the extraction results [69]. For human health risk assessments, extraction techniques that simulate the gastrointestinal digestion processes are utilised [70].

It should be noted that bioaccessibility is measurable chemically and although more conservative than bioavailability [51], which is more difficult to measure as a function of desorption, it is less conservative than the total extractable concentration. The following section briefly discusses exhaustive extractions and then focuses on HPCD and Tenax extractions, including a brief discussion of human physiologically based extraction techniques.

3.1.1 Exhaustive Measurement of HOC Bioaccessibility

Traditionally, exhaustive extraction techniques are used during routine risk assessment of contaminated soils to recover as much of sorbed HOC fractions as possible [38], regardless of bioavailability and bioaccessibility considerations. The basic principle involves the mixing of contaminated soils with 'harsh' organic solvents (e.g. dichloromethane, acetone, hexane, toluene) and extraction at high temperatures and pressures in an attempt to determine the total HOC concentrations in soils [71]. Here, total concentration refers to the dissolved, rapidly desorbing (weakly sorbed), slowly or very slowly desorbing (reversibly sorbed) and all or none or part of recalcitrant or bound HOC residues [34]. At such conditions, the solvents are highly mobile and can displace HOCs that are adsorbed to surfaces and pores, so that the HOCs are partitioned into the organic solvent phase [53, 71, 72]. Different equipment and extraction procedures have been utilised and these involve soxhlet extraction, Soxtec extraction, microwave-assisted extraction, supercritical fluid extraction, ultrasonic extraction and accelerated solvent extraction [51, 66]. Nowadays, accelerated solvent extraction techniques are used for exhaustive extractions because of its speed, solvent savings and extraction efficiency.

Total HOC concentrations from traditional exhaustive extractions generally overestimate bioavailability and bioaccessibility [28, 73]. For example, the exhaustive extraction of PAH-contaminated soils with solvents, such as dichloromethane, did not predict the bioavailability of phenanthrene and/or atrazine to bacteria and/or earthworms that were exposed to the soils that were aged for up to 320 d [73, 74]. Also, exhaustive extraction techniques are associated with high-energy inputs which can considerably change the nature of the soils being extracted

[72, 75]. In addition, the health risks and costs associated with the use and disposal of hazardous solvent wastes reinforce the unsustainability of continuous dependence on exhaustive extractions in routine risk assessments.

3.1.2 Non-exhaustive Measurement of HOC Bioaccessibility

In ecological health risk assessment, it is often considered that dissolved contaminant fractions in soil pore water are readily available for uptake by microbes, earthworms and other soil organisms and plants. However, the concentration of dissolved fractions is rarely at equilibrium, especially as surface sorbed and strongly sorbed fractions are rapidly and slowly released, respectively, into the soil solution over time [4, 5]. Hence, living organisms are ideally exposed to the bioaccessible contaminant fractions over time, that is, the dissolved plus rapidly desorbing (the sum of which is theoretically less than total contaminant concentrations). The non-exhaustive extraction techniques (NEETs) are low-energy input techniques that seek to measure bioaccessibility, rather than total contaminant concentrations (Table 1). These 'mild' solvent, hydrophobic resins or aqueous extractions are considered to better mimic the in vivo processes associated with contaminant-biota interactions, compared to exhaustive extractions. Here, extreme temperature and pressure inputs are not involved; hence, the intrinsic nature of the soil may only be minimally affected.

Mild Solvent Extractions

Mild solvent extractions involve the use of slightly polar solvent-water (e.g. methanol-water) mixtures or non-polar solvents (e.g. n-butanol). Here, an appropriate volume of solvent is added to a soil (or sediment) sample, and the slurry is gently vortexed for a period and then centrifuged. After centrifugation, the supernatant is prepared for analysis of associated HOC concentrations. Good correlations ($R^2 = 0.5$–0.9) have been reported between concentrations of mild solvent-extractable HOCs and indicators of bioaccessibility, such as microbial degradation and earthworm bioaccumulation. For instance, good positive correlations were reported between butanol-extractable phenanthrene ($y = 1.0x + 0.9$, $R^2 = 0.97$) and pyrene ($y = 1.0x - 1.0$, $R^2 = 0.99$) from artificially spiked soils and the amounts of the respective PAHs that were biodegraded [74, 76]. In a similar manner, good positive correlations were reported between butanol-extractable PAHs from an artificially spiked soil that was aged for 6 months and bioaccumulation in earthworms (*Eisenia fetida*) and ryegrass (*Lolium multiflorum*) [77]. However, the PAH profile extracted by butanol differed significantly from the profile of PAHs that were bioaccumulated, particularly for HMW PAHs [77]. There have also been reports of overestimations of PAH bioaccumulation in *Eisenia fetida* and *Lolium multiflorum* based on mild n-butanol extractions in investigations that used ten field-contaminated soils [35]. Recently, very good correlations were reported between

Table 1 An overview of developed desorption/extraction methods and optimal desorption duration (modified from Table 3 in [10])

Desorption method		Optimal desorption time	Advantages	Disadvantages	References
Non-exhaustive mild solvent extractions	n-butanol, ethyl acetate, methanol, methanol-H_2O	Up to 24 h	a. Measure microbially/earthworm available fractions b. Less conservative than exhaustive extractions c. May be useful for a detailed research study, where time efficiency is not a very important factor	a. Reportedly overestimative or underestimative of the bioavailable/rapidly desorbed fraction b. Organic solvents can cause pore swelling which may change sorbent sorption properties and behaviour, thereby, introducing a system artefact c. Operating procedures differ for different HOCs d. May not be relevant for determining desorption kinetics of residual HOCs since desorption duration may take months or years	[74, 78–83]
Supercritical Fluid (SCF)	CO_2	Up to 40 min	a. F_{rapid} correlates well with fraction biodegraded in the field b. Very fast – time efficiency is high c. at 50°C and 200 bar, PAH solubility in CO_2 mimicked aqueous solubility d. Can generate high recoveries compared to other methods	a. Different pressure and temperature conditions to ensure release of HMW and LMW PAHs, but are easily adjustable to suit different HOCs b. Aqueous desorption of phenanthrene differs from SCF c. Effectiveness for determining desorption kinetics of residual HOCs is still doubtful	[82, 84–87]

(continued)

Table 1 (continued)

Desorption method		Optimal desorption time	Advantages	Disadvantages	References
				d. May also cause swelling of organic matter, thereby influencing sorption properties and introducing a system artefact d. Procedure seems complex and may require technical expertise and equipment	
Solid phase extractions	Water/ XAD_2	Up to 4 d	a. F_{rapid} correlates well with fraction biodegraded in the field b. Fast	a. Longer desorption times can be overestimative of F_{rapid} in reality b. Tend to overestimate fractions released of 4 ring PAHs	[85]
	Tenax	264–400 h	a. Highly effective for estimating F_{rapid} and initiating the release of F_{slow}	a. To determine F_{slow} including associated kinetics, method may not be time efficient (250–640 d) b. Mostly used for sediments	[49, 52, 82, 86, 88]
Contaminant trap	PDMS/activated carbon	9–30 d	Claims to measure the desorption-resistant, after removing the bioaccessible, fractions	Complex. Further research is needed	[89]
Sink + diffusive carrier	PDMS + HPCD	Up to 14 d	Claims to measure the desorption-resistant, after removing the bioaccessible, fractions	Complex. Further research is needed	[90]

(continued)

Table 1 (continued)

Desorption method		Optimal desorption time	Advantages	Disadvantages	References
Activated energy technique	High temperature desorption (HTD) in a superheated/subcritical water system	2–3 d	a. May be useful for rapid prediction of desorption kinetics of residual HOCs at an optimum temperature of 150°C b. Desorption profiles at 25°C using Tenax/water (up to 640°C) mimicked those at 150°C using HTD after time-scaling procedures	Procedure seems complex and may require technical expertise and equipment	[82, 86, 91, 92]
Aqueous extractions	H_2O, H_2O-$CaCl_2$, SPMDs		a. Effectively measures the leachable HOC fractions (pore water phase concentrations) in OM-poor soils	a. Cannot effectively determine rapidly or slowly desorbing fractions in OM-rich and aged soils, which have implications for earthworm/plant uptake or bioaccumulation and microbial degradation b. Low extractability compared to solvent-water mixture extractability c. Underestimate actual bioaccessibility	[51] and references therein
Aqueous-based extractions	Hydroxyl-b-propyl cyclodextrin	24 h	a. Very simple b. Reproducible c. Very fast d. Highly effective for estimating F_{rapid} and initiating the release of F_{slow} e. Good	a. To determine F_{slow} including associated kinetics, method may not be time efficient as sequential extractions for a longer time	[29, 51, 69, 73, 93–101]

(continued)

Table 1 (continued)

Desorption method		Optimal desorption time	Advantages	Disadvantages	References
			correlations between F_{rapid} and microbially degradable fractions especially for LMW PAHs	period may be needed	
Persulfate oxidation	$K_2S_2O_8$	3 h	a. Quite simple b. Residual fractions of 2–6 rings and 16 EPA PAHs in soils and sediments agrees with those after microbial degradation c. Relates bioavailable and residual HOC fractions organic matter domains in a simplistic, but not robust, terms	a. Difficulty with predicting the release, not residual, of HMW PAHs (5 and 6 rings) and total petroleum hydrocarbons in aged soils	[88, 102, 103]

butanol-extractable benzo[a]pyrene in eight different artificially spiked soils and relative bioavailability using juvenile swine ($R^2 = 0.75$, $p < 0.01$) [14]. Whether similar correlations will be observed between butanol extractability and relative bioavailability of HOCs in field-contaminated soils needs to be studied. Overall, butanol extractability and HOC bioaccessibility correlation studies will need to follow set standard operating procedures to minimise variabilities from experimental and operational conditions, and supporting mechanisms also need to be investigated. Table 1 is an overview of developed desorption/extraction methods and optimal desorption duration (modified from Table 3 in [10]).

Aqueous and Aqueous-Based Non-exhaustive Extractions

Weak salt solutions that mimic the ionic strength of soil pore water, such as 0.01 M $CaCl_2$, $MgCl_2$ or $NaNO_3$, are often used to extract the labile contaminant concentrations or dissolved fractions. In a similar manner, passive samplers and semi-permeable membrane devices are used to determine freely dissolved pore water concentrations and are described elsewhere in this book. Since such aqueous extractions cover only dissolved fractions in soils, bioaccessibility may be underestimated.

Non-exhaustive extractions involve extraction of HOC-contaminated soils with aqueous solutions that incorporate materials with large hydrophobic cavities (e.g. HPCD) or a solid phase that acts as a hydrophobic sink (e.g. Tenax beads, C18 discs, XAD-2 resins, polydimethylsiloxanes rods and depletive SPME) in a soil-water slurry. Non-exhaustive extractions, such as HPCD and Tenax, are required for bioaccessibility measurements during contaminated land assessment and management [7] and are discussed in detail below. Also, an ISO standard operating procedure for HPCD and Tenax extractions are being finalised [67].

Extraction with Cyclodextrins

Cyclodextrins are cyclic oligosaccharide macromolecules resulting from enzymatic breakdown of starch by bacteria. The internal toroidal-shaped cavity of the molecule is hydrophobic and associated with varying width, and its shell is hydrophilic and exhibit high aqueous solubility as well. Commonly used cyclodextrins are the hydroxylpropyl-β-cyclodextrins (HPCD) [28, 73]. HPCD solutions can increase the aqueous solubilities of suitably shaped and sized HOCs, as well as mobilise and form inclusion complexes with HOCs in soil-HPCD slurries. General extraction procedure involves mixing an amount of spiked or field-contaminated soil with 50 mM HPCD solution (in water or 0.01 M $CaCl_2$ solution) and then simply shaking the soil-HPCD slurry for a period (20–24 h for single extractions) before centrifugation. The sorbed contaminants that become dissolved in the water phase (or $CaCl_2$ solution) become trapped in the non-polar cavity of HPCD. After centrifugation, the supernatant is collected and extracted with hexane by liquid-liquid extraction. Thereafter, the hexane phase is prepared for analysis of associated HOC concentrations. Alternately, the soil residue after HPCD extraction is sometimes subjected to exhaustive solvent extraction and associated concentrations are analysed. The HPCD-extractable concentration is then determined as a difference between HOC concentration in soils before and after HPCD extraction.

HPCD extractions have been shown to be a very valuable biomimetic approach to test the viability and efficacy of bioremediation approaches during contaminated land management. Strong correlations have been reported between HPCD extractability of HOCs in laboratory-spiked and field-contaminated soils and sediments, and microbial degradation [73, 96, 97, 104], as well as with earthworm bioaccumulation [105–107]. As shown in Fig. 7, a linear correlation approaching 1:1 ($R^2 = 0.89$, slope = 0.90) was observed between the amounts of biodegraded ^{14}C-phenanthrene and HPCD-extractable ^{14}C-phenanthrene in four dissimilar soils [104].

In Fig. 8, the robustness and reproducibility of the HPCD extraction were further confirmed in studies that showed strong correlations between the amounts of PAH in field contaminated soils that were biodegraded by indigenous microbial activities and HPCD-extractable PAH concentrations [99–101]. Further, studies show that the amounts of phenanthrene mineralised were strongly correlated with F_{rap} ($R^2 = 0.89$) [50], with considerable reduction of F_{rap} in soils that were amended with activated

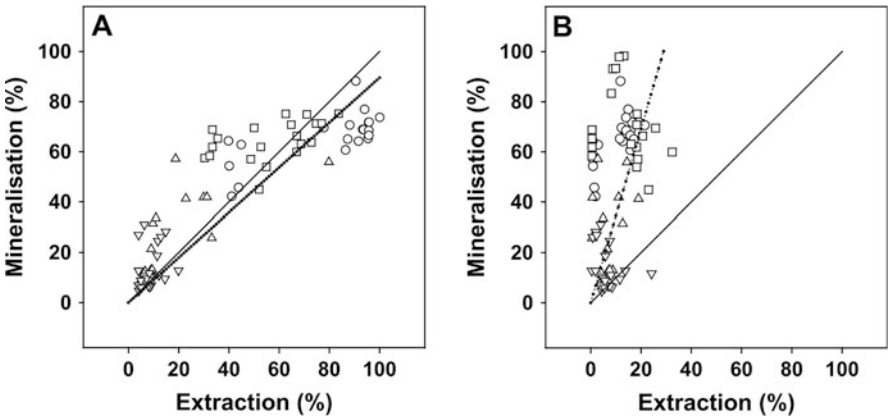

Fig. 7 Relationship between fractions of ^{14}C-phenanthrene extracted from four dissimilar soils using: (**a**) the HPCD extraction; (**b**) the water only extraction and the fraction mineralised in biodegradation assays after 1-day (○), 20 days (□), 50 days (△), 100 days (▽) contact times. Solid and dotted lines represent a 1:1 relationship and regression lines, respectively (adapted from Fig. 4. in [104])

carbon [24, 25]. Also, HPCD extractions predicted microbial degradation of different phenanthrene concentrations (0–100 mg kg^{-1}) in aged soil, including under varying pH conditions [96]. Mass transfer processes limit the desorption of HOCs in soils, and this was confirmed in a study where a single 24 h HPCD extraction of ^{14}C-phenanthrene spiked soils followed by first-order two- and three-compartment modelling showed that desorption of HOCs in soils often follows a bi- or triphasic profile [50], as in the normal profile of biodegradation of HOCs in soils [66]. Non-exhaustive extractions with HPCD followed by desorption kinetics modelling can be valuable for predicting biodegradation during risk-based contaminated land management.

However, HPCD extractions have also been reported to under-predict bioaccessibility of HMW PAHs, particularly due to steric hindrances and the presence of carbonaceous materials, such as activated carbon [24, 25, 34, 99–101]. Poor correlations have also been reported between HPCD extraction and PAH bioaccumulation in earthworms [98], benthic organisms as well as plants [35, 77]. The hindrances may be eliminated by increasing the concentration (by mass) of HPCD in the aqueous phase or by using an HPCD molecule with a larger hydrophobic cavity (e.g. HP-gamma-CD) or by adding extracellular polymeric substances (EPS) that are secreted by microbes into the extraction solution [105]. Bioaccessibility measurements can also be improved when the HPCD extraction solution incorporates a sorptive system [89]. For example, Gouliarmou and Mayer [90] developed a sorptive extraction approach incorporating silicone rod as adsorption sink for PAHs to optimise mass transfer and HPCD as the diffusive carrier phase. This approach considers that weakly sorbed HOCs are rapidly desorbed from soil and used during biodegradation, and then more strongly sorbed are slowly released to maintain the gradient resulting from the depletion of weakly

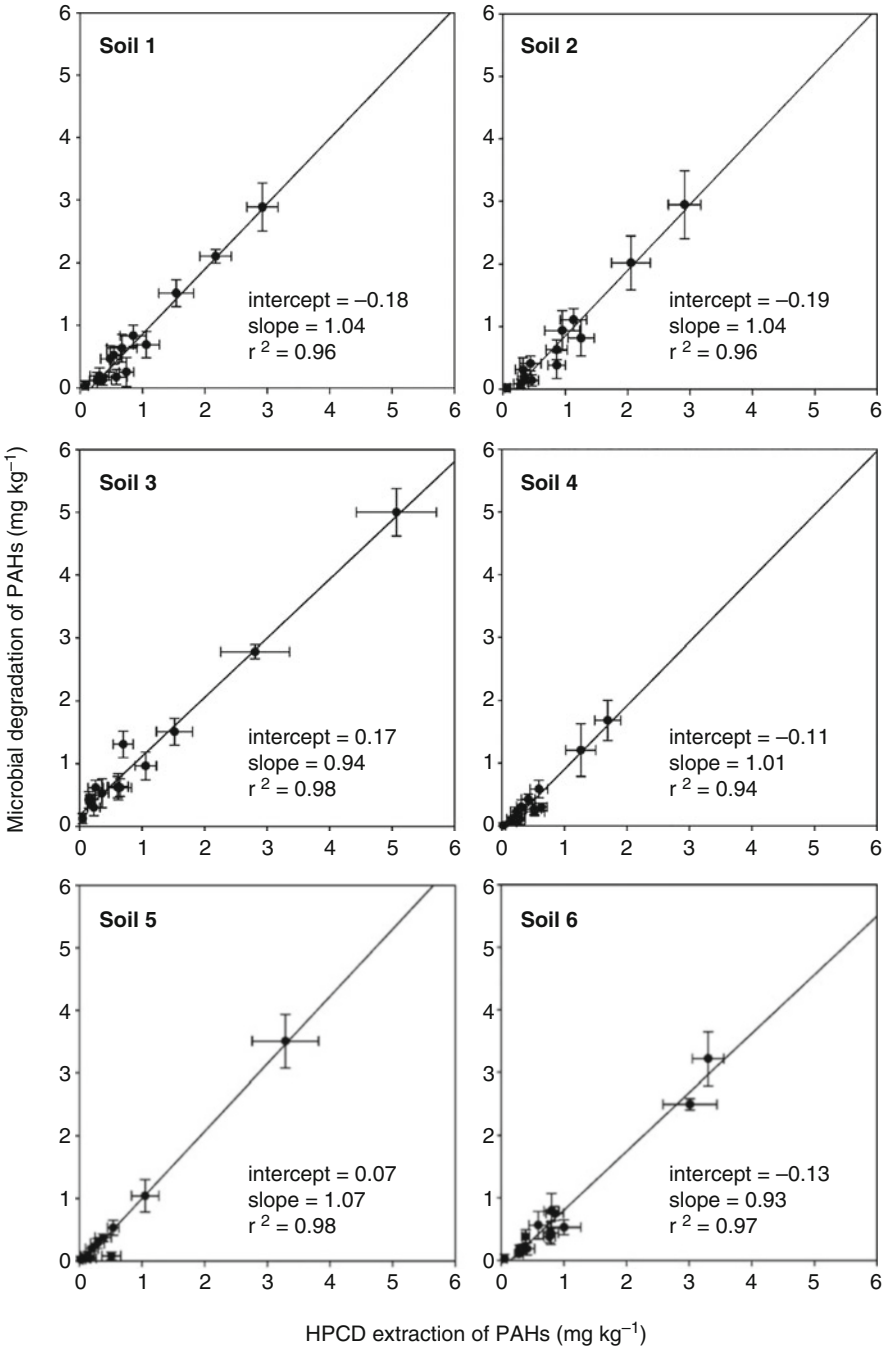

Fig. 8 Relationship between HPCD extractable and biodegradable fractions (mg/kg) of PAHs in six field-contaminated soils (1–6), with the solid lines representing the linear regression lines. Error bars are ±1 SD (adapted from Fig. 1 in [100])

sorbed HOCs. After sorption to the silicone rod is complete, the silicone rod is back extracted by exhaustive solvent extraction to determine bioaccessible HOC concentrations. The amounts of PAHs in wood soot obtained after extractions with silicone rod *plus* HPCD exceeded (up to 3–24 times) those from extractions with HPCD only [90]. The use of other sorptive materials, as intermediate or infinite sinks of HOCs in HPCD solutions, have been reported in recent studies to circumvent any or all drawbacks of existing sorptive materials, including rarity, long back-extraction time of sorptive sinks as well as the non-universality of sink dimensioning in different experiments [108, 109].

Overall, the usefulness of HPCD extractions towards predicting microbial degradation of HOCs has been demonstrated in a wide range of artificially spiked and field-contaminated soils. HPCD extraction better fits the amounts of HOCs mineralised in soils, as shown by lowest intercepts and highest slope, when compared to fittings by exhaustive and mild solvent extractions [73]. While HPCD extractions require further validation and improvement for wider applications beyond biodegradation endpoints, the simplicity, reliability, versatility and reproducibility of the extraction technique make it very valuable for bioaccessibility measurements.

Solid-Phase Extractions

Solid-phase extractions (SPEs) incorporate an infinite hydrophobic sink that depletes dissolved HOC concentrations in sediment slurries and facilitates continuous desorption of sorbed HOCs into the aqueous phase. SPEs were originally developed for bioaccessibility measurements in sediments, with associated measurements in soil slurries being tested much later. Solid phases that are often used include Tenax beads, C18 membranes, XAD-2 resins, flexible PDMS fibres, activated carbon-based traps and semi-permeable membrane devices, of which the Tenax extraction is one of the most commonly used. Tenax is a porous polymer, based on 2,6-diphenylphenol, with very strong affinities for HOCs similar to soil organic matter. Tenax extractions have been used extensively to evaluate the sizes of rapidly and slowly desorbing HOC (PAHs, and chlorobenzene compounds such as DDT and PCBs) fractions in sediments and sewage sludges (and soils) as well as associated desorption rates [37, 49, 69, 110–113]. The general extraction procedure involves mixing of a soil or sediment slurry with Tenax beads in glass or polypropylene centrifuge tubes and shaking for a period before centrifugation. The sorbed contaminants that become dissolved in the water phase (or $CaCl_2$ solution) adsorb to the Tenax beads. After centrifugation, the Tenax beads float to the surface being hydrophobic and less dense than water. The spent Tenax beads are collected by vacuum filtration or by suction and then rinsed with water to remove any adhering soil particles. It is important to ensure removal of adherent soil particles to avoid overestimation of bioaccessibility, following extraction of the Tenax beads. The rinsed Tenax beads are then back-extracted by exhaustive solvent (e.g. *n*-hexane) extraction to determine associated HOC concentrations. For desorption kinetics studies, new Tenax beads are added to the soil or sediment slurry, and the extraction

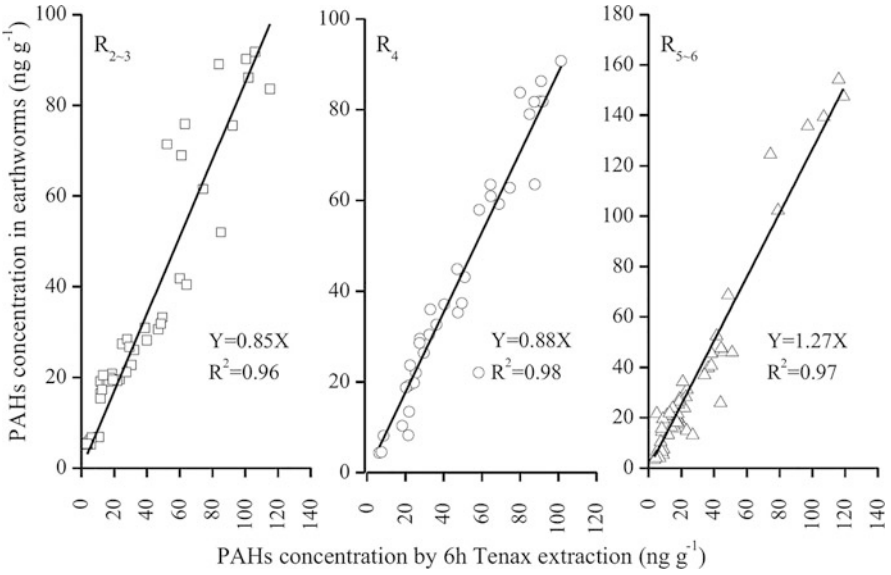

Fig. 9 The correlations between Tenax 6 h extracted PAHs concentration and earthworm accumulated concentration in 9 soils. R is the number of aromatic rings in the PAH molecule(s) (adapted from Fig. 6 in [110])

procedure is repeated. Some advantages of Tenax extractions are that the Tenax beads can be cleaned and reused, as well as that the back-extracts may not need to be cleaned up further.

Like HPCD, Tenax extraction is valuable for determining HOC bioaccessibility in soils (Figs. 9 and 10). However, very long desorption times of up to 2 years have been reported using Tenax extractions to completely capture rapid and slowly desorbing HOC fractions [49, 82, 114]. Such long extraction time in the laboratory is a major disadvantage of Tenax extraction [69], although a 6–24 h extraction time is now being used to determine the rapidly desorbing fractions (F_{rap}) [34, 37, 51, 69]. Also, phase separation of the Tenax beads from soil slurries can be challenging in the laboratory. Overall, when compared to other solid-phase extractions, such as solid-phase membrane devices and polyethylene tube dialysis, Tenax beads display better infinite sink capacities [66].

3.1.3 In Vitro Extractions to Simulate Oral Ingestion and Gastrointestinal Digestion Soil-Borne HOCs

While simple chemical extractions have been valuable in understanding complex bioavailability and bioaccessibility processes of HOCs in soils, these extractions must mimic gastrointestinal (GI) processes for realistic uses in HHRA. Since the last two decades, efforts have been put into developing complex static and dynamic

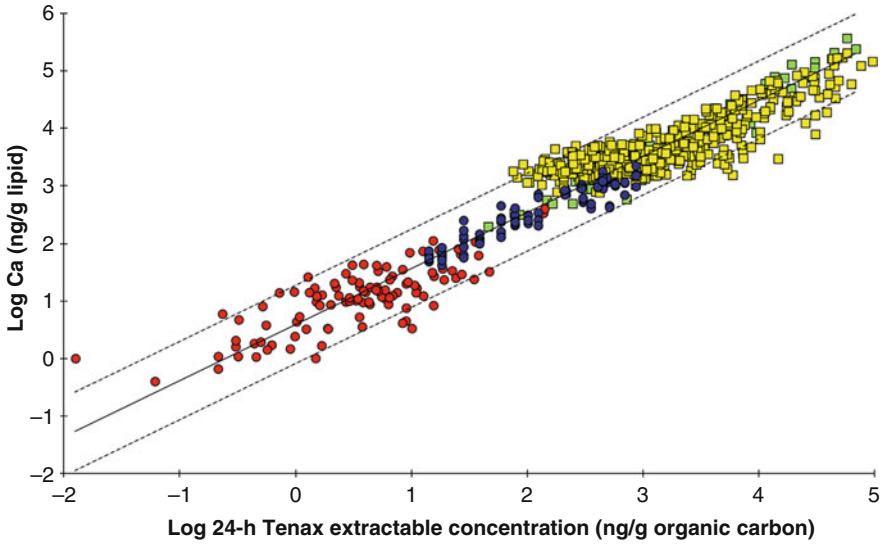

Fig. 10 Bioaccumulation Tenax model with bioaccumulation and corresponding 24 h Tenax-extractable concentrations of polychlorinated biphenyls and pyrethroids from laboratory- and field-collected sediments and organisms. The red circles and green squares represent field and laboratory data from [115–117] and from [118], respectively, used to create the bioaccumulation Tenax model; the yellow squares and blue circles represent field- and laboratory-collected data from [119, 120], respectively, used to validate the bioaccumulation Tenax model ($n = 751$, $R^2 = 0.94$). The data points represent Tenax and bioaccumulation data from multiple sediments and studies, polychlorinated biphenyls and pyrethroids and accumulation by *Lumbriculus variegatus* and *Hexagenia* (adapted from Fig. 1 in [111])

(i.e. in flowthrough or automated modes) in vitro digestion tests for measuring oral bioaccessibility of HOCs in soil (Table 2). Oral bioaccessibility refers to the dissolved contaminant concentration in the gastrointestinal fluid [55, 61] and is usually regarded as a conservative estimate of oral bioavailability [60].

As shown in Table 3, the in vitro digestion tests are designed to mimic the various compartments in the human gastrointestinal tract [31, 56, 128, 132, 133, 141, 146, 152, 153].

The bioaccessibility test system in the dynamic mode is simply automated and is used in models such as SHIME, while the static mode associated with most bioaccessibility models are non-automated (Appendix, Table 4). These static models could be batch or sequentially operated. In the former, each GI compartment is introduced and recalibrated to relevant conditions, one after the other or at the expiration of a particular GI phase in a single vessel (e.g. PBET), while different vessels are used in the latter. Flow-through models better simulate the human GI system [31, 58]; however, performance will also depend on the influent velocity into each compartment which ought to be similar to human GI system. Other common bioaccessibility models are listed in Table 2. As shown in the Appendix, Table 5, bioaccessibility models vary in the quantity and quality of compartments included.

Table 2 Bioaccessibility models for hydrophobic organic contaminants in ingested soil

Model	Acronym	References
Physiological-based extraction test	PBET	[121–128]
In vitro gastrointestinal extraction test	IVG	[64, 129–134]
In vitro digestion test	IVD	[57, 135]
Relative bioaccessibility leaching procedure	RBALP	[57, 136]
Simulator of the human intestinal ecosystem	SHIME	[137–141]
German-Deutsches Institut fur Normung	DIN	[31, 142]
Netherlands-Rijks Instituut voor Volksgezondheid and milieu	RIVM	[143–145]
Unified barge method	UBM	[146–149]
Fed ORganic estimation human simulation test	FOREhST	[56, 146]
Colon-extended physiological based extraction test	CEPBET	[121, 150–152]

In terms of quantity, most in vitro tests have only used a combination of two GI compartments: the stomach and small intestine (Appendix, Table 5). Some in vitro tests have included three compartments comprising the mouth, stomach and small intestine, while a few have included major compartments in the GI system including gastric, small intestine (which may comprise duodenum, jejunum and/or ileum) and colon (Appendix, Table 5).

Varying degrees of HOC mobilisation are associated with each compartment and a wide range of bioaccessible concentrations have been reported (Appendix, Table 6). For example, although the saliva contains certain enzymes which are capable of degrading carbohydrates (CHO) and other dietary substances which may be associated with ingested contaminated soil, transit time is very short ($\leq$2 min) to facilitate considerable enzymatic mobilisation. Greater mobilisation of HOCs, especially PAHs, has been reported within gastric and gastrointestinal compartments [31, 143] and colon [141, 152]. Bioaccessibility tests also show variations in terms of gastrointestinal composition and design (quality). Variations in pH, liquid-to-soil ratio (L/S), transit time within compartments, type of mixing/agitation used during incubation and the centrifugation speed and duration may influence bioaccessibility estimates reported (Appendix, Tables 4 and 7).

It has also been reported that for in vitro digestive tests to effectively simulate GI processes, an absorptive sink that mimics the hydrophobic characteristics of the intestinal epithelium and its facilitation of HOC desorption from ingested soils into the gut fluid needs to be incorporated [57, 60, 64, 121, 154]; else oral bioaccessibility may be underestimated [64, 154]. There is also a need to validate all the bioaccessibility models for standardised uses in risk assessment of HOCs in soils. Overall, considering the different operational conditions, soil matrix heterogeneity and HOC physicochemical properties, wide ranging bioaccessibility values (0–100%) have been reported (Appendix, Table 4); these variations make comparison between studies difficult.

Table 3 From ingestion to excretion via the in vitro gastrointestinal tract

Compartment	Activity	Enzymes and chemical composition	pH/ transit time (h)	Outcome	Remarks
Oral cavity	Simulates mastication of ingested material mixed with saliva fluid	Amylase, protease and lipase	6.5/≤ (2/60)	Increased surface area for digestion	Limited importance for hydrophobic organic contaminant mobilisation which may be due to short transit time. Hence, omitted in most models except the UBM (fed), IVD, IVG and FOREhST
Gastric	Receives masticated food from the mouth via the oesophagus. Peristalsis is simulated as shaking, mixing, or rotation. The food stimulates acid secretion by the gastric mucosal wall which then activates pepsin secretion	HCl, pepsin	1.0–4.0/ ≤3	Initial digestion of protein, chyme formation	Most in vitro models include this compartment and considerable mobilisation of hydrophobic organic contaminants begins in the stomach
Small intestine	Bicarbonates from pancreatin neutralises acidic chyme, which is further digested by pancreatic enzymes. Importantly, bile emulsifies lipids in chyme. Enterocytic absorption also occurs here	$NaHCO_3$, Pancreatin, pepsin, amylase, lipase, protease, nuclease	6.5–7.5/ ≤8	Digestion and absorption of carbohydrates, proteins, fats, H_2O and essential nutrients, e.g. Ca, Fe, vitamins	Bile mixed micelles aid the dissolution and mobilisation of hydrophobic organic contaminants [31, 141, 143]. The small intestine may comprise 3 sub-compartments, i.e. the duodenum, jejunum and ileum such as in dynamic SHIME [138]
Colon	Receives undigested residue from the small intestine, reabsorbs water and electrolytes from it and then transfers to the rectum for excretion	N/A	8.0–8.5/ ≤18	N/A	Included only in SHIME and CEPBET. Increased PAH mobilisation [139, 152] and PAH bioactivation into oestrogens [140] have been reported

Refer to Table 2 for definitions of UBM, IVD, IVG, FOREhST, SHIME, CEPBET. N/A is not applicable

4 Desorption-Resistant or Residual HOC Fractions and Associated Potential Risks

The presence of desorption-resistant or highly sequestered non-extractable residues (NERs) of PAHs in soils has raised speculations on their long-term fate, impact and potential health risks. This is a critical uncertainty constraining wide adoption of risk-based management of contaminated land. The future risk to human and ecological health following exposure to HOCs in long-term contaminated soils has not been well investigated.

A contaminant trap, consisting of a PDMS sink (with or without activated carbon) and a cyclodextrin diffusive carrier, in a custom-made reactor was developed to isolate and quantify the desorption-resistant PAH fractions from long-term contaminated soils and from wood soot [89, 90]. Similarly, methanolic saponification, alkaline hydrolysis, silylation and fumigation methods have been used to extract parent NERs and their metabolites [11, 39, 155, 156]. Where stable isotope or radiolabels are amended into contaminated soils, NERs can be quantified following combustion of soil residue and liquid scintillation counting of the resulting $^{13/14}CO_2$ activity.

A series of first-time investigations were recently published on the solvent extractability and remobilisation, bioaccumulation and sublethal genotoxicity, gastrointestinal mobilisation and oral bioaccessibility as well as potential cancer risks of PAH NERs (parent molecules only), from the perspectives of ecological and human health risk assessments [11–13, 47]. Sequential solvent (mild and exhaustive) extractions showed that the carcinogenic PAH, benzo[a]pyrene (BaP), NERs from long-term aged soils (up to 4 years) can be remobilised through an intercompartmental repartitioning mechanism during a re-equilibration period of 30 or 60 days [11, 47]. However, the amounts remobilised were extremely small with minimal or no potential to cause significant harm to human and environmental health, as the NERs were highly sequestered in soils [11, 47]. In addition, the amounts of BaP remobilised decreased substantially with time, with the implication that BaP NERs in long-term contaminated soils should not be considered in routine risk assessments [11, 47]. From the ecological risk assessment study [12], the concentrations of BaP in earthworm tissues were generally low, particularly when the soil contained highly sequestered BaP non-extractable residues, with biota-soil accumulation factors ranging from 0.6 to 0.8 kg OC/kg lipid [12]. The measurements related to genotoxicity in earthworms, that is percentage (%) of DNA in the tails and olive tail moments, were significantly greater ($p < 0.05$) in the spiked soil containing readily available BaP than in soil that did not have added BaP. There were no effects over the range of BaP concentrations (10 and 50 mg/kg soil) investigated. In contrast, DNA damage after exposure of earthworms to BaP non-extractable residues in soil did not differ from background DNA damage in the unspiked soil [12]. From the human health risk assessment study [13], PAH oral bioaccessibility approached 100% for solvent-spiked soils, but only 24–36% for long-term contaminated soils from manufactured gas plant sites. Associated cancer risks exceeded target levels (10^{-5}) from exposure to six readily available

carcinogenic PAHs in one MGP soil that was highly contaminated, particularly for 2–3-year-olds. In contrast, the amounts of associated non-extractable residues did not exceed health investigation and cancer risk target levels [13].

Overall, PAH non-extractable residues were highly sequestered in aged soils, meaning that only very small amounts could be remobilised in the soils, bioaccumulated in earthworms, or released into a simulated human gut fluid with acceptable levels of cancer risks. The direct tripartite evidence confirmed that PAH non-extractable residues in long-term contaminated soils are unlikely to cause significant harm to human or ecological health and do not need to be considered in routine risk assessments.

5 Considerations for the Development of a Simple Intelligent Desorption Extraction Scheme for the Measurement of HOCs' Bioaccessibility in Soil

The European Centre for the Ecotoxicology and Toxicology of Chemicals recently proposed the development of a simple and intelligent extraction framework that can capture the processes that control access to freely dissolved and rapidly desorbing fractions, as well as all or part of the slowly desorbing fractions in soils [38]. The framework stressed that the choice of a suitable extraction technique should arbitrarily depend on recovery strength only (i.e. how much of an HOC that can be recovered in soil). The suitability of an extraction technique should be based on detailed understanding of the physicochemical properties of the matrix and HOC of interests, as well as the biological and environmental relevance of the technique.

The nature and strength of HOC sequestration and sorption, as well as the different fractions of HOCs, in soils are critical considerations for bioaccessibility measurements during contaminated land risk assessment. Hence, an intelligent extraction technique should delineate HOC fractions in soils in relation to their sorption and desorption behaviour and associated mechanisms, and their bioaccessibility, while also considering organism, matrix and environmental factors. In the framework, bioavailable fractions refer to freely dissolved and rapidly desorbing fractions and associated measurement relate to acute exposures. The bioaccessible fractions include fractions that are bioavailable and slowly desorbing, and associated measurements are useful for chronic exposures. Particularly where qualification (other than quantification only) of each fraction is required, it is important that the extraction method should be non-destructive for the matrix.

The framework suggested that the following extraction conditions are environmentally relevant for bioaccessibility determinations: soil/extractant ratio of 1:5, minimum extraction time of 0.5 h, agitation of soil slurry with minimal energy input and extraction of soils at room temperature. The proposed framework involves a sequential extraction of the soil (or sediment) that are contaminated with labelled or unlabelled HOCs with an aqueous solution that mimics the ionic strength of soil pore water (dissolved concentration). The contaminated matrix may also be subjected to a

non-depletive SPME to determine the freely dissolved concentrations. Where the rapidly desorbing fractions are required, the soil residue that resulted after the first extraction are subjected to a non-exhaustive extraction technique in aqueous solutions incorporating a sink for HOCs, such as single HPCD or Tenax extractions lasting 20–24 h. Where more slowly desorbing fractions are required, successive extractions with HPCD or Tenax may be used. In a similar manner, water-solvent mixtures or supercritical fluid (CO_2) extraction may be used to increase HOC solubility and extractability, and this is particularly important for predicting HOC uptake by plants and soil organisms. Where the recovery of non-extractable or irreversibly sorbed residues are required for mass balance purposes or to understand the fate of NERs in soils, very harsh extraction methods under high temperature and pressure are then used. These techniques include Soxhlet, accelerated solvent, microwave-assisted extraction and digestion and combustion. It should be noted that these harsh extraction techniques have the potential to alter or destroy the soil matrix, and they are so exhaustive that associated extractions overestimate bioavailability and bioaccessibility as has been discussed earlier.

Overall, a suitable extraction technique must be one that does not underestimate the bioaccessible concentration of HOCs in soils. Hence, the biological and environmental relevance of any chosen technique will need to be validated, at least through an in vitro-in vivo correlation.

6 Conclusion and Suggestions for Further Research

The overall purpose of the risk-based approach to managing contaminated land is to minimise risks from exposure. The implication of contaminant sequestration in soils is that total contaminant residues will be segregated into freely dissolved and rapidly and slowly desorbed (reversibly sorbed) fractions, as well as irreversibly sorbed or non-extractable residues. Each of these fractions will be dependent on matrix-, site-, organism-, HOC-specific factors, including prevailing environmental conditions. Therefore, suitable extraction techniques must be chosen based on understanding the sorption and desorption behaviour of HOCs in soils, soil properties, and their biological and environmental relevance (determined by experimental validation). The intelligent extraction framework described by ECETOC is reasonable and sound, based on the critical considerations described earlier. In addition, since the desorption resistant or non-extractable residues in long-term contaminated soils are unlikely to cause harm or significant harm to human and ecological health, they do not need not to be considered in routine risk assessments and measurements, except for qualification purposes only.

Overall, there is enough information to support the consideration of bioaccessibility in routine risk assessment, and the continuous dependence on total contaminant concentrations and associated assumption of 100% bioavailability is outdated and no longer scientifically justifiable. More research should focus on developing standard operating procedures for bioaccessibility measurement, as

well as wide validation across a suite of HOCs and soils (or sediments) for specific organisms and risk assessment purposes.

Appendix

Table 4 Variations in the gastrointestinal compartmental conditions and design in different bioaccessibility models

In vitro model	Dynamic/ static	pH	L:S (mL: g) M, S, SI, C	Transit time (h)
PBET	Static	X, 1.5, 7.0, X	X, 100:1,100:1, X	X, 1, 4, X
	Static	X, 1.5, 7.0, X	X, 100:0.4, 100:0.4, X	X, 1, 7.2, X
		X, 1.5, 7.0, X	X, 100:1,100:1, X	X, 1, 4, X
	Static	X, 1.5, 7.5, X	X, 250:1, 250:1, X	X, 3, 6, X
	Static	X, 2.5, 7.0, X	X, 100:1, 100:1, X	X, 1, 4, X
CEPBET	Static	X, 2.5, 7.0, 6.5	X, 100:1,100:1, 100:1	X, 1, 4, 8
IVG	Static	6.5, 1.0, 7.8, X	X, X, X, X	0.08, 2, 4, X
	Static	6.5, 2.0, 5.5, 6.0	6:4.5, 12:4.5, 20:4.5, X	0.08, 2, 2, 24–72
	Static	X, 1.5, 7.5, X	X, 1:0.6, 6:0.6, X	X, 2 (BT at 40°C), 22, X
	Static	X, 1.5, 7.5, X	X, 5:1, 65:2, X	X, 2 (BT at 40°C), 22, X
SHIME	Dynamic	X, 1.5, 6.3, 6.3	X, (40:20[a]; 200:20[b];200:5[c]), 300:20; 500:20	X, 2, 5, 18
	Dynamic/ static	X, 1.5, 6.5, 5.9	X, 12:0.3, 6:0.3, 13:0.3	X, 2, 3.5, 18
FOREhST	Static	6.8, 1.3, 8.1–8.2, X	4.5:0.3, 9:0.3, 13.5:0.3, X	0.08, 2, 2, X
	Static	6.8, 1.3, 8.1–8.2, X	4.5:0.3, 9:0.3, 13.5:0.3, X	0.08, 2, 2, X
UBM (fed)	Static	6.5, 0.9–1.0, 6.0 ± 0.5, X	4.5:0.3, 9:0.3, 13.5:0.3, X	0.08, 2, 2, X
RBALP	Static	X, 1.5, 7.0, X	X, 100:1, 150:1, X	X, 1, 4, X
IVD	Static	X, 1.5, 6.5, X	X, 100:1, 50:1, X	X, 2, 2, X
PBET/DIN	Static	6.4, 2.0, 7.5, X	15:1; 35:1: 50:1: X	0.5, 2, 6, X
Digestive tract model/DIN	Static/ flowthrough	X, 2.0, 7.0, X	X, 120:1, 120:1, X	X, 2, 6, X
IVD + modified RIVM	Static	6.8, 2–3, 6.5–7, X	6:4.5, 12:4.5, 20:4.5, X	0.08, 2, 2, X
	Same as above	Same as above	Same as above	Same as above

Refer to Table 2 for model names. Mouth (M), stomach (S), small intestine (SI), colon/caecum (C). X refers to not provided. a, b, c, refers to different gastric L:S tested, i.e. 2, 10 and 40 respectively. BT refers to body temperature. References exactly as in Table 5

Table 5 Variations in the gastrointestinal compartmental fluid compositions in different bioaccessibility models

In vitro model	Compartmental fluid composition							Refs.
	Mouth	Stomach	Small Intestine			Large intestine	Dietary supplement	
			Duodenum	Jejunum	Ileum	Colon		
PBET	X	35.1 g NaCl +2.0 g citrate +2.0 g malate +1.7 mL lactic acid +2.0 mL acetic acid +5.0 g pepsin +4 L dH_2O + 12 M HCl	$NaHCO_3$ + 1.2 g porcine bile extract +0.36 g porcine pancreatin			X	X	[128] modified from [126]
	X	15 g glycine + I L dH_2O + HCl + I g pepsin +5 g bovine serum albumin +2.5 g type III porcine mucine	1 M NaOH +0.1 mL stock intestinal solution which is 0.1 mM mixed bile salts (23.5 mM sodium glycocholate +23.5 mM sodium glycochenodeoxycholate +16 mM sodium glycodeoxycholate +0.7 mM sodium glycolithocolate +3 mM disodium glycolithocolate sulphate +12 mM sodium taurocholate +12 mM sodium taurochenodeoxycholate +8 mM sodium taurodeoxycholate +0.3 mM sodium taurolithocholate +1 mM disodium taurolithocholate sulphate)			X	X	[157] modified from [122, 127]
	X	70.2 g NaCl +4.0 g citrate +4.0 g malate +3.4 mL lactic acid +4.0 mL acetic acid +10.0 g pepsin +8 L dH_2O + 12 M HCl	$NaHCO_3$ + 1.2 g porcine bile extract +0.36 g porcine pancreatin			X	X	[123] based on [126]
	X	**50 mL gastric solution** 0.15 M NaCl +0.05% citrate (*m/v*) + 0.05% malate (*m/v*) + 0.5% lactic acid (*m/v*) + 0.5% acetic acid (*m/v*) + 0.10% pepsin (*m/v*) + dH_2O + 12 M HCl	$NaHCO_3$ + 0.50% (*m/v*) porcine bile extract +0.018% (*m/v*) porcine pancreatin			X	X	[124] based on [125, 128]
	X	**20 mL gastric solution** 0.50 g sodium malate +0.50 g tri-sodium citrate 420 µL lactic acid +500 µL glacial acetic acid + 1.25 g pepsin (porcine)	0.035 g bile salts +0.01 g porcine pancreatin ± Tenax			X	X	[154] based on [121, 127]

CEPBET	X	0.50 g sodium malate +0.50 g tri-sodium citrate 420 μL lactic acid +500 μL glacial acetic acid + 1.25 g pepsin (porcine) 4 g L^{-1} mucin +800 mg L^{-1} cysteine hydrochloride	1.78 g bile salts +0.5 g porcine pancreatin			4.0 g type II mucin (porcine Stomach) + 4.5 g NaCl + 4.5 g KCl + 1.5 g $NaHCO_3$ + 1.25 g $MgSO_4.6H_2O$ + 800 mg cysteine HCl + 500 mg $KHPO_4$ + 500 mg K_2HPO_4 + 400 mg bile salts +189.0 mg CaCl +50.0 mg haemin (≥ 80%, bovine) + 5.0 mg $FeSO_4.7H_2O$	**Fed state (colon medium)** 5.0 g starch (potato) + 3.4 g peptone (casein) + 6.1 g tryptone (vegetable) + 4.5 g yeast extract +3.0 g casein +2.0 g pectin (citrus) + 2.0 g xylan (oat) + 2.0 g arabinogalactan (larch) + 1.0 g guar gum +1.0 g inulin (chicory)	[121, 150, 152]
	X	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	[151] Based on [152]
IVG	**5 mL salivary fluid** 0.2 M phosphate buffer +2.8 g L^{-1} alpha-amylase	**7.5 mL gastric fluid** 10.0 g L^{-1} porcine pepsin +0.2 M KCl	**20 mL small intestinal fluid** 20.0 g L^{-1} porcine pancreatin +3.00 g L^{-1} porcine lipase +14.0 g L^{-1} porcine bile extract in 0.2 M phosphate buffer	X	X	X	X	[64] Based on [129, 131]
IVG	1.80 g L^{-1} KCl + 0.40 g L^{-1} KSCN+ 1.80 g L^{-1} NaH_2PO_4 + 0.60 g L^{-1} NaCl +1.15 g L^{-1} Na_3PO_4+ 0.14 g L^{-1} NaOH +0.40 g L^{-1} urea +0.03 g L^{-1} uric acid +0.10 g L^{-1} mucin	NaCl(5.50 g L^{-1}) + NaH_2PO_4 (0.50 g L^{-1}) + KCl (1.65 g L^{-1}) + $CaCl_2$ (0.80 g L^{-1}) + NH_4Cl (0.70 g L^{-1}) + 15.0 mL L^{-1} HCl (37%, g/g) + glucose (1.30 g L^{-1}) + glucoronic acid (0.04 g L^{-1}) + urea (0.20 g L^{-1}) + 10 mL	NaCl(14.00 g L^{-1})+$NaHCO_3$ (6.80 g L^{-1}) + KH_2PO_4 (0.016 g L^{-1}) + KCl (0.72 g L^{-1}) + $MgCl_2$ (0.01 g L^{-1}) + 1.00 mL L^{-1} HCl (37%, g/g) + urea (0.20 g L^{-1}) + $CaCl_2$ (0.39 g L^{-1}) + 2.00 g L^{-1} BSA + 6.00 g L^{-1} porcine	X	X	X	Arabinogalactan (1.0 g L^{-1}) + pectin (2.0 g L^{-1}) + starch (3.0 g L^{-1}) + xylan (1.0 g L^{-1}) + glucose (0.40 g L^{-1}) + yeast extract (3.0 g L^{-1}) + proteose peptone (1.0 g L^{-1}) + mucin (4.0 g L^{-1}) + cysteine	[130] Based on [131, 138]

(continued)

Table 5 (continued)

In vitro model	Compartmental fluid composition					Large intestine	Dietary supplement	Refs.
	Mouth	Stomach	Small Intestine					
			Duodenum	Jejunum	Ileum	Colon		
	+0.30 g L^{-1} alpha-amylase	glucosamine (0.70 g L^{-1}) + 2.00 g L^{-1} BSA + 6.00 g L^{-1} porcine pepsin +2.00 g L^{-1} mucin	pancreatin +1.00 g L^{-1} porcine lipase + bile juice (10.40 g L^{-1} NaCl +11.60 g L^{-1} $NaHCO_3$ + 0.75 g L^{-1} KCl + 1 mL L^{-1} HCl (37%, g/g) + 0.50 g L^{-1} urea +0.44 g L^{-1} $CaCl_2$ + 12.0 g L^{-1} bile)				(0.50 g L^{-1}) + 0.04 g L^{-1} K_2HPO_4 + 0.08 g L^{-1} NaCl +0.40 g L^{-1} $NaHCO_3$ + 0.008 g L^{-1} $MgSO_4$ + 0.008 g L^{-1} $CaCl_2$ + 0.005 g L^{-1} haemin +500 mg/L $MnSO_4$ + 100 mg/L $FeSO_4$ + 100 mg/L $CoSO_4$ + 100 mg/L $ZnSO_4$ + 10 mg/L $CuSO_4$ + 10 mg/L AlK $(SO_4)_2$ + 10 mg/L H_3BO_3 + 10 mg/L Na_2MoO_4 + 100 mg/L $NiCl_2$ + 10 mg/L Na_2SeO_3 + 1.0 mg/L menadione +2.0 mg/L biotin +10.0 mg/L pantothenate +5.0 mg/L nicotinamide +0.50 mg/L vitamin B_{12} + 4.0 mg/L thiamin +5.0 mg/L *para*-aminobenzoic acid	

	X	1 mL gastric fluid HCl-KCl buffer +50 mg mL^{-1} pepsin	5 mL intestinal fluid (2 mL phosphate buffer with 10 mg mL^{-1} pancreatin +1 mL phosphate buffer with 20 mg mL^{-1} lipase +2 mL phosphate buffer with 20 mg mL^{-1} bile salts) + I mL *alpha*-amylase digestive fluid (Tris-HCl buffer with 50 mg mL^{-1} *alpha*-amylase)	X	X	X	X	[134] Based on [129] and described by [133, 153]
	X	10 mL gastric fluid HCl-KCl buffer +50 mg mL^{-1} pepsin	60 mL intestinal fluid (2 mL phosphate buffer with 10 mg mL^{-1} pancreatin +1 mL phosphate buffer with 20 mg mL^{-1} lipase +2 mL phosphate buffer with 20 mg mL^{-1} bile salts) + 5 mL *alpha*-amylase digestive fluid (Tris-HCl buffer with 50 mg mL^{-1} *alpha*-amylase	X	X	X	X	[132] Based on [129, 153]
SHIME	X	200 mL salt solution (0.1 M $KHCO_3$ + 0.1 M NaCl) + 1.3 mL HCl (5 M) + 10 mg porsine pepsin L^{-1}	100 mL pancreatic juice (12.5 g L^{-1} $NaHCO_3$ + 6 g L^{-1} Oxgall +0.4–0.9 g L^{-1} porcine pancreatin powder	X	X	100 mL SHIME suspension (human colon representative anaerobe and aerobe = 8.4 and 7.8 log CFU mL^{-1} respectively) e.g. *Lactobacilli*, *Bifidobacteria*, *Enterococci*, *Fungi*, *Staphylococci* and *Clostridia*	Carbohydrate based medium (5 g Nutrilon (56% lactose +12% fat +10% casein) + 3 g potato starch +1 g arabinogalactan +2 g pectin +1 g xylan +0.4 g glucose +4 g mucin +0.5 g cystein +3 g yeast extract +3 g pepton	[141] Based on [138] Further work done by [140]

(continued)

Table 5 (continued)

In vitro model	Compartmental fluid composition						Dietary supplement	Refs.
	Mouth	Stomach	Small Intestine			Large intestine		
			Duodenum	Jejunum	Ileum	Colon		
	X	12 mL gastric solution (5 g $KHCO_3$ + 3 g NaCl) + HCl (0.5 M) + 10 mg porsine pepsin L^{-1}	6 mL pancreatic solution (12.5 g L^{-1} $NaHCO_3$ + 6 g L^{-1} Oxgall +0.9 g L^{-1} porcine pancreatin powder) + 0.5 M HCl or NaOH	X	X	12 mL colon SHIME suspension from dynamic model (human colon representative anaerobe and aerobe = 1.1 x 10^8 and 2.25 x 10^7 CFU mL^{-1} respectively) e.g. *Lactobacilli*, *Bifidobacteria*, *Enterococci*, *Staphylococci* and *Clostridia*	0.5 g arabinogalactan +1 g pectin +0.5 g xylan +2 g potato starch +0.2 g glucose +1.5 g yeast extract +0.5 g pepton +2 g mucin +0.25 g cysteine	[139] Based on [137, 141]
FOREhST	**4.5 mL saliva fluid** (145–280) mg amylase + (25.0–50.0) mg mucin +15.0 mg uric acid to a 1 L Duran bottle. Then separately, 896 mg KCl + 888 mg NaH_2PO_4 + 200 mg KSCN +570 mg Na_2SO_4 + 298 mg NaCl + (1.80–2.80) mL of 1.0 M HCl (inorganic saliva components) were added into a 500 mL container made to mark with H_2O. Into a second	**9 mL gastric fluid** Add 1,000 mg bovine serum albumin + (3000–9,000) mg mucin + (1000–2,500) mg pepsin to a 1 L Duran bottle. Then, separately, 824 mg of KCl + 266 mg NaH_2PO_4 + 400 mg CaCl2 + 306 mg NH4Cl + 2,752 mg NaCl + (6.5–8.30) mL of 37% HCl (inorganic gastric components) into a 500 mL container made to mark with H_2O. Into a second 500 mL container, add 650 mg	**9 mL duodenal fluid** Add 200 mg $CaCl_2$ + 1,000 mg bovine serum albumin + (3000–9,000) mg pancreatin + (500–1,500) mg lipase to a 1 L Duran bottle. Then, separately, add 564 mg KCl + 80 mg KH_2PO_4 + 50.0 mg $MgCl_2$ + 5,607 mg $NaHCO_3$ + 7,012 mg NaCl +180 µL of 37% HCl (inorganic duodenal components) into a 500 mL container made to mark with H_2O. Into	**4.5 mL simulated bile fluid** Add 222 mg of $CaCl_2$ + 1800 mg bovine serum albumin + (6000–30,000) mg bile to a 1 L Duran bottle. Then, separately, add 376 mg KCl + 5,785 mg $NaHCO_3$ + 5,259 mg NaCl +180 µL of 37% HCl (inorganic bile components) into a 500 mL container and make to mark with	X	X	Gold toddler formula in place of HIPP organic creamy porridge which is no longer commercially available	[56] Based on [146]
UBM (fed)					X	X	Freeze-dried oat meal +0.813 g Hipp organic creamy/rice porridge infant food supplement +50 µL pure sunflower oil	[146] [148] Based on [146, 147, 149]

	500 mL container, add 200 mg urea (organic saliva component) + H_2O to mark. Then, simultaneously add the inorganic and organic saliva components into the 1 L Duran bottle.	glucose +20.0 mg glucuronic acid +85.0 mg urea +330 mg glucosamine hydrochloride (organic gastric components) and make up to mark with H_2O. Then, simultaneously add the inorganic and organic gastric components into the 1 L Duran bottle.	a second 500 mL container add 100 mg urea (organic duodenal components) was added and make up to mark with H_2O. Then, simultaneously add the inorganic and organic duodenal components into the 1 L Duran bottle.	H_2O. Into a second 500 mL container, add 100–250 mg urea (organic bile components) and make up to mark with H_2O. Then, simultaneously add the inorganic and organic bile components into the 1 L Duran bottle.				
RBALP	X	**100 mL gastric fluid** 0.4 M glycine solution	NaOH (50%, w.w.) + 175 ± 20 mg bile (bovine, mixture of 50% free and conjugated bile acids) + 50 ± 10 mg porcine pancreatin			X	X	[57] Similar to [136] but according to [158]
IVD	X	**30 mL gastric fluid** 12 M HCl in d H_2O + 0.5 M HCl	15 mL small intestinal solution (12.5 g L^{-1} $NaHCO_3$ + 6 g L^{-1} Oxgall bile +0.9 g L^{-1} porcine pancreatin powder) + 0.5 M HCl			X	X	[57] Described by [135] but based on [137]
PBET/DIN	30 mL saliva fluid	70 mL gastric fluid	100 mL intestinal fluid			X	X	[142] Based on [31] and German DIN model
Digestive tract model/DIN	X	1.8% HCl (w/w) + 10 mg pepsin +350 mg mucine +1.8% NaCl solution (w/v)	$NaHCO_3$ + 10 mg trypsin +350 mg Pancreatin +350 mg lyophilised bile			X	7 g whole milk powder or skimmed milk powder or minced beef	[31]
IVD+modified RIVM	**6 mL saliva** 10 mL KCl (89.6 g L^{-1}) + 10 mL KSCN (20 g L^{-1}) + 10 mL NaH_2PO_4	**12 mL gastric juice** 15.7 mL NaCl (175.3 g L^{-1}) + 3.0 mL NaH_2PO_4 (88.8 g L^{-1}) + 9.2 mL KCl	**12 mL duodenal juice + 6 mL bile + 2 mL HCO_3^- (1 M)** 40 mL NaCl (175.3 g L^{-1}) + 40 mL $NaHCO_3$ (84.7 g L^{-1}) + 10 mL KH_2PO_4 (8 g L^{-1}) + 6.3 mL KCl (89.6 g L^{-1}) + 10 mL $MgCl_2$ (5 g L^{-1}) + 180 μL HCl (37%, g/g) + 4 mL urea (25 g L^{-1}) + 9 mL $CaCl_2.2H_2O$			X	4.5 g infant formula (cooked) + 2 mL sunflower oil per 100 g	[144] Based on [145]

(continued)

Table 5 (continued)

In vitro model	Compartmental fluid composition							
			Small Intestine			Large intestine	Dietary supplement	Refs.
	Mouth	Stomach	Duodenum	Jejunum	Ileum	Colon		
	(88.8 g L^{-1}) + 10 mL Na_2SO_4 + 1.7 mL NaCl (175.3 g L^{-1}) + $NaHCO_3$ (84.7 g L^{-1}) + 8 mL urea (25 g L^{-1}) + 290 mg amylase +15 mg uric acid +25 mg mucin	(89.6 g L^{-1}) + 18 mL $CaCl_2.2H_2O$ (22.2 g L^{-1}) + 10 mL NH_4Cl (30.6 g L^{-1}) + 6.5 mL HCl (37%, g/g) + 10 mL glucose (65 g L^{-1}) + 10 mL glucoronic acid 2 g L^{-1} + 3.4 mL urea (25 g L^{-1}) + 10 mL glucoseamine hydrochloride (33 g L^{-1}) + 1 g BSA + 2.5 g pepsin +3 g mucin	(22.2 g L^{-1}) + 1 g BSA + 9 g pancreatin +1.5 g lipase + bile juice (30 mL NaCl, 175.3 g L^{-1} + 68.3 mL $NaHCO_3$ (84.7 g L^{-1}) + 4.2 mL KCl (89.6 g L^{-1}) + 150 µL HCl (37%, g/g) + 10 mL urea (25 g L^{-1}) + 10 mL $CaCl_2.2H_2O$ (22.2 g L^{-1}) + 1.8 g BSA +30 g bile					
	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	4.5 g infant formula (blended chicken + mashed potatoes. Nutritional content per 100 g: 3.5 g protein: 8.5 g carbohydrates:3.5 g fat)	[143] Based on [145]

Refer to Table 2 for model names. X refers to not provided. Refs refers to references. The letters printed in bold represent gastric or intestinal solutions and the associated composition is outlined thereafter

Table 6 Variations in Initial PAH concentrations (∑PAH), particle size and bioaccessibility estimates from different bioaccessibility models

In vitro model	Initial [∑PAH]	Soil ingested (g) and particle size	Contaminated matrix	Bioaccessibility of ∑PAHs (M:S:SI:C) %	Study objectives
PBET	0.112 μg g^{-1} to 27.8 μg g^{-1}	6 (< 250 mm)	Soils from kindergarten, primary and middle schools, university and residential areas, bus stop, gas stations and roadside	X: 3.9–54.9: 9.2–60.5: X	To determine PAH bioaccessibility in 13 composite soil samples from different sources (ranging from recreational and residential areas to motor highways) within Beijing
	100 μg g^{-1} pyrene	0.4 (< 2 mm)	Near surface sandy loam field soils (freshly spiked and aged)	X: X: <0.5–12: X	To show the capacity of engineered natural organic sorbents to reduce pyrene in vitro bioaccessibility in freshly spiked and aged (105 d) sterile soil.
	1,503–3,369 μg kg^{-1} for Tianjin soils, and 2,954 to 6,892 μg kg^{-1} for Beijing soils	6 (X)	Soils from long-term wastewater irrigated sites in Tianjin and Beijing parts of northern China	X: 20.1–46.0: 27.4–52.8: X (for ∑PAHs). X: 3.6–54.6: 6.1–68.9: X (for individual PAHs from Tianjin). X: 5.8–64.8: 3.4–58.6: X (for individual PAHs from Beijing	To evaluate PAH levels, sources and bioaccessibility in soils from long-term wastewater irrigated sites in Tianjin and Beijing, China
	83–8,845 μg kg^{-1}	0.2 (< 250 μm)	Soils from 20 sites representing different functional areas (6 residential, 3 industrial, 2 business districts, 4 recreational/scenic, 2 agricultural and 3 public areas)	X: 4.9–21.8: 14.6–63.2: X	To investigate PAH levels and bioaccessibility in soils from different functional areas in Xiamen city, Southwest China so as to provide data regarding risk from exposure to PAHs in

(continued)

Table 6 (continued)

In vitro model	Initial [∑PAH]	Soil ingested (g) and particle size	Contaminated matrix	Bioaccessibility of ∑PAHs (M:S:SI:C) %	Study objectives
					various urban settings, which may be useful for HHRA
	10 mg kg^{-1} (∑PAHs) for spiked soils. For field contaminated soils: PYR (609 µg kg^{-1}) FLT (818 µg kg^{-1}) CHR (282 ug kg^{-1}) BbF (491 ug kg^{-1}) BKF (219 ug kg^{-1}) B[a]P (391 ug kg^{-1}) BPY (551 ug kg^{-1})	0.2 (< 250 µm)	5 pristine soils different provinces in China artificially spiked with 16 USEPA priority PAHs, and a field-contaminated agricultural soil from Wuxi, Jiangsu, China	X: X: 8.25–20.8 and 3.70–6.92 (in artificially spiked and field contaminated soils respectively without Tenax), 55.7–65.9 and 16.3–31.0 ((in artificially spiked and field-contaminated soils respectively with Tenax): X	To demonstrate the potential of Tenax as a sorption sink to facilitate and enhance PAH mobilisation in in vitro bioaccessibility studies
CEPBET	460 µg/g naphthalene +94 µg/g acenaphthalene +91 µg/g fluorene +95 µg/g phenanthrene +93 µg/g anthracene +96 µg/g fluoranthene +95 µg/g pyrene	1 (X)	Artificially spiked OECD standard soil containing milled moss peat, 50–200 µm sand, kaolin clay in 10%, 70%, 20% proportions respectively. Adjusted to pH 6 with $CaCO_3$. Concentrations spiked typical for the upper 15 cm soil of heavily contaminated old gasworks sites in the UK	X: X: X: X	To develop a colon-included PBET model for PAH bioaccessibility determination in artificially spiked and field contaminated soil. However, Collins et al. [150] included an activated carbon on a PDMS prepolymer as a contaminant trap/sink and obtained greater bioaccessibility values

	Same as above	(X)	10 red shale samples representative of red shale materials considered for reuse within a residential development platform	X: X: X: 5.3–28.4	To derive site specific assessment criterion (SSAC) for B[a]P in a red shale at a former coking works. As a result, an SSAC of 2.5 mg kg^{-1} was derived
IVG	73.4 + 1.1 μg g^{-1}	0.05 g soot (<75μm)+2g silicone sheet	Fuel soot	X: X: 84. 8 (for 11 PAHs), 69.2–89.3 (for individual PAHs): X	To investigate the effectiveness of including a silicone sheet as an absorptive sink to increase PAH bioaccessibility in fuel soot matrix
	X	5 (< 250 μm)	Artificially spiked plant biopolymers	X: X: 39–94 (cutin-cutan): X	To assess the bioaccessibility of phenanthrene sorbed to 2 cutin and cutan and assess the influence of their structural differences on sorption/desorption behaviour and bioaccessibility
	X	0.6 (X)	A soil sample from Changping, Beijing artificially spiked and aged for 15 months with PAHs and DDXs	X: X: 86 ± 6.8: X	To provide evidence for increased bioaccessibility or mobilisation of PAHs and DDX from soil in the SI fluid when the fraction dissolved in the fluid and weakly sorbed in the digestive residue in the GI compartment is considered

(continued)

Table 6 (continued)

In vitro model	Initial [∑PAH]	Soil ingested (g) and particle size	Contaminated matrix	Bioaccessibility of ∑PAHs (M:S:SI:C) %	Study objectives
		2 (X)	3 soil samples from a heavily contaminated northern China plain		To investigate PAH bioacessibility in 3 field contaminated soils by a conventional solvent extraction method and compared with an in vitro model The study found that for all the soils assessed, the measured PAH concentration after digestion was greater than that from normal solvent extractions. Hence the proposition of bound residue mobilisation in vitro
SHIME	49 ± 1.5 mg kg^{-1}	20 (X)	Soil from a contaminated recreational area in Belgium, having received 2 decades old atmospheric deposition from nearby industrial activities	X: (0.05[a], 0.44[b], 0.83[c]): 0.12: 0.3	To investigate the in vitro release/mobilisation of PAHs and associated complexation processes
	0.6 (bulk soil 1.e 4 mm sieved) – 3.7 (45 μm sieved mg kg^{-1}	0.3 (< 45 μm)	18 bulk soil samples from an arctic brownfield in Nunavut, Canada	X: X: 1–10: 1.2–21	To investigate PAH enrichment and bioaccessibility in 18 soils, and also estimate cancer risks associated with

					ingestion and absorption of soil fractions
FOREhST	For bulk soil (< 2 mm): < 0.5 (naphthalene) – 196.0 (chrysene) mg kg^{-1}; ∑PAHs 1070.4 mg kg^{-1} for <250 μm: < 0.5 (naphthalene) – 191 (chrysene) mg kg^{-1}. ∑PAHs 871. 1 mg kg^{-1}	0.3 (< 250 μm)	Creosote-contaminated soil (7,767 ± 1,286 mg kg^{-1} ∑PAHs) recovered after 100 d of enhanced natural attenuation treatment	X: X: < 4: X	To investigate bioavailability and bioaccessibility following accidental ingestion of residual PAHs-laden-creosote contaminated soil following 1,000 d of enhanced natural attenuation
	2–68 mg kg^{-1} B[a]P or 9.9–286.6 mg kg^{-1} ∑PAHs	0.3 (< 250 μm)	11 soil samples collected from disused gas work sites within the UK	X: X: BaA (31–61%); BbF (30–55%); BkF (19–38%); B[a]P (21–50%); dBahA (12–30%); indeno [1,2,3-cd] pyrene (30–59%); X	To compare a static but robust FOREhST test with dynamic SHIME for PAH bioaccessibility in 11 soil samples from disused gas work sites within the UK. No significant difference in both results, although FOREhST over predicted compared to SHIME
UBM (fed)	9.0–1,404 mg kg^{-1})	0.3 ((< 250 μm)	6 soil samples collected from a historically tar works contaminated site which has been left derelict for over 3 decades	X:35–83.9 (across individual PAHs in gastric + intestinal juice (median = 63.9)); X	To investigate PAH bioaccessibility in soils from a former industrial tar site within the UK. An interlaboratory comparison of the method was conducted and achieved good results

(continued)

Table 6 (continued)

In vitro model	Initial [∑PAH]	Soil ingested (g) and particle size	Contaminated matrix	Bioaccessibility of ∑PAHs (M:S:SI:C) %	Study objectives
RBALP	0.17–650 μg B[a]P equivalents g^{-1} soil	1 (< 45 μm)	Eight contaminated soils from sites (wood preservation sites, gas work sites, petroleum hydrocarbon sites in Sweden and Canada	X: X: 0.14 and 0.67 μg B[a]P equivalents or < 7.9% (with and without C-18 respectively): X	To provide evidence on increased PAH bioaccessibility in 8 different soils, using 2 different bioaccessibility models plus lipid sink in the SI compartment
IVD	0.17–650 μg B[a]P equivalents g^{-1} soil	0.3 (< 45 μm)	Eight contaminated soils from sites (wood preservation sites, gas work sites, petroleum hydrocarbon sites in Sweden and Canada	X: X: 6.6E-3 and 0.29 μg B[a]P equivalents or < 13% (with and without C-18 respectively): X	This study provided evidence on the increased bioaccessibility of PAHs in 8 different soils, using two different in vitro bioaccessibility models in the presence of a lipid sink introduced into the small intestinal medium
PBET/DIN	10 mg kg^{-1} for individual PAHs (FLA-d_{10}, PYR-d_{10}, BaA-d_{12}, CHR-d_{12}, BbF-d_{12}, BkF-d_{12}, B[a]P-d_{12}, indeno [1,2,3-*cd*] pyrene-d_{12}, DbahA-d_{14}, and BghiP-d_{12}) and 100 mg kg^{-1} for ∑PAHs	2 (< 60 μm)	PAH spiked reference geosorbents (quartz sand <60 μm, montmorillonite clay, charcoal or BC < 60 μm, and peat <60 μm) representing common natural geosorbents	X: X: For single PAH compounds, quartz sand (12.6–50.7), Na-montmorillonite clay (2.3–10.5), Pahokee peat (3.3–10.5) and charcoal (0.0–0.37%). For ∑PAHs, quartz sand (26.9 ± 7.5), Na-montmorillonite clay (4.8 ± 1.1), Pahokee peat (6.4 ± 2.2) and charcoal (0.1 ± 0.1): X	This study investigated the influence of commonly occurring geosorbents in soil on PAH bioaccessibility

Digestive tract model /DIN	189–3,800 μg g^{-1}	1 (X)	Untreated, air dried or lyophilises contaminated material	X: 0.4–75: 5–66: X	This study made use of a simple in vitro digestive model to simulate and examine the mobilisation of PAH and PCBs from 31 different contaminated soils which also included contaminated soils of technogenic origin
IVD + modified RIVM	2.25–17.3 mg kg^{-1}	0.4–4.5 (X)	5 samples each from Danish contaminated sites: Urban soil from an old city area (loamy sand >100 years), heavy traffic contaminated soil (sandy loam) and ash contaminated soils (sandy loam) from a porcelain factory	X: X: 13–36: X	This study assessed the oral bioaccessibility of PAHs from 15 soil samples from 3 different Danish sites
	0.31–4.7 mg kg^{-1} for B[a]P and 0.08–0.99 mg kg^{-1} for DbaA	0.4 (X)	Soil samples from an area with >100 years of urban history, traffic highway soil, rural area with fishing net tarring and urban soil with ashes from porcelain factory	X: X: (5.7–38 for B[a]P, and 12–40 for DbahA): X	To predict B[a]P and DbaA bioaccessibility in soils from 4 Danish-contaminated sites and validated with soils whose bioavailability have been determined from studies with mice and minipigs

Refer to Table 2 for model names. M:S:SI:C refers to mouth/stomach/small intestine/colon/caecum respectively. X refers to not provided. a, b, c, refers to results associated with different gastric L:S tested, i.e. 2, 10 and 40 respectively. References exactly as in Table 5

Table 7 Variations in extraction and analytical techniques in different in vitro bioaccessibility studies

In vitro model	Centrifuge	Mixing type (Speed)	Extraction method		Analytical technique				
			Pellets	Supernatant	Instrument used	Internal standard	Detection limit	Quantification limit	Recovery (%)
PBET	7,000 × *g* (10 min)	Paddle stirrers (ca. 100 rpm)	Soxhlet extraction with DCM/ACE (7:9, v/v)	Separatory funnel shake (using 40 mL 1:3 ACE: *n*-HEX, v/v) + back extraction with *n*-HEX	GC-MS	X	X	X	85–110
	X	Gyratory shaker (50 rpm)	Combustion and ^{14}C-radioactivity analysis	^{14}C-radioactivity analysis using a liquid scintillation counter	Biological oxidiser and liquid scintillation counter	X	X	X	X
	7,000 × *g* (10 min)	Paddle stirrers (ca. 100 rpm)	X	Separatory funnel shake (using 40 mL 1:3 ACE: *n*-HEX, v/v) 3x	GC/MS-SIM	X	0.001–0.01 mg L^{-1}	X	90.8 ± 6.3
	4,000 × *g* (10 min)	Agitation (100 rpm)	X	C-18 cartridge extraction using 6 mL HEX/DCM/ACE (45:45:10) mixture	GC/MS-SIM	X	1 µg L^{-1}	X	89.2–103.5
	3,000 × *g* (5 min)	X	Harvesting of Tenax beads by filtration using filter paper and washed by dH_2O (3x). Filter paper containing Tenax was air-dried overnight and	Liquid-liquid (L-L) extraction using 10 mL DCM (3x) in a separatory funnel	HPLC-florescence spectrophotometer	X	0.10–0.42 µg L^{-1}	X	53.5–75.3 (for L-L extraction) and 79.0–93.1 (for recovery of soil samples spiked with individual

			extracted as in supernatant						standards of the 7 PAHs)
CEPBET	3,000 × *g* (10 min)	X	4 mL ACE/HEX (5:4) on a roller shaker for 20 min	4 mL ACE/HEX (5:4) and vortexed at 1600 rpm (1 min)	GC-FID (PAHs in pellets)/GC/MS-SIM (PAHs in supernatant)	500 μg mL^{-1} biphenyl	5 μg g^{-1} – 22 μg g^{-1} (GC-FID) and 100–400 μg L^{-1} (GC-MS)	X	50.1–96.0
	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above	Same as above
IVG	3,000 rpm (20 min, at 20 °C)	End-over-end shaker (60 rpm)	Acetone extraction (20 mL, 3x) used for silicone sheet. Toluene-methanol (20 mL, 1:6, v/v) extraction through a microwave accelerated extraction used for soot residue	*n*-hexane (30 mL, 2x) extraction used for digestive fluid	GC-MS	NAP-d8, ACE-d_{10}, PHE-d_{10}, CHR-d_{12} and Perylene-d_{12}	Digestive fluid (0.0046–0.070 ng mL^{-1}); silicone sheet (0.11–1.05 ng g^{-1}) soot residue (3.10–52.2 ng g^{-1})		Method recoveries: Digestive fluid (69.2–141%); digestion residue (67.7–112%). Spike recovery or surrogate: Digestive fluid (80–95%); digestion residue (77–89%); silicone sheet (96–105%)

(continued)

Table 7 (continued)

In vitro model	Centrifuge	Mixing type (Speed)	Extraction method		Analytical technique				
			Pellets	Supernatant	Instrument used	Internal standard	Detection limit	Quantification limit	Recovery (%)
	3,000 × *g* (5 min)	Head-over-heels (55 rpm)	C-18 membrane extraction with methanol and sonication		HPLC-PDA (photodiode array detector)	X	X	X	X
	7,600 × *g* (10 min)	Rotator (100 rpm)	20 mL n-HEX/ACE (1:1, v/v)	Separating funnel using 30 mL n-HEX extraction (2x) on a shaker (300 rpm)	GC/MS-SIM	X	0.85–6.8 ng g^{-1}	X	72–121 (fluid); 67–139 (residue)
	7,601 × *g* (10 min)	Rotator (100 rpm)	Microwave accelerated reaction system using 20 mL mixed n-HEX:Ace (1:1, v/v)	Separating funnel using 30 mL n-HEX extraction (2x) on a shaker (300 rpm)	GC/MS-SIM	2- fluoro-1, Í – biphenyl and p-terphenyl-$_{d14}$	0.11–0.29 ng g^{-1}	X	72–121 (fluid); 67–139 (residue)
SHIME	1,500 × *g* (5 min)[i], 3,000 × *g* (10 min)[ii]	Stirrer (150 rpm)	Accelerated solvent extraction (using 1:1 ACE/HEX)	DCM	GC-MS (USEPA method 8,270)	X	0.2 µg L^{-1}	0.4 µg L^{-1}	80–100
	Filtration by 2 µm pre-filter and 0.45 µm, Whatmann 13 mm GD/X disposable PTFE filter	Rotary mixing (200 rpm)	Accelerated solvent extraction (using 4:1 HEX/DCM)	Solid phase extraction with 2 washes of 1.5 mL acetonitrile	LC-FD (USEPA method 8,270)	9-phenylanthracene	X	X	63–72

FOREhST	3,000 × *g* (5 min)	End-over-end rotation (30 rpm)	Pressurised fluid extraction system (ACE/DCM, 50:50, v/v; 2000 psi pressure; 100 °C); 10 min	Methanolic saponification using ca. 5.6 M KOH (3 mL) and SPE with DCM: Tetrahydrofuran (1:1, v/v)	GC-MS	10–20 μL of a 100–1,000 μg mL^{-1} 4,4́-difluorobiphenyl solution per GC-MS sensitivity	X	X	82–104 (for B[a]P across soil, FOREhST and faecal matrices)
	3,000 × *g* (5 min)	End-over-end rotation (30 rpm)	Pressurised fluid extraction system (ACE/DCM, 50:50, v/v; 2000 psi pressure; 100 °C); 10 min	Methanolic saponification using ca. 5.6 M KOH (3 mL) and then SPE with DCM:Tetrahydrofuran (1:1, v/v)	GC-MS	10–20 μL of a 100–1,000 μg mL^{-1} 4,4́-difluorobiphenyl solution according to sensitivity required of GC-MS	X	X	90
UBM (fed)		End-over-end shaker (30 rpm)					1.1–2.3 mg kg^{-1} (solution) and 1.4–2.8 mg kg^{-1} (soil and residue)	X	63.0–114.0
RBALP	X	End-over-end rotation (30 rpm)	X	Solid phase extraction	HPLC-FD	X	0.07–0.64 pg	X	92
IVD	X	Shake (70 rpm)	X	Solid phase extraction	HPLC-FD	X	0.07–0.64 pg	X	92
PBET/DIN	1,500 × *g* (10 min), vacuum filtration (0.45 μm membrane filter)	Magnetic stirrer	X	Separatory funnel shake (using 1:3 ACE:*n*-HEX): 3x	GC-MS	X	0.05–0.09 μg L^{-1}	X	X
Digestive tract model /DIN	7,000 × *g* (10 min 2x),	Tangential shaker (200 rev/min, 0.75 cm)	Ultrasonication (using 1:1 ACE: *n*-HEX)	Separatory funnel shake (using 1:3 ACE:*n*-HEX)	HPLC-FD	X	X	X	X

(continued)

Table 7 (continued)

In vitro model	Centrifuge	Mixing type (Speed)	Extraction method		Analytical technique				
			Pellets	Supernatant	Instrument used	Internal standard	Detection limit	Quantification limit	Recovery (%)
IVD + modified RIVM	2,750 × *g* (5 min)	Head-over-heels (55 rpm)	Aqueous solution of pyrophosphate and DCM	DCM	GC/MS-SIM	X	X	X	X
	Same as above	End over end mixing	Same as above	Same as above	Same as above	Deuterated standards	0.005 mg kg^{-1} dw for soil/residue, and 0.05 μg L^{-1}	X	95–105 for soil/residue and 98–116 for supernatant

Refer to Table 2 for model names. X refers to not provided. 3x, 2x, etc. refers to 3 times, 2 times (i.e. frequency) etc. References exactly as in Table 5 above

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Passive Sampling for Determination of the Dissolved Concentrations and Chemical Activities of Organic Contaminants in Soil and Sediment Pore Waters

Kilian E. C. Smith

Contents

Abstract The freely dissolved concentrations of organic contaminants in soil and sediment pore waters are relevant for the wide range of fate processes where compound bioavailability plays a role but also for several abiotic processes. However, determining these is challenging due to their low levels and sorption to dissolved organic matter. Here, passive sampling can play a role and involves bringing an inert polymer into direct contact with the soil or sediment matrix such that the dissolved contaminant molecules partition into the polymer until a partitioning equilibrium is reached. Passive sampling has been applied to determine the freely dissolved concentrations of a range of mainly neutral organic contaminants in soils and sediments. For this, a range of formats using different polymers

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and architectures have been developed, some targeted towards equilibrium and others towards kinetic passive sampling. The most common polymers for neutral hydrophobic organics include various silicones, polyethylene and polyoxymethylene. However, for the passive sampling of polar and ionic compounds, different polymers with a higher affinity for these compounds are required. For kinetic sampling, in situ calibration methods are needed to account for variations in the uptake kinetics.

Keywords Dissolved concentrations, Passive sampling, Pore water, Sediment, Soil

1 Fate of Organic Contaminants in Soils and Sediments

In soils and sediments, organic contaminants exist associated with the matrix material, sorbed to dissolved organic matter or as freely dissolved molecules in the pore water [1]. This distribution is determined by the contaminant properties [2], the physical and chemical characteristics of the sorbing matrices [3, 4], environmental conditions [5] as well as contact time [6].

Hydrophobic organic compounds (HOCs) have the tendency to partition out of the aqueous phase and become sorbed to the solid (e.g. organic matter, combustion residues, mineral material) and nonaqueous liquid (e.g. oils) components of the soil or sediment [1]. A fraction of the organic matter in the soil or sediment also exists dissolved in the pore water, providing an additional sorptive phase for the HOCs that is also relatively mobile [7]. Despite their higher aqueous solubilities, polar and ionic organic contaminants can also appreciably partition to mineral surfaces or to the functional groups of the organic matter [8, 9]. Therefore, both the composition and also the relative abundances of the different sorbing phases making up the soil or sediment matrix play a fundamental role in the determining the solid/water partitioning of organic contaminants. This partitioning is further influenced by environmental conditions such as temperature (e.g. reduced partitioning is observed at higher temperature [5]) or in soils the moisture content (e.g. partitioning of both hydrophobic and polar compounds is orders of magnitude higher in dry soils [10]). Finally, sorption is time dependent, and partitioning generally increases with longer contact times between the organic contaminant and solid matrix in a process known as ageing [6].

2 The Role of Dissolved Organic Contaminants in Soil and Sediment Pore Water

The distribution of an organic contaminant between the solid and aqueous phases in soils and sediments (see Fig. 1) plays a key role in a wide range of environmental fate processes. The mass fraction existing in the aqueous phase (either freely dissolved or associated with dissolved organic matter) is subject to leaching to deeper soil layers or groundwaters [7]. Furthermore, as discussed in chapter "Why Biodegradable Chemicals Persist in the Environment?: A Look at Bioavailability", microbial biodegradation remains an important removal mechanism for organic contaminants in many ecosystem compartments [11]. This biodegradation is partly determined by the freely dissolved concentrations, since these determine diffusive uptake by the degrading microorganisms [12]. Partitioning of an organic contaminant into the organisms inhabiting soils or sediments is largely determined by their freely dissolved pore water concentrations. This is relevant for ecotoxicity but also for food chain accumulation and secondary poisoning [13–15]. Whether a polluted sediment acts as a contaminant source or sink to the overlying bulk water depends on the dissolved concentration gradient of the pollutant between the sediment pore water and the water column [16, 17]. Finally, for some organic contaminants, soil-air exchange remains an important secondary source to the atmosphere from where they can be transported over (long) distances. Here, partitioning between the solid matrix, pore water and soil air determines the direction and magnitude of any soil-air exchange fluxes [18].

Therefore, the freely dissolved concentrations of an organic contaminant in the soil or sediment pore water are a key phase for numerous biotic as well as abiotic fate processes. Understanding the sorptive properties of the individual components making up the soil or sediment matrix is challenging, and the analytically simpler approach is to develop methods aimed at measuring these dissolved concentrations in the pore water.

3 Measuring Dissolved Concentrations of Organic Contaminants in Pore Water

Given the central role that the freely dissolved pore water concentrations of organic contaminants have in soils and sediments, much effort has been put into the development of analytical methods for measuring these. However, this is difficult given the low concentrations and the relatively small volumes or pore water that can be isolated. Concentrations might be directly determined in the pore water after centrifugation or filtering to first separate the solid and liquid phases [4]. However, the collection and separation process can itself lead to a disturbance in the partitioning. Moreover, the isolated pore water can also contain dissolved organic matter which appreciably sorbs particularly HOCs. This leads to an overestimation

of their aqueous concentrations, as well as their bioavailability for processes such as bioconcentration or microbial degradation [19]. Furthermore, in unsaturated soil, only small amounts of soil pore water can be isolated which can lead to analytical difficulties. Circumventing this by simply adding excess liquid to create a suspension can change the partitioning distribution between the solid and liquid phases [20].

4 Passive Sampling for Measuring Dissolved Concentrations of Organic Contaminants

Passive sampling is one approach that avoids some of these challenges. This involves bringing an inert polymer into direct contact with the soil or sediment matrix. Dissolved contaminant molecules in the pore water spontaneously partition into the polymer due to the gradient in chemical activity existing between both phases. This diffusive uptake continues until a partitioning equilibrium is reached, after which the polymer concentrations remain constant (see Fig. 1) [21–23]. Passive sampling can be performed in situ or the soil or sediment samples first collected, and this then done in vessels in the laboratory. Whereas the in situ approach more accurately depicts the dynamic situation as this occurs in the field, the latter is more commonly applied due practical considerations (e.g. homogenization, the possibility of speeding up sampler equilibration via additional mixing). However, the laboratory approach leads to measurements on a sample that is no longer part of the natural environment and may therefore not accurately reflect processes such as biodegradation, bioirrigation due to benthic organisms flushing their burrows, etc. On the other hand, the small size of passive samplers means that they can really only sample at relatively small spatial scales, and with in situ passive sampling, it can be challenging to adequately capture heterogeneity as this occurs in the field.

Provided the amount of organic contaminant that is take up by the passive sampling polymer does not significantly disturb the equilibrium distribution existing between the sorbed and dissolved phases in the bulk soil or sediment, then passive sampling can be used to determine the freely dissolved concentrations in the pore water [21–23]. Measurement of the sorbed polymer concentrations allows for calculation of the freely dissolved concentrations via an equilibrium partitioning ratio (for equilibrium passive sampling) or a sampling rate (for kinetic passive sampling) (see Fig. 1) [22, 24]. Measurement of the passive sampler-sorbed amounts can be performed directly via thermal desorption or by re-extraction of the analyte into solvent followed by analysis. Other advantages of passive sampling include its simplicity (e.g. no power source is needed), relatively low costs and the significant up-concentration of the organic contaminants into the sampling polymer which simplifies analysis of the often low freely dissolved concentrations of organic contaminants present in pore water.

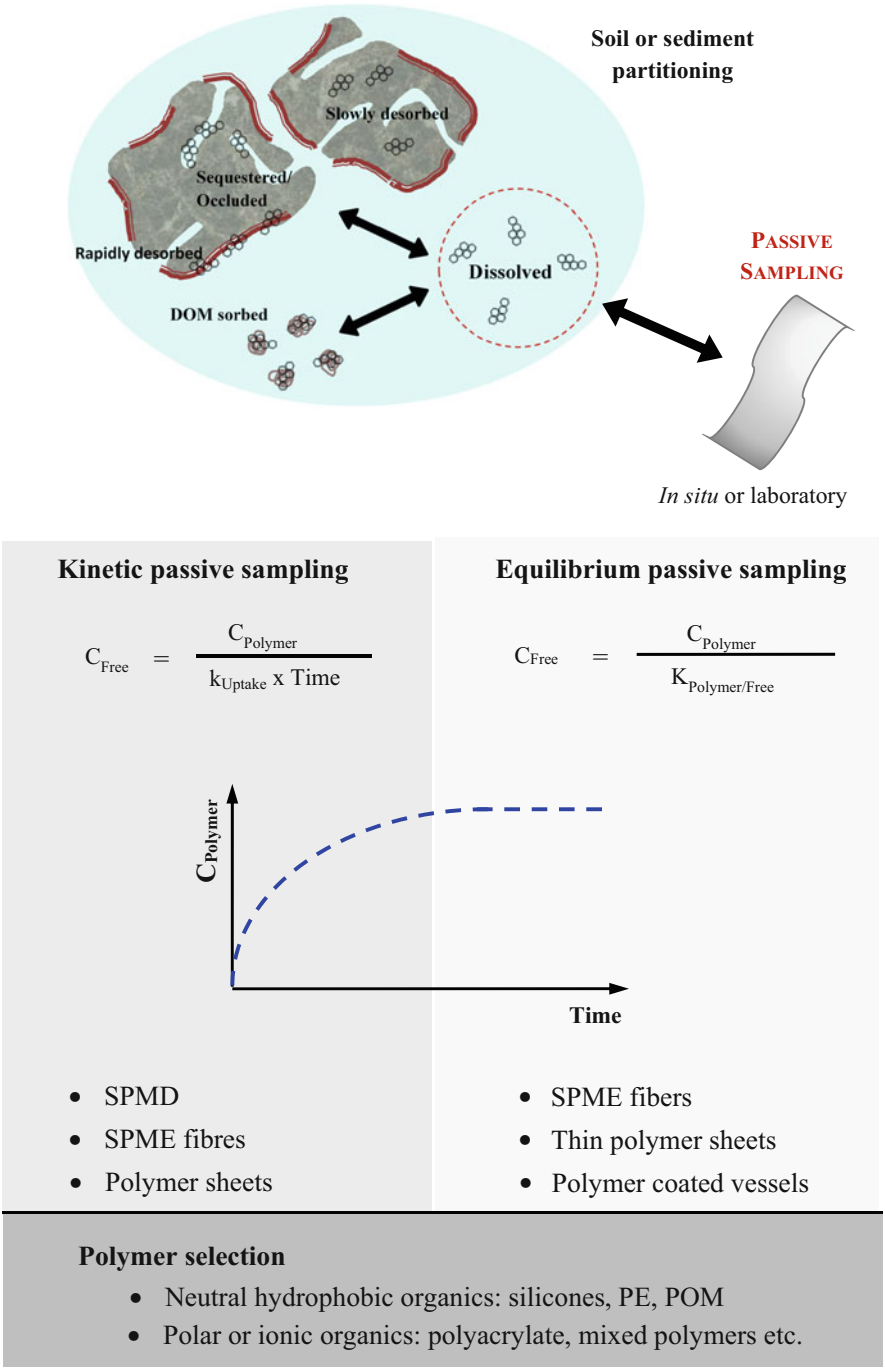

Fig. 1 Passive sampling in soils and sediments. Freely dissolved concentrations (C_{Free}, µg/L) in the pore water are (partly) determined by partitioning from the different sorbed phases. Bringing the passive sampling polymer into contact with the soil or sediment leads to diffusive accumulation of

In most cases passive sampling is used to obtain freely dissolved concentrations which can then be used in different ways. The most straightforward is their comparison to known effect concentrations or regulatory thresholds (although the latter are still mainly based on total soil or sediment concentrations). For example, the application of passive sampling in a sediment toxicity test provides a more accurate picture of the bioavailable contaminant exposure profile. Since passive samplers target the bioavailable contaminant fraction in the soil or sediment, they can also be directly used to predict internal effect concentrations of non-metabolized compounds [25]. In other words, the accumulated amount in the passive sampler can serve as a surrogate estimate for organism accumulation. However, caution is needed here since passive sampling cannot perfectly mimic all relevant processes such as contaminant intake with food (i.e. biomagnification), digestive processes, active uptake/elimination mechanisms or biotransformation.

A contaminant's freely dissolved concentration is the effective concentration for processes such as diffusion, partitioning and organism bioconcentration [26]. However, they are not so relevant in terms of the total contaminant mass present in the soil or sediment. They can also be related to chemical activity, chemical potential or fugacity via either linear or logarithmic relationships [26]. These multimedia parameters give a direct indication of the potential of an analyte for spontaneous processes such as diffusion or partitioning. For example, chemical activity has been used in assessing the toxic potential of sediments [17]. Here, passive sampling was used to determine the sum chemical activity of bioavailable PAHs at different depths in sediment cores. These measured chemical activities could then be compared to the range corresponding to baseline toxicity. Conversion of freely dissolved concentrations into these different multimedia parameters is described in the literature [22, 26].

When immersing the passive sampler directly into the soil or sediment, there is the possibility of fouling of the surface by the sample constituents with resultant changes in the sorption kinetics or partitioning equilibrium. For equilibrium passive sampling, fouling can be relatively easily minimized by careful cleaning of the sampler surfaces prior to extraction and analysis. However, for kinetic sampling, such fouling can in some cases alter the uptake kinetics, and this then needs to be accounted for (see section on kinetic sampling below). Instead of directly inserting the passive sampler into the soil or sediment, more volatile compounds can also be passively sampled by placing the polymer in the headspace above the sample

Fig. 1 (continued) the freely dissolved molecules until a partitioning equilibrium is reached. The freely dissolved concentrations can then be determined from the polymer concentrations ($C_{Polymer}$, µg/L or µg/kg): for kinetic passive sampling using an uptake rate constant (k_{uptake}, 1/t) and for equilibrium passive sampling using a compound-specific equilibrium partitioning ratio ($K_{Polymer/Dissolved}$, L/L or L/kg). Common formats for kinetic passive sampling include semi-permeable membrane devices (SPMDs), solid phase microextraction (SPME) fibres or polymer sheets. Common formats for equilibrium passive sampling include SPME, thin polymer sheets or vessels coated with a thin polymer film. Polymers for neutral hydrophobics include various silicones, polyethylene (PE) or polyoxymethylene (POM). Other polymers are needed for polar and ionic organic contaminants

[27]. The organic contaminant then approaches a partitioning equilibrium between the sample, headspace and polymer. This approach is particularly advantageous for those sample types that result in significant fouling of the sampler surface, e.g. soils or sediments with large amounts of oily phases or combustion residues [28].

In the following sections, the underlying principles, formats and selected examples for equilibrium and kinetic passive sampling are covered in more detail.

5 Non-depletion Passive Sampling

An important consideration for passive sampling is the non-depletion criterion [22, 24]. Accumulation of the organic contaminant in the passive sampler unavoidably leads to depletion of the freely dissolved pool in the pore water. When this is significant, the amount of contaminant accumulated in the polymer can no longer be related back to the original undisturbed freely dissolved concentrations that were present in the sample. This is most relevant for the passive sampling of HOCs in aqueous systems containing only low amounts of sorbing matrices. Here, the high $K_{\text{Polymer/Free}}$ values of HOCs means that large sample volumes are needed to avoid significant depletion of the dissolved pool. For equilibrium passive sampling of such aqueous samples in the laboratory, this can be a limitation, since there are practical constraints on the volumes than can be handled. However, this is less of an issue with field passive sampling, where the sample can be considered as being infinite. In contrast, soil and sediment contain an appreciable sorbed pool of HOCs. In this case, any depletion of the dissolved pool due to uptake in the sampling polymer can be replenished by these sorbed HOCs. This confers more flexibility in the amount of sampling polymer that can be added, but the kinetics of replenishment of the dissolved pool always need to be considered. The non-depletion concept for matrix rich samples such as soils and sediments has been formalized in the literature [22, 24]. For example, if the objective is to have less than 1% depletion of the contaminant mass in the soil or sediment sample, then using mass balance considerations, it can be shown that the maximum polymer mass (M_{Polymer}, kg) that should be used for equilibrium passive sampling is

$$\frac{M_{\text{Polymer}} \times K_{\text{Polymer/Free}}}{M_{\text{OC}} \times K_{\text{OC}}} < 0.01 \tag{1}$$

Here, $K_{\text{Polymer/Free}}$ (L/kg) is the compound-specific equilibrium partitioning ratio between the polymer and freely dissolved phases, M_{OC} (kg) is the mass of OC in the soil or sediment sample, and K_{OC} (L/kg) is the compound-specific equilibrium partitioning ratio between the sample organic carbon and freely dissolved phases. Assuming that the sediment organic carbon and polymer matrices have similar partitioning characteristics, a general rule is that a ratio of 1:100 polymer mass to organic carbon mass should reduce any depletion to an acceptable value of around

1%. Note that some studies set a less stringent depletion criterion than the 1% used in the example above.

The non-depletion consideration applies equally to equilibrium and kinetic passive sampling, with the caveat that depletion of the dissolved pool is somewhat reduced in the latter mode of sampling because the maximal equilibrium levels are not reached in the polymer. When the non-depletion criterion is met, one speaks about non-depletion (nd) passive sampling. Non-depletion conditions can be confirmed by varying the passive samplers volume and verifying that the concentration in both is the same [29, 30]. In this case, where depletion is to be an issue, then the sampler with the larger volume would accumulate lower amounts since depletion is more significant.

6 Equilibrium Passive Sampling in Soils and Sediments

With equilibrium passive sampling, sufficient contact time between the polymer and soil or sediment is required to ensure that a partitioning equilibrium is established. The freely dissolved concentration (C_{Free}, μg/L) can then be calculated from the measured equilibrium polymer concentrations ($C_{\text{Polymer, equilibrium}}$, μg/L or μg/kg) using $K_{\text{Polymer/Free}}$ (L/L or L/kg depending on the units of $K_{\text{Polymer/Free}}$)

$$C_{\text{Free}} = \frac{C_{\text{Polymer,equilbrium}}}{K_{\text{Polymer/Free}}} \tag{2}$$

$K_{\text{Polymer/Free}}$ describes the equilibrium partitioning distribution of an organic contaminant between the freely dissolved and polymer phases for a specific set of conditions (temperature and ionic strength are especially important here). Values of $K_{\text{Polymer/Free}}$ for the most commonly applied passive sampling polymers and for a wide range of organic contaminants can be found in the literature [31–34]. These data sets are complemented by increasingly reliable predictive approaches (e.g. http://www.ufz.de/lserd).

Equilibrium passive sampling has certain advantages. It is relatively robust, since the numerous factors that affect the uptake process (e.g. mixing regime, temperature) no longer apply when equilibrium has been reached. Furthermore, contaminant accumulation is maximized at equilibrium which leads to lower detection limits. However, a number of criteria need to be met for this mode of passive sampling. Partitioning into the sampling polymer must follow a linear isotherm, such that a single $K_{\text{Polymer/Free}}$ characterizes the enrichment of the analyte over the relevant range in C_{Free}. This is generally the case for absorptive partitioning and can be confirmed by varying the surface-area-to-volume ratio and showing that the concentration in all samplers is the same [29]. Significant surface sorption would show up as a higher concentration in the passive sampler with the higher surface-area-to-volume ratio. The sampling polymer should also retain its partitioning properties when

immersed in the sample matrix. For example, this has been shown to be the case for polydimethylsiloxane (PDMS) silicone in a wide range of complex matrices including sediment [35]. In general, polymers with adsorptive partitioning are more susceptible to artefacts including saturation effects, analyte competition effects, sample matrix effects and surface-catalysed reactions which makes them less suited for equilibrium passive sampling.

Various polymers have been used for the passive sampling of HOCs, with the most widely applied being silicones, polyethylene (PE) and polyoxymethylene (POM) [22]. Which of these polymers is used is often the result of personal preference, and they all share in common a high affinity for the HOCs. Concentration-independent partitioning has been demonstrated for these polymers [36]. Whereas PE and POM are more often deployed as thin sheets with large surface areas, PDMS and other silicones have been deployed as coatings on a thin glass fibre and thin sheets or as coatings inside vials. These formats are discussed in more detail below. Some important differences between the various polymers exist, impacting their application in passive sampling. Silicones including PDMS generally have the highest internal diffusion coefficients. These are typically several orders of magnitude higher than those of low-density PE, which in turn has higher values than POM [37, 38]. Higher internal diffusivities speed up the equilibration process and help avoid the development of internal concentration gradients within the polymer. The latter is important since the correct application of $K_{\text{Polymer/Free}}$ to calculate C_{Free} requires a homogenous distribution of the compounds in the polymer at equilibrium. On the other hand, it has been suggested that POM is less prone to fouling by black carbon particles and nonaqueous phase due to its smooth surface properties [28, 39]. POM also has a repeating ether group (-CH2-O-CH2-), resulting in a higher affinity for polar compounds compared to PE and PDMS [34].

A range of equilibrium passive sampling formats have been used to measure the C_{Free} of organic contaminants in soil or sediment pore water [16, 17, 21–23, 40–48]. Most of the early passive sampling work focussed on more persistent HOCs such as PAHs, PCBs, PCDD/Fs and organochlorine pesticides in sediments. These organic contaminants accumulate to high levels in soils and sediments due to their persistence and/or hydrophobicities. Although their C_{Free} levels in pore waters are often very low and in the ng/L range, their hydrophobic nature means they can nevertheless reach high levels in soil or sediment dwelling organisms via partitioning. The low levels found in pore water immediately make analytical determination challenging. This is further complicated by small amounts of dissolved organic matter (DOM) existing in the pore water that can sorb significant HOC amounts. The latter leads to significant artefacts when applying pore water extraction and analysis approaches, since the measured aqueous concentrations comprise both freely dissolved and DOM-sorbed contaminant molecules [19].

One of the earlier passive sampling formats routinely applied in soils and sediments was solid-phase microextraction (SPME). SPME is based on polymer-coated fibres and was originally introduced as a sample preparation method taking advantage of the selective up-concentration of analytes in the polymer [49]. The thin coating and high surface-area-to-volume ratio of polymer in SPME are advantageous

in terms of reducing equilibration times but can be a limitation with regard to detection limits. This is because the polymer volume (in the range of tens of μL for SPME) is directly proportional to the amount of organic contaminant that is accumulated. A variety of SPME fibres are commercially available with coatings of various thicknesses and composed of different polymers [50]. Whereas PDMS silicone coatings, for example, are particularly suited for HOCs, other polymer types and even mixed polymer phases are more appropriate for polar or ionic organic contaminants [51, 52]. SPME with mainly PDMS silicone coatings have been used to determine the C_{Free} of a wide range of HOCs including PAHs, PCBs and organochlorines in marine and freshwater sediments as well as soils. These studies have focussed on investigating various aspects including the partitioning behaviour of different geosorbents in relation to the contaminant properties, sediment-water exchange, bioconcentration in organisms and even uptake by plants [13, 42, 53].

SPME can also be used to simultaneously measure the total concentrations and C_{Free} in aqueous samples containing a sorbing matrix such as DOM [54, 55]. For this, isotopically labelled surrogates of the target analytes are added to the sample prior to the SPME measurement. Since both the surrogate and target compounds behave identically, total concentrations (i.e. freely dissolved plus sorbed) can be determined via internal calibration of the SPME-sorbed target analyte versus the isotopically labelled surrogate. C_{Free} for the target analyte is calculated using $K_{\text{Polymer/Free}}$ as described above. For the correct application of this method, both the target and surrogate compounds need to behave identically with respect to their partitioning distribution in the sample. In practice, this limits the approach to aqueous samples where a partitioning equilibrium is rapidly attained, e.g. those containing DOM or small organisms such as bacteria. For bulk soil and sediment samples, where equilibration processes occur over month to year timescales, the approach is not appropriate.

Subsequently, other passive sampling formats have emerged. These include thin polymer sheets made of various silicones, PE or POM which have been applied in a wide range of field and laboratory studies with both soils and sediments [40–42]. Such sheets are commercially available at low cost and can be cut up and arranged as required to meet the study aims. Often these are simply inserted directly into the sediment or soil bed, sometimes inside some sort of a protective casing. However, more elaborate arrangements have also been used. For example, a series of PE sheets were vertically arranged within a frame to obtain a depth profile of freely dissolved DDT and its metabolites from the overlying water column and down into the upper layers of the sediment bed [46].

Another widely applied passive sampling format consists of a thin polymer layer coating the inside of glass or other inert vessels [29, 30, 56]. To date, mainly silicones have been used for this purpose due to their easy handling during the coating process. Advantages of this format include the ability to coat thin silicone layers of varying thicknesses ranging from a few to tens of micrometres. This format has a very high polymer surface-area-to-volume ratio and thus relatively short equilibration times, particularly when rolling or tumbling the vessel. Another benefit is that the sample of soil or sediment can be directly collected and stored in the

passive sampling vessel to minimize the number of handling steps. For example, glass vessels were coated with 2 to 8 μm thick layers of PDMS silicone and used to passively sample PCBs in Baltic Sea sediments [30]. With this format, equilibrium was reached within 2 weeks, and dissolved PCB levels down to fg/L levels could be determined. A variation of the above format is to cast a thicker layer of polymer into the base of the vessel. Although this takes longer to reach equilibrium due to the reduced surface area, the advantage is the increased amounts of organic contaminant that is sampled by the lager mass of polymer.

By far the majority of passive sampling studies have focussed on neutral and relatively hydrophobic organic contaminants. The above silicone, PE and POM polymers have all proved well-suited for this group of contaminants but have too low affinities for more polar and ionic contaminants. This results in low up-concentration in the passive samplers and thus poorer detection possibilities. As a result, recently there have been efforts invested in identifying polymers more suited for polar and ionic compounds [51, 52]. These have mainly been centred around the SPME format, and a selection of single and even mixtures of polymers identified for this purpose. Several studies have applied these novel SPME types for measuring the partitioning behaviour of polar and ionic organic contaminants in sediments. SPME using polyacrylate as the extraction phase was applied to measure the sorption to marine sediments of three alcohol ethoxylates (AE) commonly found in laundry cleaning products. In this study, adsorption was found to dominate over absorption at low aqueous concentrations leading to higher distribution coefficients [57]. The effect of the molecular structure and salinity on the non-linear sorption of the anionic surfactant linear alkylbenzene sulfonate (LAS) to marine sediment was studied using the same polyacrylate-coated SPME fibres [58]. Sorption of polar and ionic organic contaminants to dissolved organic carbon (DOC) was also studied using the same type of SPME fibres [52]. The compounds investigated in this study covered a log K_{OW} range of 2.5 to 7.5 and consisted of pharmaceuticals, industrial chemicals, hormones and pesticides. These included representatives of neutral, anionic and cationic structures.

7 Confirming the Equilibrium in Equilibrium Passive Sampling

Obviously, the pre-requisite for this mode passive sampling is that a partitioning equilibrium is attained. Equilibration times span a wide range from days, to weeks or even months with the time needed depending on the physico-chemical properties of the analyte, the architecture of the sampling device and the conditions that apply [24]. Generally, longer equilibration times are required for very hydrophobic contaminants, samplers with a low surface-area-to-volume ratio and when there is little mixing (the latter is particularly an issue for field passive sampling). For the simultaneous measurement of multiple contaminants that cover a large

hydrophobicity range, individual compounds may therefore come to a complete equilibrium whereas for others equilibrium is not reached even after prolonged times. In slurries of soils or sediments, equilibration times generally decrease in line with increased suspension concentrations [59]. This is due to the more effective resupply via desorption of contaminant in the immediate vicinity of the sampler surface at the higher concentrations. All this has important practical implications, when the time to reach equilibrium is longer than the fluctuations in C_{Free}, and then equilibrium passive sampling is not so well-suited. Here, recourse should be made to kinetic passive sampling (see below).

The attainment of equilibrium can be confirmed in several ways: firstly, by performing a time series of measurements of passive samplers deployed under the same conditions to observe when constant passive sampler concentrations are reached [24]. Although simple, this approach is costly in terms of the preparative and analytical effort involved. Another way to confirm equilibrium is to prepare passive samplers of the same material but with different surface-area-to-volume ratios. The passive sampler with the lower ratio approaches equilibrium more slowly, and thus when the analysed concentrations in both passive samplers are the same, then this provides unambiguous confirmation that both have reached a partitioning equilibrium. This approach has been successfully applied for the determination of the C_{Free} of a range of HOCs in soil and sediment slurries [29, 30]. Other advantages of using different surface-area-to-volume ratios include confirming the absence of sample depletion, surface artefacts (e.g. fouling, abrasion) as well as adsorption (see above). A final but less commonly applied approach is the simultaneous deployment of two passive samplers – one unspiked and the other spiked with the target contaminant at levels above the expected equilibrium concentration. Convergence of measured concentrations in the two samplers confirms equilibrium.

8 Non-equilibrium Passive Sampling in Soils and Sediments

Particularly when passive sampling HOCs under undisturbed conditions in field sediments and soils, the lack of mixing means that local depletion at the sampler surface due to uptake needs to be replenished by slow molecular diffusion via the soil or sediment matrix. When compounds take impractically long times to attain equilibrium, passive sampling can be applied in kinetic mode for measuring C_{Free} [22–24]. Kinetic passive samplers are deployed during the initial linear stage of the uptake process (see Fig. 1). For first-order uptake, a near-linear response between the passive sampler accumulation versus time is maintained up until approximately 40% of the analyte's equilibrium concentration is reached. One advantage is the shorter sampling times, because equilibrium does not have to be attained. Moreover, kinetic sampling provides a time-weighted average of C_{Free} for the sampling period, which includes information on variations in environmental dissolved levels [60]. However, kinetic sampling requires knowledge of the sampling rate, which is influenced by numerous environmental factors such as mixing, temperature or biofouling of the sampler surface. Therefore, uptake kinetics calibrated in the laboratory cannot

simply be extrapolated to the field, and other approaches are needed to calibrate these in situ.

Perhaps the most well-established in situ calibration method is the use of performance reference compounds (PRCs). These are compounds that are not found in the environment and are spiked into the sampling polymer prior to deployment. Subsequent determination of the in situ losses of the PRCs allows the analyte uptake kinetics to be determined, provided both show isotropic exchange behaviour [47, 61]. Therefore, a good PRC should permit precise measurement of its loss and follow the same exchange kinetics as the target analyte but also not be present in the environment. Commonly a selection of PRCs covering a range of properties is used and the exchange kinetics of the target compounds determined by interpolation. The PRC approach is well-established and robust and has been successfully applied for kinetic sampling using single-phase samplers in waters and sediment slurries [16, 40, 41, 47]. However, particularly in unmixed systems, uptake of target compound/loss of PRCs involves diffusion over increasing further distances. This can lead to a lack of isotropy in contaminant uptake and PRC loss and requires the application of diffusion models to account for this. Furthermore, processes such as biodegradation in the pore water can lead to enhanced losses of labile PRCs compared to more persistent ones. This phenomenon has in fact been utilized to measure in situ biodegradation rates of labile compounds [62].

Semipermeable membrane devices (SPMDs) are a passive sampler type based on low-density PE tubes filled with triolein. These were first developed for passive sampling in waters but have also been used to a more limited extent in sediments [63, 64]. Their relatively low surface-area-to-volume ratio means that SPMDs seldom reached a partitioning equilibrium (particularly for HOCs), and they are thus used in kinetic sampling mode. One disadvantage to using SPMDs in soils and sediments is the relatively long exposure time needed to reach equilibrium, which can lead to problems such as biofouling. Single-phase sheets made of polymers such as silicone, PE or POM have also been widely applied for kinetic sampling in particularly sediments but also soils. As for equilibrium sampling above, the resulting information on C_{Free} has been used to investigate partitioning, sediment-water exchange, organism uptake and ecotoxicity, etc.

9 Passive Sampling in Soil Slurries Versus Dry Soil

Wet field sediments with a high water content can be used directly as slurries for passive sampling. However, sometimes additional water or medium with the same ionic strength as the pore water is added to facilitate slurry formation and good mixing. For compounds with a high affinity for the sediment material, the mass fraction that is transferred to this additional aqueous phase remains very small compared to the fraction that is sorbed. Addition of some sort of a biocide is necessary for contaminants that are biodegradable (e.g. PAHs).

Therefore, adding excess water to a soil or sediment to create a slurry provides a homogenous suspension for simplifying passive sampling in the laboratory.

However, there remains a number of drawbacks with this approach. Firstly, the in situ C_{Free} reflects a dynamic balance between desorption and (a)biotic losses. Thus, particularly in combination with a biocide, the slurry approach aims more towards measuring the maximum equilibrium rather than the in situ steady-state C_{Free}. Secondly, soils are typically unsaturated, and adding excess water to create a slurry dramatically alters this. In fact, several studies have shown that the partitioning properties of soils change depending on their hydration state [20].

Relatively few studies have applied passive sampling in unsaturated soils at or below their water holding capacity. Equilibrium SPME coated with PDMS silicone was used to measure how changes in PAH C_{Free} in soil amended using activated charcoal, biochar or compost influenced uptake by radish [53]. Silicone passive samplers were used to measure uptake kinetics and equilibrium kinetics as a function of the soil water content of a series of PAHs spiked into an artificial soil, and marked changes in the partitioning behaviour depending on the hydration state were observed [20]. In a greenhouse experiment, in situ passive sampling was performed using low-density PE strips as well as SPME coated with PDMS silicone to analyse PAH C_{Free} in a skeet shooting range soil and an uncontaminated control soil under different water saturation conditions [43]. Equilibrium with the SPME fibres was only reached after periods of more than 3 months. In water-saturated soil, in situ (i.e. in the greenhouse pots) and ex situ (i.e. a soil slurry in the lab under tumbled conditions) treatments with similar dissolved levels were measured. In contrast, under unsaturated conditions, this was not the case indicating that the degree of water saturation of the soil influences the PAH partitioning behaviour.

10 Outlook for Passive Sampling in Soils and Sediments

Passive sampling has been successfully applied in both laboratory and in situ settings to determine C_{Free} of a range of mainly neutral organic contaminants in soil and sediments. The ability to differentiate between the total and dissolved concentrations of organic contaminants opens up a window to observe the mechanisms controlling a wider range of abiotic (e.g. sorption, sediment-water exchange) and biotic (e.g. biodegradation, ecotoxicity) processes in soils and sediment. More recently, studies using appropriate polymers have focussed on measuring C_{Free} for polar and ionic compounds. Here, identifying further polymer types with optimal partitioning affinities for the full range of organic contaminants (neutral, polar, non-polar, ionic, etc.) would be important. This is particularly relevant for issues such as mixture toxicity, where simultaneously identifying the full bioavailable mixture profile is important to adequately quantify this. For kinetic sampling, when first-order kinetics describe the exchange processes, in situ calibration with PRCs is well-established. This is often the case for well-mixed systems (e.g. tumbled sediments). However, for static incubations such first-order kinetics no longer describe the observed sampler exchange, and more involved diffusion models are required. These account for the diffusion occurring over increasing length scales, and further investigations into their

wider applicability are needed to identify those key factors that need to be considered (e.g. biodegradation). Relatively few studies have applied passive sampling in unsaturated soils, despite partitioning being dependent on the water content. Here, future work could concentrate on passive sampling formats with shorter equilibration times and also testing the established diffusion models to better account for the lack of first order exchange kinetics that are often observed at below saturation levels.

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Microbial, Plant, and Invertebrate Test Methods in Regulatory Soil Ecotoxicology

Jörg Römbke and Fabrice Martin-Laurent

Contents

Abstract Standard tests have been used in soil ecotoxicology for about 40 years, but there is still room for improvement, such as (1) increased use of such tests because of regulatory requirements, in particular for the risk assessment of chemicals (mainly pesticides) and, to a lesser extent, of contaminated soils; (2) increased efforts regarding the standardization of ecotoxicological methods, handled either by OECD (Organization for Economic Cooperation and Development) prospectively for individual chemicals or by ISO (International Organization for Standardization) retrospectively for contaminated soils; (3) increased inclusion of ecological aspects, i.e., by performing higher-tier tests under semi-field and field conditions. However, until quite recently, nominal concentrations of the stressors are used, meaning that their bioavailability was not taken into account. We are providing an overview on currently required and/or proposed ecotoxicological effect tests, covering OECD and ISO methods for main soil organism groups (microbes, invertebrates, and plants). Based on this overview, we discuss how the current set of test methods could be improved, trying to capture ecological reality by addressing issues such as

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different soils, species, endpoints, and exposure (i.e., bioavailable instead of nominal/total concentrations). The TRIAD approach is highlighted as an example how bioavailability could be implemented in soil quality regulations.

Keywords Bioavailability, Ecotoxicology, Invertebrates, Microorganisms, Organic pollutants, Plants, Soil, Standard tests

1 Introduction

Standard tests have been used in soil ecotoxicology for about 40 years. Actually, the "earthworm acute toxicity" and the "Seedling Emergence and Seedling Growth" tests are seen as the first regulatory important documents for soil invertebrates and plants, respectively [1, 2]. About 10 years later, test methods to assess the effects of chemicals on soil organisms started to develop in different directions which can be characterized in short as follows:

- Regulatory requirements, both on the national (Germany, France, USA, Canada) and the international level (mainly the European Union), increased with a strong focus on assessing the risks of chemically active ingredients (i.e., that part of the pesticide formulation which is biologically potent), mainly plant protection products (PPPs, also called pesticides) (e.g., [3]), while a biological assessment of contaminated soils was – in contrast to setting up chemical target values – rarely required.
- Standardization of ecotoxicological method efforts was, due to the legal requirements, handled either by the Organisation for Economic Co-operation and Development (OECD; prospectively for individual chemicals) or by the International Organization for Standardization (ISO; retrospectively for contaminated soils), meaning that the same method (but with slight adaptations) could be published by both organizations.
- Scientific work supported the inclusion of further test species (microbes (e.g., [4]) or more invertebrates in addition to the currently used earthworms, springtails, and predatory mites but also more sensitive endpoints (e.g., reproduction in contrast to mortality) in soil ecotoxicology; even first ideas for the use of soil microcosm tests were developed (e.g., [5]), culminating today in requirements for assessing the effects of stressors on the biodiversity of soil organism communities (e.g., [6]).

Today, there is a high number of ecotoxicological standardized test methods available, both prospectively for individual chemicals (or mixtures) and retrospectively for contaminated soils. Due to organizational reasons, the number of OECD guidelines is increasing more slowly than the number of ISO documents, partly because the latter are more focused on the respective environment on a global scale.

For example, ISO discusses intensively the identification of test species occurring in the respective region.

All these requirements – until quite recently – focused on the effects of the respective stressor on soil organisms, using the nominal added concentration in standard tests as a measure for the exposure of the tested organisms. However, the measurement of the actual concentration of the tested substance(s) in the test soil has been asked for (e.g., [7]), but it is not yet implemented in the respective guidelines (in contrast to aquatic ecotoxicological tests). Strictly speaking, these are effect tests, but of course they also indicate whether the tested stressors are (bio)available or not. In fact, this information cannot be gained by using chemical extraction methods, due to the following reasons (e.g., [8]):

- Each chemical extraction method does extract a certain level of the overall chemical load out of soil – and no method extracts precisely the amount which is causing the observed effects on the different organisms living in soil.
- The results of each chemical extraction method depend not only on the method used but also, to a (often unknown) degree, on environmental conditions such as soil properties, most notably but not exclusively pH, organic matter content, or clay content.
- The measurement of the action of a stressor in the organisms themselves, i.e., at the site (e.g., organ) where an effect is caused, is technically impossible, especially when this site is not known beforehand.

In addition, organisms can – depending on species, life cycle, physiological status, and sensory abilities – react to chemical stressors in different ways, either eliminating (by biodegradation, mainly microbes), avoiding (e.g., by escape reactions, mainly invertebrates), or accumulating them via different uptake routes (again, mainly invertebrates). So, in short for the time being, measuring the effects of stressors on organisms using (at best) different species, exposure pathways, effect mechanisms, etc. is still the best way to assess the bioavailability of chemicals for individual organisms, species, or even communities as defined in ISO 17402 [9], slightly adapted: *Degree to which chemicals present in the soil may be absorbed or metabolised by ecological receptors or are available for interaction with biological systems.* Other ISO guidelines (e.g., ISO (International Organization for Standardization) [10]) also provide definitions, but they do not refer to the specific way chemicals and soil-living organisms interact.

In this contribution, we are providing an overview on currently required and/or proposed ecotoxicological effect (i.e., including bioaccumulation) test methods, covering mainly OECD and ISO methods due to their regulatory relevance. In order to facilitate comparability, we tried to use a standard format for each individual method. Based on this overview, we will discuss whether (actually, how) further ecotoxicological test methods are necessary or not.

Finally, it has to be mentioned that we do not discuss in detail the bioaccumulation of chemicals in soil organisms despite the fact that this process is part of the whole complex of bioavailability [11]. This is due to the very limited number of appropriate test systems (mainly one, the oligochaete bioaccumulation

test, using both lumbricid earthworms and enchytraeids as test organisms, has been standardized by OECD (Organisation for Economic Co-operation and Development) [12]. In comparison to effect, data information on the bioaccumulation of chemicals in soil invertebrates is still limited, probably because it is rarely required for the regulation of PPPs, veterinary drugs, or biosolids [13].

2 Overview on Soil Ecotoxicological Test Methods

In the following section, the available test guidelines relevant for the assessment of the bioavailability of chemicals in soil are listed, and their usage is revised. It should be kept in mind that OECD guidelines, used mainly for the registration of individual chemicals such as pesticides or veterinary pharmaceuticals, are not regularly revised (only when a mistake has been identified), meaning that they keep their original publication date. In contrast, ISO standards – used for the retrospective assessment of contaminated soils – are regularly (i.e., every 5 years) revised, and the date of the last revision is used as the publication date.

2.1 Soil Properties

Any determination of the effects of a stressor in soil needs the best possible description of the soil properties since these properties can affect the availability quite strongly. This requirement does not refer to the OECD standard artificial soil. This soil substrate is a mixture of 70% air-dried fine quartz sand, 10% sphagnum peat (as close to pH 5.5 to 6.0 as possible, no visible plant remains), 20% kaolin clay (kaolinite content preferably above 30%), and 0.3 to 1.0% calcium carbonate (CaCO3, pulverized, analysis grade to obtain an initial pH of 6.0 $\pm$ 0.5). This mixture has to be finely ground (dried to measured moisture content). In all the other cases, at least the following soil properties are known to influence the availability of chemicals for soil organisms: pH value ($CaCl_2$, KCl) [14], organic and total carbon contents [15, 16], cation exchange capacity (CEC) [17], soil dry mass [4], texture [18], as well as the C/N ratio are of special interest.

2.2 Biological Test Methods

In this part the individual methods to be reviewed are listed in a tabular format, separated according to the main soil organism groups. Please remember that OECD standards are only used for the testing of chemicals (mainly individually, but mixtures, especially in the form of plant protection formulations, are possible). In contrast, ISO standards were initially only intended for the testing of (potentially,

i.e., unknown) contaminated soil samples. ISO standards are also frequently used in regulatory risk assessments of chemicals. The reverse situation is rare, since ISO is quickly adapting new OECD methods for their own purposes.

2.2.1 Soil Microorganisms

Microbial ecology and microbial ecotoxicology are still constantly evolving young sciences, which are very active in the development of up-to-date omics methodologies (meta-genomics, meta-transcriptomics, meta-proteomics, and meta-bolomics) to characterize the abundance, composition, and diversity of the microbial community [19]. Most of these methodologies improve on a day-to-day basis because of technological breakthrough and consequently are not yet ready to be standardized. That is the reason why current standardized methods focus on the quantification of the abundance and the activity of the microbial community (Table 1). It is noteworthy that most of the standards measuring the microbial abundance and activity target the entire microbial community because of its intrinsic complexity. In any case, the soil to be tested has to be stored and handled in a way that the microbes inhabiting it are not disturbed (ISO (International Organization for Standardization) [35]).

Different standards are available to measure the microbial biomass of soils (Table 1): substrate-induced respiration to estimate the active aerobic microbial biomass of soils (ISO (International Organization for Standardization) [21] and OECD 216) and fumigation-extraction method to quantify extractable microbial C biomass of soils (ISO (International Organization for Standardization) [21]). Phospholipid fatty acids (PLFA) and phospholipid ether lipids (PLEL) analyses (ISO (International Organization for Standardization) [24] and 29843-2 [25], respectively) permit not only the estimation of the abundance but also the composition of bacterial and fungal communities of soils. Similarly, qPCR assays (ISO 17601 [36]) performed on soil DNA extracts prepared according to ISO/DIS 11063 [37] allow the quantification of the abundance of microbial groups.

A series of standards aim to measure the activity of soil microbial community (Table 1). Among them, three concern N cycle; two measure the mineralization of N and the nitrification in soils (ISO 14238 [28] and OECD 217), and the last determines the potential nitrification and the inhibition of nitrification via the measure of the oxidation of ammonium (ISO 15685). Two additional standards allow the measurement of soil enzyme activity patterns using either fluorogenic (ISO 22939 [29]) or colorimetric (ISO 20130 [30]) substrates. Two other standards contribute to the assessment of dehydrogenase activity using either 2,3,5-triphenyltetrazolium chloride (TTC) or 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) as substrates (ISO 23753-1 [31] and 23753-2 [32], respectively). Another one measures the dehydrogenase activity of *Arthrobacter globiformis* in a contact test with various contaminants to assess their ecotoxicity (ISO 18187 [33]. Two standards allow the estimation of the biodegradability of organic chemicals under aerobic conditions (ISO 14239 [23] and OECD 301/310). The last (ISO/CD 23265), which is still under development, will permit to measure the mineralization of organic matter decomposition in contaminated soils.

Table 1 Overview on important (OECD and ISO) standard tests with microorganisms

Name	Species/ communities	Test endpoints	Guideline	Comments
Microbial biomass and respiration				
Carbon transformation test	Natural microbial community	Respiration rate 28 d; extension to 100 d possible	OECD 216 [20]	Used for the assessment of the microbial toxicity of pesticide registration dossiers at EU level (EU Regulation 1107/2009/EC)
Carbon transformation test	Natural microbial community	Respiration rate 28 d; extension to 100 d possible	ISO 14240-1 [21]	Used for the measurement of the activity of soil microbial communities to biodegrade various carbon sources
Fumigation-extraction method	Natural microbial community	Microbial carbon biomass (in mg C per kg of soil)	ISO 14240-2 [22]	Frequently used to measure the microbial C biomass in arable soils
Quantification of microbial groups	Natural microbial community	Abundance of gene sequence in soil DNA extracts	ISO 17601 [23]	Used for the quantification of the abundance of microbial groups using as template soil DNA extracts
PLFA and PLEL analysis	Natural microbial community	Quantification of the abundance of microbial groups. Part 1: Method by phospholipid fatty acid analysis (PLFA) and phospholipid ether lipids (PLEL) analysis	ISO/TS 29843-1 [24]	Used for the characterization of the abundance and composition of soil microbial communities Also used for the characterization of the abundance and the diversity of soil microbial communities
PLFA and PLEL analysis	Natural microbial community	Quantification of the abundance of microbial groups. Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	ISO/TS 29843 -2 [25]	
Microbial activity measurements				
Nitrogen transformation test	Natural microbial community	Ammonium oxidation; 4 d	OECD 217 [26]	Used for the assessment of the microbial toxicity of pesticides for the preparation of the dossier to ask for registration at EU

(continued)

Table 1 (continued)

Name	Species/ communities	Test endpoints	Guideline	Comments
				level (EU Regulation 1107/2009/EC)
Nitrogen transformation test (potential nitrification and inhibition of nitrification)	Natural microbial community	Ammonium oxidation; 4 d	ISO 15685 [27]	Used for the assessment of the impact agricultural practices (including agrochemicals) on N cycle catalyzed by the soil microbial community
Nitrogen transformation test (nitrogen mineralization and nitrification)	Natural microbial community		ISO 14238 [28]	Used for the assessment of the effect of chemicals on nitrogen mineralization and nitrification by soil microorganisms
Extracellular enzyme activity	Natural microbial community	Fluorescence measurement; 3 h	ISO/TS 22939 [29]	Used for the assessment of the effect of chemicals and of anthropogenic actions on extracellular enzyme activities used as proxies of soil biological quality
Extracellular enzyme activity	Natural microbial community	Colorimetric measurement; 3 h	ISO 20130 [30]	
Dehydrogenase activity in soils	Natural microbial community	Colorimetric measurement with triphenyltetrazolium chloride (TTC) or iodotetra-zolium chloride (INT)	ISO/TS 23753-1 [31] and 23753-2 [32]	Used for the measurement of dehydrogenase activity in soils
Soil contact test	*Arthrobacter globiformis* (bacterial strain)	Dehydrogenase activity	ISO 18187 [33]	Short-term test (6 h), mainly used for (almost) on-site assessment of soil quality
Ready biodegradability tests	Natural microbial community of activated sludge	O_2, CO_2, or inorganic C	OECD 301/310	Used for the assessment of the microbial toxicity of pesticides for the preparation of the dossier to ask for registration at EU level (EU Regulation 1107/2009/EC)
Mineralization of organic chemicals	Natural soil microbial community	CO_2, $^{14}CO_2$	ISO 14239 [23]	Description of incubation systems that can be used to measure the

(continued)

Table 1 (continued)

Name	Species/ communities	Test endpoints	Guideline	Comments
				mineralization of organic compounds to carbon dioxide by soil microorganisms
Mineralization of organic matter	Natural soil microbial community	CO_2, $^{13}CO_2$, $^{14}CO_2$	ISO/CD 23265 [34]	Still under development, this standard will describe a test to measure organic matter decomposition in contaminated soils

2.2.2 Soil Invertebrates

In the following, several aspects of the current tests with soil invertebrates will be addressed, in particular the number and selection of species, endpoints, and substrates, i.e., soils (Table 2).

Species Traditionally, soil ecotoxicology focuses on earthworms (Lumbricidae) and springtails (Collembola) as test organisms, representing both macro- and mesofauna as well as soft- and hard-bodied species. By doing so, different (and probably the most important) exposure pathways are covered, especially in conjunction with the predatory mite test (i.e., the uptake of chemicals via contaminated food). However, according to the recent EFSA opinion [6] on the future of pesticide testing and risk assessment, the following seven soil organism groups should be covered: besides earthworms and springtails, also potworms (Enchytraeidae); woodlice (Isopoda), representing macro-arthropods and terrestrial gastropods (slugs and snails); and round worms (Nematoda).

Endpoints Since acute (i.e., mortality) endpoints are not suitable for protective risk assessment schemes (both prospective and retrospective), reproduction has been used successfully due to its high ecological relevance for almost two decades, but it is often (e.g., due to time requirements) not very practical. Thus, behavioral endpoints, especially avoidance, have been proposed as an addition/and or replacement. In parallel, there is an urgent need to use endpoints directly related to the functions and services of soil organisms, such as degradation, organic matter, or maintenance of the soil structure [6, 48].

Soils Currently, all tests required are usually performed with OECD artificial soil (a mixture of sand, clay, grounded peat, and water) [1]. Despite the fact that this mixture is suitable for testing many different soil organisms, its properties (especially the high amount and specific characteristics of the organic matter (10%), sphagnum or coconut peat) differ quite a lot from natural soils. Therefore, a list of

Table 2 Overview on important (OECD and ISO) standard tests with soil invertebrates

Name	Species etc.	Test endpoints	Guideline	Comments
Soil invertebrates				
Acute earthworm test	*Eisenia fetida*, *E. andrei*	Biomass, survival; 14 d	OECD 207 [1]	First soil invertebrate test; rarely required today
Chronic earthworm test	*Eisenia fetida*, *E. andrei*	Biomass, reproduction; 56 d	OECD 222 [38]	By far the most relevant soil test; in all EU regulations listed
Chronic earthworm test	*Eisenia fetida*, *E. andrei* (field-relevant species added)	Biomass, reproduction; 56 d	ISO 11268-2 [39]	Often most sensitive soil test
Earthworm avoidance test	*Eisenia fetida*, *E. andrei*	Behavior, esp. focusing on avoidance; 48 h	ISO 17512-1 [40]	Short test, results partly comparable to those chronic tests
Chronic enchytraeid test	*Enchytraeus albidus*, *E. crypticus*	Reproduction; 42 and 28 d (depending on species)	OECD 222 [38], ISO 16387 [41]	Rarely required in EU dossiers but regularly used in science (as ISO)
Chronic Collembola test	*Folsomia candida*, *F. fimetaria*	Reproduction; 21 and 28 d (depending on species)	OECD 232 [42]	Often required in EU regulations (e.g., pesticides, veterinary drugs)
Chronic Collembola test	*Folsomia candida*	Reproduction, 28 d	ISO 11267 [43]	Representative for soil arthropods in contaminated land evaluation
Collembola avoidance test	*Folsomia candida*	Behavior, esp. focusing on avoidance; 48 h	ISO 17512-2 [44]	Potential alternative but not required legally so far
Chronic predatory mite test	*Hypoaspis* (*Geolaelaps*) *aculeifer*	Reproduction, survival	OECD 226 [45], ISO 21285 [46]	Required for PPP assessment, but not in other regulations
Chronic moss mite test	*Oppia nitens*	Reproduction, survival	ISO 23266 [47]	Not required regulatorily in the EU, but for boreal forests in Canada

regionally representative and well-characterized natural soils has to be identified for testing purposes (e.g., for the Mediterranean region, see Chelinho et al. [49] or for Central Europe Smolders et al. [50]).

Regions Traditionally, test conditions (both in the laboratory and when selecting field study sites) are reflecting the conditions of the temperate zones of the world, in particular those of Europe. However, pesticides and other agrochemicals are used intentionally in (almost) all ecological zones. The environmental conditions (i.e., temperature, soil properties, and soil moisture) of these zones differ quite

considerably; these conditions influence not only the fate of the pesticides (e.g., in terms of degradation and/or bioavailability) but also the occurrence and behavior of soil organisms. In addition, the different application patterns and especially agricultural practices in regions outside of the Northern temperate zones have to be considered, such as the (often) higher application rates and also higher number of applications per crop cycle. However, these issues are not covered in the current risk assessment schemes and, thus, are also not addressed in the testing requirements.

Consequences for Test Development and Application In summary, there are various research needs which have to be addressed, preferably (at least in the European Union) before new requirements will be put in practice. In general, testing schemes have to be more adapted to reality – and preferably the most important standardization organizations such as OECD and ISO but also national organizations such as Environment Canada should be involved. For example, right now the earthworm reproduction test [16] is modified in a way that the current standard test species (*Eisenia fetida*/*Eisenia andrei*) is exchanged by a battery of test species which could be used when addressing the respective environment, e.g., *Dendrobaena rubidus* (for mainly Northern boreal forest soils) (e.g., [51]; Environment Canada ([52]; currently under revision) or *Aporrectodea caliginosa* (for temperate anthropogenically used grassland and crop sites) (e.g., [53]).

2.2.3 Plants

In comparison to the number and complexity of microbial as well as invertebrate tests available today (plus the amount of work currently done with these organisms (see, e.g., [54])), only a few new developments occur in the area of plant testing (Table 3). Almost all methods available today have been developed a long time ago – and they are still in use for different reasons. However, there is one exception: all common plant species used for testing purposes today are crop species, mainly because they are well-known (e.g., regarding their growth conditions). Another issue might be even more relevant: for these species defined, seeds are available commercially – and these seeds do not differ much, i.e., variability is low. In contrast, ecologically more relevant "wild species" show natural variability in their reaction to environmental factors (e.g., light, moisture etc.) but – at least potentially – also to stressors. For these reasons testing with wild species is limited (e.g., [61]) (Tables 2 and 3).

Table 3 Overview on important (OECD and ISO) standard tests with plants

Name	Species etc.	Test endpoints	Guideline	Comments
Root length test	*Hordeum vulgare*; rarely *Triticum aestivum* or *Avena sativa*	Root length; 5–7 d	ISO 11269-1 [55]	Often used for the assessment of contaminated land due to the short duration
Seedling emergence test	*Lactuca sativa*	Emergence rate; 5 d	ISO 17126 [56]	Screening test; so far not required for regulatory purposes
Seedling emergence and seedling growth test	*Avena sativa*, *Brassica rapa*; other crop species		OECD 208 [57]	Standard test for environmental risk assessment of PPPs and other chemicals
Emergence and growth test	*Avena sativa*, *Brassica rapa*; other crop species	Emergence rate, biomass, shoot length, early growth, ca. 14–21 d	ISO 11269-2 [58]	Test required regularly for the assessment of contaminated land
Vegetative vigor test	*Avena sativa*, *Brassica rapa*; other crop species		OECD 227 [59]	Test specifically required for PPPs which are sprayed on emerging/standing crops
Chronic plant test	*Avena sativa*, *Brassica rapa*	Biomass, no. of buds and seed pods; ca. 35–42 d	ISO 22030 [60]	Not required; rarely used since sensitivity seems similar to emergence/growth tests

2.3 Bioavailability in Prospective and Retrospective Risk Assessment

2.3.1 General Considerations

The decision whether a chemical, in particular pesticides or veterinary pharmaceuticals, can be marketed in the European Union if data (in fact a detailed dossier) is provided by the manufacturer of that chemical. This dossier includes data on the basic properties, of this chemical, information on its fate in soil (and other environmental compartments) as well as test results addressing its ecotoxicological effects are provided to the respective governmental agencies following very specific rules (e.g., [62]). These quite exhaustive documents do not require regularly the inclusion of the concept of bioavailability to be taken into account when calculating the exposure of a chemical. Only in the case of industrial chemicals (in fact, mainly for metals) the bioavailability concept has been implemented in EU regulatory frameworks. Actually, metals such as copper or nickel are by far the best-studied chemicals in the context of their bioavailability in soils, including the derivation of soil values to be used in regulatory soil protection activities (e.g., [50, 63]).

The exposure of soil organisms to pesticide residues has been discussed for more than a decade [7]. For example, it has been stated that for soft-bodied soil organisms and plants, pore-water-mediated uptake is mainly responsible for the observed

effects [64]. These authors also observed that toxicity could decrease over time, even though soil residues remained constant. Despite the fact that this discussion did not (yet) lead to a modification of the actual testing requirements for PPPs, the need for more guidance on how to consider bioavailability became obvious, as highlighted in the scientific opinion on the state of the art of risk assessment in soil-living organisms [6]. For example, scientific concepts focusing on the bioavailability of organic chemicals are presented in this document, especially the one written by Ortega-Calvo et al. [8]. In addition, changes of current test methods are recommended, for example, exchanging the standard artificial soil with a natural soil being representative for agricultural sites (e.g., both in terms of the type and the amount of organic matter content). Actually, already today many invertebrate effect tests are performed with the LUFA (**L**andwirtschaftliche **U**ntersuchungs- und **F**orschungsanstalt, Speyer) Soil 2.2, a commercially available standard soil representative for many Central European crop sites which is already recommended in some ISO standard test guidelines (e.g., the predatory mite test [29]). In contrast, a non-treated field soil is used as a reference or control soil. This would also improve the comparability between effect and exposure data (for the latter already up to five natural soils are used). In this context, it should also be mentioned that so far, no standard "mild" chemical extraction method has been identified which could be used to measure the bioavailable fraction of the test chemical in soil. In any case, these changes would improve the interpretation of the results of current effect tests. In addition, the current practice of dividing toxicity endpoints by a factor of two in order to account for the bioavailability of the test substance when tests have been performed with an artificial soil containing 10% peat would be obsolete or could be exchanged with a substance-dependent scaling factor (see EFSA PPR Panel (Panel on Plant Protection Products and their Residues (PPR)) [6] for details).

Actually, the best way of incorporating bioavailability into current risk assessment procedures would be to measure the actual available concentration of the test chemical directly in the ecotoxicological test, i.e., in parallel to measuring the effects of that chemical. Until now, and despite the impressive number of chemicals and biological methods, it is not an easy task to assess the bioavailability of contaminants in soil [65]. Being site specific, bioavailability needs to combine both traditional (analytical chemistry) and novel (biotest, biosensor) methods. By doing so, it could be shown that such chemical measurements allow characterizing the scenario of exposure responsible for the observed impact on biological processes (see ISO 17402 [9] and Ortega-Calvo et al. [8] for details). In any case, several tests covering various putative exposure pathways as well as biological endpoints (i.e., test species) are necessary. In other words, more scientific studies addressing basic relationships between soil properties, the bioavailability of the chemicals to be assessed and soil organisms to be protected, represented by a battery of test species (microbes, plants, and invertebrates), are needed (e.g., [66–68]).

2.3.2 Case Study

Actually, we are not aware that the principles laid down in this contribution have been applied in a case study with an organic chemical (e.g., a pesticide). However, there is experience with the implementation of the bioavailability of certain metals when performing a risk assessment for them. Interestingly, two metals have been also used as pesticides in the environment: copper and arsenic. Copper is extremely data-rich, both regarding laboratory tests and, partly, even field studies. Its bioavailability for soil organisms has been taken into account when preparing a REACH dossier but also in the context of its use as a fungicide all over Europe (e.g., [50, 69]). More recently, the German government supported the derivation of "precautionary soil values" for arsenic, which, if exceeded, indicate that concern for harmful effects on soil organisms and their functions exists [70]. Such soil quality thresholds – expressed as single total metal concentrations – cannot reflect the variation in bioavailability of (any) metal across soils. Therefore, in order to improve this derivation (i.e., to get a realistic risk assessment of metals in soil), their bioavailability must be considered. In this project, bioavailable fractions of arsenic were linked with ecotoxicological effect concentrations, taking into account properties (texture, pH, organic matter content, etc.) of six different soils, the results of six different extraction methods (there is, as expected, no best chemical method, since they depend on soil and organismic properties), and the specific reactions of eight different test species (microbes, plants, and invertebrates).

2.3.3 Prospective Risk Assessment

In the European Union, for the (prospective) regulation of pesticides and, to a lesser extent, veterinary pharmaceuticals or industrial chemicals, various soil organism tests (usually according to OECD guidelines) have to be performed, such as reproduction tests with earthworms [1, 38], collembolans [42], predatory mites [45], plants [57, 59], and microorganisms [20]. These tests cover main organism groups (invertebrates, plants, and microbes) and different exposure pathways (i.e., via pore water, soil, air, and food as well as morphological/physiological (i.e., hard- and soft-bodied) groups. However, some important organism groups (see [6]) are missing in this list, such as Enchytraeidae, Nematoda, and soil macro-arthropods or mycorrhizal fungi that are forming symbiosis with almost all higher plants. For some of these groups, standard guidelines do exist (e.g., [71, 72]). Maybe even more important is the lack of functional tests, especially in the light of the increasing regulatory importance of the Ecosystem Services (ESS) concept, which has been highlighted in particular by the European Food Safety Authority (EFSA) in recent years [6, 73]. Besides the protection of biodiversity in general, these documents highlight the necessity of determining directly the functions and services provided by, in the context of this document, soil-living organisms.

2.3.4 Applications of Bioavailability in Retrospective Risk Assessment

Problems with contaminated soils are known in Europe and North America since the 1970s, but except (total) concentrations of metals, PAHs, polychlorinated biphenyls (PCBs), or pesticides, not much was known on their fate and effects on soil organism communities and their functions. However, the risks tend to be overestimated when total extractable concentrations have been used, resulting in the remediation of potentially contaminated sites that did not pose significant risk to biological endpoints. In fact, this became only obvious when taking the bioavailability of these contaminants into account. For example, in the Netherlands, maximum allowable concentrations for specific land uses, such as natural areas, agriculture, living, playgrounds, and industrial sites, were defined. These values were defined for a standard soil having 10% organic matter (OM), and measured values are required to be corrected by the actual % OM of the soil to accommodate different soil types. This correction step was – probably not even intentionally – the first step to apply standard soil protection values based on their bioavailable fractions. In fact, in combination with the respective land use at these sites, these values are more risk-based. However, due to the limited knowledge on sorption processes in different soils at that time, bioavailability and risks were not always understood. Based on these experiences, a general protocol for considering bioavailability in a higher-tier risk evaluation was agreed upon by experts in the Netherlands and has been applied to specific sites such as a large area (450 ha) of diffuse contaminated soil (mainly hydrophobic persistent chemicals like PAHs, PCBs, and/or mineral oil) using desorption extraction and/or passive sampling methods [74]. A proposal for the inclusion of bioavailability in the generic regulation requirements in retrospective RA has not yet been implemented. However, these Dutch experiences were used, primarily to compile the information gained in a more generally relevant national standard, bringing together not only data from practical measurements and remediation efforts but the knowledge on how to organize such a complex effort, including the cooperation between landowners, engineers, and scientists, and the general public also proved instructive. This document, known today as one of the first examples of the application of the three-tiered TRIAD approach in the terrestrial environment, was subsequently used to prepare an international ISO standard 19204 [23].

Today, this standard which explicitly includes bioavailability applications in remediation projects is used all over the world (although it is not yet the most common one). Practical experiences are available from, for example, Denmark [75], Brazil (Niemeyer et al. [76, 77]), or Poland (Klimkowicz-Pawlas et al. [78]). In these case studies under realistic conditions, it could be shown that the TRIAD approach (and thus the inclusion of bioavailability) is scientifically sound, efficient, and helpful in building up good relations with the respective landowners, neighbors, and other relevant stakeholders.

3 Concluding Remarks and Future Prospects

This overview on existing standard test methods in the area of terrestrial ecotoxicology proves that a wide range of organism communities, populations, species, and (often chronic) endpoints has already been covered. There is still room for improvements: e.g., most of these organisms do occur in the field in temperate regions, meaning that others (e.g., tropical or boreal) are not (yet) well-represented. All of these methods are based on the working hypothesis that ecotoxicological impact results from the exposure of organisms to the bioavailable fraction of chemicals. This means that the prerequisite is to measure the bioavailable part of the total concentration of a chemical in soil to be able to interpret endpoints measured in response to acute or chronic exposure. Some attempts (so far mainly with metals) have been made to measure the bioavailable fraction of pollutants in different soils and for different organisms. This effort is important to increase our understanding of the processes driving bioavailability (e.g., in the context of developing tools for predicting bioavailability in different environmental scenarios), but the most important is to be able to predict if a given pollutant in a given situation will cause ecological damage or not. Keeping in mind the hundred thousand of chemicals that are on the market, it is not possible (i.e., in terms of efforts and resources) to test all of them on all living organisms under a wide range of environmental conditions (e.g., soil type, temperature, etc.). Therefore, one needs to predict the bioavailable part of chemicals (and thus the effects) to minimize as much as possible ecotoxicological testing. This is especially true for current laboratory tests, since it is hard to imagine that the effects of chemicals in the field on whole communities, taking into account the numerous interactions between quite different species, variable environmental conditions, and – not yet considered in environmental legislation – complex exposure scenarios (both chemical and other), regulated stressors and others into consideration (some recent documents such as from EFSA [6] do move already in that direction).

Thus, understanding the processes determining the bioavailability of chemicals is an important step to improve future environmental risk assessment. One way to support this process is to perform a series of laboratory tests, in which a battery of test species, a representative number of soil types (geographically and pedologically), as well as different chemical extraction methods are considered at the same time. Predictions regarding the respective bioavailability (and thus effects) could be made based on the outcome of such tests (currently only known for a few metals). Ideally, such results could be examined under real conditions, either as part of the current environmental risk assessment required for pre-registration or as part of post-registration monitoring.

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Part V
Bioavailability in Chemical Risk Assessment

Implementation of Bioavailability in Prospective and Retrospective Risk Assessment of Chemicals in Soils and Sediments

Willie J. G. M. Peijnenburg

Contents

Abstract The quality of soils and sediments is commonly evaluated on the basis of total contaminant concentrations within prospective as well as in retrospective risk assessment. From common practice, the perception has arisen that performing risk evaluation by measuring or modelling total concentrations often leads to either over- or underestimation of the risks: too often there is an indication of risk whereas in reality the ecosystem seems to be unaffected, whereas on the other hand, there are examples in which adverse effects are observed in realistic field settings at levels

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well below toxicity levels generated in a laboratory setting. This calls for properly accounting for the impact of the binding capacity of soils and sediments on the availability and risks of contaminants in soils and sediments.

Building upon an analysis of common approaches in prospective and retrospective risk assessment, an overview is given of options for inclusion of the encompassing bioavailability assessment in risk assessment. The overview culminates in a recommendation of chemical and analytical methods suited to mimic bioavailability, and a regulatory-oriented framework for actual implementation of these methods in effect-oriented risk assessment is depicted. Given pragmatic considerations (including the obvious desire of continued use of the current set of toxicity data), the regulatory framework boils down to directly relating measured/modelled bioavailable concentrations to either toxicity data for soil or sediment organisms or to toxicity data for aquatic biota.

Keywords Accumulation, Bioavailability, Bioconcentration, Ecotoxicity, Hydrophobicity, Organic compounds, Sediment, Soil, Sorption, Toxicity, Trophic transfer

1 Introduction

Environmental risk assessment basically aims to assess the likelihood of chemicals causing harm to man and the environment to an extent that exceeds acceptable limits. Environmental risk assessment includes the description of potential hazards and impacts before taking precautions to reduce the risks. As such, environmental risk assessment basically comprises five key steps:

1. Identification of possible hazards, i.e. identification of the possible sources of harm.
2. Description of the harm these chemicals might cause: hazard assessment.
3. Evaluation of the risk of occurrence and identification of precautions to be put in place to minimize the risks. This basically boils down to quantifying exposure of man and the environment to potentially hazardous contaminants in occupational or environmental settings.
4. Recording of the results of the assessment and implementation of precautions in case of unacceptably high risk.
5. Reviewing the assessment at regular intervals, amongst other incorporating the latest scientific insights in hazards and exposure assessment of chemicals.

Typically, there are two major types of environmental risk assessment. The first type is predictive and often associated with the authorization and handling of hazardous substances like pesticides, or like new and existing chemicals as,

for instance, regulated in the European Union by means of the regulation on the Registration, Evaluation, Authorisation and Restriction of Chemicals [1]. This type of environmental risk assessment is ideally undertaken prior to environmental release of the substance and is termed prospective risk assessment. The second type of environmental risk assessment, termed either retrospective risk assessment or ecological risk assessment, is a description or estimation of impacts on human health or of changes in populations or ecosystems at specific sites or areas already polluted. Retrospective assessment should hence be conveyed as impact assessment rather than risk assessment. The principles of ecological risk assessment have been described over two decades in various review papers and books, e.g. Ferguson et al. [2], Lanno [3], Suter et al. [4], Thompson et al. [5], US-EPA [6], and Weeks et al. [7].

The assessment of the risks to man and the environment as associated with the presence of chemical contaminants in our environment has traditionally been based on the total amount of contaminant present in any of the environmental compartments. This is commonly done on the basis of comparing the total concentrations with (standardized) toxicity testing. As a result of investigations by environmental scientists, the awareness amongst especially the scientific community has increased that realistic assessment of the risk posed by contaminated ecosystems should include considerations of bioavailability. Practical experiences have given rise to the perception that performing risk evaluation based on (measured) total concentrations may lead to an incorrect assessment of the actual risks. There is (too) often an indication for risks, whilst the ecosystem is not affected. On the other hand there are examples in which adverse effects are observed in realistic field settings at levels well below toxicity levels generated in a laboratory setting [8]. A false indication of risk could lead to the application of remediation measures and associated expenses that do not result in an improved ecosystem. On the other hand, in some cases, there might be an underestimation of the actual effects (false-negative indication), resulting in insufficient protection of the ecological functioning of the ecosystem. This is against the basic principles of environmental policy in Europe of protection of 95% of the species potentially present in an ecosystem.

Regulators have only recently realized the need to consider bioavailability in risk assessment and have started to accept the implementation of bioavailability in prospective and retrospective risk assessment. In common practice, this means that more realistic decisions can be made with regard to potentially and actually occurring adverse effects in polluted ecosystems, compared to the traditional approach of using total concentrations. The basic problem is that the mere presence of a contaminant does not by definition mean that there is an actual risk or measurable effect on the ecosystem. Actually occurring effects are directly related to the binding capacity of the soil or sediment and the speciation of the contaminant in the pore water, as affected by the physical-chemical properties of the soil and the contaminant, and specific properties of the biota that are actually exposed to contaminants. This implies in the case of solid matrices like soils and sediment the assessment of the environmental impacts of total extractable concentrations of contaminants, as, for instance, exemplified in a case study on discharges of the oil and gas industry in Norway [9]. The various processes and concepts underlying

the identification and quantification of differences in bioavailability in between different environmental compartments of varying composition and in between biological receptors within different biota are schematically depicted in Fig. 1.

By properly taking bioavailability of contaminants into account during risk assessment, the amount of false risk evaluations will significantly be reduced. This allows for effective expenditure of the scarce economic means available for soil management and soil remediation. Up till now, no systematic application or implementation of bioavailability in soil and sediment risk assessment has been performed, although a number of pragmatic approaches have been implemented in some countries. An ISO working group (ISO/DIS 17402) provided guidance for the selection and application of methods for the assessment of bioavailability in soil and soil materials. This guidance provides an overview of methods that are potentially ready for implementation of bioavailability in soil quality assessment. Further action is however needed to perform the actual implementation.

As reflected in the titles of the paragraphs to follow, three issues are essential ingredients in implementing bioavailability in risk assessment: proper *consideration* of the key issues allowing for implementation of bioavailability in risk assessment, assessment of the options for actual *inclusion* of bioavailability in risk assessment, and performing the actual *implementation* of bioavailability in risk assessment. The latter includes the operationalization of the methods developed so far for this purpose as well the development of a proper reference framework. The three highlighted key issues are discussed in more detail in Sects. 2, 3, and 4, after which issues to be considered in future are discussed in the concluding paragraph.

2 Consideration of Bioavailability in Prospective Risk Assessment

2.1 General

Environmental risk assessment is commonly performed in phases or tiers, each of which may include predictive (modelling) as well as descriptive methods. The successive tiers require increasing information needs, and thus, as a rule of thumb, they require more time and effort. Regulators dealing with the prospective risk assessment of chemicals usually require manufacturers, processors and handlers, and distributors of chemical substances to provide a minimum set of data on the basic substance properties to allow for a simplified first-tier risk assessment. Commonly, a worst-case approach is applied in this first tier that does not require significant amounts of detailed information. This worst-case approach includes, for instance, the assumption of persistence of the chemical under consideration (lack of degradation), the use of total concentrations as a first estimation of exposure, and the assumption of lack of thermodynamic equilibration (limited or even no inter-compartmental transport). Prospective risk assessment (like, for instance, within REACH, within the European Water Framework Directive, or within national

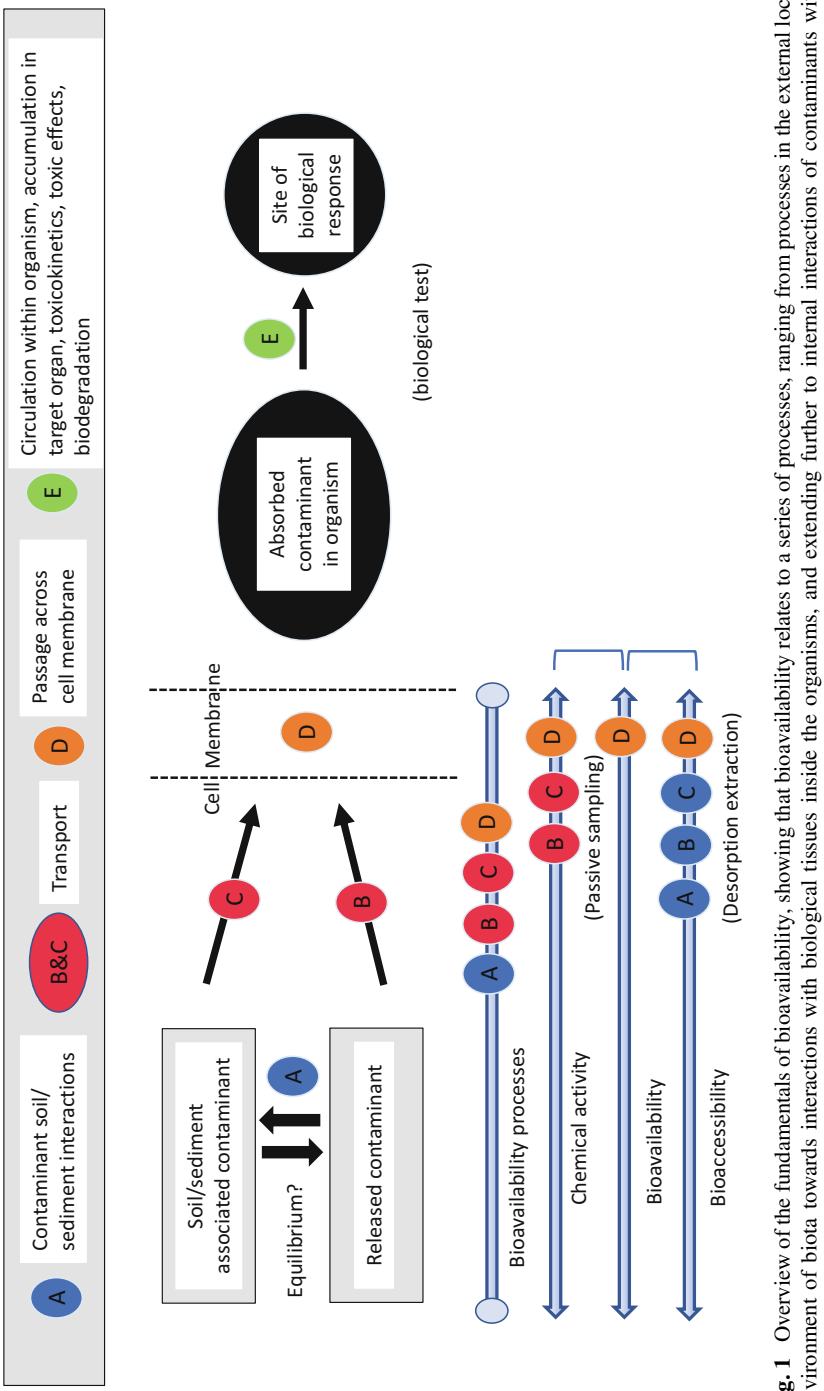

Fig. 1 Overview of the fundamentals of bioavailability, showing that bioavailability relates to a series of processes, ranging from processes in the external local environment of biota towards interactions with biological tissues inside the organisms, and extending further to internal interactions of contaminants with biological response sites. Redrawn from Ortega-Calvo et al. [10] by Wilma IJzerman

legislations) is commonly performed on the basis of the risk paradigm of risk being proportional to the extent in which exposure concentrations exceed concentrations considered safe for either man or the environment. In practical terms, this implies comparing predicted environmental concentrations (PECs) to predicted no effect concentrations (PNECs). In PEC derivation, fate modelling is applied especially in lower tiers of assessment, as done, for instance, in REACH on the basis of the multimedia fate model SimpleBox [11]. In multimedia fate models, partitioning of a chemical between the solid and the liquid phase (pore water) is in the case of organic chemicals properly accounted for, commonly on the basis of organic carbon-based correction factors that account for the hydrophobicity of the chemical of interest as quantified on the basis of the octanol-water partition coefficient (K_{ow}): in the case of ionic organic chemicals, a pH-dependent adjustment is commonly applied. Also, soil-type-specific correction factors have been derived that indirectly incorporate bioavailability considerations in risk assessment.

In the first tier of prospective assessment, basic substance properties required include vapour pressure, aqueous solubility, octanol-water partition coefficient (K_{ow}), melting point, and boiling point. In addition, basic fate properties as preferably derived according to widely accepted testing guidelines, like the OECD guidelines and ISO standards, are required as well as information on (eco)toxicity and exposure based on estimated emissions. The required basic fate properties include rates of hydrolysis, potential for (bio)degradability, and potential for bioaccumulation. The basic data set is then used to assess the risks that a chemical may pose for human health or the environment. In the specific case of the European REACH regulation [2], companies are made responsible for providing information throughout the supply chain regarding the hazards, exposure, risks, and safe use of chemical substances that they manufacture or import. As risk, as stated above, is proportional to both exposure and hazard of a chemical, the information needs within REACH are also tiered with respect to the tonnage manufactured or imported. Furthermore, additional information requirements are in place in case of substances of high or very high concern, or substances expected to be persistent, bioaccumulative, and/or toxic (PBT substances).

It is common for regulatory frameworks on chemical safety to allow for weight-of-evidence approaches and/or the use of several lines of evidence. The options to be used include modelling tools like (quantitative) structure-activity relationships ((Q)SARs) and grouping and read-across approaches as these facilitate the optimal use of existing information on structurally similar chemicals. Apart from the optimal use of scarcely available experimental data and cost considerations, animal welfare considerations also play an important role in this respect. In addition, considerations of reduced bioavailability may be included in higher-tier assessments. These considerations typically consist of substance-specific approaches that are allowed to be used by registrants to adapt the standard information requirements to their substance. The key issue in this respect is that the registrant needs to be able to justify to regulators of the appropriateness and correctness of the adaptations proposed. Hence, the adaptations must be scientifically valid, well-documented, and justified, whereas the associated uncertainties need to be described and, as much as possible, quantified.

2.2 Derivation of Risk Limits/Quality Standards

There is a long history of environmental quality standards and standard setting in various countries. The first Dutch official (water) quality standards date, for instance, back to the late 1960s of the past century [12]. Over the years, scientific developments and policy needs have influenced the methodology to set standards. At the same time, the regulatory context for risk assessment of substances shifted from a national to a European level. In doing so, terminology also shifted, and it is typical that different terms are used for similar risk levels. Whilst (as explained above) the terms target value and intervention value are often used in management frameworks, these terms are often substituted by the negligible concentration (NC) and the serious risk concentration (SRC), respectively. Below the NC, negligible risks are considered to be present and no action is needed. The SRC is used as input for the derivation of the so-called intervention values. Intervention values are concentrations in soil, sediment, or (ground)water above which measures should be taken. The intervention values are based on a combination of human toxicological and ecotoxicological risk limits. In between the NC and the SRC, the maximum permissible concentration (MPC) is defined as the concentration above which intolerable risks are expected and action is prescribed. Between NC and MPC, there is room for improvement of environmental quality, and policy should be aimed at ultimately reaching the NC.

The NC and the MPC are defined with respect to human health and with regard to the protection of ecosystems in terms of composition and functioning. For compounds for which a threshold level for adverse health effects can be determined, the MPC for humans is set at the acceptable daily intake (ADI) or tolerable daily intake (TDI). For substances without a threshold (genotoxic carcinogens), the MPC is commonly set to an increased probability of death of 10^{-6} per year (10^{-4} on a lifetime basis). The NC was defined as 1% of the MPC, taking account of the fact that, whilst setting standards for single compounds, combination toxicity may in reality occur due to simultaneous exposure to multiple substances. The MPC for the environment is defined as the concentration which protects at least 95% of the species in an ecosystem, thereby protecting the functioning of the ecosystem. The MPC is also termed 'HC5' to indicate the hazardous concentration that affects 5% of the species. Similarly to the human risk assessment, the NC for the ecosystem is commonly set to 1% of the MPC. The 95% protection level is associated with the use of the species sensitivity distribution (SSD) model [13]. For humans, the intervention value uses the MPC level according to the definition given above, whilst for ecosystems the SRC is used. The SRC for ecosystems is defined as the concentration at which 50% of the species is potentially affected.

An SSD represents the cumulative frequency distribution of results of toxicity testing of apical endpoints of individual species (Fig. 2; Posthuma et al [14]). As reflected in Fig. 2, SSDs can be used retrospectively to derive EQCs as based on

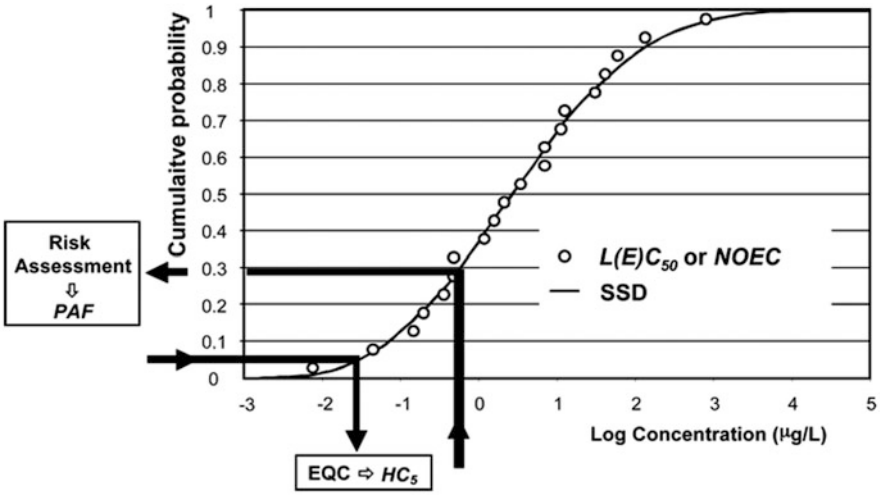

Fig. 2 The species sensitivity distribution (SSD) concept. Each circle represents laboratory ecotoxicity data for a compound for a specific organism; (—) represents the fitted SSD. A potentially affected fraction of species (PAF) is derived from an ambient concentration x (x→y). Environmental quality criteria (EQC) are derived from a chosen value of y (y→x, here HC_5). $L(E)C_{50}$ = lethal (or effect) concentration yielding 50% effect; NOEC = no-observed-effect concentration; HC_5 = hazardous concentration for 5% of the species. Redrawn from Posthuma et al. [14]

the common regulatory acceptance of 5% of species potentially being at risk due to exposure to chemicals (HC5 or MPC). In addition, the SSD concept allows the diagnostic assessment of the fraction of species potentially affected.

The SSD method is used to predict the sensitivity of a whole community on the basis of the results of laboratory data on individual species and enables estimation of the fraction of species in the community that is potentially affected given a certain exposure level. The initially proposed 5% cut-off level as the basis for standard setting is generally adopted in both prospective and retrospective risk assessment. In the various guidance documents for standard setting that have been published, application of the SSD method is advised when an extensive set of toxicity data is available of at least ten endpoints for at least eight different taxonomic groups. If properly justified, ecotoxicity data on microbial or enzymatic processes (functional endpoints) and data reflecting the effects on species or populations (structural endpoints) are allowed to be combined in one data set instead of deriving separate risk limits for processes and species, respectively. If fewer data are available, assessment factors are applied to the lowest ecotoxicity endpoint, assuming that this would at least guarantee a similar level of protection [15].

In addition to information on direct (apical) adverse effects as deduced by means of standard laboratory testing (see, for instance, Løkke and van Gestel [16] for a review on toxicity test methods available for soil organisms), secondary poisoning is

commonly included in the final standards for soil or sediments. The focus is on the potential risks for birds and mammals due to consumption of water and/or soil organisms, as based on the pioneering work of Romijn et al. [17]. Using this method, critical toxicity data for birds and mammals were back-calculated to safe concentrations in prey based on assumptions on daily food intake. The concentrations in prey are in turn recalculated into corresponding MPCs in soil and/or sediments using information on bioconcentration and bioaccumulation.

Actual implementation of bioavailability considerations in the derivation of risk limits/quality standards is on the one hand restricted to the assumption that aquatic laboratory ecotoxicity tests represent the effects of dissolved concentrations instead of total concentrations. In case of organic chemicals, correction on the basis of the concentration of dissolved organic matter in aquatic environments is used to take differences in bioavailability between laboratory test systems and realistic aquatic environments into account. In the case of chemicals for which limited toxicity data are available for soil and sediment, the basic assumption of predominant exposure of soil- and sediment-dwelling organisms via pore water is used to calculate risk limits for soil and sediment on the basis of aquatic toxicity data, assuming chemical equilibration between the solid and liquid phases. The equilibrium partitioning method (EqP method) can be used to calculate soil quality standards (expressed in mg kg^{-1}) from aquatic quality standards (expressed in μg L^{-1}) using a partitioning coefficient. The validity of this application of the EqP method was studied by Van Beelen et al. [18] by comparing aquatic with terrestrial toxicity data. For ten organic substances (chlorpyrifos, atrazine, carbofuran, pentachlorophenol, chlordane, aldrin, trichlorobenzene, heptachlor, trichlorophenol, and trichloroethene) and for eight metals, sufficient data were available to allow for a proper comparison. The aquatic toxicity data were multiplied by the partitioning coefficient in order to obtain aquatic data expressed in mg kg^{-1}. For some compounds, the terrestrial toxicity data were significantly higher than the aquatic data, whereas for other compounds, it was the other way around. These differences indicate that the EqP method can give significant over- or underestimations, due to inaccurate partitioning coefficients or differences in sensitivities between aquatic and terrestrial organisms. These over- or underestimations can have an impact on the setting of environmental quality standards (HC5 values). The HC5 values derived using the EqP method were in 5% of the cases more than 20 times higher than the corresponding HC5 values that were derived directly from soil toxicity tests. Despite this uncertainty, the use of the EqP method can still be advocated for setting soil quality guidelines when only a very limited number of terrestrial toxicity data are available.

3 Inclusion of Bioavailability in Retrospective Risk Assessment

3.1 General

Whereas it is typical nowadays for prospective risk assessment to consider both actual exposure and hazards, retrospective risk assessment in one way or the other focuses on setting environmental quality standards, on warranting compliance to these standards, and on inclusion of a tiered approach in (site-specific) risk assessment in case of exceedance of the standards [19]. This tiered approach allows for inclusion of increased realism in risk assessment. An example of a management framework for the case of contaminated soil, in which retrospective risk assessment plays a pivotal role in the initial management stages, is shown in Fig. 3. In this figure, background values relate to chemicals of natural origin and are commonly set to the natural background concentration. Soils are considered contaminated once target values are exceeded, whereas management actions are needed in case of exceedance of intervention values.

In retrospective risk assessment, three basic activities play a central role:

1. Derivation of risk limits like standards and trigger values, as commonly done on the basis of total concentrations.
2. A tiered approach for human and ecological risk assessment in which the focus is on management and remediation and which is triggered by exceedance of standards and trigger values.
3. Site-specific risk assessment in which various lines of evidence are brought together and in which the focus is on environmental realism. Especially in this stage, there usually is ample room for inclusion of bioavailability considerations, as operationalized by means of bioassays, modelling tools, and analytical methods for bioavailability assessment.

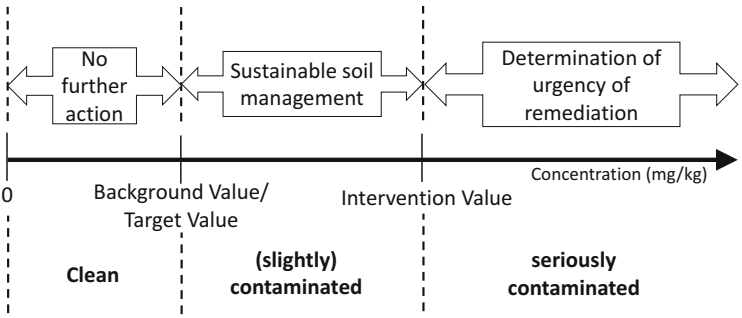

Fig. 3 General outline of a management framework for contaminated soil. Retrospective risk assessment is operationalized in this framework by means of target and intervention values, which are indicated by means of the vertical dotted lines

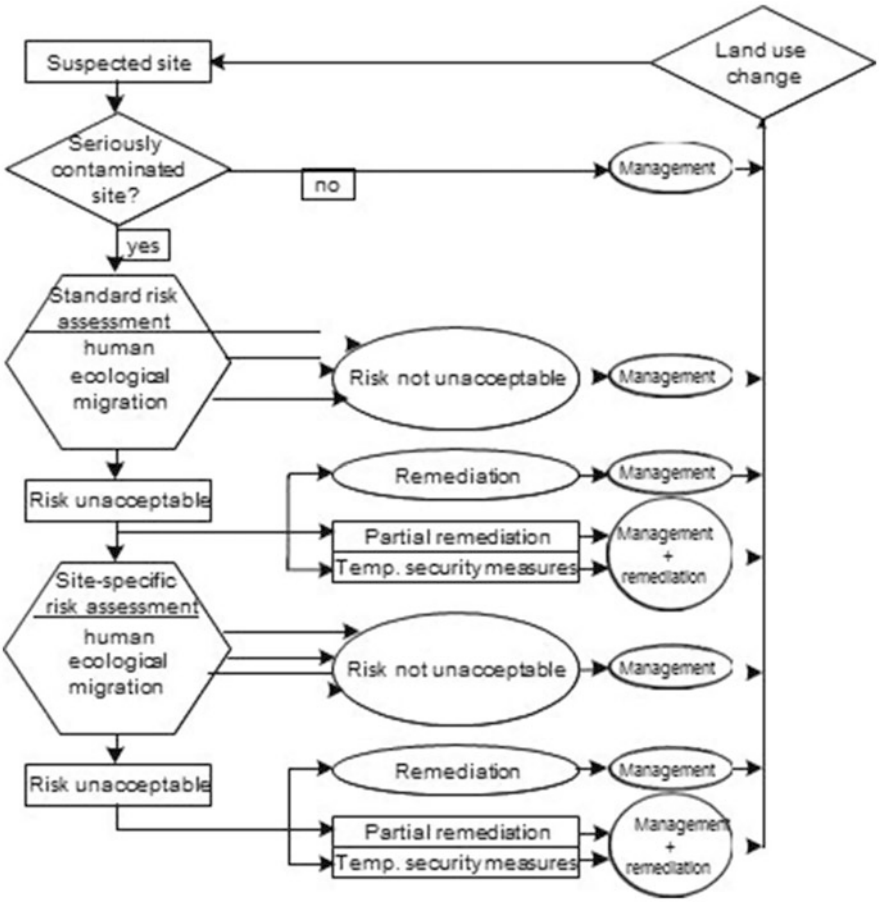

Fig. 4 A typical example of a regulatory framework for contaminated site management building upon a tiered approach. Redrawn from INERIS [20]

A typical example of a regulatory framework for contaminated site management is given in Fig. 4. The example builds upon a tiered approach, as triggered by the exceedance of standards like target and intervention values shown in Fig. 3. The three basic activities within retrospective risk assessment are discussed in more detail below.

3.2 Tiered Approaches to Human and Ecological Risk Assessment

Risk assessment of contaminated sites is based on exposure and transport modelling of contaminants in soil, sediment, and groundwater. The risk assessment encloses

risks to humans, ecosystems, and risks due to contaminant migration. Also, current and intended (or future) land uses are commonly considered. Figure 5 shows an example of a flowchart of decisions to be taken once a trigger value (like an intervention value) is exceeded at a large scale. Once the trigger value is exceeded, a standard risk assessment has to be carried out. When, according to the standard risk assessment, unacceptable risks cannot be excluded, remediation of the site is necessary or, in cases of uncertainty, a site-specific risk assessment might be carried out.

A tier-based approach is commonly proposed for decisions about remediation. It is required that such a tiered approach is suitable to be applied for different goals, like:

1. Site-specific risk assessment of contaminated sites, or 'case-by-case' site-specific human and ecological risk assessment of contaminated land.

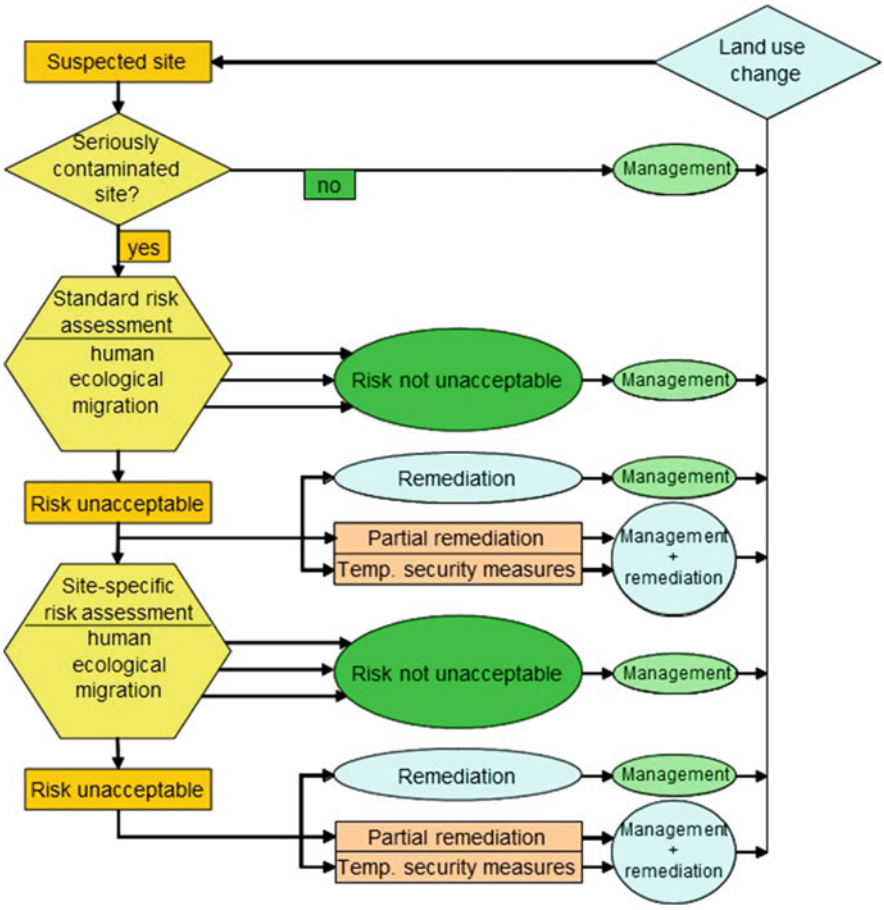

Fig. 5 Example of a flowchart of a management approach for seriously contaminated sites

2. Advising (local) authorities about human and ecological health risks. In both cases, it is important to take all exposure routes into account including exposure from other sources (like air) and including considerations of bioavailability.
3. Deriving remediation objectives, including the derivation of remediation objectives for different land uses and corresponding exposure and effect scenarios. As an example: for deriving critical soil concentrations for carcinogenic contaminants, a higher protection level is used in general. For noncarcinogenic contaminants, the background exposure (exposure from other sources) is also taken into account.

In tier 0 (preselection phase), the possible exposure pathways are considered together with relevant contaminant concentrations and the objectives of the assessment.

A generic assessment is carried out in tier 1, based on a realistic worst-case exposure scenario. Critical soil concentrations for these scenarios are derived, after which the measured concentrations in soil and groundwater can be compared with these concentrations.

When risks cannot be excluded in tier 1, site-specific and land-use-specific exposure and risks can be assessed in tier 2. Exposure scenarios are used in this tier that relate to the current or intended land use. In tier 3, site-specific data, e.g. through measurements in contact media, can be used to make to optimize the site specificity of the risk assessment and to decrease uncertainties.

Although any site-specific risk assessment implies uncertainties and always can be improved on specific aspects, a tier-based approach and the estimation of the different exposure routes are an efficient and solid base for risk-based land management and possible international harmonization of site-specific risk assessment in the future.

Depending on the outcome and the defined goal of the risk assessment of seriously contaminated soil, a decision has to be made by the competent authorities. When unacceptable risks cannot be excluded, the competent authority can decide to:

- Do (partial) remediation.
- Take temporal security measures.
- Take soil management measures (e.g. restriction on soil use).

Figure 6 shows an example of a framework for the risk assessment, in this case human risk assessment. The framework consists of three tiers headed by a preselection (tier 0). In the preselection in Fig. 6, it should be made clear which measured concentrations should be used for the risk assessment. When these concentrations are higher than a specific level (e.g. intervention values), tier 1 should be initiated. However, in specific situations, an unacceptable risk can be present below this level. An example is a site for which volatile compounds are found in shallow groundwater. Furthermore, the goal of the risk assessment has to be set in this tier.

For tier 1 (simple human risk assessment in this example), critical concentrations in soil and groundwater need to be either collected or derived, as upper limit for human risks equal to the maximum permissible risk for humans (MPR_{human}).

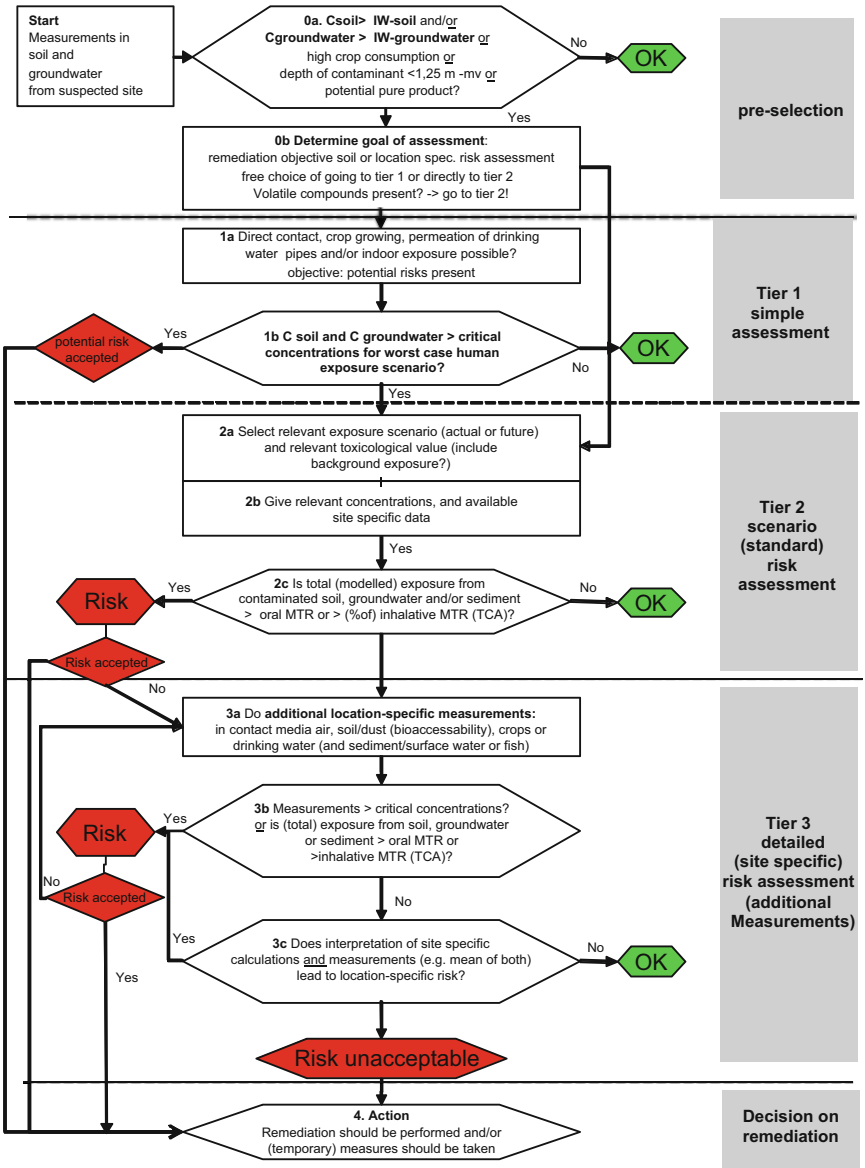

Fig. 6 Example of a framework for site-specific (human) risk assessment

In tier 1, relevant exposure pathways are determined (ingestion of soil and dust and dermal contact with soil and dust, vegetable consumption, inhalation of indoor air, permeation of drinking water mains, and consumption of fish, meat, and milk originating from the site). Different sensitive soil uses or situations can be identified. Based on this information, it can be concluded whether potential risks are present for a compound or for a given set of compounds.

In tier 2, a more profound site-specific risk assessment is carried out. To this purpose, choices have to be made about the relevant toxicological risk levels and the relevant exposure scenario (related to the land use). The selected exposure scenarios, model concepts, and toxicological reference values are important for the risk assessment. For each land use, an exposure scenario is defined which comprehends soil ingestion rates, contact time, and consumption rates. Each land use is made specific with an exposure scenario for human risk assessment. For this purpose, a number of land uses might be distinguished, like, for instance:

- Residential with garden, possibly including the sub-scenario 'Residential with garden without consumption of vegetables from own garden'
- Children playgrounds and places where children play
- Residential with kitchen or vegetable garden
- Agriculture use (excluding the residential farm with premises)
- Nature areas
- (Urban) green areas with nature values (including sport areas, recreational areas, and municipal parks)
- Other green areas, infrastructure, buildings, and industry
- Agricultural scenarios:
  - Agriculture without crop production for human consumption
  - Grasslands and meadows with consumption of own meat and milk products
  - Agriculture with crop production and consumption of own crops/fruits

An overview of the exposure pathways for each land use is given in Table 1.

In the standard site-specific risk assessment, the maximum permissible risk (MPR_{human}) may be used as the toxicological risk level. Below the MPR_{human}, it is considered that no risks for noncarcinogenic compounds are present, whereas the risk for carcinogenic (or non-threshold) compounds is acceptable. The MPR_{human} is expressed as either a tolerable daily intake (TDI) or an excess carcinogenic risk via oral intake (CR_{oral}), both covering exposure by oral ingestion. For exposure by inhalation, the MPR_{human} is derived from a tolerable concentration in air (TCA) or an excess carcinogenic risk via air (CR_{inhal}). A procedure to derive $MPRs_{human}$ was outlined in detail by Janssen and Speijers [21].

For the critical exposure level in the risk assessment of soil contamination, two aspects can be taken into account:

1. Exposure from other sources than soil contamination can be included ('background exposure'). This aspect is not commonly included in standard risk assessment, because it is commonly decided that remediation should only be carried out when the risk is caused by the soil contamination and not due to exposure from other sources. When the goal of the assessment is to derive a level for good soil quality, background exposure is found relevant.
2. Selection of a more protective risk level. For carcinogenic risk, it is a common policy decision that the MPR is set at the level of an additional cancer risk of 10^{-4} at lifelong exposure to soil contaminants. For the level of good soil quality, the

Table 1 Overview of the relevant human exposure pathways per land use (+ = present; +/− = lowered compared to maximum; − = not included; na = not applicable)

	Parameter	Land-use scenarios									
		1. Residential with garden	2. Places were children play.	3. Residential with kitchen, — vegetable garden	4. Agriculture use (excluding the residential farm with premises)	5. Nature areas	6. (Urban) green areas with nature values	7. Other green areas, infrastructure, buildings and industry	8. Agriculture (general)	9. Agriculture, grasslands	10. 'Agriculture with crop production'
Ingestion	Ingestion soil and dust outside	+	+	+	+	+/−	+/−	+/−	+	+	+
	Ingestion soil and dust inside	+	+	+	+	−	−	+/−	+	+	+
	Intake surface water	na	na	na	na	na	na	na	na	na	na
Dermal	Dermal contact outside	+	+	+	+	+	+	+	+	+	+
	Dermal contact inside	+	+	+	+	−	−	+	+	+	+
	Dermal contact surface water	na	na	na	na	na	na	na	na	na	na
Consumption	Fraction contaminated leafy veg.	+/−	−	+	+/−	−	−	−	−	−	+
	Fraction contaminated leafy veg.	+/−	−	+	+/−	−	−	−	−	−	+
	Fraction contaminated meat	−	−	−	−	−	−	−	−	+	−
	Fraction contaminated milk	−	−	−	−	−	−	−	−	+	−
	Fraction contaminated fish	na	na	na	na	na	na	na	na	na	na

Inhalation	Inhalation inside	+	+	+	+	–	–	+/–	+	+	+	
	Inhalation outside	+	+	+	+	+/–	+/–	+/–	+/–	+/–	+/–	
	Time sleeping	+	+	+	+	–	–	–	+	+	+	
	Time stay at other location	–	–	–	–	+	+	+	–	–	–	

acceptable risk is commonly set at an additional risk of 10^{-6}. This is in accordance with the risk levels set for environmental risk assessment, as stated above.

In specific cases (e.g. during excavation activities), it is relevant to assess risks due to (repeated) short-term exposure. Temporary higher exposure can be tolerated but within certain limits and not for acute effects at the level of the MPR. For frequently found compounds, it could be further investigated if short-term exposure at a higher level has no negative effect. Another objective of a risk assessment can be that concentrations in contact media should not be influenced by the contamination of soil or groundwater. In case of volatile compounds, this objective is sometimes set.

When, based on the tier 2 assessment, the risks are not unacceptable, the assessment can be stopped, and a decision can be made by the competent authority. When unacceptable risks cannot be excluded, it is recommended to carry out a tier 3 risk assessment, or it can be decided to start remediation (or take measures).

In tier 3, more site-specific data should be obtained and used in the risk assessment. Especially field data and measured concentrations in (soil) air, crops, soil pore water, and groundwater are important for reducing the uncertainties of the tier 2 risk assessment. Typically, at this stage, considerations of bioavailability start to play an increasingly important role. The risk assessment can be made more precise and site specific by performing measurements in contact media. It should be noted on the other hand that results of measurements are time dependent and dependent on spatial heterogeneity.

With regard to the implementation of human risk assessment for estimation risks of soil pollution, it can be concluded that:

- A lot of new information has become available during the last decades about the behaviour of contaminants and the human health risks involved. Still many uncertainties are involved in estimating the risks, as partly related to the typical heterogeneity of the contamination in the environment and the heterogeneity of the soil itself.
- Different objectives can be the start of risk assessment. It is therefore important to set the goal of the risk assessment in advance.
- Exposure scenarios depend on the goal of an assessment and there should be a limited amount of scenarios. It is therefore essential that the exposure scenarios are supported by a political decision.
- To allow as much as possible for comparison of data and comparison of approaches, it is important to standardize the use of measured data of soil and groundwater concentrations as much as possible.

3.3 Site-Specific Risk Assessment: The Triad Approach

In addition to the tiered approach towards retrospective risk assessment of contaminated sites discussed in Sect. 3.2, the Triad approach has been developed for assessing site-specific ecological risks at contaminated sites with specific consideration of environmental realism [22]. The Triad approach can be applied when there

are indications that there is an unacceptable ecological risk associated with the current use or anticipated use of contaminated soils or sediments. This is, for instance, the case when:

- Biodiversity is likely to be affected (protection of biological species)
- Natural recycling processes or soil/sediment functions like nutrient cycling or natural biodegradation are affected (protection of processes)
- Bioaccumulation and secondary poisoning are enhanced

As schematically shown in Fig. 7, environmental realism is quantified in the Triad approach by bringing together three distinct lines of evidence originating from three fields of research:

1. *Chemistry*: quantification of either the total or the bioavailable concentration of contaminants at the site of interest, accumulated in biota, or modelled via food chains. This total or bioavailable concentration is used for calculating risks on the basis of toxicity data from literature.
2. *Toxicology*: performing bioassays with biota across genera in order to measure the actual toxicity present in environmental samples from a contaminated site.
3. *Ecology*: performing field ecological observations at the contaminated site and comparing the observations to similar assessments at a non-contaminated reference site. The observed deviations from the reference site, which can be plausibly attributed to the contaminant levels present, are included in the Triad. Obviously, a key challenge lies in identifying a suited reference site.

The crux of the Triad is related to the integration of three distinct fields of knowledge which allows to efficiently reduce the overall uncertainties. This is basically done by combining multiple lines of evidence. The Triad can in turn be applied within a tiered approach, often comprising three tiers. This offers the

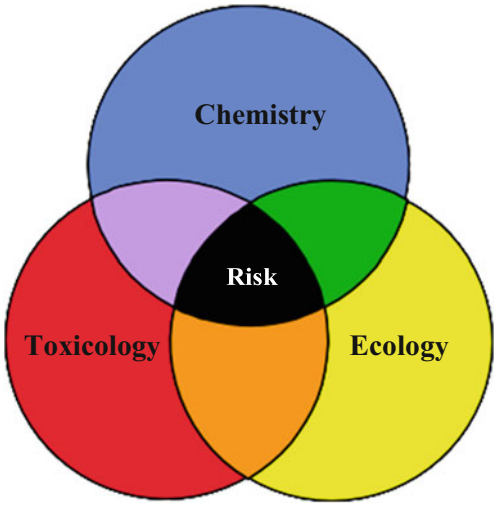

Fig. 7 A schematic representation of the lines of evidences underpinning site-specific risk assessment

opportunity to use a standardized set of methods and tools to perform an initial assessment and prevents unnecessary research. In case of large uncertainties due to contradictory outcomes of the three legs of the Triad, additional assessment methods can be applied.

Along the three tiers of the Triad, there is increasing room for inclusion of bioavailability considerations, as operationalized by means of bioassays, modelling tools, and analytical methods for bioavailability assessment. The Triad typically starts with a simple evaluation of the status of the contaminated site at the screening level and basically is equal to tier 3 of the framework of human and ecological risk assessment discussed in Sect. 3.2. At the very first stage of the Triad (also termed the simple screening stage), total concentrations of all relevant chemicals are used in order to evaluate or confirm the need for conducting a site-specific assessment. Total concentrations of the contaminants are individually compared to soil quality objectives, soil screening levels, target values, etc. At this stage, bioavailability is not explicitly accounted for, although a simple bioassay might be performed which will by definition directly or indirectly express the bioavailability of the contaminants present.

In the second tier of the Triad (also termed the refined screening stage), a first refinement of the exposure measurements is performed, which at the same time provides further insight into the toxicological and ecological properties of the contaminated soil. Tier 2 deviates from the conservatism that is normally associated with the use of total concentrations in risk assessment by taking (rough) estimates of bioavailability into consideration. The reduced conservatism in exposure assessment is compensated by a first screening of the toxicological and ecological properties of the soil. The key objective of bioavailability assessment in this tier is to make the site-specific exposure estimation and the exposure conditions created in most laboratory studies more comparable. To this end, a number of non-exhaustive extraction procedures aimed at providing a more accurate estimate of the actual exposure (or the bioavailable fraction) can be applied. The extracted concentration, expressed in units of mg of chemical kg^{-1} dry soil, is directly compared to soil screening levels. It is therefore a prerequisite of this comparison that the extractability in the laboratory tests is close to 100% by the most used methods. In most short-term laboratory tests, with typical exposure times <4 weeks, it will be reasonable to assume that only little ageing or strong sequestration occurs and hence most of the spiked chemicals are still extractable with mild (organic) solvents. Strictly speaking, this still has to be validated for most methods. Organic solvents most frequently used include different ratios of low molecular weight alcohols and water, ethyl acetate, and tetrahydrofuran. Selection of these mild extractants builds upon pioneering work on bioavailability assessment of organic contaminants, demonstrating the environmental relevance of bioavailability [23].

Later on, it was decided that these extraction methods should be standardized via two possible approaches:

1. Methods based on desorption of the target chemicals from soil or sediment by an extractant acting as an infinite sink. Some of these methods are discussed by Umeh et al. [24].
2. Methods that measure the freely dissolved chemical concentration in the aqueous phase of soil or sediment.

These two possible approaches are used as the basis for more in-depth bioavailability assessment of pollutants in historically contaminated soil or sediments in the third tier of the Triad (also termed the detailed screening stage). Obviously, the various methods have their strengths and weaknesses depending on the mix of substances in question. The weakness of lack of guidelines for extracting methods and methods for measuring freely dissolved concentrations is expected to be solved by currently ongoing standardization efforts. In the Triad tier 3, the estimated pore water concentrations (either the dissolved or the extractable concentration, expressed in units of $\mu g\ L^{-1}$) are compared to water quality standards.

When needed, for instance, still due to lack of conclusive evidence, a fourth stage assessment may be performed: in-depth assessment to answer any remaining questions. Although no methods or procedures are prescribed for this tier of the Triad, various options are available including any alternative chemical simulation method for bioavailability, long-term bioaccumulation studies, collection of bioconcentration factors, and collection of monitoring data from biota or target organs of biota collected at the contaminated site in order to model food web effects and dispersion of the pollution and to get a true estimate of the truly bioavailable fractions of the pollutants present.

4 Implementation of Bioavailability in Risk Assessment

4.1 Why Implementation of Bioavailability?

As already indicated above, there is increasing awareness of the fact that performing risk evaluation on the basis of measured total concentrations may lead to an incorrect assessment of the actual risks, be it with regard to false-positive indications of risk or with regard to the less common case of false-negative assessments in which soil ecosystems are negatively affected at concentrations below the soil quality standards. If bioavailability is properly taken into account during a risk evaluation, the number of false positives and false negatives can be reduced, thus properly protecting the ecosystem and allowing for effective expenditure of the in general scarce means available for soil management and soil remediation. The basic issue is that the mere presence of a contaminant does not by definition mean that there is an actual risk or a measurable effect on an ecosystem. Actually occurring effects are directly related to the binding capacity of the soil or the sediment and the speciation in the pore water, as affected by the physical-chemical properties of the soil or sediment and the contaminants and the specific properties of the biota that are

actually exposed to the contaminants. Binding of contaminants to the solid matrix in combination with the composition of the pore water can reduce the concentrations of the contaminants in the ecosystem to which biota are effectively exposed to. In other words, depending on the solid matrix, organisms may actually experience less impacts of contaminants than potentially expected from total soil concentrations. Therefore, it is only the bioavailable concentration that is able to exert adverse effects in terrestrial and benthic ecosystems. Hence, the key issue in operationalizing bioavailability is to improve the correlation between bioavailable fractions (as determined by means of an extensive set of exhaustive extraction methods and equilibration-based methods mimicking pore water concentrations of contaminants) and actually or potentially occurring effects.

In establishing such correlation, it is to be realized that various processes can effectively affect the bioavailability in the field. The first type of process is physicochemically driven and relates to sorption, precipitation, and occlusion in organic matter and in mineral particles. Factors influencing this process are soil-specific and substance-specific and include aqueous solubility, hydrophobicity, dissociation constant, pH, clay content, organic matter type and organic matter content, and cation exchange capacity of the solid phase. Finally, the chemical composition of the pore water is important as it determines the speciation of a chemical. Jointly, these factors determine the actual exposure of an organism. In addition, physiologically driven uptake processes are of importance, as controlled by biological species-specific parameters like surface-volume ratio, anatomy, feeding strategy, and related uptake routes of nutrients and contaminants, as well as by preferences in habitat. These parameters determine the biological availability of a chemical for a specific organism. Finally, internal allocation process as controlled by organisms determines the toxicological bioavailability. Relevant allocation processes include metabolism and other means of detoxification like excretion, storage capacity, and energy sources [25]. All processes have in common that they are time and space dependent. This creates a complex system that is difficult to understand and difficult to mimic. This complexity is one of the main reasons why legislators have been reluctant to effectively implement bioavailability in risk assessment in general or even in specific risk assessment procedures. Nevertheless, it is important to realize that great improvements have been made during the last decades in increasing and in quantifying our understanding of the chemical and biological mechanisms responsible for the availability of chemicals for uptake and toxicity.

An important obstacle hindering implementation of methods developed to quantify the bioavailable fraction is probably the large number of methods that are available. Most, if not all, methods have in common that they were developed without proper consideration of the need to develop the corresponding reference system for linking chemical availability to biological and toxicological availability. Furthermore, field validation and extrapolation of testing results typically obtained in a laboratory setting to realistic, varying, field conditions have in general also been ignored. As discussed above, bioavailability considerations are taken into account in, for instance, tiered approaches towards site-specific risk assessment. In such cases,

often biota-specific and chemical-specific information on the link between chemical availability and biological/toxicological bioavailability is used to deduce whether the ecosystem or part of the ecosystem is at risk. Up till now, large-scale applications of such approaches are still virtually non-existing.

4.2 Experimental Methods Available for Implementation of Bioavailability in (Tiered) Risk Assessment

As already indicated by Harmsen et al. [26], bioavailability is in itself the outcome of a series of dynamic processes that occur in soils and sediments and in biota associated with the solid phase. As chemical as well as biological aspects are included in the dynamics of bioavailability, there are two complementary ways to assess bioavailability: either by biological or by chemical measurements. Biological measurements (bioassays) have the advantage of directly displaying the actual impact of the truly bioavailable fraction of a contaminant or of a mixture of contaminants. Although (as advocated by [16]) it in principle is well possible to standardize terrestrial bioassays, their role in risk assessment is in general limited to application in a weight of evidence approach in the higher tiers of site-specific risk assessment. A key issue hindering the implementation of bioassays in risk assessment is the inability of including the dynamics of bioavailability in the evaluation of the outcome of bioassays which in turn hinders the extrapolation of biological measurements from one soil ecosystem to another.

An alternative to biological measurements is the critical body burden residue (or CBR) approach. Critical body burden is a concept that examines the relationship between the accumulation of a toxicant in an organism and its effects on an organism. It is based on the premise that a toxicant must reach a critical concentration within an organism before an adverse effects is observed [27]. These effects are not necessarily lethal but can range from the lowest observed adverse effect to mortality. The threshold concentration is the critical or lethal body burden. If the critical body burden is exceeded, effects on organisms are imminent. The CBR allows to determine effects on soil organisms, if the freely dissolved pore water concentrations and some site-specific data (like organic matter content) are known. The CBR approach is suited for effect prediction of hydrophobic contaminants that induce toxicity via the mechanism of non-polar narcosis, but it is not suited to predict toxicity of contaminants that have a different mode (or even modes) of action as in case of different mode(s) of action, the toxicity is equal to the summed impacts of the non-polar narcotic pathway and the impact of the specific mode(s) of action. The suitability of the CBR for risk assessment purposes is therefore limited.

Aside from biological testing, chemical testing can be used to quantify the bioavailability of contaminants across terrestrial and benthic systems. According to ISO/DIS 17402 [28], chemical testing can be used if the bioavailability for an organism in soil can be mimicked by a chemical process. In such a case, a

Table 2 Overview of chemical methods potentially suited to mimic bioavailability

Organic contaminants
• Passive sampling methods including
– Solid-phase micro-extraction (SPME)
– Semipermeable membrane devices (SPMD)
– Polyoxymethylene solid-phase extraction (POM-SPE)
– Empore discs
– Silicone rubber
• Tenax extraction
• Cyclodextrin extraction
• Supercritical fluid extraction
Heavy metals
• Acid extractions
• Extractions with chelating agents
• Weak extractions
• Donnan membrane technique
• Diffusive gradient in thin films
• Sequential extractions
• Models to calculate metal speciation and/or partitioning in the soil solid phase and soil solution
– Mechanistic assemblage models
– Empirical models such as transfer functions
– Models to calculate internal concentrations and effect levels
Terrestrial biotic ligand models (BLM)
Empirical effect models

(quantitative) relationship between the bioavailability as expressed in terms of actually occurring adverse effects and the chemical test that estimates the bioavailability is required. A number of non-exhaustive extraction procedures aimed at providing a more accurate estimate of the actual exposure (or the bioavailable fraction) can be applied. These chemical methods have in common that they determine the fraction of contaminants assumed to be available for specific biological receptors. If a correlation between the chemical measurement and biological effects or accumulation can be demonstrated, these chemical measurements may replace biological testing in a routine assessment of soil quality. There is a large diversity of methods potentially suited to mimic bioavailability. The most common methods are listed in Table 2.

Brand et al. [29] used a set of practical considerations and a set of selection criteria to prioritize the methods that were deemed to be the most promising for implementation in a regulatory framework:

Practical Considerations

– Which method(s) are considered to be the most promising for the implementation of bioavailability?

– How can the selected methods be implemented into policy or in which framework can they be used?
– Under which preconditions could these methods be implemented?
– Which knowledge is still missing and therefore which further research should be performed?

Criteria

1. Wide ranging applicability, i.e.
   (a) The possibility to perform the technique in a standard laboratory
   (b) The possibility to assess more than one type of organism
   (c) The possibility to assess more than one type of soil
   (d) The possibility to assess more than one type of contaminant
2. Practical use
3. Added value compared to determination of total content
4. Validity for ecotoxicity
5. Applicability beyond ecotoxicity assessment

Based on the practical considerations and an expert assessment of the criteria defined, the following methods were considered to be the most promising:

4.2.1 Organic Contaminants

Measuring actually bioavailable concentrations:

– Passive sampling with either SPME, POM-SPE, or silicone rubber, as discussed in detail by Smith [30]

Measuring potentially bioavailable concentrations:

– Tenax extraction
– Cyclodextrin extraction

4.2.2 Heavy Metals

Measuring actually bioavailable concentrations:

– Weak extraction with 0.01 M $CaCl_2$

Measuring potentially bioavailable concentrations:

– Acid extraction with 0.43 M HNO_3

The methods considered for measuring potentially bioavailable concentrations take explicitly into consideration that a large fraction of the organics and metals present in soil or sediment are either irreversibly sequestered in the solid matrix or strongly absorbed in or adsorbed to the solid phase. These sorbed parts are in

principle either unavailable for rapid exchange in the soil or sediment solution and thus unavailable for degradation and transport processes or unavailable for interactions and uptake by organisms including man. They may, however, provide a potentially bioavailable pool from which molecules are depleted and end up in the liquid phase.

4.3 Reference Framework as the Basis for Implementation of Bioavailability

The best way of implementing bioavailability in current frameworks for risk assessment is by relating measured bioavailable concentrations in the field to toxicity data for soil or sediment biota that are based on bioavailable contaminant concentrations. These toxicity data are preferably measured with the same analytical method as the field data, using the methods recommended in the previous paragraph. Most toxicity data for soils and sediments are derived from experiments where contaminants have been spiked. Spiked contaminants can be considered to be totally bioavailable because they did not have the time to bind to sites that are not accessible to organisms (also called ageing of soils). If there is a desire to implement bioavailability by relating the measured bioavailable concentrations to toxicity data based on bioavailable contaminant concentrations determined with the same analytical methods, 'new' soil toxicity data and, hence, soil quality standards need to be derived. Because of the obvious restriction that bioavailability should be implemented in the existing regulatory framework, deriving new bioavailability-based quality standards is not a realistic option.

Two alternative options stand out:

1. Relating actually bioavailable concentrations to the toxicity of aquatic biota
2. Relating potentially bioavailable concentrations to the toxicity of soil biota

4.3.1 Relating Actually Bioavailable Concentrations to the Toxicity of Aquatic Biota

The key issues with regard to this option are on the one hand the assumption of similar intrinsic sensitivity of terrestrial and aquatic organisms to chemicals, whereas on the other hand, it is assumed that exposure to the pore water of soil or sediment is the dominant exposure route for most terrestrial organisms, as confirmed by Peijnenburg et al. [31]. The latter assumption boils down to the bioavailability, bioaccumulation, and toxicity being closely related to pore water concentrations. Examples of aquatic toxicity data to relate the measured concentrations to could be the HC_5 level, the HC_{50}, or any other HC_x level for surface water. As a matter of course, the final decision on which risk limit should be chosen as a standard still has to be made and would ultimately be a policy decision.

This approach is a quick, transparent, reproducible, and relatively representative way of implementing bioavailability for organic contaminants in risk assessment. It essentially eliminates the need for toxicity testing when extracted bioavailable concentrations do not exceed the water standards. As aquatic toxicity data are expressed as μg L^{-1}, measured bioavailable pore water concentrations should preferably also be expressed in μg L^{-1}. The passive samplers SPME, POM-SPE, and silicone rubber indeed measure the concentration of the contaminant that is freely dissolved as μg L^{-1} pore water. Therefore, measured concentrations can directly be compared to aquatic toxicity data, and no conversion is needed. The bioavailable concentration either does or does not exceed the pre-set aquatic risk level. If the risk level is exceeded, effects on the ecosystem are imminent. This outcome can then be used for short-term decision-making like determining the remediation urgency of contaminated sites.

Technically, it is also possible to translate potentially bioavailable concentrations (given in units of mg kg^{-1}) as measured with Tenax and cyclodextrin into a concentration in μg L^{-1} by using the distribution coefficient (either K_{oc} or K_d) between soil and water. However, this calculation introduces an extra uncertainty due the uncertainty in the chosen K_{oc} value. It is therefore preferable to relate potentially available concentrations directly to soil toxicity data, which are also given in units of mg kg^{-1}.

4.3.2 Relating Potentially Bioavailable Concentrations to the Toxicity of Soil Biota

A second approach is to relate the measured bioavailable concentrations to toxicity data for soil organisms, as discussed by Römbke and Martin-Laurent [32]. Examples of these toxicity data are again the HC_5 or HC_{50} values for soil. As with the actually bioavailable concentrations, a final decision on which risk limit to be chosen still needs to be made. This approach resembles the current way of risk assessment because the measured concentrations are compared with the common set toxicity data of soil organisms, as typically expressed on the basis of measured total concentrations. The results of Tenax and cyclodextrin extractions are reported as concentrations in soil in units of mg kg^{-1}. Therefore, no conversion of the results is required, and the outcome of these methods can be directly related to terrestrial toxicity data.

To demonstrate how the above procedures for implementing bioavailability can be used for decision-making concerning contaminated soils, a semi-quantitative case study is schematically presented in Fig. 8.

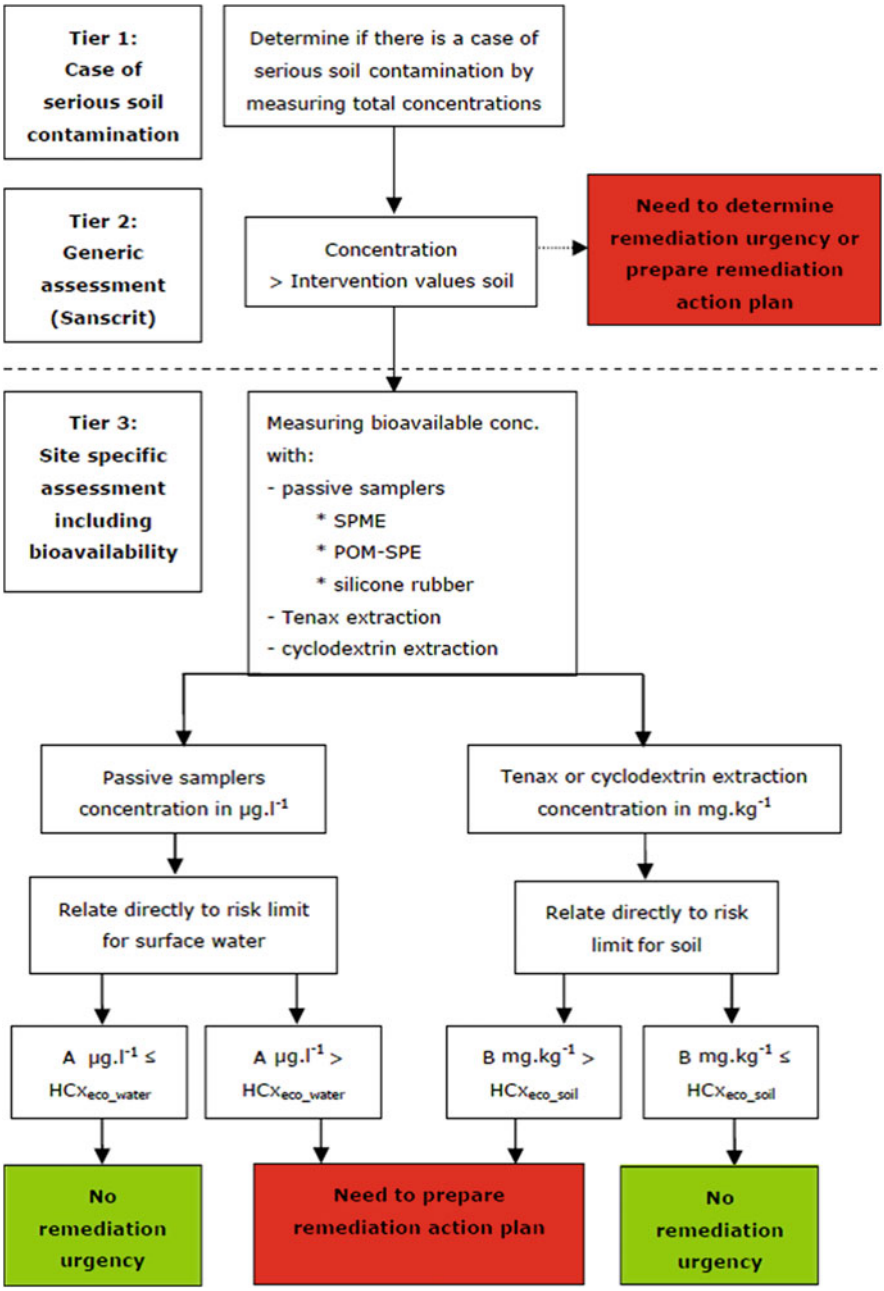

Fig. 8 A hypothetical, qualitative case study exemplifying the procedures for implementation of bioavailability in decision-making concerning contaminated soils. The values A and B refer to the hypothetical analytical results obtained using the methods recommended for measuring bioavailability, using either passive samplers or Tenax/cyclodextrin, respectively. Sanscrit is an example of a decision support system suited to determine the urgency of remediation of contaminated soils [33]

5 Perspective

Some policy questions require further attention to be able to decide on implementation of bioavailability. These include issues like:

- What is the aim of implementation of bioavailability in a regulatory framework? As a matter of course, various aims can be strived for, including a more accurate assessment of subsequent (remediations) activities and a first assessment of the potential costs and benefits of performing a bioavailability assessment, and to do so within the options offered within a specific framework. Important considerations with regard to the need of more accurate bioavailability and cost-benefit assessment are the intended land use and the size of the site to be assessed. Thereupon, feasibility of specific future remediations is to be considered in general terms at this stage. Furthermore, specific in-depth questions need answering, like:
- Is implementation of bioavailability possible within the various approaches that are available to determine the remediation urgency of a contaminated site?
- Is site-specific use of bioavailability approaches possible in sustainable land management?
- Is the implementation of bioavailability as presented in this contribution possible or are there (non-scientific) issues that hamper its use?
  Amongst others, the acceptability of the outcome of the assessment is an important consideration in this respect, especially when interests of stakeholders diverge. Although it is obvious that the science underlying the bioavailability concept and its means of implementation requires continuous updating in order to encompass the latest insights and the latest techniques, the current state of the science is such that this in itself is not likely to be a sound argument in non-scientific discussions on the use of bioavailability.
- What are the consequences of the implementation of bioavailability?
- For future risk assessments and the amount of urgent sites?
- For past risk assessments of contaminated sites?

Answering these questions requires knowledge of the options available to reduce risks and/or to either control or remediate the site in case of unacceptably high risks as determined by the bioavailability assessment. In case of lack of any options or in case the (worst-case) consequences of bioavailability assessment requiring immediate action can on forehand not be implemented, the added value of such an assessment is very limited.

– Is additional information needed to come to a decision on the implementation of bioavailability in the current framework?

Furthermore, some additional scientific underpinning is needed. Amongst others, the following actions will have to be carried out:

1. To investigate the consequences of the required ecological protection limits, in which a distinction needs to be made between the framework of soil remediation and sustainable land management. A crucial issue within risk assessment is the selected risk limit and the level of actual protection provided to ecosystems by this protection level. This immediately raises the following key question: at which risk level is an ecosystem sufficiently protected without having too strict limitation on soil (re)use? This (in part policy-related) issue includes the scientifically relevant research question of actually occurring adverse effects in highly complex ecosystems and requires field validation of the environmental realism of the risk limits set.
2. To determine whether to use potentially bioavailable concentrations or actually bioavailable concentrations (or a combination of both) in prospective and in retrospective risk assessment.

   – In prospective risk assessment, this question boils down in selecting the optimal combination of methods suited for assessing actual and potential bioavailable concentrations and selecting the proper reference framework as explained above.
   – With regard to retrospective assessment, this issue could be different between the framework of soil remediation and sustainable land management. More information and more insights need to be generated about the relationship between effects on an ecosystem and the actually or potentially bioavailable concentrations. 'Actual concentrations' represent current risks, whilst the 'potential concentrations' also address potential risks in the future. Again considering the differences between the framework of soil remediation and sustainable land management, a proposal is needed on how to integrate actually bioavailable and potentially bioavailable concentrations.

Clearly, the quest for optimal risk assessment based on proper incorporation of bioavailability and based on a thorough evaluation of the linkage between measured bioavailable concentrations and actually occurring adverse impacts on ecosystems that are typically affected by a large number of external and internal stressors is by far not finished yet.

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Concluding Remarks and Research Needs

Jose J. Ortega-Calvo and John R. Parsons

Contents

Abstract A key motivation for this handbook arose from the different perceptions of scientists and regulators of the scientific advances made during the last 30 years in the field of bioavailability of organic chemicals in soil and sediment. This last chapter provides a distillation of the general messages extracted from the individual chapters from this handbook, by answering the following questions: (1) Is bioavailability science ready for use in regulation? (2) How should bioavailability be measured? And (3) how should it be implemented? We conclude the chapter with the research needs covering the knowledge gaps that still remain after this effort, focusing on the methodologies for measuring bioavailability, the environmental risks of non-bioavailable compounds, and the innovative remediation of contaminated waters, sediments, and soils.

Keywords Communication, Methods, Regulation, Remediation, Risk

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Over the last 30 years, numerous studies have established a solid knowledge about the bioavailability of organic chemicals in soil and sediment. However, this knowledge has not always been used to provide relevant and measurable data to support risk assessment (RA) and remediation. This uncertainty and the missing integrated approach for implementation have fueled the reluctance of the regulatory/RA community to include bioavailability within RA and management procedures and, ultimately, legislation. It is important to be aware of the differences between scientific and regulatory perceptions; these differences served as the key motivation for this handbook. In regulatory decision-making scenarios, a greater degree of clarity and predictability and, perhaps, greater simplicity are required compared with those required in science. However, other factors influence the decision-making process by regulators, for example, the costs, the uncertainties, the purpose of the site to be used, who is responsible, and whether the adapted risk results in any damage. Scientific developments need specific adaptation measures, leading to practical approaches ready to be used in this context.

For bioavailability to be accepted by environmental regulators and incorporated into RA frameworks for organic chemicals, three questions must be addressed: (1) Is bioavailability science ready for use in regulation? (2) How should bioavailability be measured? And (3) how should it be implemented? We honestly think that this handbook, contributed by key actors in their respective research fields, gives updated answers to all of them. Let us summarize below a few of the general lessons learnt already and propose, on the basis of these, the knowledge gaps that still remain after this effort.

1 Is Bioavailability Science Ready for Use in Regulation of Organic Chemicals?

Our conclusion is yes. Generally, the three major coordinates defining in the chemical space of bioavailability are the physicochemical characteristics of the chemical(s), the composition of the soil/sediment matrix, and the ecophysiological, morphological, and metabolic complexities of the organisms. Making special emphasis on the latest advances from the last 5 years, these coordinates have been examined in this handbook, either by focusing on the chemical distribution in soil and sediment (Sect. 1), on bioaccumulation (Sect. 2), or toxicity, persistence, and remediation (Sect. 3). All of these contributions share a common message: total pollutant concentrations lead to overestimation of risks, but more realistic assessments can be done by incorporating bioavailability.

As a starting point in the present handbook, the bioavailability concept considered the importance of an organism's cell membrane [1]. Only the molecules of the chemical that can interact with or pass across a biological membrane are considered to be bioavailable. In the integrated bioavailability approach for implementation into the RA and management of contaminated systems, the following considerations are relevant: (1) organic chemicals are sorbed to soil/sediment, and sorption becomes

stronger with time (aging); (2) desorption and remobilization from these sites will take more time; therefore, putative toxicity will decrease; and (3) only the rapidly desorbing molecules in the soil or sediment and the molecules of the contaminant that are dissolved in water can interact with the organism and are indicators for bioavailability. In that concept, we considered that slowly desorbing chemicals are not bioavailable over a relevant period. This consideration is a simplification in terms of science but less so in practice. The time scale of bioavailability is the crucial factor in this regard. Most of the existing methods are designed for short time scales (days or weeks at most) that do not represent potential long-term organism exposure that could occur by slow desorption and bioaccumulation. However, this simplification is powerful because it enables the regulator to prioritize risks. The reader of this handbook may perceive that the concept has stood up well during the last 5 years, even observed it to be a precedent for an ISO standard on bioavailability, published in 2018 [2]. Long-term desorption can be predicted using kinetic models possessing (at least) two compartments and considering rapid and slow desorption in parallel, as well as gradients of the chemical activity and sinks [3]. We recognize, however, that the potential long-term persistence and impact of slowly desorbing chemicals is a research issue that must be considered in the future, as it is explained below.

2 How Should Bioavailability of Organic Chemicals Be Measured?

One section of this handbook, consisting of three chapters, provides a comprehensive update of the different chemical and biological methods in this regulatory context. They also determine whether the current set of methods requires improvements to evaluate a wide range of contaminants and bioavailability scenarios. The existing chemical methods include the use of mild solvents and infinite polymer sinks to extract the bioavailable fractions of contaminants and the use of passive samplers to measure the freely dissolved concentration (C_{free}). Some of these methods have been recently standardized and adopted in guidelines [2, 4]; however, some debate remains about their respective advantages and disadvantages when applied, respectively, to soils and sediments. Biological methods employ a variety of organisms (including vertebrates, invertebrates, plants, and microorganisms) to measure the accumulation, effects, and biodegradation of chemicals as measures of their bioavailability. Typically, chemical methods focus on individual chemicals or specific classes of chemicals, whereas biological methods commonly integrate the effect of mixtures of chemicals to which an organism is exposed. A representative number of bioassays should be used to facilitate standardization and broad adoption. In particular, transparent criteria, commonly defined beforehand by risk assessors and stakeholders, must be used when selecting the most appropriate biological test methods. The methods and approaches available for the implementation of

bioavailability in RA must be applicable to large sets of structurally different contaminants and their mixtures common in the environment. Fate and effect models need to be evaluated that relate the available fractions of organic contaminants to actually occurring adverse effects and internal concentrations of the contaminants in the biota.

3 How Should Bioavailability Be Implemented into Regulation of Organic Chemicals?

A weight-of-evidence approach should be used to include the results of tests on bioavailability in decision-making. To date, the TRIAD approach (a subject of standardization within ISO), which consists of three lines of evidence, namely, environmental chemistry, (eco)toxicology, and ecology, represents the most consistent approach. In different tiers that follow, a decision is made on whether further investigation is necessary. According to this scheme, bioavailability can be included at a higher tier to provide additional site-specific data. Under the new paradigm proposed generally in this handbook, and discussed in detail in the last chapter, bioavailability should be part of a second-tier of assessment.

The implementation of bioavailability science into RA will also require the identification of the most important communication bottlenecks in incorporating bioavailability science in RA, in coordination with relevant stakeholders. The projections of bioavailability research will specially impact the prospective RA scenarios that address the approval and regulation of new organic chemicals, where bioavailability is completely absent. To introduce a more realistic system of RA based on bioavailability, clear advantages for regulators and industry must be apparent. Industry tends to minimize incidental negative effects, whereas regulators have a more precautionary approach. These efforts should provide the best case studies of the implementation of bioavailability in legislation. The conditions needed for bioavailability to be implemented within regulatory frameworks (at the national or international level) in a manner that it is workable and logical for industry should be identified.

4 Research Needs in Bioavailability

From the point of view of implementing bioavailability into regulation, three major research areas can be identified:

1. Methodologies for measuring bioavailability. Although standard biological and chemical methods are already in use, method development and refinement are in progress. New or modified methods may be required to consider new and/or emerging contaminants and pollutant mixtures. Research is needed to determine the adequacy and required improvements of the current methods to evaluate a

wide range of environmental conditions and contaminants (including polar and ionic chemicals): How should chemical methods based on extraction and passive sampling methods based on the C_{free} concept be used in a complementary manner, and how do these fit into biological measurements? An analysis is needed on the similarities and the pros and cons of the different methods used for measuring bioavailability, delivering comparison tools and pathways for standardization (ISO, CEN). These research efforts should also define "quality assurance/quality control" with ring trials, the definition of figures-of-merits, and good laboratory practices. The time scale that is relevant for bioavailability measurements should also be determined, as well as the comparability of methods applied in different laboratories, the need for reference materials with known bioavailable and total pollutant concentrations, and the bioavailability component of mixture toxicity.

2. Environmental risks of non-bioavailable compounds. The potential limitations of the focus on relatively short-term bioavailability measures need also to be explored. There is a risk that regulators may hesitate to integrate the concept of bioavailability into RA. This risk is most likely associated with uncertainties about long-term predictions, such as the possibility of desorption-resistant pollutants becoming available in the future. Therefore, this major scientific gap should be covered by the development of methods that challenge desorption-resistant fractions, enabling the possibility of long-term predictions. Topics foreseen are how to reliably characterize non-bioavailable and non-extractable residues (NBRs and NERs, respectively), developing methods for parameterization of kinetic models and long-term predictions for bioaccumulation, biodegradation and toxicity for a variety of compounds and matrices, the stability and reversibility of the aging process and NER formation, and the time scales that should be considered. Temporal bioavailability shifts should also be evaluated, providing suggestions on how to address these shifts using established methods (e.g., monitoring). How bioavailability increases should be considered in RA, for example, by resuspension of bound substances, changing environmental conditions due to climate change, and biota-driven shifts in bioavailability.
3. Bioavailability in the remediation of waters, sediments, and soils. An evaluation is needed for the most innovative technologies for using bioavailability to reduce the risk from pollutants, for example, by immobilization and modern biological treatment. A systemic perspective on bioavailability in RA would be part of this evaluation, for example, to determine whether bioavailability to some (micro)organisms helps to reduce bioavailability to other organisms and the long-term safety of remediating polluted soils and sediments by inducing the formation of NBRs and/or NERs or by promoting bioremediation to exhaust the bioavailable contaminant pool.

5 Conclusions

Currently, risk characterization, which is based on total contaminant concentrations, is a conservative approach that minimizes liability. In some cases, no actual risks are associated with the presence of contaminants at elevated total levels that are

non-bioavailable. Despite the recent shift to a more risk-based assessment strategy, which is beneficial to a large number of stakeholders, such as regulators, industries, and owners of contaminated sites, the implementation of bioavailability knowledge in a more pragmatic, site-specific approach remains uncommon. However, fully realistic impact and risk assessments must include the consideration of bioavailability. For enabling sound regulatory decisions, the concentration of a chemical present in soil or sediment that is bioavailable for uptake from the environmental matrix and that can cause adverse effects to biota within a given period must be explicitly determined. Moreover, these decisions must rely on measurements obtained using established and, preferably, standardized methods. These standardized methods will enable the adoption of bioavailability concepts independent of the specific matrix composition, the contaminants, and other constituents in more complex mixtures found at some contaminated sites. These methods will also enable the development of model approaches to predict bioavailability, based on the physicochemical properties of the compound and on matrix characteristics.

As the scientific knowledge of bioavailability continues to grow, the potential for implementation of an integrated approach is expanding. Given the significance of the chronic exposure of biota to individual organic chemicals and chemical mixtures, such approach to account for general bioavailability in decision-making is vital to properly protect the environment and human health. Thus, the implementation of bioavailability will save financial and societal resources by using a real risk-based approach. This approach will enable a more appropriate assessment of the environmental risks of chemicals and the prioritization and design of improved remediation measures and will have an enormous impact on the sustainable protection of the environment.

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