Residual hydrophobic organic contaminants in soil: are they a barrier to risk-based approaches for managing contaminated land?

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List of Abbreviations

BR	Bound Residue
CSM	Conceptual Site Model
DCM	Dichloromethane
DCM/ACE	Dichloromethane/Acetone
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EET	Exhaustive Extraction Technique
EHRA	Ecological Health Risk Assessment
ER	Extractable Residue
EVA	Ethylene Vinyl Acetate
GI	Gastrointestinal
HHRA	Human Health Risk Assessment
HOC	Hydrophobic Organic Contaminant
HPCD	Hydroxypropyl-β-Cyclodextrin
HPLC-FD	High-Performance Liquid Chromatography with Fluorescence Detection
MGP	Manufactured Gas Plant
NEET	Non-Exhaustive Extraction Technique
NEPM	National Environment Protection Measure
NER	Non-Extractable Residue
OC	Organic Carbon
PDMS	Polydimethylsiloxane
PED	Polyethylene devices
POM	Polyoxymethylene
RA	Risk Assessment
RBA	Relative Bioavailability
RBLM	Risk-Based Land Management
SARA	Soil-sediment Availability Ratio
SBE	Silicon-Based Extraction
SFE	Supercritical Fluid Extraction
SGV	Soil Guideline Value
SOP	Standard Operating Procedure
SPE	Solid Phase Extraction
SPMD	Semipermeable Membrane Devices
SPME	Solid Phase Micro-Extraction
TR	Total Residue
USEPA	United States Environmental Protection Agency

Abstract

Risk-based approaches to managing contaminated land, rather than approaches based on complete contaminant removal, have gained acceptance as they are likely to be more feasible and cost effective. Risk-based approaches aim to minimise risks of exposure of a specified contaminant to humans. However, adopting a risk-based approach over alternative overly-conservative approaches requires that associated uncertainties in decision making are understood and minimised. Irrespective of the nature of contaminants, a critical uncertainty is whether there are potential risks associated with exposure to the residual contaminant fractions in soil to humans and other ecological receptors, and how they should be considered in the risk assessment process. This review focusing on hydrophobic organic contaminants (HOCs), especially polycyclic aromatic hydrocarbons (PAHs), suggests that there is significant uncertainty on the residual fractions of contaminants from risk perspectives. This is because very few studies have focused on understanding the desorption behaviour of HOCs, with few or no studies considering the influence of exposure-specific factors. In particular, it is not clear whether the exposure of soil-associated HOCs to gastrointestinal fluids and enzyme processes release bound residues. Although, *in vitro* models have been used to predict PAH bioaccessibility, and chemical extractions have been used to determine residual fractions in various soils, there are still doubts about what is actually being measured. Therefore it is not certain which bioaccessibility method currently represents the best choice, or provides the best estimate, of *in vivo* PAH bioavailability. It is suggested that the fate and behaviour of HOCs in a wide range of soils, and that consider exposure-specific scenarios, be investigated. Exposure-specific scenarios are important for validation purposes, which may be useful for the development of standardised methods and procedures for HOC bioaccessibility determinations. Research is needed to propose the most appropriate testing methods and for assessing potential risks posed by residual fractions of HOCs. Such investigations may be useful for minimising uncertainties associated with a risk-based approach, so that consideration may then be given to its adoption on a global scale. This review critically appraises existing information on the bioavailability of HOC residues in soil to establish whether there may be risks from highly sequestered contaminant residues.

Keywords: Risk-Based Approach, Residual HOC Fractions, Bioavailability and Bioaccessibility, Sorption-Desorption, Standard Operating Procedures, soil-HOC-organism interactions

1. Introduction

“To forget how to tend soils is to forget ourselves” (Mahatma Gandhi).

Chemical contamination of soils is a global problem [1] and is arguably of similar significance as other major environmental challenges such as climate change and biodiversity loss [2]. Sustainable soil management has a vital role for addressing the challenge of widespread contamination, especially with regard to attaining the United Nations ‘Sustainable Development Goals’ [3]. Among the anthropogenic contaminants of soils, hydrophobic organic contaminants (HOCs) require attention as they are persistent, they bioaccumulate, and are toxic and potentially carcinogenic [4-6]. Examples of important HOCs include PAHs, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), dichloro-diphenyl-trichloroethane (DDT) and other chlorinated pesticides. The risk of soil contamination by HOCs is a matter of great concern for human and ecological health [7]. Human exposure to HOCs in soils may be by oral ingestion, inhalation, and/or dermal routes [7, 8]. Ingestion has been regarded as very important for HOCs, such as the PAHs [7, 9].

Assessment of HOC contamination in soils is currently based on the measurement of total concentrations and the assumption that the measured HOCs are 100% bioavailable [10-13]. It has been reported that total contaminant concentrations in ingested soil or food do not provide a good measure of the contaminant’s absorption via the organism’s gut membrane or that absorbed into its systemic circulation [11, 14-18]. This may be due to the interactions between the contaminant and the soil. Wide variations in soil and HOC properties may significantly affect bioaccessibility (bioavailability + potential bioavailability) of HOCs in humans [14, 19-21]. For example, when HOCs enter soil, sequestration processes such as diffusion of molecules into inaccessible micro- and nano-pores, as well as sorption to soil components, are known to affect bioavailability and bioaccessibility [19, 22-27]. Consequently, varying soil-organism-HOC interactions may influence the significance of potential harm that may result from exposure. Hence, the bioaccessible contaminant concentration rather than the total concentration in soil is more important for realistic risk assessment (RA) purposes.

Risk-based approaches to contaminant management and remediation offer feasible remediation practices in that they recognise that complete removal of a contaminant is likely to be technically very difficult, expensive, and sometimes unnecessary [28-31]. Risk-based approaches need to be underpinned by a thorough understanding of the chemical behaviour of HOCs in soils, and in particular definition of the fraction of the total concentration that is relevant to biological or environmental impacts, i.e. bioaccessibility. Adoption and application of risk-based approaches face a significant hurdle due to the lack of regulatory recognition of the bioavailability concept which underpins the approach, although it is perceived as an important concept for RA within certain sections of the regulatory [32] and scientific [11-13, 15, 33] communities. This is related to the lack of standard operating procedures (SOPs) for measuring the bioavailable fractions of HOCs in soil, as such measurements are still in their infancy. Although validated SOPs for bioavailability of thoroughly studied inorganic contaminants such as lead in soil have gained regulatory approval and are currently in use in the USA [34], there is no generally accepted SOPs for determining HOC bioavailability in soil. In addition, information on the bioavailability and risks associated with 'residual' HOCs in soil is limited.

Although bioavailable fractions of HOCs are by definition of most concern in assessing their environmental and health risks in soil, it is yet to be demonstrated conclusively whether residues that remain following removal of the bioavailable fractions pose ongoing risk from long-term exposure, especially in historically contaminated soils [29, 35-39]. In the absence of conclusive evidence regarding potential risk arising from long-term exposure to residual fractions, the adoption of the risk-based approach for HOCs is often challenged. There have been arguments regarding the significance of non-extractable residual (NER) fractions, defined in this review as highly sequestered residues, to ongoing risks in the long term [35, 36, 39-45], because changes in environmental or exposure conditions may mobilise residual pools in soil [30, 43, 46-48]. A number of papers have argued that long-term exposure to NER fractions in soil poses little or no risk because of the strong interactions between HOCs and soil components especially organic matter (OM), resulting in very slow remobilisation HOCs, mainly through desorption [25, 26, 30, 31, 43, 49-51]. Desorption rates of HOCs from historically contaminated soils and sediments have been reported to be slow or very slow (10^{-2} to 10^{-4} h^{-1}) [26, 49], and with half-lives that could last decades [50]. For example, a study reported that the slowly desorbing fraction of ^{14}C-phenanthrene slightly increased between 2 and 10 d in 4 different artificially-spiked soils (4.8 - 10.25 % OM), with the soil richest in OM showing more pronounced effects with ageing [52]. Other studies have corroborated these findings, with increases in the desorption-resistant (i.e. residual) fraction by up to 2 - 10 fold with ageing [26, 53, 54]. The implication is that with increase in the residual (or strongly-sorbed) fraction as ageing progresses, desorption of contaminants becomes increasingly difficult. Even when desorption does occur, it does so at a very slow rate such that the risk is acceptable, or that no risk is likely to be posed [55]. However, this argument is mainly derived from indirect empirical relationships between contaminant sorption behaviour and soil OM structure [56]. Although the afore-mentioned studies have been useful for understanding soil-HOC interactions on a macroscopic scale, more convincing molecular-level based approaches which directly depicts these interactions are lacking, especially given the current availability of advanced analytical techniques such as the nuclear magnetic spectroscopy (NMR) among others [56-58]. While such advanced approaches may not be needed for routine assessment of residual fractions, detailed investigations of residual HOCs in a wide range of long-term contaminated soils will provide much needed data to regulators on potential long-term risks arising from such fractions remaining in soils.

In contrast, it has been argued that long-term exposure to NER in soil may be harmful to receptors. For instance, enhanced PAH mobilisation has been speculated in long-term aged (50 years) soils from manufactured gas plant sites (MGP) under anoxic conditions and other changing environmental conditions such as temperature [55, 59, 60]. Some studies also reported that higher organisms may have the capacity to access HOC residues, which were hitherto classified as inaccessible by microbial processes; this is due to differences in uptake mechanisms and exposure conditions such as gut conditions of specific organisms, including humans [38, 47, 61-65]. However, none of these approaches would be a measure of the bioaccessible fraction. Mayer et al. [65] also noted that it was important to investigate the potential availability of strongly-sorbed contaminant fractions to non-microbial receptors such as humans using *in vitro* gastrointestinal (GI) models, to further support the consideration of bioavailability and the risk-based approach within regulatory structures. In addition, it has been advised that regardless of whether risk-based land management (RBLM) approaches are adopted, monitoring will be useful to check contaminant remobilisation should there be changes in land use or environmental conditions [32]. Such an approach is commonly used as part of monitored natural attenuation of groundwater or contaminated soils [66-73].

These contrasting arguments raise the questions:

(a) should RA be about the present, rather than the future?
(b) what are the residual and bioaccessible HOC fractions in soils?
(c) how they should be defined, classified, and measured? and
(d) whether remobilisation of residual HOCs in soil and its associated potential risks are exposure-specific, for example, upon contact of residues with the GI fluid.

To date, there have been only a few published studies that have reported potential remobilisation of HOC residues in long-term aged or historically contaminated soils, and potential risks to receptors [50, 55]. These studies have mostly focused on ecological receptors, especially microbes and earthworms. Other sensitive receptors, especially those that simulate human physiology, are yet to be studied. The focus of much of the studies using animal models has been the assessment of bioavailable fractions rather than the more tightly bound residual fractions. While there have been studies that have attempted to elucidate soil-contaminant relationships using advanced molecular based techniques, such as the NMR, they have not focused on the dynamics of release or retention of residual HOCs in soil with respect to the combined effects of ageing, varying soil properties, environmental conditions and realistic exposure scenarios, or the controlling mechanisms [56, 58].

To minimise uncertainties associated with risk-based approaches, understanding the influence of soil properties, ageing and exposure-specific factors on the potential release and harmful effects of HOC residues in soil is necessary. Importantly, understanding the mechanisms controlling underlying processes, especially at a molecular scale, may complement previous empirical relationships describing the fate and behaviour of HOCs in soil. Within the context of RBLM approaches for soils contaminated with HOCs, the objective of this article is to critically review existing information on the bioaccessibility of HOC residues in an attempt to establish whether there is a need to further investigate potential, or the likelihood of harm being caused by highly sequestered residues in soil.

2. Fate and behaviour of HOCs in soil

The fate and behaviour of HOCs, such as PAHs, in soil has been studied for more than two decades [21, 29, 74, 75]. Following their addition to soil, PAHs are subjected to various physio-chemical, biological, and environmental processes that are summarised in Fig. S1 in the supplementary material [21, 29, 74, 76, 77]. In the longer term, PAHs in soil are subject to the 'ageing effect' [19, 22, 75, 78-81] that results in significant partitioning of PAHs within the soil leading to a reduction in mobility, extractability (bioaccessibility), and bioavailability. Over time, HOC in soil becomes less available to exposed biological membranes (Fig. 1). The ageing effect is due to the increasing HOC interactions with heterogeneous soil matrix components, including mineral and organic matter and their associated pore structures and spaces [27, 82, 83]. Persistent HOCs diffuse into micro- and nano-interstitial spaces within the soil matrix and interact through a number of potential physical and physiochemical processes with soil components [25, 27, 54]. The behaviour is termed 'sorption' and results in weak or strong HOC retention in soil [22, 82], depending on the key soil properties that influence the sorption at a particular time [26, 27, 75, 84-89]. Not only do soil-related properties influence sorption, but the physicochemical properties of the HOCs (Table S1 in supplementary material) may also influence the process as well [26, 89]. In addition, the degree of hydrophobicity or aqueous solubility may indicate whether HOCs associate with the organic or mineral solid phases of the soil matrix, and could influence associated rates and extents of sorption and desorption in soil.

2.1. Mechanisms controlling sequestration of HOCs in soils: Implications for mobility and bioaccessibility

The heterogeneity of soils at macro- (aggregate) and micro-(particle) scales may influence HOC accessibility and potential risk upon biological exposure [19, 27, 82, 85, 87, 88, 90]. However, modifying processes may influence risks due to exposure (Fig. S1 in supplementary material). Sorption and desorption play major roles in controlling HOC bioaccessibility in soil [26, 27, 60, 82, 91, 92]. Soil organic matter and mineral heterogeneity play significant roles in dictating mechanisms of sorption and desorption of contaminants, as they may control rates of diffusion of the contaminants in the soil matrix [27, 93].

Isotherms fitted to sorption models (e.g. Freundlich and Langmuir, among other models) have shown that sorption is mostly non-linear as solution concentrations increase [85]. The non-linearity index (n) is reported as less than unity, indicating heterogeneity of sorption sites in soil [88, 94]. Two sequestration phases are likely within soil, i.e. external (as influenced by advection and diffusion) and internal (influenced by diffusion and adsorption) [82]. While the former is unlikely to be rate-limiting in determining slow sorption and especially slow desorption, the latter is likely [27, 82, 84, 85, 95, 96]. In a particle of soil, a HOC molecule could be small enough that it fits into minute pore spaces, following diffusion through the dissolved water phase. Such a tortuous journey occurs with increased ageing such that contaminant remobilisation is unlikely [84], unless influenced by changing environmental conditions. Soil conditions such as absence of oxygen, increased temperature, and soil mixing may potentially influence the release of previously sorbed HOCs via a tortuous and reverse-phase journey (i.e. from the sorption sites within the soil matrix back to the dissolved water phase or soil surface) [59, 60, 97].

Pignatello and Xing [82] explained that to better understand mechanisms of slow sorption, it was important to consider the following:

(a) That mass transfer processes including associated rate constants differ tortuous diffusion. This may be related to the diverse activation energies associated with different sorption sites within the soil matrix [84, 85, 98];

(b) It appears that sorption becomes stronger with decreasing sorbate concentration. Sorption equilibrium of HOCs in soil is concentration-dependent [99, 100]; and

(c) The kinetic-hysterical nature of sorption-desorption processes, whereby the rate constants during slow sorption appear greater than slow desorption for the same HOC and soil. Hence, the rate and extent of formation of strong HOC-soil bonds becomes faster compared to breaking such bonds [84, 85].

Models describing sorption and desorption mechanisms include diffusion in organic matter, sorption-retarded pore diffusion, intra-particle organic matter diffusion, pore deformation, and pore swelling, among others. These mechanisms may be stand-alone or synergistic in different situations; detailed reviews have been presented elsewhere [27, 38, 39, 82, 101-104].

Many researchers have focused on sorption and associated mechanisms. Of more importance, however, is desorption which dictates contaminant mobility, accessibility and potential risks. Hence, understanding desorption of HOCs in soil, especially from residual pools, including associated mechanisms is important. Nonetheless, because of the interplay between both processes in defining rates and extents of HOC release from soil, and hence, potential risks, both processes are discussed in detail in subsequent sections.

2.1.1. Sorption of HOCs by soil and contaminant pool classification

Sorption plays an important role in influencing HOC bioavailability, and hence, associated mobility, transport, loss, and biological effects such as bioaccumulation, biomagnification and biotransformation [82, 84, 105, 106]. HOC pools in soil can be segregated into fractions ranging from that which is weakly sorbed to the strongly sorbed [22, 27]. The continuum may comprise dissolved, weakly sorbed and rapidly desorbed, the slowly desorbed, and the strongly bound fractions (Fig. 2). Although, this contaminant sorption continuum is a simplification considering the complex and dynamic nature of soil-contaminant interactions, it helps understanding of complex processes [25, 54, 82]. These pools presents different barriers for HOC desorption, mobility, and availability. For example, PAHs in dissolved and weakly sorbed (rapidly desorbing) pools are likely to be readily accessible to exposed organisms [10, 12, 15, 107, 108]. However, the strongly sorbed and bound (slowly desorbing) pool is presumed to be less bioavailable [43, 50, 109].

Earlier studies have mostly focused on using sorption isotherms (e.g. Freundlich and Langmuir) and distribution coefficients (K_d) in describing the mostly non-linear HOC sorption behaviour in soil [85, 110]. These have resulted in proposing empirical relationships between distribution and partitioning coefficients (Log K_d *or* Log K_{oc}) of different HOCs and soil properties [88, 94, 111-114]. However, the sole use of Log K_{oc} or octanol-water partition coefficient (Log K_{ow}) to describe HOC sorption in soil should be used with caution, as other intrinsic soil properties such as SOM-clay mineral interactions may play greater roles in influencing sorption processes [113, 115]. In addition, while these empirical relationships are valuable and have formed the basis for emerging investigations, inferences regarding the relationship between soil properties and HOC sorption behaviour have been based on indirect observations [56].

Of the current advanced analytical techniques, NMR has been reported to provide mechanistic information on molecular-scale soil-contaminant interactions at the solid, liquid, and soil-water (gel) phases [56, 57]. In many NMR investigations, the soil is physically or chemically fractionated into operationally defined pools in an attempt to obtaining direct molecular information about SOM composition [57, 116-120] and understand precise HOC sorption behaviours in soil [99]. Although, the actual nature of the SOM is still contentious [121], these investigations provide detailed understanding on the dynamics of SOM-HOC interactions. However, physical or chemical fractionation is likely to change the behaviour of HOCs. Some NMR investigations of soil-contaminant interactions have been reviewed elsewhere [56, 58]. However, while applications of NMR spectroscopy in soil science are emerging, investigations towards understanding HOC sorption mechanisms especially for historically contaminated soils are still lacking. Therefore, molecular level understanding of the long-term stability of strongly sorbed HOC residues in historically contaminated soils would help minimise uncertainties relating to risk based approaches to managing contaminated soils. Such investigations should employ both empirical and advanced molecular-based techniques in a complementary and multi-tiered manner to provide robust evidence on the long-term stability of highly sequestered contaminant residues in soil.

2.1.1.1. HOC residual pools in soils

The total contaminant pool is made up of potentially labile and non-labile pools (Figs. 1 and 2). While the labile fraction consists of the dissolved and rapidly desorbable fractions, the non-labile component is comprised of the non-desorbable and irreversibly sorbed or recalcitrant fractions [10, 79, 107, 122, 123]. There are no methods for the determination of non-labile residual HOC fractions, especially relating to changing exposure scenarios and estimations of harm. Furthermore, a residue which may not be accessible to microorganisms may be accessible to other living organisms such as earthworms, plants and other higher forms including humans due to differences in the mechanisms of contaminant uptake and release [32, 62, 124]. Specifically in relation to HHRA, that fraction of the mobilised or dissolved contaminant in the gut, following ingestion, which is absorbed and may cause harm is the bioavailable fraction [28] and the un-mobilised fraction is referred to as the residue [125]. However, there seems to be confusion regarding what the residual fraction in soil really is. It could be the fraction remaining after the complete release of the labile fraction (measured by an *in vitro* technique) or the residues from historically contaminated soils. The NER, especially in EHRA, is mostly regarded as the fraction that is left behind after solvent extraction [126]. Tao et al. [61, 127] suggested that the difference between the concentration of PAH and organochlorine pesticides mobilised by an *in vitro* digestion model (which was greater) and that extracted by accelerated solvent extraction represents a part of the residual fraction. On the other hand, Juhasz et al. [62] suggested that the residual fraction was that which remained after long-term ongoing bioremediation of a field-contaminated soil; this description appears similar to that in Thavamani et al. [30]. While the former description of the residual fraction may be more relevant for HHRA purposes, the extracting medium should mimic the GI fluid.

A residual fraction from an EHRA perspective may differ from an HHRA perspective with respect to concentrations and specific receptors. Hence, while the use of highly sequestered fraction may be relevant for EHRA purposes [107], its relevance for HHRA purposes must also be demonstrated [19]. To the regulator, a remediated soil is one in which risk from exposure to remaining or residual fraction is reduced to an acceptable level which may depend on ultimate end use.

2.1.2. Desorption of HOCs in soil

2.1.2.1. Slow desorption of HOCs in long-term contaminated soils

The bioavailability of HOCs in soil following exposure is limited by its desorption kinetics [97, 128]. Desorption rates and extents may influence various contaminant loss processes such as biodegradation, uptake and bioaccumulation among others, and are therefore key factors for RA. Carmichael and others investigated whether microbial mineralisation of PAHs in soil is limited by their desorption rates [91]. Using ^{14}C-radiolabelling, they examined the desorption kinetics and microbial mineralisation kinetics of freshly spiked ^{14}C- and aged ^{12}C-phenanthrene and chrysene in two soils from a tar and oil-recycling sites. The key findings were that the rates of desorption were faster than mineralisation rates in ^{14}C-PAH spiked contaminated soils, however, the mineralisation rates of native PAHs in the organic carbon-rich contaminated soil were faster than or equal to their desorption rates. Hence, the rates and extents of PAH desorption in historically-contaminated soils may represent a limitation to their biodegradation. This may be one reason why PAHs persist in microbially-active soils [129]. Based on their results, the sole use of ^{14}C-radiolabels in describing fate and behaviour of PAHs in soil should be applied with caution [91].

One study combined both ^{14}C- and ^{12}C- techniques to monitor the long-term fate and behaviour of HOCs in soil [50]. Extractability was observed to be low and desorption very slow (calculated half-lives of up to 38 years for fluoranthene), which supports the notion that risks from exposure will be minimal [31, 55]. However, it was pointed out that the results were only applicable to contaminated agricultural soils used. They recommended that similar investigations be conducted on historically-contaminated soils from different sources [50]. It is further suggested here that different exposure scenarios and other key risk endpoints, such as earthworm bioaccumulation, plant uptake, and animal-based bioavailability and *in vitro* bioaccessibility assays, should be considered in addition to microbial degradation and chemical extractability for a robust conclusion. For instance, following maximum desorption of labile HOCs in field-contaminated sediments, slow intercompartmental reorganisation and remobilisation of HOCs have been observed [46]. It is also known that gastric, and intestinal fluids especially bile and other intestinal enzymes, can influence the solubility and mobilisation of soil-associated HOCs [61, 125, 130]. Such gastrointestinal effects may influence HOC desorption behaviour from residual pools and their remobilisation. Hence, considering other exposure scenarios, other than microbial-specific ones, may further demonstrate and authenticate the fact that historically aged HOCs in field-contaminated soils are mostly residual and slowly desorbing, and as a result, pose minimal risks. Again, application of direct molecular-level tools in this regard may be useful.

2.1.2.2. Desorption phases of HOCs and associated kinetics in soil

Studies using artificially-spiked and historically-contaminated soils have described HOC desorption in two or three-phases [26, 49, 52, 60, 83, 91, 131-133]. While biphasic desorption comprise both rapid (F_{rapid}) and slowly (F_{slow}) desorbing fractions including associated rate constants (k_{rapid} and k_{slow} respectively), triphasic desorption includes a very slowly desorbing fraction and rate constant ($F_{very\ slow}$ *and* $k_{very\ slow}$). However, considering the heterogeneity and complexity of soil-HOC interactions in the field, it is likely biphasic and triphasic models are simplifications of multiple desorption phases in reality [134].

To describe desorption kinetics of HOCs in different soils and humic materials, various models that assume different desorption phases have been used [26, 49, 83, 134, 135]. The key observations from these studies were that the 3-parameter biphasic (i.e. one that assumes quick equilibration for F_{rapid}) and 3-parameter biphasic polymer diffusion models were recommended as preferable for predicting HOC desorption kinetics in contaminated soils compared to others, although rate limiting site-specific conditions must be considered. Importantly, these studies revealed that rates of slow desorption (k_{slow}) from strongly sorbed pools could range from between 10^{-2} to 10^{-4} h^{-1} [26, 49, 134], depending on stage of SOM diagenesis and hydrophobicity of the HOCs of concern [37, 136, 137]. Generally, k_{slow} was reported to be up to 2 orders of magnitude lower than k_{rapid} [50]. Similarly, the highly condensed soil or humic material (e.g. kerogen-like shale) tends to retain a greater percentage (up to 70%) of the residual HOC fractions [49, 134]. This residual fraction may increase by up to 2 - 10 folds with ageing [26, 53, 54]. These observations support the argument that long-term exposure to NER fraction may pose no risk [31]. However, a complementary molecular-scale approach to providing such evidence for different exposure scenarios is still necessary [56, 57].

2.2. Biodegradation of HOCs in historically contaminated soils

Biodegradation influences the fate and behaviour of HOCs in soil [12, 138]. However, HOCs sequestration happens along with the biodegradation process. Biodegradation is often observed as a biphasic process which occurs in two phases, i.e. fast and slow loss phases. Due to its biphasic nature, biodegradation may not necessarily lead to the total removal of contaminants in soil, but may result in transformations that lead to reductions in initial concentrations, mobility and toxicity of contaminants, thereby, reducing the level of harm to flora and fauna [139-141]. Bacteria, fungi and algae play important roles in the biotransformation and biodegradation of contaminants in soil [142-144]. Generally, for biodegradation to occur, microorganisms with sufficient capacity to degrade the contaminant of concern (i.e. possesses relevant degradation enzymes) should be present in soil; the contaminant must be bioavailable or become bioavailable, and environmental factors must permit the biodegradation process [133, 142, 145, 146]. Microbial mineralisation of HOCs is limited by HOCs' desorption kinetics, especially in long-term contaminated soils, thereby supporting its biphasic nature [91]. Therefore, bioremediation (cost-effective remediation technique relying on optimising contaminant biodegradation) is sometimes constrained due to sorption limitations which limits microbial accessibility to HOCs in aged soils leaving behind residual contamination in soil or sediment [29, 47]. Different mechanisms through which microorganisms circumvent accessibility limitations include attachment to soil surfaces to allow HOCs partitioning from soil into microbial cell membrane, co-metabolism, and production of extracellular enzymes and biosurfactants to enhance HOCs mobilisation from sorbed phases to microbially-accessible spaces in soil [145, 147].

The lingering question is whether the residual contaminant fraction (i.e. the fraction that is not biodegradable or extractable/mobile/bioaccessible) in soil could potentially cause harm? With regards to biological uptake of contaminants, microbial inaccessibility of HOCs in historically contaminated soil may not mean inaccessibility by other exposed higher organisms, as contaminant accessibility is species and matrix dependent [62, 133]. A contaminant must desorb or be bioaccessible to allow biological uptake. Hence, like sorption/desorption processes, different soil and organism properties may modify contaminant uptake in soil. Other abiotic loss processes such as volatilisation, photolysis, leaching, and chemical transformations may also affect fate and behaviour of HOCs in historically contaminated soils (Fig. S1 in supplementary material). However, these effects are mostly insignificant compared to losses by biodegradation, especially for very hydrophobic HOCs [29].

3. Bioavailability and bioaccessibility of HOCs in soil

The concept of HOC availability has increasingly attracted diverse discussions within the scientific and regulatory community [11, 107, 148]. The concept has been used differently in specific fields such as toxicokinetics, pharmacokinetics, agriculture, and environmental science among others [11, 22, 77, 149]. The terms 'bioavailability' and 'bioaccessibility' have mostly been used interchangeably in the literature [150, 151], within EHRA and HHRA. However, the terms are different but related measures [15, 77]. Specifically for HHRA, Hack and Selenka [125] and Ruby et al. [151] considered the bioaccessible contaminant fraction as the dissolved and mobilisable fraction within simulated GI fluid, following ingestion of HOC-laden soil. This bioaccessible fraction, therefore, represents the contaminant fraction maximally available for intestinal absorption (Fig. 3). For EHRA, the different definitions proposed for bioavailability and bioaccessibility has been compared and reviewed elsewhere [77, 149].

A quick citation analysis of the definitions by Peijnenburg and Jager [152], Semple et al. [15] and Reichenberg and Mayer et al. [153] which are mostly used for EHRA is presented in Fig. 4. The analysis was performed by comparing the number of times each definition was cited as revealed from Web of science, Scopus, and Google Scholar on 02 December 2015. The definitions provided by Semple et al. and Reichenberg and Mayer had been more cited. However, this analysis does not suggest actual uses of the bioavailability definitions within the scientific and regulatory communities. The recent European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) workshops [35, 36, 107] adopted the bioavailability and bioaccessibility concept by Semple and others [15]. Recently, the mostly used definitions of contaminant availability in RA were articulated [10], as shown in Fig. 5. Hence, bioavailability and bioaccessibility in this article and with respect to EHRA follows definitions by Semple et al. and Reichenberg and Mayer.

3.1. Measurement techniques for bioaccessible and residual HOC fractions in soil

Various techniques have been developed for HOC bioavailability or bioaccessibility measurement, and are presented in Table 1. However, there is no consensus on the choice technique. While it has been documented that a few methods are being standardised for ecological receptors such as microbes [10], there is an urgent need to develop and validate SOPs for HOC bioaccessibility in soil, especially for HHRA.

Ultimately, a biological experiment is needed to study HOC bioavailability in soil. For example, if humans are the most sensitive receptors for a particular RA procedure, then a human or higher vertebrate model will be most appropriate test organism. However, this approach is unethical and in fact, there have been moves away from animal-based testing [154, 155]. Also, animal-based studies are expensive, time consuming, and may have challenges with reproducibility [33]. Hence, over the past few decades, focus has shifted to development and validation of simple and complex *in vitro*, including *in silico*, approaches for contaminant bioavailability or bioaccessibility assessments. Ideally, the expectation for these *in vitro* approaches are that they serve as uncomplicated bioavailability surrogates for the environmental receptor of concern. This implies that for any realistic applications in RA, these alternative approaches must be validated and standardised. However, while much efforts has been focused on the development side, less efforts has been devoted to validation of *in vitro* approaches for use in reality. It is therefore suggested that while development of innovative bioavailability techniques are necessary, validation and standardisation of existing promising *in vitro* methods are important for minimising uncertainties in RA.

3.1.1. Brief classification of techniques

The approaches for determining bioavailability and bioaccessibility can either be classified as biological (*in vivo*), chemical extraction (*in vitro*) or computer modelling (*in silico*). A clear distinction has to be made between the actual bioaccessible fraction and the residual fraction as has been previously emphasised. Most currently developed *in vitro* methods have focused on microbial degradation and earthworm bioaccumulation as ecological endpoints, hence, various biodegradation and ecotoxicity assays are available [30]. However, significant efforts at developing *in vitro* bioaccessibility assays for HOCs, such as PAHs, in soil especially for HHRA is only beginning to gain attention.

A detailed review of simple chemical extraction techniques for measuring bioaccessibility for specified ecological endpoints can be found elsewhere [12, 77, 133, 156, 157]. Some of these methods include Soxhlet extraction, solid-phase extraction (SPE), negligibly depleting solid-phase micro-extraction (nd-SPME), hydroxypropyl-β-cyclodextrin (HPCD) extraction, supercritical fluid extraction (SFE), use of complexation or solubilisation agents and surfactants, microwave extraction, persulphate oxidation, thermodesorption, soil-sediment availability ratio (SARA), use of polyethylene devices (PEDs), semipermeable membrane devices (SPMDs), polyoxymethylene (POM) sampler, and thin ethylene vinyl acetate (EVA). While the *in silico* approaches for bioaccessibility calculations and predictions with computer-based programs could be potentially useful [158], significant developments may be limited by the quality and quantity of reliable bioavailability data from laboratory or field experiments.

None of the methods summarised above provide a clear measure of residual fraction. Given this scenario, the most likely approach to assessing potential risks arising from such a fraction may be via toxicological studies that expose sensitive receptors to such fractions. Whether these highly sequestered fractions do get released in the gut or are bioaccumulated in organisms including earth worms or plants may then be investigated.

3.1.2. Exhaustive extraction techniques (EETs) versus non-exhaustive extraction techniques (NEETs)

The feasibility of using chemical extractions to predict the bioavailability or bioaccessibility of HOCs and to determine associated residues in soil has been proposed and widely discussed. Mostly, apolar and polar solvents of varying extraction strengths have been used. Harsh extractions (or EETs) use strong solvents such as dichloromethane (DCM), hexane, acetone, and toluene [159]. Mild extractions (or NEETs) make use of weaker solvents (or mild solvent - water mixture) such as methanol, *n*-butanol or propanol [160]. The extraction with EETs and NEETs simply involves thorough mixing of a contaminated soil sample with solvent for a given duration. Analysis of the extract is then performed by chromatography (HPLC or GC), mass spectrometry, spectrophotometry, or radio-isotopic analysis [22, 133, 159, 161, 162].

Exhaustive extractions generally overestimate the bioavailable contaminant fractions. As a result, they are over-predictive of actual risks and often seen as too conservative and as a consequence are likely to result in a technically difficult and expensive remediation of contaminated sites [75, 159, 161, 163, 164]. The effect of ageing (120 d) on HOCs extractability was unchanged using DCM, while it is well known that ageing results in a decrease in bioavailability of HOCs in soil [161]. Kelsey et al. [159] showed that DCM extractions failed to predict the availability of phenanthrene and atrazine to specialized bacteria and earthworms over a 120 d period, while better prediction was achieved when mild extractions (*n*-butanol and methanol-H_2O) were used [159]. Similarly, a strong correlation was reported between butanol extractability and microbial bioavailability for phenanthrene (R^2 = 0.971; y = 0.952x + 0.943) and pyrene (R^2 = 0.994; y = 0.998x - 0.990) in artificially spiked soil [165]. These studies support the use of NEETs, rather than EETs, to predict HOCs bioavailability in soil. In addition, the use of harsh and mild solvents for HOC bioaccessibility determination in RA has been deemed unreliable, for example in Germany, since they do not possess a clear mechanistic basis [108].

Advanced NEET methods exploit HOCs' hydrophobicity to allow their partitioning or mobilisation towards a hydrophobic aqueous or solid phase within a soil or sediment suspension. Advanced NEETs include an aqueous-based technique such as hydroxylpropyl-beta-cyclodextrin (HPCD) for extraction of HOCs in soil especially low molecular weight (LMW) PAHs [164, 166, 167]. They also include solid-phase adsorbents, such as SPME or Tenax, for extraction of HOCs in sediments and soil-water suspensions [26, 64, 168]. Various studies have shown that HOC extractability from a wide range of contaminated soils using these techniques often correlates with different ecological endpoints such as microbial degradation, earthworm bioaccumulation, and plant uptake [53, 64, 169, 170]. Nonetheless, these techniques have also shown poor HOCs bioaccessibility correlations especially when a non-microbial receptor is considered [19, 64, 79, 171]. Factors which may affect bioavailability such as ageing, soil properties among others are presented in Table S2 in the supplementary material and have been reviewed in detail elsewhere [75, 77, 133, 161, 172, 173].

3.1.3. Can the desorption-resistant fraction be measured or estimated?

Desorption techniques are presented in Table 1 and are further discussed below. These techniques have different guiding principles and none has been standardised to date. They have mostly been devoted to measuring the leachable, extractable, bioaccessible fractions using microbes and earthworms among other ecological receptors, as sensitive endpoints. However, measurements of the bioavailable and residual pools, as currently delineated, is problematic [43]. The residual fraction may not simply be the total *minus* the bioavailable or bioaccessible fraction. In a study by Cuypers et al. [129], it was observed that persulphate oxidation of soils (n = 7) and sediments (n = 7) under-predicted the biodegradable fractions of 5 and 6 rings PAH. In contrast, the residual fractions after oxidation and biodegradation agree with an almost 1:1 correlation. It is not clear if desorption and bioavailability which impact biodegradation are similar to those for a toxic endpoint. According to ISO 17402 [174] and as documented by Kordel et al. [108], there are practical differences between ‘environmental availability, environmental bioavailability, and toxicological bioavailability’. If these do differ, it may imply that residual fractions may also vary for biodegradation and toxicological endpoints, and between EHRA and HHRA. However, to predict the significance of harm that may be caused from exposure, desorbable concentration from residual fractions in soil must then be considered.

Recently, Mayer and others developed an isolation and quantification technique for the so called ‘desorption-resistant’ or residual fraction using a contaminant trap method [65]. The contaminant trap was simply a PDMS-activated carbon sink and a cyclodextrin diffusive carrier in a custom-made un-agitated reactor. In this study, 12 PAHs in two industrial-contaminated soils from different sources were studied; a contaminated material dumpsite in Austria (4.6% organic carbon, OC, w/w) and a former historically-contaminated MGP site (1.5% OC w/w). The soils were incubated in the trap for 1, 7, 31 and 92 d, and for 4, 7, 14, 28, 56, 77 and 92 d for desorption and biodegradation experiments respectively. After soil slurry filtration following desorption and biodegradation, the filtrate and residual samples were exhaustively extracted and analysed for PAH concentrations using HPLC-FLD and reverse phase HPLC-FLD respectively, and then compared. The elimination kinetics and trapping efficiency of the contaminant trap for the PAHs in soil were reportedly adequate, and improved if the traps were agitated [65]. Although, the residual concentrations of individual and total PAHs quantified by the trap in the first soil were less than those analysed after biodegradation, desorption limits by the trap and biodegradation limits plateaued similarly above the Danish regulatory limit of 40 mg/kg dry weight of soil. However, PAH desorption and biodegradation in the second soil was hardly noticed, as both processes were in good agreement. The PAHs were strongly sorbed in both soils, however, the soil with lower amounts of OC exhibited stronger sorption resulting in smaller biodegradative losses and greater residual PAH concentration, compared to the soil richer in OC. The authors noted that the quality (origin, type, and composition) of OC and pollution history of soil, rather than quantity of OC determines HOCs sorption behaviour [65]. This observation further revealed the importance of using direct molecular-level techniques with the capacity for such information to complement empirical approaches, for a robust description of HOC sorption-desorption behaviour in soil.

An improved extraction method was developed which included a silicone rod as an adsorption sink to optimise mass transfer, and HPCD as diffusive carrier [175]. This silicone-based extraction (SBE) method was based on the principle that after desorption from the solid to aqueous phase in soil, uptake by a microbial receptor creates a gradient which facilitates mass transfer processes until equilibrium is reached. In the SBE method, the silicone rod acted as a quasi-reservoir, rather than a trap, such that sorbed residues could be back-extracted. Using the SBE method, the researchers evaluated desorption of PAHs from wood soot (50 mg), as the sorbent matrix, with initial PAH concentration (mg/kg) ranging from 7.02 ± 0.22 - 108.54 ± 3.36 (median and total PAHs concentration = 55.47 ± 1.42 mg/kg and 646.96 ± 19.95 mg/kg respectively). The mobilised and desorbed fraction were compared in the presence and absence of sink (i.e. HPCD only). In comparison with the HPCD only extraction, SBE extracts contained up to 3 - 25 times greater individual PAH concentrations. Although, extractions using the SBE were compared with HPCD extractions, direct comparison of SBE extractable and associated residue with the microbially degradable fractions and residues may be more useful, as in Mayer et al. [65]. However, the SBE procedure seems complex and may be technically demanding, and does not demonstrate the relevance of the derived residual fractions to HHRA.

It appears that only a few studies have focussed on understanding HOCs desorption or extractability from residual pools in soil, especially with respect to HHRA [61, 62, 127, 176]. A reliable measurement approach for the desorption-resistant fraction may be very useful in RA. For example, such methods may be needed to test the effectiveness of techniques which are based on extraction of the labile pools, in that the residue remaining after extraction is investigated for further extractability. Such approach, may then reveal whether the residual contaminants are actually bound in soil or could be potentially mobilised. Hence, there is a need for further investigation into the development of a simple, reliable, robust, adjustable, and exposure-relevant technique for delineating and quantifying residual HOC pools in a wide range of soils.

3.1.3.1. Can chemical extractions be used to classify HOC residues in soil?

In a recent ECETOC report, extractions using mild extractions (including NEETs) and EETs were denoted as extractable and NER fractions [35]. The ‘NER’ term as used implies not extractable by mild extractions, but by exhaustive extractions. However, this ‘NER’ term could be a misnomer and its use is confusing as a contaminant fraction is either extractable or not. Hence, NER is referred to as a highly sequestered contaminant fraction in this article. The residues left after exhaustive extractions which can only be assessed using ^{14}C-radiocombustion methods were referred to as bound residues [35]. Although the residue classification scheme was only relevant for EHRA, it appears superficial and may not even be appropriate for all ecological receptors. This is because the proposed scheme did not fully consider issues such as relationships between reversibility of sorbed HOCs, physico-chemical properties of HOCs and the soil matrix, and physiological variations within complex organisms such as plants and aquatic organisms, let alone humans. Therefore, the development of an ‘intelligent extraction strategy framework’ which considers some of the aforementioned issues has been proposed [107]. Such an approach must enable risk assessors including regulators to predict with confidence whether highly sequestered fractions do pose ongoing risks from long-term exposure to historically contaminated soils. It is therefore important to re-emphasise the need to investigate the differences in mechanisms of desorption and uptake from residual HOC pools in soils by key organism classes, and to conclude whether these differences can be measured and/or described using chemical extractions. Such investigations may help in properly delineating residual and non-residual HOC pools in soil, and help minimise uncertainties associated with RA.

3.2. General comments on the bioavailability or bioaccessibility of highly sequestered residues in soil and their implications for RA

There are obvious challenges associated with the incorporation of bioavailability or bioaccessibility concepts within current regulatory structures including the lack of precision and inconsistency in the use of the bioavailability and bioaccessibility terms; confusion regarding what is actually being measured; inadequate systematic comparisons to reveal the most reliable prediction methods for bioavailability or bioaccessibility after considering contaminant-organism-matrix differences; contaminant availability prediction methods that are not validated; and scarcity of clear guidance on how the contaminant availability concept can be incorporated into current RA frameworks. While bioavailability underpins a risk based approach, most regulators and risk assessors persist with total extractable concentration. Unlike toxic metals for which the Australian National Environment Protection Measure (NEPM) and the United States Environment Protection Agency (USEPA) includes standard operating procedures for assessment of the bioavailable fraction [34, 177], there are still no SOPs for HOCs. Comparison of studies may be difficult and caution must be applied when interpreting HOCs bioavailability data generated from the different techniques. However, both HPCD and TENAX extractions seem promising for standardised use for HOC bioavailability determinations in soil and/or sediments for EHRA purposes [10]. The former is simpler and time-efficient compared to the latter [53]. On the other hand, only a few studies have investigated PAH *in vitro* bioaccessibility for HHRA purposes and have been reviewed in detail elsewhere [178-182]. In addition, *in vitro* bioaccessibility-*in vivo* bioavailability correlations for HOCs, such as PAHs, in soil have not been established. More so, comparisons between PAH *in vitro* GI bioaccessibility and *in vivo* bioavailability are scarce, revealing the paucity of data to allow such comparisons. However, these are likely to become subjects of future investigations [19, 62, 183-186].

It may be worthwhile to subject a wide range of soils to single or sequential extractions using aqueous, aqueous-based, solid-phase extraction, and sink-included *in vitro* GI digestion techniques to differentiate between the labile and non-labile HOC fractions, as validated using relevant receptors. Similarly, the non-labile/residual fractions obtained from methods such as persulphate oxidation, SFE, and contaminant trap among other relevant methods could be tested for remobilisation under weak to strong extraction regimes and varying exposure conditions, for example under the influence of *in vitro* gut fluid. Further, the labile and residual HOC fractions obtained using different techniques could be compared to confirm if they had been obtained from the same (or similar) pools in soil. The use of advanced analytical and empirical methods to understand the mechanisms controlling soil-HOC-organism interactions in these investigations should be key, and efforts should be made to validate and standardise the choice methods. Such investigations may contribute to understanding the kinetics, risks and associated significance of potentially desorbing HOC fractions from non-residual and residual pools in soil, which may in turn be useful for RA.

4. Does exposure to residual HOC fractions in soil pose risks to receptors?

A recent study using a series of multi-species ecotoxicity assays revealed that exposure to residual total petroleum hydrocarbons in field contaminated soils had no environmental risk [30]. Similarly, ecological risk from exposure to pesticide residues in soil is deemed minimal [43, 44]. In addition, HOCs associated with diagenetically-advanced and recalcitrant humic materials (e.g. black carbon and biochar) in soil are unlikely to be remobilised [51]. One of the most important reasons advocated for such minimal or no risks is that aged HOCs are highly sequestered within the soil matrix [21, 25, 31, 133]. Further, it is interesting that in most previous studies no significant relationship was reported between bioavailability and total PAH concentrations (extractable) in soil, while a recent study that investigated the relative bioavailability (RBA) of B[a]P in juvenile swine using 8 soils, and silica sand as reference material, both spiked at the same B[a]P concentration found significant correlation between the RBA of B[a]P in spiked soils and DCM/ACE extractable fraction ($R^2 = 0.67$, $p < 0.05$) and butanol extractable fraction ($R^2 = 0.75$, $p < 0.01$), but not HPCD or Milli-Q water extractable fractions [19]. Although, RBA of contaminants as measured by time-based blood concentrations following soil ingestion has been criticised [178, 180], it may still be a reliable estimate of oral bioavailability compared to other bioavailability endpoints since systemic toxicity is mostly due to the effect of parent PAHs in systemic circulation [183].

For clarity, it is important to carefully consider the following, bearing in mind previous discussions in this article. By ECETOC's classification, the total HOC residue (TR) comprises the extractable (ER) + non-extractable residue (NER) + bound residue (BR), which is based on Zarfl et al. delineation of TR in soil [35, 122]. According to the bioavailability framework suggested by Ortega-Calvo and others for use in regulation and modified from an earlier study [187], the total extractable residue (TER) = ER + NER [10]. Further, the TER comprise the slowly and very slowly desorbing + rapidly desorbing + dissolved fractions [188]. Also note that a single HPCD or Tenax extraction of ≤ 20 h measures the rapidly desorbing fraction [49, 108], whereas sequential extractions can also access the slowly desorbing pool [49, 83]. However, the TER has been defined as the fraction measurable by EETs, while an apparent earthworm- or microbial-bioavailable fraction is measurable by NEET [79]; and it has been frequently reported that RA which assumes that TER is bioavailable overestimate risks to humans, plants, and animals [11, 15, 43]. Hence, the implications of the reported correlation between RBA and EETs, but not NEETs [19] are 3-fold:

(a) That the so called 'highly sequestered residue' may still be accessible upon human or animal exposure to aged HOC-laden soil;

(b) That chemical stability of residues must be complemented by biological stability. There may be a need to support the wide use of simple chemical extractions for classifying bioavailable and residual HOC pools in soil, to EHRA and HHRA. Perhaps, through validation with biological experiments and the complementary use of advanced molecular techniques, while simple chemical extractions may be restricted to uses in the EHRA, rather than the HHRA; and that

(c) There is a need for clearer characterisations of the residual and non-residual HOC pools in soil, and further investigations into the potential risks associated with consequent exposure to these residues.

5. Risk assessment framework for contaminated sites

The general RA framework for contaminated sites consist of a series of preliminary and detailed phases of investigation which must be completed to determine the certainty of risk from exposure to a contaminated site [108, 189, 190]. The preliminary investigation phase (Tier 1) includes site reconnaissance; design of a conceptual site model (CSM); and confirmation of potential contamination by sampling soil and analysing its total extractable contaminant concentrations (TER) for comparison with soil guideline values (SGVs). The detailed investigation phases (Tiers 2 and 3) usually proceeds when TER exceeds the SGV considered as safe. A detailed investigation of the sources, pathways, and receptor relationships (or pollutant linkage) is then conducted and the CSM is subsequently modified to reflect realistic exposure scenarios on the site. Establishing a significant pollutant linkage implies that there is a risk, hence, such sites must be managed or remediated to minimise the risks, protect receptors of concern, or permit proposed changes to land uses.

While such pollutant linkages could be established, associated human or ecological health risks are frequently overestimated. This is because the total contaminant load quantified on a site, at every point in time, is traditionally equated to potential risks that may result following exposure [10, 187]. In fact, the criteria for developing SGVs do not consider contaminant-matrix-organism dynamics [191] which may modify biological exposure conditions and consequent risks. This traditional approach for risk estimation by measuring total contaminant load in soil (including overly-conservative regulatory guidance) has resulted in high costs and slow pace of remediation, including dereliction of potentially reusable lands [32]. These challenges are constraints to efficient contaminated land management [30].

Risk-based approaches can ensure cost- and time-effectiveness in contaminated land management and may have positive socio-economic implications such as rapid redevelopment of derelict sites, but its regulatory adoption is largely constrained by uncertainties as described previously. Perhaps, the most confounding problem may be the unclear fate associated with the residual contaminant fraction in soil.

Bioavailability research has gained increasing attention, and the determination of bioavailability and its consideration for incorporation into risk assessment policies is considered essential [192]. Clear framework guidance documents to support wide regulatory adoption of the bioavailability concept have recently been published [10, 32, 108, 187, 189, 190]. It still appears unlikely that regulators are ready to make the change from the traditional to the risk-based approach for contaminated site land management. Some researchers have recently argued that while current available evidence may not be sufficient for such departure from the status quo, the bioavailability concept is sufficiently developed for inclusion into existing RA framework, as part of higher tier steps (i.e. Tiers 2 and 3) to modify exposure scenarios and risk estimations [10, 187, 189]. Perhaps, the ideal but unlikely situation would be to consider contaminant bioavailability at the initial stages of developing SGVs, and throughout the preliminary and detailed RA phases. While the proposed frameworks may ensure a realistic RA process, uncertainties with the risk-based approach need to be considerably minimised.

5.1. Could the residual fraction be the defining point for risk based land management?

The importance of the risk-based approach to contaminated land management cannot be over-emphasised. There has been moves to include bioaccessibility or bioavailability into RA to reduce the overprotective nature of RA caused by the use of total extractable concentrations. By including an extractable fraction (bioaccessible) which is less than the total extractable concentration as part of site-specific RA, and thus reducing remedial concentration endpoints, site management becomes more achievable and less expensive. Further, by considering bioaccessibility in RA rather than bioavailability, there is still a degree of overprotection, although it is not just as conservative as using total concentrations. However, should the associated uncertainties especially those related to the fate of residual contaminant fractions be overlooked, or resolved to allow seamless incorporation of the risk-based approach into existing regulatory structures? Exposure to the rapidly desorbing contaminant fractions (i.e. the bioavailable) in soil defines realistic risks, therefore understanding the dynamic processes that control bioavailable fractions may be more critical than the residual fractions, as suggested in the pesticides literature [43]. Whether pesticide residual fractions should be included in RA is still being debated, including clear guidance on how they should be considered [40, 126]. Arguably, bringing 'residual HOC fractions' into the RA debate, may negatively impact on RA making it more expensive and time consuming to remediate contaminated land, thereby, making pragmatic remediation to an acceptable level almost impossible. Nonetheless, regulatory confidence in adopting the risk-based approach may be significantly increased when convincing evidence shows that contaminant residues in soil are not taken up by a wide range of receptors, and in the event they are taken up, consequent biological effects are non-existent or negligible [48]. In other words, the so called 'contaminant residues' must be demonstrated to pose minimal or no risks, from exposure, in the short- and long-term. It is suggested that future investigations should focus on clearly answering the key question: how bioavailable are soil-associated HOC residues to key receptors and are there potential risks from exposure? Finally, whether the need for investigations into the likely role played by residual fractions in potentially defining risk based land management, is merely a routine academic endeavour or one which is necessary for pragmatic and realistic RA would be beyond the scope of this review and is thus subject to discussions in the future.

6. Conclusion and future research prospects

The overall purpose of the risk-based approach to managing contaminated lands is to minimise risks from exposure. However, adopting the risk-based approach within current regulatory structures over the overly-conservative traditional approach, requires that associated uncertainties are understood and minimised. One such uncertainty is whether there are potential risks associated with exposure to the residual contaminant fractions in soil and how they should be considered in the risk assessment process. There have been arguments for and against the certainty of risks in this regard. However, evidence supporting these arguments is based on empirical observations which may not be completely convincing. The review of the literature suggests that the exclusion of the residual fractions appears inappropriate since very few studies have focused on systematically understanding desorption behaviour of residual hydrophobic organic contaminants in soil, with few studies considering the influence of exposure-specific factors on residual contaminant mobilisation. For example, the exposure of soil-associated hydrophobic organic contaminants to gastrointestinal fluids and enzyme processes may elicit dynamic contaminant release responses, which may affect remobilisation of highly sequestered residues. Although, *in vitro* models have been used to predict PAH bioaccessibility and chemical extractions used to determine different residual fractions in soils, there are still doubts as to what is actually being measured given variations in results generated. Therefore, it is not certain which bioaccessibility model currently represents the best choice, or provides the best fit for *in vivo* bioavailability determinations. Neither, has the relevance of current definitions of the residual contaminant fractions to ecological and human health risk assessments been convincingly demonstrated. How should the residual and bioaccessible contaminant pools be defined? Would it be prudent to define the residual contaminant fractions in soil based on exposure-specific scenarios? How should these highly sequestered contaminant residues in soil be isolated and quantified? Is it possible to predict potential risks from exposure to these residual fractions? And in fact, could there be any scientific, regulatory and/or pragmatic basis for these investigations? These remain questions of concern which may hamper the adoption of the risk based approach. To provide convincing evidence, it is suggested that desorption behaviour of hydrophobic organic contaminants in a wide range of aged historically contaminated soils should be investigated, considering exposure-specific scenarios and also bearing in mind the importance of mechanistic molecular-level data to supporting empirically-derived data. Investigations to develop and validate standard operating procedures for hydrophobic organic contaminants bioavailability determinations are required, and inter-laboratory research efforts may be needed to propose the most appropriate testing method(s). Such investigations may be useful for minimising uncertainties associated with the risk-based approach, so that due consideration may then be given to its regulatory adoption on a wider scale.

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