

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

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1 List of Abbreviations

2	BR	Bound Residue
3	CSM	Conceptual Site Model
4	DCM	Dichloromethane
5	DCM/ACE	Dichloromethane/Acetone
6	ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
7	EET	Exhaustive Extraction Technique
8	EHRA	Ecological Health Risk Assessment
9	ER	Extractable Residue
10	EVA	Ethylene Vinyl Acetate
11	GI	Gastrointestinal
12	HHRA	Human Health Risk Assessment
13	HOC	Hydrophobic Organic Contaminant
14	HPCD	Hydroxypropyl- β -Cyclodextrin
15	HPLC-FD	High-Performance Liquid Chromatography with Fluorescence Detection
16	MGP	Manufactured Gas Plant
17	NEET	Non-Exhaustive Extraction Technique
18	NEPM	National Environment Protection Measure
19	NER	Non-Extractable Residue
20	OC	Organic Carbon
21	PDMS	Polydimethylsiloxane
22	PED	Polyethylene devices
23	POM	Polyoxymethylene
24	RA	Risk Assessment
25	RBA	Relative Bioavailability
26	RBLM	Risk-Based Land Management
27	SARA	Soil-sediment Availability Ratio
28	SBE	Silicon-Based Extraction
29	SFE	Supercritical Fluid Extraction
30	SGV	Soil Guideline Value
31	SOP	Standard Operating Procedure
32	SPE	Solid Phase Extraction
33	SPMD	Semipermeable Membrane Devices
34	SPME	Solid Phase Micro-Extraction
35	TR	Total Residue
36	USEPA	United States Environmental Protection Agency

37 Abstract

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

64 global scale. This review critically appraises existing information on the bioavailability of
65 HOC residues in soil to establish whether there may be risks from highly sequestered
66 contaminant residues.

67

68

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

72 1. Introduction

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

74 Chemical contamination of soils is a global problem ¹ and is arguably of similar
75 significance as other major environmental challenges such as climate change and
76 biodiversity loss ². Sustainable soil management has a vital role for addressing the challenge
77 of widespread contamination, especially with regard to attaining the United Nations
78 ‘Sustainable Development Goals’ ³. Among the anthropogenic contaminants of soils,
79 hydrophobic organic contaminants (HOCs) require attention as they are persistent, they
80 bioaccumulate, and are toxic and potentially carcinogenic ⁴⁻⁶. Examples of important HOCs
81 include PAHs, polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs),
82 dichloro-diphenyl-trichloroethane (DDT) and other chlorinated pesticides. The risk of soil
83 contamination by HOCs is a matter of great concern for human and ecological health ⁷.
84 Human exposure to HOCs in soils may be by oral ingestion, inhalation, and/or dermal routes
85 ^{7,8}. Ingestion has been regarded as very important for HOCs, such as the PAHs ^{7,9}.

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

99 Risk-based approaches to contaminant management and remediation offer feasible
100 remediation practices in that they recognise that complete removal of a contaminant is likely
101 to be technically very difficult, expensive, and sometimes unnecessary²⁸⁻³¹. Risk-based
102 approaches need to be underpinned by a thorough understanding of the chemical behaviour
103 of HOCs in soils, and in particular definition of the fraction of the total concentration that is
104 relevant to biological or environmental impacts, i.e. bioaccessibility. Adoption and application
105 of risk-based approaches face a significant hurdle due to the lack of regulatory recognition of
106 the bioavailability concept which underpins the approach, although it is perceived as an
107 important concept for RA within certain sections of the regulatory³² and scientific^{11-13, 15, 33}
108 communities. This is related to the lack of standard operating procedures (SOPs) for
109 measuring the bioavailable fractions of HOCs in soil, as such measurements are still in their
110 infancy. Although validated SOPs for bioavailability of thoroughly studied inorganic
111 contaminants such as lead in soil have gained regulatory approval and are currently in use in
112 the USA³⁴, there is no generally accepted SOPs for determining HOC bioavailability in soil.
113 In addition, information on the bioavailability and risks associated with 'residual' HOCs in soil
114 is limited.

115 Although bioavailable fractions of HOCs are by definition of most concern in assessing
116 their environmental and health risks in soil, it is yet to be demonstrated conclusively whether
117 residues that remain following removal of the bioavailable fractions pose ongoing risk from
118 long-term exposure, especially in historically contaminated soils^{29, 35-39}. In the absence of
119 conclusive evidence regarding potential risk arising from long-term exposure to residual
120 fractions, the adoption of the risk-based approach for HOCs is often challenged. There have
121 been arguments regarding the significance of non-extractable residual (NER) fractions,
122 defined in this review as highly sequestered residues, to ongoing risks in the long term^{35, 36,}
123³⁹⁻⁴⁵, because changes in environmental or exposure conditions may mobilise residual pools
124 in soil^{30, 43, 46-48}. A number of papers have argued that long-term exposure to NER fractions
125 in soil poses little or no risk because of the strong interactions between HOCs and soil
126 components especially organic matter (OM), resulting in very slow remobilisation HOCs,

127 mainly through desorption^{25, 26, 30, 31, 43, 49-51}. Desorption rates of HOCs from historically
128 contaminated soils and sediments have been reported to be slow or very slow (10^{-2} to 10^{-4} h-
129 1)^{26, 49}, and with half-lives that could last decades⁵⁰. For example, a study reported that the
130 slowly desorbing fraction of ¹⁴C-phenanthrene slightly increased between 2 and 10 d in 4
131 different artificially-spiked soils (4.8 - 10.25 % OM), with the soil richest in OM showing more
132 pronounced effects with ageing⁵². Other studies have corroborated these findings, with
133 increases in the desorption-resistant (i.e. residual) fraction by up to 2 - 10 fold with ageing^{26,}
134 ^{53, 54}. The implication is that with increase in the residual (or strongly-sorbed) fraction as
135 ageing progresses, desorption of contaminants becomes increasingly difficult. Even when
136 desorption does occur, it does so at a very slow rate such that the risk is acceptable, or that
137 no risk is likely to be posed⁵⁵. However, this argument is mainly derived from indirect
138 empirical relationships between contaminant sorption behaviour and soil OM structure⁵⁶.
139 Although the afore-mentioned studies have been useful for understanding soil-HOC
140 interactions on a macroscopic scale, more convincing molecular-level based approaches
141 which directly depicts these interactions are lacking, especially given the current availability
142 of advanced analytical techniques such as the nuclear magnetic spectroscopy (NMR) among
143 others⁵⁶⁻⁵⁸. While such advanced approaches may not be needed for routine assessment of
144 residual fractions, detailed investigations of residual HOCs in a wide range of long-term
145 contaminated soils will provide much needed data to regulators on potential long-term risks
146 arising from such fractions remaining in soils.

147 In contrast, it has been argued that long-term exposure to NER in soil may be harmful to
148 receptors. For instance, enhanced PAH mobilisation has been speculated in long-term aged
149 (50 years) soils from manufactured gas plant sites (MGP) under anoxic conditions and other
150 changing environmental conditions such as temperature^{55, 59, 60}. Some studies also reported
151 that higher organisms may have the capacity to access HOC residues, which were hitherto
152 classified as inaccessible by microbial processes; this is due to differences in uptake
153 mechanisms and exposure conditions such as gut conditions of specific organisms, including
154 humans^{38, 47, 61-65}. However, none of these approaches would be a measure of the

155 bioaccessible fraction. Mayer et al. ⁶⁵ also noted that it was important to investigate the
156 potential availability of strongly-sorbed contaminant fractions to non-microbial receptors such
157 as humans using *in vitro* gastrointestinal (GI) models, to further support the consideration of
158 bioavailability and the risk-based approach within regulatory structures. In addition, it has
159 been advised that regardless of whether risk-based land management (RBLM) approaches
160 are adopted, monitoring will be useful to check contaminant remobilisation should there be
161 changes in land use or environmental conditions ³². Such an approach is commonly used as
162 part of monitored natural attenuation of groundwater or contaminated soils ⁶⁶⁻⁷³.

163 These contrasting arguments raise the questions:

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

169 To date, there have been only a few published studies that have reported potential
170 remobilisation of HOC residues in long-term aged or historically contaminated soils, and
171 potential risks to receptors ^{50, 55}. These studies have mostly focused on ecological receptors,
172 especially microbes and earthworms. Other sensitive receptors, especially those that
173 simulate human physiology, are yet to be studied. The focus of much of the studies using
174 animal models has been the assessment of bioavailable fractions rather than the more
175 tightly bound residual fractions. While there have been studies that have attempted to
176 elucidate soil-contaminant relationships using advanced molecular based techniques, such
177 as the NMR, they have not focused on the dynamics of release or retention of residual
178 HOCs in soil with respect to the combined effects of ageing, varying soil properties,
179 environmental conditions and realistic exposure scenarios, or the controlling mechanisms ⁵⁶.
180 ⁵⁸.

181 To minimise uncertainties associated with risk-based approaches, understanding the
182 influence of soil properties, ageing and exposure-specific factors on the potential release

183 and harmful effects of HOC residues in soil is necessary. Importantly, understanding the
184 mechanisms controlling underlying processes, especially at a molecular scale, may
185 complement previous empirical relationships describing the fate and behaviour of HOCs in
186 soil. Within the context of RBLM approaches for soils contaminated with HOCs, the objective
187 of this article is to critically review existing information on the bioaccessibility of HOC
188 residues in an attempt to establish whether there is a need to further investigate potential, or
189 the likelihood of harm being caused by highly sequestered residues in soil.

190 2. Fate and behaviour of HOCs in soil

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

210 2.1. Mechanisms controlling sequestration of HOCs in soils: Implications for mobility and
211 bioaccessibility

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

219 Isotherms fitted to sorption models (e.g. Freundlich and Langmuir, among other models)
220 have shown that sorption is mostly non-linear as solution concentrations increase⁸⁵. The
221 non-linearity index (n) is reported as less than unity, indicating heterogeneity of sorption sites
222 in soil^{88, 94}. Two sequestration phases are likely within soil, i.e. external (as influenced by
223 advection and diffusion) and internal (influenced by diffusion and adsorption)⁸². While the
224 former is unlikely to be rate-limiting in determining slow sorption and especially slow
225 desorption, the latter is likely^{27, 82, 84, 85, 95, 96}. In a particle of soil, a HOC molecule could be
226 small enough that it fits into minute pore spaces, following diffusion through the dissolved
227 water phase. Such a tortuous journey occurs with increased ageing such that contaminant
228 remobilisation is unlikely⁸⁴, unless influenced by changing environmental conditions. Soil
229 conditions such as absence of oxygen, increased temperature, and soil mixing may
230 potentially influence the release of previously sorbed HOCs via a tortuous and reverse-
231 phase journey (i.e. from the sorption sites within the soil matrix back to the dissolved water
232 phase or soil surface)^{59, 60, 97}.

233 Pignatello and Xing⁸² explained that to better understand mechanisms of slow sorption,
234 it was important to consider the following:

- 235 (a) That mass transfer processes including associated rate constants differ tortuous
236 diffusion. This may be related to the diverse activation energies associated with
237 different sorption sites within the soil matrix ^{84, 85, 98};
- 238 (b) It appears that sorption becomes stronger with decreasing sorbate concentration.
239 Sorption equilibrium of HOCs in soil is concentration-dependent ^{99, 100}; and
- 240 (c) The kinetic-hysterical nature of sorption-desorption processes, whereby the rate
241 constants during slow sorption appear greater than slow desorption for the same HOC
242 and soil. Hence, the rate and extent of formation of strong HOC-soil bonds becomes
243 faster compared to breaking such bonds ^{84, 85}.

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

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

254 2.1.1. Sorption of HOCs by soil and contaminant pool classification

255 Sorption plays an important role in influencing HOC bioavailability, and hence, associated
256 mobility, transport, loss, and biological effects such as bioaccumulation, biomagnification
257 and biotransformation ^{82, 84, 105, 106}. HOC pools in soil can be segregated into fractions ranging
258 from that which is weakly sorbed to the strongly sorbed ^{22, 27}. The continuum may comprise
259 dissolved, weakly sorbed and rapidly desorbed, the slowly desorbed, and the strongly bound
260 fractions (Fig. 2). Although, this contaminant sorption continuum is a simplification
261 considering the complex and dynamic nature of soil-contaminant interactions, it helps

262 understanding of complex processes^{25, 54, 82}. These pools presents different barriers for HOC
263 desorption, mobility, and availability. For example, PAHs in dissolved and weakly sorbed
264 (rapidly desorbing) pools are likely to be readily accessible to exposed organisms^{10, 12, 15, 107,}
265¹⁰⁸. However, the strongly sorbed and bound (slowly desorbing) pool is presumed to be less
266 bioavailable^{43, 50, 109}.

267 Earlier studies have mostly focused on using sorption isotherms (e.g. Freundlich and
268 Langmuir) and distribution coefficients (K_d) in describing the mostly non-linear HOC sorption
269 behaviour in soil^{85, 110}. These have resulted in proposing empirical relationships between
270 distribution and partitioning coefficients (Log K_d or Log K_{oc}) of different HOCs and soil
271 properties^{88, 94, 111-114}. However, the sole use of Log K_{oc} or octanol-water partition coefficient
272 (Log K_{ow}) to describe HOC sorption in soil should be used with caution, as other intrinsic soil
273 properties such as SOM-clay mineral interactions may play greater roles in influencing
274 sorption processes^{113, 115}. In addition, while these empirical relationships are valuable and
275 have formed the basis for emerging investigations, inferences regarding the relationship
276 between soil properties and HOC sorption behaviour have been based on indirect
277 observations⁵⁶.

278 Of the current advanced analytical techniques, NMR has been reported to provide
279 mechanistic information on molecular-scale soil-contaminant interactions at the solid, liquid,
280 and soil-water (gel) phases^{56, 57}. In many NMR investigations, the soil is physically or
281 chemically fractionated into operationally defined pools in an attempt to obtaining direct
282 molecular information about SOM composition^{57, 116-120} and understand precise HOC
283 sorption behaviours in soil⁹⁹. Although, the actual nature of the SOM is still contentious¹²¹,
284 these investigations provide detailed understanding on the dynamics of SOM-HOC
285 interactions. However, physical or chemical fractionation is likely to change the behaviour of
286 HOCs. Some NMR investigations of soil-contaminant interactions have been reviewed
287 elsewhere^{56, 58}. However, while applications of NMR spectroscopy in soil science are
288 emerging, investigations towards understanding HOC sorption mechanisms especially for
289 historically contaminated soils are still lacking. Therefore, molecular level understanding of

290 the long-term stability of strongly sorbed HOC residues in historically contaminated soils
291 would help minimise uncertainties relating to risk based approaches to managing
292 contaminated soils. Such investigations should employ both empirical and advanced
293 molecular-based techniques in a complementary and multi-tiered manner to provide robust
294 evidence on the long-term stability of highly sequestered contaminant residues in soil.

295 2.1.1.1. HOC residual pools in soils

296 The total contaminant pool is made up of potentially labile and non-labile pools (Figs. 1
297 and 2). While the labile fraction consists of the dissolved and rapidly desorbable fractions,
298 the non-labile component is comprised of the non-desorbable and irreversibly sorbed or
299 recalcitrant fractions ^{10, 79, 107, 122, 123}. There are no methods for the determination of non-labile
300 residual HOC fractions, especially relating to changing exposure scenarios and estimations
301 of harm. Furthermore, a residue which may not be accessible to microorganisms may be
302 accessible to other living organisms such as earthworms, plants and other higher forms
303 including humans due to differences in the mechanisms of contaminant uptake and release
304 ^{32, 62, 124}. Specifically in relation to HHRA, that fraction of the mobilised or dissolved
305 contaminant in the gut, following ingestion, which is absorbed and may cause harm is the
306 bioavailable fraction ²⁸ and the un-mobilised fraction is referred to as the residue ¹²⁵.
307 However, there seems to be confusion regarding what the residual fraction in soil really is. It
308 could be the fraction remaining after the complete release of the labile fraction (measured by
309 an *in vitro* technique) or the residues from historically contaminated soils. The NER,
310 especially in EHRA, is mostly regarded as the fraction that is left behind after solvent
311 extraction ¹²⁶. Tao et al. ^{61, 127} suggested that the difference between the concentration of
312 PAH and organochlorine pesticides mobilised by an *in vitro* digestion model (which was
313 greater) and that extracted by accelerated solvent extraction represents a part of the residual
314 fraction. On the other hand, Juhasz et al. ⁶² suggested that the residual fraction was that
315 which remained after long-term ongoing bioremediation of a field-contaminated soil; this
316 description appears similar to that in Thavamani et al. ³⁰. While the former description of the

317 residual fraction may be more relevant for HHRA purposes, the extracting medium should
318 mimic the GI fluid.

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

325 2.1.2. Desorption of HOCs in soil

326 2.1.2.1. Slow desorption of HOCs in long-term contaminated soils

327 The bioavailability of HOCs in soil following exposure is limited by its desorption kinetics
328 ^{97, 128}. Desorption rates and extents may influence various contaminant loss processes such
329 as biodegradation, uptake and bioaccumulation among others, and are therefore key factors
330 for RA. Carmichael and others investigated whether microbial mineralisation of PAHs in soil
331 is limited by their desorption rates ⁹¹. Using ¹⁴C-radiolabelling, they examined the desorption
332 kinetics and microbial mineralisation kinetics of freshly spiked ¹⁴C- and aged ¹²C-
333 phenanthrene and chrysene in two soils from a tar and oil-recycling sites. The key findings
334 were that the rates of desorption were faster than mineralisation rates in ¹⁴C-PAH spiked
335 contaminated soils, however, the mineralisation rates of native PAHs in the organic carbon-
336 rich contaminated soil were faster than or equal to their desorption rates. Hence, the rates
337 and extents of PAH desorption in historically-contaminated soils may represent a limitation to
338 their biodegradation. This may be one reason why PAHs persist in microbially-active soils
339 ¹²⁹. Based on their results, the sole use of ¹⁴C-radiolabels in describing fate and behaviour of
340 PAHs in soil should be applied with caution ⁹¹.

341 One study combined both ¹⁴C- and ¹²C- techniques to monitor the long-term fate and
342 behaviour of HOCs in soil ⁵⁰. Extractability was observed to be low and desorption very slow
343 (calculated half-lives of up to 38 years for fluoranthene), which supports the notion that risks

344 from exposure will be minimal^{31, 55}. However, it was pointed out that the results were only
345 applicable to contaminated agricultural soils used. They recommended that similar
346 investigations be conducted on historically-contaminated soils from different sources⁵⁰. It is
347 further suggested here that different exposure scenarios and other key risk endpoints, such
348 as earthworm bioaccumulation, plant uptake, and animal-based bioavailability and *in vitro*
349 bioaccessibility assays, should be considered in addition to microbial degradation and
350 chemical extractability for a robust conclusion. For instance, following maximum desorption
351 of labile HOCs in field-contaminated sediments, slow intercompartmental reorganisation and
352 remobilisation of HOCs have been observed⁴⁶. It is also known that gastric, and intestinal
353 fluids especially bile and other intestinal enzymes, can influence the solubility and
354 mobilisation of soil-associated HOCs^{61, 125, 130}. Such gastrointestinal effects may influence
355 HOC desorption behaviour from residual pools and their remobilisation. Hence, considering
356 other exposure scenarios, other than microbial-specific ones, may further demonstrate and
357 authenticate the fact that historically aged HOCs in field-contaminated soils are mostly
358 residual and slowly desorbing, and as a result, pose minimal risks. Again, application of
359 direct molecular-level tools in this regard may be useful.

360 2.1.2.2. Desorption phases of HOCs and associated kinetics in soil

361 Studies using artificially-spiked and historically-contaminated soils have described HOC
362 desorption in two or three-phases^{26, 49, 52, 60, 83, 91, 131-133}. While biphasic desorption comprise
363 both rapid (F_{rapid}) and slowly (F_{slow}) desorbing fractions including associated rate constants
364 (k_{rapid} and k_{slow} respectively), triphasic desorption includes a very slowly desorbing fraction
365 and rate constant ($F_{very\ slow}$ and $k_{very\ slow}$). However, considering the heterogeneity and
366 complexity of soil-HOC interactions in the field, it is likely biphasic and triphasic models are
367 simplifications of multiple desorption phases in reality¹³⁴.

368 To describe desorption kinetics of HOCs in different soils and humic materials, various
369 models that assume different desorption phases have been used^{26, 49, 83, 134, 135}. The key
370 observations from these studies were that the 3-parameter biphasic (i.e. one that assumes
371 quick equilibration for F_{rapid}) and 3-parameter biphasic polymer diffusion models were

372 recommended as preferable for predicting HOC desorption kinetics in contaminated soils
373 compared to others, although rate limiting site-specific conditions must be considered.
374 Importantly, these studies revealed that rates of slow desorption (k_{slow}) from strongly sorbed
375 pools could range from between 10^{-2} to 10^{-4} h^{-1} ^{26, 49, 134}, depending on stage of SOM
376 diagenesis and hydrophobicity of the HOCs of concern ^{37, 136, 137}. Generally, k_{slow} was
377 reported to be up to 2 orders of magnitude lower than k_{rapid} ⁵⁰. Similarly, the highly
378 condensed soil or humic material (e.g. kerogen-like shale) tends to retain a greater
379 percentage (up to 70%) of the residual HOC fractions ^{49, 134}. This residual fraction may
380 increase by up to 2 - 10 folds with ageing ^{26, 53, 54}. These observations support the argument
381 that long-term exposure to NER fraction may pose no risk ³¹. However, a complementary
382 molecular-scale approach to providing such evidence for different exposure scenarios is still
383 necessary ^{56, 57}.

384 2.2. Biodegradation of HOCs in historically contaminated soils

385 Biodegradation influences the fate and behaviour of HOCs in soil ^{12, 138}. However, HOCs
386 sequestration happens along with the biodegradation process. Biodegradation is often
387 observed as a biphasic process which occurs in two phases, i.e. fast and slow loss phases.
388 Due to its biphasic nature, biodegradation may not necessarily lead to the total removal of
389 contaminants in soil, but may result in transformations that lead to reductions in initial
390 concentrations, mobility and toxicity of contaminants, thereby, reducing the level of harm to
391 flora and fauna ¹³⁹⁻¹⁴¹. Bacteria, fungi and algae play important roles in the biotransformation
392 and biodegradation of contaminants in soil ¹⁴²⁻¹⁴⁴. Generally, for biodegradation to occur,
393 microorganisms with sufficient capacity to degrade the contaminant of concern (i.e.
394 possesses relevant degradation enzymes) should be present in soil; the contaminant must
395 be bioavailable or become bioavailable, and environmental factors must permit the
396 biodegradation process ^{133, 142, 145, 146}. Microbial mineralisation of HOCs is limited by HOCs'
397 desorption kinetics, especially in long-term contaminated soils, thereby supporting its
398 biphasic nature ⁹¹. Therefore, bioremediation (cost-effective remediation technique relying on

399 optimising contaminant biodegradation) is sometimes constrained due to sorption limitations
400 which limits microbial accessibility to HOCs in aged soils leaving behind residual
401 contamination in soil or sediment^{29, 47}. Different mechanisms through which microorganisms
402 circumvent accessibility limitations include attachment to soil surfaces to allow HOCs
403 partitioning from soil into microbial cell membrane, co-metabolism, and production of
404 extracellular enzymes and biosurfactants to enhance HOCs mobilisation from sorbed phases
405 to microbially-accessible spaces in soil^{145, 147}.

406 The lingering question is whether the residual contaminant fraction (i.e. the fraction that is
407 not biodegradable or extractable/mobile/bioaccessible) in soil could potentially cause harm?
408 With regards to biological uptake of contaminants, microbial inaccessibility of HOCs in
409 historically contaminated soil may not mean inaccessibility by other exposed higher
410 organisms, as contaminant accessibility is species and matrix dependent^{62, 133}. A
411 contaminant must desorb or be bioaccessible to allow biological uptake. Hence, like
412 sorption/desorption processes, different soil and organism properties may modify
413 contaminant uptake in soil. Other abiotic loss processes such as volatilisation, photolysis,
414 leaching, and chemical transformations may also affect fate and behaviour of HOCs in
415 historically contaminated soils (Fig. S1 in supplementary material). However, these effects
416 are mostly insignificant compared to losses by biodegradation, especially for very
417 hydrophobic HOCs²⁹.

418 3. Bioavailability and bioaccessibility of HOCs in soil

419 The concept of HOC availability has increasingly attracted diverse discussions within the
420 scientific and regulatory community^{11, 107, 148}. The concept has been used differently in
421 specific fields such as toxicokinetics, pharmacokinetics, agriculture, and environmental
422 science among others^{11, 22, 77, 149}. The terms 'bioavailability' and 'bioaccessibility' have mostly
423 been used interchangeably in the literature^{150, 151}, within EHRA and HHRA. However, the
424 terms are different but related measures^{15, 77}. Specifically for HHRA, Hack and Selenka¹²⁵
425 and Ruby et al.¹⁵¹ considered the bioaccessible contaminant fraction as the dissolved and

426 mobilisable fraction within simulated GI fluid, following ingestion of HOC-laden soil. This
427 bioaccessible fraction, therefore, represents the contaminant fraction maximally available for
428 intestinal absorption (Fig. 3). For EHRA, the different definitions proposed for bioavailability
429 and bioaccessibility has been compared and reviewed elsewhere^{77, 149}.

430 A quick citation analysis of the definitions by Peijnenburg and Jager¹⁵², Semple et al.¹⁵
431 and Reichenberg and Mayer et al.¹⁵³ which are mostly used for EHRA is presented in Fig. 4.
432 The analysis was performed by comparing the number of times each definition was cited as
433 revealed from Web of science, Scopus, and Google Scholar on 02 December 2015. The
434 definitions provided by Semple et al. and Reichenberg and Mayer had been more cited.
435 However, this analysis does not suggest actual uses of the bioavailability definitions within
436 the scientific and regulatory communities. The recent European Centre for Ecotoxicology
437 and Toxicology of Chemicals (ECETOC) workshops^{35, 36, 107} adopted the bioavailability and
438 bioaccessibility concept by Semple and others¹⁵. Recently, the mostly used definitions of
439 contaminant availability in RA were articulated¹⁰, as shown in Fig. 5. Hence, bioavailability
440 and bioaccessibility in this article and with respect to EHRA follows definitions by Semple et
441 al. and Reichenberg and Mayer.

442 3.1. Measurement techniques for bioaccessible and residual HOC fractions in soil

443 Various techniques have been developed for HOC bioavailability or bioaccessibility
444 measurement, and are presented in Table 1. However, there is no consensus on the choice
445 technique. While it has been documented that a few methods are being standardised for
446 ecological receptors such as microbes¹⁰, there is an urgent need to develop and validate
447 SOPs for HOC bioaccessibility in soil, especially for HHRA.

448 Ultimately, a biological experiment is needed to study HOC bioavailability in soil. For
449 example, if humans are the most sensitive receptors for a particular RA procedure, then a
450 human or higher vertebrate model will be most appropriate test organism. However, this
451 approach is unethical and in fact, there have been moves away from animal-based testing
452^{154, 155}. Also, animal-based studies are expensive, time consuming, and may have challenges

453 with reproducibility³³. Hence, over the past few decades, focus has shifted to development
454 and validation of simple and complex *in vitro*, including *in silico*, approaches for contaminant
455 bioavailability or bioaccessibility assessments. Ideally, the expectation for these *in vitro*
456 approaches are that they serve as uncomplicated bioavailability surrogates for the
457 environmental receptor of concern. This implies that for any realistic applications in RA,
458 these alternative approaches must be validated and standardised. However, while much
459 efforts has been focused on the development side, less efforts has been devoted to
460 validation of *in vitro* approaches for use in reality. It is therefore suggested that while
461 development of innovative bioavailability techniques are necessary, validation and
462 standardisation of existing promising *in vitro* methods are important for minimising
463 uncertainties in RA.

464 3.1.1. Brief classification of techniques

465 The approaches for determining bioavailability and bioaccessibility can either be
466 classified as biological (*in vivo*), chemical extraction (*in vitro*) or computer modelling (*in*
467 *silico*). A clear distinction has to be made between the actual bioaccessible fraction and the
468 residual fraction as has been previously emphasised. Most currently developed *in vitro*
469 methods have focused on microbial degradation and earthworm bioaccumulation as
470 ecological endpoints, hence, various biodegradation and ecotoxicity assays are available³⁰.
471 However, significant efforts at developing *in vitro* bioaccessibility assays for HOCs, such as
472 PAHs, in soil especially for HHRA is only beginning to gain attention.

473 A detailed review of simple chemical extraction techniques for measuring bioaccessibility
474 for specified ecological endpoints can be found elsewhere^{12, 77, 133, 156, 157}. Some of these
475 methods include Soxhlet extraction, solid-phase extraction (SPE), negligibly depleting solid-
476 phase micro-extraction (nd-SPME), hydroxypropyl- β -cyclodextrin (HPCD) extraction,
477 supercritical fluid extraction (SFE), use of complexation or solubilisation agents and
478 surfactants, microwave extraction, persulphate oxidation, thermodesorption, soil-sediment
479 availability ratio (SARA), use of polyethylene devices (PEDs), semipermeable membrane

480 devices (SPMDs), polyoxymethylene (POM) sampler, and thin ethylene vinyl acetate (EVA).
481 While the *in silico* approaches for bioaccessibility calculations and predictions with computer-
482 based programs could be potentially useful ¹⁵⁸, significant developments may be limited by
483 the quality and quantity of reliable bioavailability data from laboratory or field experiments.

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

489 3.1.2. Exhaustive extraction techniques (EETs) versus non-exhaustive extraction 490 techniques (NEETs)

491 The feasibility of using chemical extractions to predict the bioavailability or bioaccessibility
492 of HOCs and to determine associated residues in soil has been proposed and widely
493 discussed. Mostly, apolar and polar solvents of varying extraction strengths have been used.
494 Harsh extractions (or EETs) use strong solvents such as dichloromethane (DCM), hexane,
495 acetone, and toluene ¹⁵⁹. Mild extractions (or NEETs) make use of weaker solvents (or mild
496 solvent - water mixture) such as methanol, *n*-butanol or propanol ¹⁶⁰. The extraction with
497 EETs and NEETs simply involves thorough mixing of a contaminated soil sample with
498 solvent for a given duration. Analysis of the extract is then performed by chromatography
499 (HPLC or GC), mass spectrometry, spectrophotometry, or radio-isotopic analysis ^{22, 133, 159,}
500 ^{161, 162}.

501 Exhaustive extractions generally overestimate the bioavailable contaminant fractions. As
502 a result, they are over-predictive of actual risks and often seen as too conservative and as a
503 consequence are likely to result in a technically difficult and expensive remediation of
504 contaminated sites ^{75, 159, 161, 163, 164}. The effect of ageing (120 d) on HOCs extractability was
505 unchanged using DCM, while it is well known that ageing results in a decrease in
506 bioavailability of HOCs in soil ¹⁶¹. Kelsey et al. ¹⁵⁹ showed that DCM extractions failed to

507 predict the availability of phenanthrene and atrazine to specialized bacteria and earthworms
508 over a 120 d period, while better prediction was achieved when mild extractions (*n*-butanol
509 and methanol-H₂O) were used ¹⁵⁹. Similarly, a strong correlation was reported between
510 butanol extractability and microbial bioavailability for phenanthrene ($R^2 = 0.971$; $y = 0.952x +$
511 0.943) and pyrene ($R^2 = 0.994$; $y = 0.998x - 0.990$) in artificially spiked soil ¹⁶⁵. These studies
512 support the use of NEETs, rather than EETs, to predict HOCs bioavailability in soil. In
513 addition, the use of harsh and mild solvents for HOC bioaccessibility determination in RA
514 has been deemed unreliable, for example in Germany, since they do not possess a clear
515 mechanistic basis ¹⁰⁸.

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

529 3.1.3. Can the desorption-resistant fraction be measured or estimated?

530 Desorption techniques are presented in Table 1 and are further discussed below. These
531 techniques have different guiding principles and none has been standardised to date. They
532 have mostly been devoted to measuring the leachable, extractable, bioaccessible fractions
533 using microbes and earthworms among other ecological receptors, as sensitive endpoints.
534 However, measurements of the bioavailable and residual pools, as currently delineated, is

535 problematic ⁴³. The residual fraction may not simply be the total *minus* the bioavailable or
536 bioaccessible fraction. In a study by Cuypers et al. ¹²⁹, it was observed that persulphate
537 oxidation of soils ($n = 7$) and sediments ($n = 7$) under-predicted the biodegradable fractions
538 of 5 and 6 rings PAH. In contrast, the residual fractions after oxidation and biodegradation
539 agree with an almost 1:1 correlation. It is not clear if desorption and bioavailability which
540 impact biodegradation are similar to those for a toxic endpoint. According to ISO 17402 ¹⁷⁴
541 and as documented by Kordel et al. ¹⁰⁸, there are practical differences between
542 'environmental availability, environmental bioavailability, and toxicological bioavailability'. If
543 these do differ, it may imply that residual fractions may also vary for biodegradation and
544 toxicological endpoints, and between EHRA and HHRA. However, to predict the significance
545 of harm that may be caused from exposure, desorbable concentration from residual fractions
546 in soil must then be considered.

547 Recently, Mayer and others developed an isolation and quantification technique for the
548 so called 'desorption-resistant' or residual fraction using a contaminant trap method ⁶⁵. The
549 contaminant trap was simply a PDMS-activated carbon sink and a cyclodextrin diffusive
550 carrier in a custom-made un-agitated reactor. In this study, 12 PAHs in two industrial-
551 contaminated soils from different sources were studied; a contaminated material dumpsite in
552 Austria (4.6% organic carbon, OC, w/w) and a former historically-contaminated MGP site
553 (1.5% OC w/w). The soils were incubated in the trap for 1, 7, 31 and 92 d, and for 4, 7, 14,
554 28, 56, 77 and 92 d for desorption and biodegradation experiments respectively. After soil
555 slurry filtration following desorption and biodegradation, the filtrate and residual samples
556 were exhaustively extracted and analysed for PAH concentrations using HPLC-FLD and
557 reverse phase HPLC-FLD respectively, and then compared. The elimination kinetics and
558 trapping efficiency of the contaminant trap for the PAHs in soil were reportedly adequate,
559 and improved if the traps were agitated ⁶⁵. Although, the residual concentrations of individual
560 and total PAHs quantified by the trap in the first soil were less than those analysed after
561 biodegradation, desorption limits by the trap and biodegradation limits plateaued similarly
562 above the Danish regulatory limit of 40 mg/kg dry weight of soil. However, PAH desorption

563 and biodegradation in the second soil was hardly noticed, as both processes were in good
564 agreement. The PAHs were strongly sorbed in both soils, however, the soil with lower
565 amounts of OC exhibited stronger sorption resulting in smaller biodegradative losses and
566 greater residual PAH concentration, compared to the soil richer in OC. The authors noted
567 that the quality (origin, type, and composition) of OC and pollution history of soil, rather than
568 quantity of OC determines HOCs sorption behaviour⁶⁵. This observation further revealed the
569 importance of using direct molecular-level techniques with the capacity for such information
570 to complement empirical approaches, for a robust description of HOC sorption-desorption
571 behaviour in soil.

572 An improved extraction method was developed which included a silicone rod as an
573 adsorption sink to optimise mass transfer, and HPCD as diffusive carrier¹⁷⁵. This silicone-
574 based extraction (SBE) method was based on the principle that after desorption from the
575 solid to aqueous phase in soil, uptake by a microbial receptor creates a gradient which
576 facilitates mass transfer processes until equilibrium is reached. In the SBE method, the
577 silicone rod acted as a quasi-reservoir, rather than a trap, such that sorbed residues could
578 be back-extracted. Using the SBE method, the researchers evaluated desorption of PAHs
579 from wood soot (50 mg), as the sorbent matrix, with initial PAH concentration (mg/kg)
580 ranging from 7.02 ± 0.22 - 108.54 ± 3.36 (median and total PAHs concentration = $55.47 \pm$
581 1.42 mg/kg and 646.96 ± 19.95 mg/kg respectively). The mobilised and desorbed fraction
582 were compared in the presence and absence of sink (i.e. HPCD only). In comparison with
583 the HPCD only extraction, SBE extracts contained up to 3 - 25 times greater individual PAH
584 concentrations. Although, extractions using the SBE were compared with HPCD extractions,
585 direct comparison of SBE extractable and associated residue with the microbially degradable
586 fractions and residues may be more useful, as in Mayer et al.⁶⁵. However, the SBE
587 procedure seems complex and may be technically demanding, and does not demonstrate
588 the relevance of the derived residual fractions to HHRA.

589 It appears that only a few studies have focussed on understanding HOCs desorption or
590 extractability from residual pools in soil, especially with respect to HHRA^{61, 62, 127, 176}. A

591 reliable measurement approach for the desorption-resistant fraction may be very useful in
592 RA. For example, such methods may be needed to test the effectiveness of techniques
593 which are based on extraction of the labile pools, in that the residue remaining after
594 extraction is investigated for further extractability. Such approach, may then reveal whether
595 the residual contaminants are actually bound in soil or could be potentially mobilised. Hence,
596 there is a need for further investigation into the development of a simple, reliable, robust,
597 adjustable, and exposure-relevant technique for delineating and quantifying residual HOC
598 pools in a wide range of soils.

599 3.1.3.1. Can chemical extractions be used to classify HOC residues in soil?

600 In a recent ECETOC report, extractions using mild extractions (including NEETs) and
601 EETs were denoted as extractable and NER fractions³⁵. The 'NER' term as used implies not
602 extractable by mild extractions, but by exhaustive extractions. However, this 'NER' term
603 could be a misnomer and its use is confusing as a contaminant fraction is either extractable
604 or not. Hence, NER is referred to as a highly sequestered contaminant fraction in this article.
605 The residues left after exhaustive extractions which can only be assessed using ¹⁴C-
606 radiocombustion methods were referred to as bound residues³⁵. Although the residue
607 classification scheme was only relevant for EHRA, it appears superficial and may not even
608 be appropriate for all ecological receptors. This is because the proposed scheme did not
609 fully consider issues such as relationships between reversibility of sorbed HOCs, physico-
610 chemical properties of HOCs and the soil matrix, and physiological variations within complex
611 organisms such as plants and aquatic organisms, let alone humans. Therefore, the
612 development of an 'intelligent extraction strategy framework' which considers some of the
613 aforementioned issues has been proposed¹⁰⁷. Such an approach must enable risk
614 assessors including regulators to predict with confidence whether highly sequestered
615 fractions do pose ongoing risks from long-term exposure to historically contaminated soils. It
616 is therefore important to re-emphasise the need to investigate the differences in mechanisms
617 of desorption and uptake from residual HOC pools in soils by key organism classes, and to
618 conclude whether these differences can be measured and/or described using chemical

619 extractions. Such investigations may help in properly delineating residual and non-residual
620 HOC pools in soil, and help minimise uncertainties associated with RA.

621 3.2. General comments on the bioavailability or bioaccessibility of highly sequestered
622 residues in soil and their implications for RA

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

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

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

661 A recent study using a series of multi-species ecotoxicity assays revealed that exposure
662 to residual total petroleum hydrocarbons in field contaminated soils had no environmental
663 risk³⁰. Similarly, ecological risk from exposure to pesticide residues in soil is deemed
664 minimal^{43, 44}. In addition, HOCs associated with diagenetically-advanced and recalcitrant
665 humic materials (e.g. black carbon and biochar) in soil are unlikely to be remobilised⁵¹. One
666 of the most important reasons advocated for such minimal or no risks is that aged HOCs are
667 highly sequestered within the soil matrix^{21, 25, 31, 133}. Further, it is interesting that in most
668 previous studies no significant relationship was reported between bioavailability and total
669 PAH concentrations (extractable) in soil, while a recent study that investigated the relative
670 bioavailability (RBA) of B[a]P in juvenile swine using 8 soils, and silica sand as reference
671 material, both spiked at the same B[a]P concentration found significant correlation between
672 the RBA of B[a]P in spiked soils and DCM/ACE extractable fraction ($R^2 = 0.67$, $p < 0.05$) and

673 butanol extractable fraction ($R^2 = 0.75$, $p < 0.01$), but not HPCD or Milli-Q water extractable
674 fractions ¹⁹. Although, RBA of contaminants as measured by time-based blood
675 concentrations following soil ingestion has been criticised ^{178, 180}, it may still be a reliable
676 estimate of oral bioavailability compared to other bioavailability endpoints since systemic
677 toxicity is mostly due to the effect of parent PAHs in systemic circulation ¹⁸³.

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

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

694 (b) That chemical stability of residues must be complemented by biological stability.

695 There may be a need to support the wide use of simple chemical extractions for
696 classifying bioavailable and residual HOC pools in soil, to EHRA and HHRA. Perhaps,
697 through validation with biological experiments and the complementary use of
698 advanced molecular techniques, while simple chemical extractions may be restricted
699 to uses in the EHRA, rather than the HHRA; and that

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

703 5. Risk assessment framework for contaminated sites

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

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

725 Risk-based approaches can ensure cost- and time-effectiveness in contaminated land
726 management and may have positive socio-economic implications such as rapid

727 redevelopment of derelict sites, but its regulatory adoption is largely constrained by
728 uncertainties as described previously. Perhaps, the most confounding problem may be the
729 unclear fate associated with the residual contaminant fraction in soil.

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

743 5.1. Could the residual fraction be the defining point for risk based land management?

744 The importance of the risk-based approach to contaminated land management cannot be
745 over-emphasised. There has been moves to include bioaccessibility or bioavailability into RA
746 to reduce the overprotective nature of RA caused by the use of total extractable
747 concentrations. By including an extractable fraction (bioaccessible) which is less than the
748 total extractable concentration as part of site-specific RA, and thus reducing remedial
749 concentration endpoints, site management becomes more achievable and less expensive.
750 Further, by considering bioaccessibility in RA rather than bioavailability, there is still a degree
751 of overprotection, although it is not just as conservative as using total concentrations.
752 However, should the associated uncertainties especially those related to the fate of residual
753 contaminant fractions be overlooked, or resolved to allow seamless incorporation of the risk-

754 based approach into existing regulatory structures? Exposure to the rapidly desorbing
755 contaminant fractions (i.e. the bioavailable) in soil defines realistic risks, therefore
756 understanding the dynamic processes that control bioavailable fractions may be more critical
757 than the residual fractions, as suggested in the pesticides literature ⁴³. Whether pesticide
758 residual fractions should be included in RA is still being debated, including clear guidance on
759 how they should be considered ^{40, 126}. Arguably, bringing 'residual HOC fractions' into the
760 RA debate, may negatively impact on RA making it more expensive and time consuming to
761 remediate contaminated land, thereby, making pragmatic remediation to an acceptable level
762 almost impossible. Nonetheless, regulatory confidence in adopting the risk-based approach
763 may be significantly increased when convincing evidence shows that contaminant residues
764 in soil are not taken up by a wide range of receptors, and in the event they are taken up,
765 consequent biological effects are non-existent or negligible ⁴⁸. In other words, the so called
766 'contaminant residues' must be demonstrated to pose minimal or no risks, from exposure, in
767 the short- and long-term. It is suggested that future investigations should focus on clearly
768 answering the key question: how bioavailable are soil-associated HOC residues to key
769 receptors and are there potential risks from exposure? Finally, whether the need for
770 investigations into the likely role played by residual fractions in potentially defining risk based
771 land management, is merely a routine academic endeavour or one which is necessary for
772 pragmatic and realistic RA would be beyond the scope of this review and is thus subject to
773 discussions in the future.

774 6. Conclusion and future research prospects

775 The overall purpose of the risk-based approach to managing contaminated lands is to
776 minimise risks from exposure. However, adopting the risk-based approach within current
777 regulatory structures over the overly-conservative traditional approach, requires that
778 associated uncertainties are understood and minimised. One such uncertainty is whether
779 there are potential risks associated with exposure to the residual contaminant fractions in
780 soil and how they should be considered in the risk assessment process. There have been

781 arguments for and against the certainty of risks in this regard. However, evidence supporting
782 these arguments is based on empirical observations which may not be completely
783 convincing. The review of the literature suggests that the exclusion of the residual fractions
784 appears inappropriate since very few studies have focused on systematically understanding
785 desorption behaviour of residual hydrophobic organic contaminants in soil, with few studies
786 considering the influence of exposure-specific factors on residual contaminant mobilisation.
787 For example, the exposure of soil-associated hydrophobic organic contaminants to
788 gastrointestinal fluids and enzyme processes may elicit dynamic contaminant release
789 responses, which may affect remobilisation of highly sequestered residues. Although, *in vitro*
790 models have been used to predict PAH bioaccessibility and chemical extractions used to
791 determine different residual fractions in soils, there are still doubts as to what is actually
792 being measured given variations in results generated. Therefore, it is not certain which
793 bioaccessibility model currently represents the best choice, or provides the best fit for *in vivo*
794 bioavailability determinations. Neither, has the relevance of current definitions of the residual
795 contaminant fractions to ecological and human health risk assessments been convincingly
796 demonstrated. How should the residual and bioaccessible contaminant pools be defined?
797 Would it be prudent to define the residual contaminant fractions in soil based on exposure-
798 specific scenarios? How should these highly sequestered contaminant residues in soil be
799 isolated and quantified? Is it possible to predict potential risks from exposure to these
800 residual fractions? And in fact, could there be any scientific, regulatory and/or pragmatic
801 basis for these investigations? These remain questions of concern which may hamper the
802 adoption of the risk based approach. To provide convincing evidence, it is suggested that
803 desorption behaviour of hydrophobic organic contaminants in a wide range of aged
804 historically contaminated soils should be investigated, considering exposure-specific
805 scenarios and also bearing in mind the importance of mechanistic molecular-level data to
806 supporting empirically-derived data. Investigations to develop and validate standard
807 operating procedures for hydrophobic organic contaminants bioavailability determinations
808 are required, and inter-laboratory research efforts may be needed to propose the most

809 appropriate testing method(s). Such investigations may be useful for minimising
810 uncertainties associated with the risk-based approach, so that due consideration may then
811 be given to its regulatory adoption on a wider scale.

812 7. Acknowledgments

813 The authors acknowledge the Cooperative Research Centre for Contamination
814 Assessment and Remediation of the Environment (CRC CARE) and Global Centre for
815 Environmental Remediation (GCER), University of Newcastle (UoN) Australia for providing
816 financial and infrastructural support. AU is grateful to UoN and CRC CARE for awarding him
817 the International Postgraduate Research and CRC CARE scholarships.

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