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Critical Review

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Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil—State of the Science

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Abstract

This article reviews the state of the science regarding oral bioavailability, bioaccessibility, and dermal absorption of carcinogenic polycyclic aromatic hydrocarbons (cPAHs) in soil by humans, and discusses how chemical interactions may control the extent of absorption. Derived from natural and anthropomorphic origins, PAHs occur in a limited number of solid and fluid matrices (i.e., PAH sources) with defined physical characteristics and PAH compositions. Existing studies provide a strong basis for establishing that oral bioavailability of cPAHs from soil is less than from diet, and an assumption of 100% relative bioavailability likely overestimates exposure to cPAHs upon ingestion of PAH-contaminated soil. For both the oral bioavailability and dermal absorption studies, the aggregate data do not provide a broad understanding of how different PAH source materials, PAH concentrations, or soil chemistries influence the absorption of cPAHs from soil. This article summarizes the existing studies, identifies data gaps, and provides recommendations for the direction of future research to support new default or site-specific bioavailability adjustments for use in human health risk assessment.

Introduction

This article reviews the state of the science regarding oral bioavailability, bioaccessibility, and dermal absorption of carcinogenic polycyclic aromatic hydrocarbons (cPAHs) in soil and discusses how chemical interactions may control the extent of absorption. The focus of this review is on the potential exposures that may be incurred by humans; the article does not attempt to characterize exposures by receptors of ecological interest, such as soil invertebrates. Of particular interest are the seven priority pollutant cPAHs (benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, dibenzo[*a,h*]anthracene, and

23 indeno[1,2,3-*cd*]pyrene) currently regulated by the U.S. Environmental Protection Agency
24 (EPA) as carcinogens, as these cPAHs drive human health–based cleanup goals at PAH-
25 contaminated sites. Attention is given to benzo[*a*]pyrene (BaP) because the toxicity of BaP has
26 been better characterized than that of other cPAHs. Studies using naphthalene, considered to be a
27 carcinogen by the inhalation route of exposure, are not reviewed because its high volatility and
28 water solubility renders it chemically distinct from the cPAHs associated with human exposures
29 via oral and dermal routes of exposure. Studies of noncarcinogenic PAHs such as pyrene and
30 phenanthrene (PN) are reviewed to the extent that they provide information about important
31 precedents, inform the discussion of research methods and study design, or elucidate the
32 processes that control oral bioavailability, bioaccessibility, or dermal absorption of cPAHs
33 from soil.

34 Publications regarding dermal absorption of PAHs from soil began to appear in the late 1980s
35 and early 1990s. These were followed by studies of oral bioavailability in animal models and the
36 development of physiologically based extraction tests (PBETs) to measure PAH bioaccessibility,
37 with the latter topic yielding most of the publications in the last 10 years. To date, 67
38 publications or abstracts have been identified for review regarding the oral bioavailability,[1-22]
39 bioaccessibility,[16,17,20,21,23-52] and dermal absorption[2,11,53-67] of PAHs in soil or PAH source
40 materials (soot, char, coal, coke, coal tar, pitch, creosote, and petroleum products).

41 The bulk of this article discusses the sources and chemistry of PAHs in soil; *in vivo* and *in vitro*
42 models that have been developed to assess the oral bioavailability and bioaccessibility,
43 respectively, of cPAHs; and dermal absorption of cPAHs from soil. This article concludes with
44 recommendations for future research that would fill data gaps and yield studies that are readily
45 applicable to human health risk assessment.

46 **Definitions**

47 Because the terms "bioavailability" and "bioaccessibility" are sometimes defined in different
48 ways by different authors, the following definitions, which are standard in the fields of
49 mammalian toxicology and environmental exposure assessment for humans, are used throughout
50 this article.

51 ***Oral Bioavailability***

52 "Oral bioavailability is defined as the fraction of an ingested dose that crosses the gastrointestinal
53 epithelium and becomes available for distribution to internal target tissues and organs."[68] This is
54 commonly referred to as absolute oral bioavailability.

55 ***Relative Oral Bioavailability***

56 Relative oral bioavailability refers to comparative bioavailabilities of different forms of a
57 substance or for different exposure media containing the substance; it is expressed herein as
58 relative bioavailability (RBA).[68,69] For the studies reviewed in this article, the exposure medium
59 of interest is soil and the appropriate reference medium is BaP in rodent chow, because this is the
60 medium that was used for dosing in the critical toxicity studies.

61 ***Bioaccessibility***

62 Bioaccessibility is a measure of the physiological solubility of a chemical at the portal of entry
63 into the body.[68-70] In this article, bioaccessibility refers to the solubility of PAHs in benchtop
64 extraction tests (or "*in vitro*" extraction tests) conducted to estimate the relative oral
65 bioavailability that might be measured in an animal study. Bioaccessibility is an operationally

66 defined measure, dependent on parameters such as extraction fluid pH and chemical
67 composition, extraction time, and temperature.

68 ***Dermal Absorption***

69 Dermal (or percutaneous) absorption of a chemical in soil describes the transport from soil
70 through the skin to subcutaneous circulation. In this discussion, it is assumed to include cPAHs
71 remaining in or on the skin after washing, because lipophilic chemicals in the skin could
72 eventually be systemically absorbed.[71]

73 Sources of PAHs to Soil and Chemical Interactions

74 PAHs are deposited in soil from different source materials (e.g., soot, char, coke, coal, pitch, coal
75 tar, creosote, oil tar, crude oil, or petroleum products) and subsequently interact with soil
76 components, which could affect their oral bioavailability and dermal absorption. Because
77 research on this topic specific to human exposures is limited, the concepts presented here have
78 been formulated from research on chemical interactions of PAHs with carbonaceous materials
79 and the published literature on how these interactions control uptake in benthic invertebrates and
80 earthworms.

81 PAH Source Materials

82 Carcinogenic PAHs are emitted into the environment either as by-products of incomplete
83 combustion and pyrolysis processes (pyrogenic PAHs), or when released from petroleum
84 products or coal (petrogenic PAHs). Petrogenic PAHs are generally released within nonaqueous-
85 phase liquid (NAPL) matrices such as crude oil or petroleum distillates, while pyrogenic PAHs
86 are generally emitted within and sorbed to the surface of a matrix of tar, pitch, or black carbons

87 such as soot and char. Table 1 summarizes the PAH sources of natural, industrial, and
88 nonindustrial origins and the primary PAH-bearing materials produced by these sources. Black
89 carbon from both natural and industrial origins is ubiquitous in soil and is particularly elevated at
90 specific types of industrial sites, such as manufactured gas plants (MGPs) and coking operations.

91 **PAH Sorption and Desorption from Different Forms of Organic Carbon**

92 Within the soil environment, sorption of PAHs can be broadly described as a combination of
93 sorption to natural organic matter (NOM) and black carbon domains.[72] While NOM typically
94 displays linear and noncompetitive *ab*sorption or partitioning,[73] black carbon typically displays
95 nonlinear and competitive surface *ad*sorption.[74,75] The PAH fraction weakly absorbed within
96 NOM or petroleum, or on mineral surfaces, can be defined as the *rapidly desorbing* fraction[76]
97 and is widely regarded as the PAH fraction potentially available for uptake by organisms living
98 in soil or sediment. PAHs strongly adsorbed to the surface or residing within narrow nanopores
99 of more carbonized materials have enhanced sorption to the carbon phase, diminishing their
100 tendency to partition out of the sorbed phase into the aqueous phase. These PAHs are considered
101 to be part of the *slowly desorbing* fraction.[76] This fraction includes *strongly bound/recalcitrant*
102 PAHs that are regarded as unavailable for degradation by soil organisms and are only extractable
103 from the soil matrix using harsh solvents.[77] Some PAHs can be so tightly bound or entrapped
104 within these carbonized materials[78,79] that they cannot be removed by vigorous solvent
105 extractions; these are considered to be in the *irreversibly bound* or *nonextractable* fraction.[77]
106 These distinctions are depicted conceptually in Figure 1, in which certain types of organic
107 carbon, like NOM and NAPL (e.g., crude oil or petroleum products), contain only rapidly
108 desorbing PAHs. Black carbon materials contain primarily slowly desorbing and irreversibly
109 bound PAHs, and some types of organic carbon, such as pitch, contain a mixture of rapidly

110 desorbing, slowly desorbing, and irreversibly bound PAHs (depending on the production process
111 and extent of weathering).

112 Black carbons such as soot and char have been shown to provide strong sorption domains for
113 PAHs.[72,74,80-82] In the presence of these strong binding domains, the sorption of organic
114 contaminants to soils and sediments can be up to two orders of magnitude higher than that
115 predicted for NOM.[74,83] A number of studies have shown how this enhanced sorption can reduce
116 the fraction of PAHs available for uptake by earthworms and benthic invertebrates.[84-86] While the
117 oral bioavailability of PAHs ingested by a human is complex, it is likely that strong sorption,
118 especially to black carbon domains, may limit the release of PAHs in the gastrointestinal tract
119 environment. For dermal absorption to occur, the sorbed PAHs must be released at the skin
120 surface. Thus, it is likely that the presence of slowly desorbing PAHs reduces the dermal
121 absorption of PAHs from soil, although this has yet to be demonstrated. This chemical model
122 suggests that the PAH source material or the organic carbon form into which the PAHs have
123 predominantly partitioned will act as controlling factors in determining the relative oral
124 bioavailability and dermal absorption of PAHs.

125 **Competition and Saturation Effects**

126 Another important issue to consider when examining the interaction between PAHs and soils is
127 that adsorption to black carbons is competitive and nonlinear, so the lower the concentration of
128 PAHs in soil, the more likely that black carbons will dominate sorption.[74] However, at higher
129 organic compound concentrations, which include not only PAHs but also other organic
130 contaminants and native organic compounds in soils (e.g., natural aromatic acids), competition
131 effects can saturate or block the available surface adsorption sites.[74,87] Absorption into NOM

132 may therefore gain increasing importance at high PAH concentrations. Whether PAH
133 partitioning is governed by adsorption to black carbon or by absorption in NOM can result in as
134 much as two orders of magnitude difference in aqueous equilibrium partitioning of PAHs.[74]
135 These differences suggest that studies of oral bioavailability or dermal absorption conducted at
136 elevated PAH concentrations (tens to thousands of milligrams per kilogram of BaP) may
137 overestimate oral bioavailability or dermal absorption compared to what would be seen at more
138 environmentally relevant concentrations (e.g., in the range of soil cleanup goals of approximately
139 0.1 to 1 mg/kg as BaP equivalents).

140 **Effects of Aging or Weathering on PAH–Soil Interactions**

141 It is also important to consider the processes that occur during weathering of PAHs in soils over
142 many decades in the natural environment. In this context, weathering is associated with losses by
143 biodegradation, leaching, or volatilization of the rapidly desorbing fraction of PAHs, and the
144 continuous diffusion and retention of PAH molecules into remote and inaccessible regions within
145 the soil matrix.[88] The diffusion of PAHs into less accessible regions over time—from less
146 strongly sorbing NOM or NAPL into more strongly sorbing black carbon phases, or into even
147 more inaccessible nanopores within the black carbon particles—is likely to reduce the oral
148 bioavailability and dermal absorption of PAHs from soil. Thus, studies that rely on soils that
149 have been spiked with PAHs, including those in which the spiked PAHs have been artificially
150 weathered in the laboratory, may lead to oral bioavailability or dermal absorption measurements
151 that are biased higher than would be seen with PAHs weathered into soils in the environment.
152 Experiments utilizing spiked soils may be appropriate for providing initial insights into
153 bioavailability and bioaccessibility processes, making preliminary comparisons across soil types

154 or concentrations, or evaluating the effects of mixtures. However, the limitations of utilizing
155 spiked soils should be acknowledged in any interpretation of the data resulting from such studies.

156 Oral Bioavailability of PAHs from Soil

157 Several approaches can be used for estimating the oral bioavailability of a chemical in soil to
158 laboratory animals, including measurement of the parent chemical and/or metabolite(s) in blood,
159 tissue, or excreta (urine or feces). These approaches have been used in a number of studies to
160 assess the relative oral bioavailability of PAHs from soil. Table 2 summarizes the key
161 experimental parameters of these studies, and Table S1 describes the soils, experimental
162 conditions, and results for studies on the oral bioavailability of PAHs from soil that have been
163 conducted to date. All of these approaches have theoretical rationales, and if their underlying
164 assumptions are met, can yield reasonable estimates of bioavailability. However, many of the
165 assumptions are difficult to satisfy, especially when evaluating the bioavailability of PAHs *in*
166 *vivo*. Because of this, there are substantial practical limitations in the choice of methods, and
167 these limitations must be considered carefully when designing or interpreting results from studies
168 of the bioavailability of PAHs from soil. The following sections describe the different
169 fundamental approaches to assessment of the oral bioavailability of PAHs from soil; Figure S1
170 provides a schematic of the absorption, distribution, metabolism, and excretion pathways for
171 PAHs in mammals to illustrate the concepts described below.

172 Measurement in Blood

173 In classical pharmacological terminology, the bioavailability of a chemical is the fraction of an
174 administered dose that is absorbed into systemic circulation.[89] Bioavailability is calculated from

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175 the concentration of the chemical in blood (whole blood, serum, or plasma) over time, and
176 reported as the *area under the curve* (AUC) from the blood concentration versus time profile.
177 The AUC captures the rise and subsequent decline in concentration following dosing and is
178 assumed to be proportional to the amount of chemical absorbed systemically. *Absolute* oral
179 bioavailability is derived from the ratio of the AUCs following matched doses administered
180 orally versus intravenously.[90] *Relative* oral bioavailability (expressed as the RBA) of a chemical,
181 for use in risk assessment, is derived from the ratio of the AUC following an oral dose of the
182 chemical in the medium of interest (e.g., soil) versus the AUC from an equivalent oral dose of
183 the chemical in the medium used in the critical toxicity study[69] (e.g., rodent chow for BaP). Note
184 that doses do not have to be equivalent as long as they are in the linear pharmacokinetic range
185 and the AUCs are corrected by the ratio of the doses administered. In the case of PAHs, the
186 critical studies that currently form the basis of EPA's cancer potency estimate used BaP provided
187 to animals in their diet (rodent chow),[91,92] and EPA has recently proposed a potency estimate
188 based on BaP dietary exposure from a different rodent study.[93] Therefore, absorption of PAHs
189 from soil relative to absorption from diet is the appropriate metric for determining RBA for use
190 in human health risk assessment. (As noted in Tables 2 and S1, absorption from the diet is not
191 always selected as the basis for calculating RBA values reported in the literature.)

192 The AUC can be determined for blood concentrations of a parent chemical, one or more
193 metabolites, or the parent chemical plus metabolites. Among the published studies evaluating
194 RBA of PAHs using blood measurements, most have measured the parent chemical (usually
195 BaP),[2,6,21] while one has measured the parent chemical plus metabolites by measuring
196 radioactivity in blood following a dose of radiolabeled BaP (Goon et al.,[1] as described in Magee
197 et al.[3]). The AUC of parent PAH after an oral dose is dependent on not only the amount of PAH

198 that is absorbed and enters the systemic circulation, but also the rate of removal of the PAH from
199 the blood, either through metabolism, excretion (biliary and urinary), or deposition into tissues.
200 In order for the ratio of AUCs of parent PAH from soil versus diet to reflect RBA, rates of
201 removal from the blood must be the same under both dosing conditions. This is a difficult
202 condition to meet when bioavailability is measured subsequent to multiple PAH doses.

203 Carcinogenic PAHs, such as BaP, are potent inducers of cytochrome P450 (CYP) enzymes,
204 including enzymes that mediate their own metabolism.[94] Unless the extent of enzyme induction
205 is equivalent following both soil and diet doses, measurement of RBA is confounded by
206 differential extents of PAH metabolism. In theory, administered doses from soil and food could
207 be adjusted so that the internal dose of BaP to the liver is the same and the induction state is
208 equivalent. This can be accomplished by bracketing the estimated internal dose from test soils
209 with multiple doses of reference material and monitoring hepatic enzyme activities.[95] We note
210 that this approach can be challenging, in that substantial variability in induction can occur among
211 animals in the same treatment group, making comparisons difficult. This problem can be avoided
212 by assessing RBA in naïve animals after a single dose. As long as the animals for each of the
213 treatment groups have been housed under the same conditions with the same diet, interference
214 with RBA measurement from differences in CYP activity and BaP clearance should be minimal.
215 Although the dosing regimen does not mimic environmental exposures, in that it is not repeated
216 over time, it is well suited for measuring the extent to which PAHs in a soil matrix have
217 diminished gastrointestinal absorption relative to PAHs in diet.

218 As noted above, the fundamental assumption underlying blood measurements to establish RBA
219 values is that the AUC is directly proportional to the amount of chemical that has been absorbed
220 from the gastrointestinal tract and that reaches systemic circulation. While this assumption is

221 generally valid over a limited oral dose range, chemical-specific saturable processes affecting
222 absorption or metabolism can cause the relationship between absorbed dose and AUC to be
223 nonlinear. Ideally, when assessing the RBA of a chemical from soil, it should be demonstrated
224 that the doses of PAH administered are in the range of linear pharmacokinetics, although most
225 studies simply select similar doses to administer from the media being compared and assume that
226 the basic pharmacokinetics (other than fraction absorbed) will be the same.

227 To measure an AUC with reasonable accuracy, blood concentrations at several time points are
228 needed. At progressively lower PAH concentrations in soil and diet, blood concentrations can
229 decrease until most are below analytical detection limits and the error in estimating the AUC
230 becomes unacceptably high. Based on studies published to date (e.g., van Schooten et al.[6] and
231 Duan et al.[21]), soil BaP concentrations need to be minimally in the tens of milligrams per
232 kilogram to produce blood concentrations sufficient to determine BaP bioavailability (Table S1).
233 In comparison, EPA's current screening levels for BaP in residential and commercial soils are
234 0.015 and 0.21 mg/kg, respectively;[96] in practice, site-specific cleanup goals tend to be in the
235 range of 0.1 to 1 mg/kg BaP equivalents. Thus, there is a wide range of BaP concentrations in
236 soil for which bioavailability information might be useful but cannot be quantified by direct
237 measurement of BaP in blood. At present, it is unclear whether RBA measurements obtained for
238 highly contaminated soil (i.e., soil with BaP concentrations in the range of 50 to 200 mg/kg) can
239 be assumed to apply to soil with lower, more environmentally relevant concentrations (e.g., 0.1
240 to 1 mg/kg). As discussed above, some of the processes that control the binding of PAHs to soil
241 are concentration dependent, so bioavailability may also be concentration dependent, and the
242 extrapolation of RBA values from soil with high PAH concentrations may overestimate RBA for
243 soils with lower PAH concentrations.

244 Rather than measuring an AUC, some bioavailability studies have measured the blood
245 concentration of BaP at a single time point as an indicator of absorbed dose. This approach is
246 valid only if the time course of increasing and decreasing blood concentrations is identical
247 following administration in soil and diet, so that the ratio of blood concentrations at any single
248 time point reflects the comparative fraction of dose that is absorbed from these two dosing
249 media. Any shift in the blood concentration versus time profile (e.g., if absorption from soil
250 occurs more slowly than from diet) can cause blood concentrations at a given time point to be
251 different even if the total absorbed dose is the same. If blood concentrations are measured at only
252 one time point, there is no way to determine whether a shift has occurred, making this approach
253 unreliable under most circumstances.

254 **Measurement in Urine**

255 PAH metabolites, and to some extent parent PAHs, are excreted in urine.[97-99] If the amount
256 excreted is proportional to the absorbed dose, then urinary excretion can be used as a quantitative
257 measure of absorption. Previous attempts to use urinary excretion to measure bioavailability
258 have focused on metabolite excretion, e.g., 3-hydroxybenzo[*a*]pyrene following exposure to BaP
259 in soil[9] or 3-hydroxypyrene following exposure to pyrene in soils.[5,7] A principal problem caused
260 by using urinary metabolites as an indicator of PAH absorption stems from the fact that urinary
261 excretion is a minor pathway of elimination. For example, Jacob et al.[100] observed that only 0.4%
262 of an oral dose of pyrene in rats was excreted in urine as pyrene plus 1-hydroxypyrene, and
263 Jongeneelen et al.[101] observed that only 0.22% to 0.35% of an oral dose of BaP in rats was
264 excreted as 3-hydroxybenzo[*a*]pyrene (parent BaP was not detectable). In studies by Ounnas et
265 al.[19] and Costera et al.,[102] goats were given daily oral doses of soil spiked with 100 mg/kg of PN,
266 pyrene, and BaP (each) for 10 days, and the predominant hydroxylated metabolite for each was

267 measured in urine. Goats excreted 20% to 32% of the pyrene dose as 1-hydroxypyrene and 5% to
268 7% of the PN dose as 3-hydroxyphenanthrene, but 3-hydroxybenzo[*a*]pyrene concentrations in
269 urine were too low to quantify. Finally, in rats dosed with a mixture of PAHs, including 35
270 mg/kg pyrene and 9.2 mg/kg BaP, only 0.2% of the pyrene in soil was excreted in urine as
271 1-hydroxypyrene, and 3-hydroxybenzo[*a*]pyrene was not detected.[6]

272 The very low urinary excretion rates for larger PAHs like BaP, particularly after doses in soil,
273 create three limitations that are related to 1) analytical detection limits, 2) contamination by fecal
274 matter, and 3) signal-to-noise ratio. Because the fraction excreted in urine is low, doses of PAH
275 administered must be high (relative to environmental doses) to be able to detect and reliably
276 measure the metabolite in urine. For example, as noted above, in studies involving goats, rats,
277 and mice, excretion of 3-hydroxybenzo[*a*]pyrene in urine following doses of BaP in soil with
278 concentrations ranging from 10 to 100 mg/kg was vanishingly small, if quantifiable at all.
279 Analytical data close to the practical detection limit is prone to high uncertainty. The second
280 limitation is the potential for contamination from feces. Specialized metabolism cages for
281 rodents allow for separation of urine and feces, but none are completely effective in this regard.
282 Extensive biliary excretion of PAHs and metabolites means that both absorbed and unabsorbed
283 PAHs are eliminated predominantly in feces. As an example, Grimmer et al.[103] found that the
284 hydroxylated metabolite profile for chrysene administered orally to rats was very similar
285 between urine and feces, but that feces contained 100-fold higher concentrations. Because of the
286 much higher concentrations of PAHs and metabolites in feces relative to urine, even transient
287 contact of urine with fecal matter as they are separated in the metabolism cage can confound
288 measurements of urinary concentration and result in overestimates of bioavailability due to
289 PAHs and metabolites detected in urine but actually excreted in feces. Lastly, estimates

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290 regarding the extent of absorption based on measurement of urinary metabolites must be made
291 on changes in very small numbers, which is inherently prone to error—a classic signal-to-noise
292 problem.

293 **Measurement in Feces**

294 Ingested PAHs that are not absorbed from the gastrointestinal tract are eliminated in feces, either
295 as parent compounds or as metabolites formed by gut microflora. PAHs absorbed from the
296 gastrointestinal tract are largely metabolized and returned to the gut through biliary excretion.
297 For PAHs with four to six rings (i.e., the cPAHs), biliary excretion is the predominant route of
298 elimination of metabolites.[97-99] For example, Foth et al.[97] found that approximately 40% of an
299 intravenous dose of BaP in rats was excreted in bile as metabolites within four hours of dosing.
300 As a result, fecal contents reflect both absorbed and unabsorbed PAHs, and distinguishing
301 between the two for the purpose of estimating bioavailability is difficult. Both hepatic
302 metabolism of absorbed PAHs and microbial metabolism of unabsorbed PAHs produce
303 hydroxylated metabolites.[90,104] While it might be possible to identify distinctive metabolite
304 profiles from the two sources so that they can be individually quantified from fecal
305 measurements, this has never been demonstrated. Studies of gut microbial metabolism of PAHs
306 are limited, but it is reasonable to speculate that PAH metabolism patterns are dependent on the
307 specific microflora present, which in turn would be expected to vary with host species (e.g., rat,
308 mouse, or human), diet, and other factors.

309 An additional confounding factor is the enterohepatic recirculation of PAHs. Glucuronide and
310 sulfate conjugates of PAHs excreted in bile can be cleaved by intestinal microbial flora,
311 facilitating reabsorption of the PAH or metabolite from the intestine. Subsequent conjugation

312 and biliary excretion followed by microbial deconjugation and reabsorption continues the cycle,
313 delaying elimination from the body. Enterohepatic recirculation has been demonstrated for a
314 variety of PAHs, including pyrene and BaP.[6,90,105,106] Enterohepatic recirculation (Figure S1)
315 affects not only the time course over which PAHs appear in feces but the form in which they
316 appear. Conceivably, the complicated nature of these processes would be immaterial in
317 determining RBA if microbial metabolism and enterohepatic recirculation apply equally to doses
318 from any medium (i.e., if the amount of PAH or metabolite excreted in feces is directly
319 proportional to absorbed dose), but this has not been demonstrated.

320 Collection of bile directly (rather than feces) would capture the primary route of excretion for
321 PAHs while avoiding confounding effects from microbial metabolism in the gut and
322 enterohepatic recirculation, and the amount of PAH metabolite eliminated in the bile should be
323 proportional to the absorbed dose. However, the presence of bile salts in the intestinal lumen is
324 very important for the absorption of PAHs, particularly four- and five-ring PAHs.[107] As a
325 consequence, interruption of bile flow by cannulation of the bile duct and collection of bile
326 samples creates a model in which PAH absorption is artificially diminished and its reliability in
327 determining RBA is untested (and could result in a low bias for estimates of absorbed dose).

328 **Measurement in Tissue**

329 With repeated doses, and once a steady state has been achieved between blood and tissues, the
330 concentration of a PAH or metabolite in tissues will be proportional to the absorbed dose. Thus,
331 tissue concentrations could be used to estimate RBA by comparing results from animals given
332 the same dose (e.g., from soil versus food). The relationship between tissue concentration and
333 absorbed dose is more tenuous when not at steady state. To achieve steady state, multiple doses

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334 must be given over time. As discussed above, self-induction of metabolism that occurs with
335 repeated doses can produce differences in metabolic clearance among animals ingesting PAHs in
336 soil versus diet, and the direct proportionality between tissue concentrations and absorbed dose
337 needed for bioavailability determination may be lost. Although difficult to address
338 experimentally, this problem can be approached in the same manner as described above
339 ("Measurement in Blood") if differences in metabolic clearance from multiple doses are likely.

340 **Measurement of Bioavailability Using Biomarkers**

341 The use of biomarkers as endpoints for bioavailability measurements has appeal because it can
342 potentially provide highly relevant indicators of the internal dose of a chemical. Although
343 biomarkers as endpoints may not fit the classical definition of bioavailability, they offer an
344 alternative and potentially informative view of differential absorption of environment chemicals.
345 There is particular interest in biomarkers related to critical toxic effect(s). For PAHs, limited
346 studies have attempted to assess bioavailability using CYP induction[8,15,108] and PAH–DNA
347 adducts[5,7,10,12] as endpoints. CYP metabolism of PAHs produces reactive, genotoxic
348 metabolites;[90] DNA adducts are biomarkers because they are considered precursor events leading
349 to PAH carcinogenesis.[109] As long as a biomarker is a better quantitative indicator of toxicity
350 than simply measuring a chemical or its metabolite(s) in the body, there is a logical basis to use it
351 for bioavailability assessment. Establishing a quantitative relationship between a biomarker and
352 toxicity is difficult, particularly for cancer risk in the case of PAHs. For example, while CYP
353 activity is clearly an important factor in PAH carcinogenesis, there is currently no established
354 quantitative relationship between CYP activity and cancer risk. Similarly, while the presence of
355 DNA adducts is considered necessary for PAH carcinogenesis and is associated with increased
356 cancer risk in humans, DNA adducts do not always correlate well with tumor formation. In mice

357 treated with BaP, DNA adducts are found in tissues that do not develop tumors as well as those
358 that do.[110] Therefore, unless the relationship between the selected biomarker and the risk or
359 incidence of the toxic effect is well established, this approach may be unreliable. Further,
360 because biomarkers usually result from the culmination of a number of biological processes, the
361 likelihood that they are linearly related to dose over the entire exposure range of interest is small.
362 As noted by Godschalk et al.,[109] DNA adduct formation from PAHs does not display a strong
363 proportional relationship to exposure in humans. Hence, an RBA value generated using
364 biomarkers may be dose dependent. In other words, RBA will depend on the PAH concentration
365 in soil, along with other variables, making it not only site-specific but concentration-specific.
366 This greatly complicates its use for RBA assessment.

367 Finally, there is the issue of using an RBA based on internal dose metrics (i.e., biomarkers) but
368 with a toxicity value based on external doses, such as a cancer slope factor. If the RBA is based
369 on something other than a difference in absorption from the exposure medium and incorporates
370 other biological processes, then it is addressing a fundamentally different form of "dose" than the
371 one used to derive the toxicity value. An exposure estimate from a biomarker-based RBA would
372 be incompatible with a standard cancer slope factor or reference dose for risk estimation.

373 **Review of Existing *In Vivo* Studies**

374 Table S1 provides a summary of some of the key parameters reported in various *in vivo*
375 models/studies that have been conducted to evaluate the oral bioavailability of PAHs, including
376 BaP. The table shows 1) studies that have appeared in peer-reviewed publications and for which
377 enough information is provided that the quality of the study can be evaluated, 2) studies that
378 have appeared in peer-reviewed publications but with insufficient information to fully evaluate

379 their quality, and 3) studies that are available only as abstracts or are referred to in other
380 publications. Studies published only in non-English languages are excluded from this review.

381 The 20 studies summarized in Table S1 used a variety of animal models to evaluate the RBA of
382 various PAHs, including mice, rats, mini pigs, juvenile swine, and goats, and have attempted to
383 evaluate the RBAs of BaP, dibenzo[*a,h*]anthracene, benzo[*a*]anthracene, pyrene, PN, anthracene,
384 cPAHs, and total PAHs. Measurement endpoints have included the AUC of BaP and metabolites
385 in blood (based on radiolabel), the AUC of BaP in blood and plasma, excretion of hydroxylated
386 PAH metabolites in urine, excretion of parent PAHs in feces, DNA adduct formation in lung and
387 liver tissue, concentrations of parent PAHs in various tissues, and liver enzyme induction. Most
388 of the studies (17 of 20) report the soil particle size dosed, but most of them dosed soil particles
389 much larger than those that adhere to human hands and may be incidentally ingested (<150 to
390 <250 μm).[111,112] Given this array of animal models, PAHs evaluated, measurement endpoints,
391 and soil particle sizes dosed, it is difficult to compare results directly across studies. Of the 20
392 studies reviewed in Table S1, RBA values that could be used in human health risk assessment
393 were either not reported, or could not be calculated from the data presented in the publication, for
394 nine of them.

395 Together, these studies provide a general basis for establishing that the bioavailability of PAHs
396 from soil is reduced relative to absorption from diet, and that the default assumption of 100%
397 RBA likely overestimates actual exposure to cPAHs from soil. The studies do not, however,
398 provide a strong basis to support conclusions regarding the specific reduction in bioavailability
399 or allow for further understanding beyond the individual samples tested. Because of the limited
400 scope of each individual study and the large variability in animal models and study designs
401 utilized, the aggregate data do not indicate what soil chemical conditions will yield a particular

402 RBA estimate, nor do they provide a broad understanding of how different PAH source materials
403 (e.g., soot, char, coal, coke, coal tar, pitch, creosote, petroleum products) influence the RBA of
404 cPAHs from soil.

405 In general, the studies used test materials with BaP concentrations in the 20 to 120 mg/kg range,
406 well above the soil cleanup goals utilized by regulatory agencies (0.1 to 1 mg/kg, as BaP
407 equivalents). As discussed in "Competition and Saturation Effects," above, it appears that studies
408 conducted in the higher BaP concentration range may tend to overestimate RBA for soils in the
409 1 mg/kg concentration range.

410 In the four studies that evaluated the effect of aging on RBA, one showed a slight reduction in
411 RBA values (14% to 27% reduction after 6 to 12 months of aging)[1] and three showed no
412 reduction in RBA values after weathering for 3 to 12 months.[10,13,21] Only nine of the studies
413 reported the total organic carbon (TOC) of the soils they tested, and none of them characterized
414 the types of organic carbon in the test soils. These data are needed to fully understand the effects
415 of PAH source materials and different forms of organic carbon on cPAH RBA values. There is
416 some indication that soil TOC is inversely related to the RBA of BaP,[1,21] but the data are very
417 limited. If true, this would be consistent with results from PBET studies, as described below.

418 Bioaccessibility of PAHs from Soil

419 *In vitro* extraction tests are defined herein as any benchtop chemical extraction test that is
420 designed to measure the bioaccessibility of PAHs. The focus of our analysis has been on studies
421 that use bioaccessibility as a surrogate to understand oral bioavailability to human receptors. This
422 is to be distinguished from studies related to bioaccessibility to ecological receptors, such as
423 earthworms or benthic communities, for which a separate body of literature exists. To date, these

424 tests have been almost entirely PBETs, developed for two general reasons: 1) to obtain a simple,
425 inexpensive tool to predict the RBA of PAHs from soil; or 2) as a tool to study the chemistry of
426 PAHs in a simulated gastrointestinal system. Whether development of a PBET that correlates to
427 *in vivo* RBA measurements across a wide range of PAH sources and soil types is feasible has not
428 yet been resolved. Although this has been achieved for arsenic and lead in soil using relatively
429 simple *in vitro* tests,[113,114] PAHs present a more complex system in which solubilization in the
430 small intestine, absorption mechanisms, metabolism, and elimination are likely to come into
431 play. However, based on the success of *in vitro* systems in evaluating the absorption of lipophilic
432 pharmaceuticals,[115] the development of a reliable *in vitro* test for PAHs in soil should be feasible.

433 From the time that the first article regarding a PBET for measuring PAH bioaccessibility from
434 soil appeared in the peer-reviewed literature,[23] there have been 33 additional articles addressing
435 some aspect of PBET test development and/or application for PAHs. This has caused a
436 proliferation of methods, most of which are permutations of earlier PBET methods for PAHs and
437 other contaminants.[23,116,117] PBETs originating in Europe have tended to be more complex
438 because of an attempt to mimic gastrointestinal-tract chemistry as closely as possible, based on
439 the assumption that this will yield an extraction system that more accurately predicts uptake in
440 humans. In contrast, tests originating in Canada, the U.S., and Australia have tended to rely on
441 correlation with *in vivo* data, to try to establish that they are accurately predicting the
442 bioavailable fraction as measured in animal models. As a result, these tests can be less complex
443 because they are focused on capturing the critical test components that allow for a correlation
444 with animal data. Most recently, simple extractions using chemical solvents and PAH
445 partitioning approaches have also been evaluated for their ability to predict the oral
446 bioavailability of BaP[21] and other PAHs.[22]

447 Only the key findings from available *in vitro* studies and the attempts to validate an *in vitro* test
448 against *in vivo* RBA data are discussed below. Table 3 summarizes the key PBET parameters
449 addressed in the published literature on the bioaccessibility of PAHs from soil, and the ranges of
450 values that have been used to date. Table S2 describes the soils, experimental conditions, and
451 results in detail for the PBET studies conducted to date.

452 **Key Findings of Bioaccessibility Research**

453 Reported values for the bioaccessibility of PAHs from soil are highly variable, depending on the
454 PBET method used and the substrates evaluated; however, in general, reported bioaccessibility
455 values are <50% for BaP and other cPAHs. Overall, the available studies we identified indicate
456 that the TOC content of soils is inversely related to bioaccessibility.[16,25,26,32,37,39,40]

457 Bile salts in the small intestine act as the primary agent for solubilization of PAHs from soil and
458 greatly increase the bioaccessibility of PAHs.[23,26,27,40,118] Consistent with this observation,
459 Rahman et al.[107] demonstrate that in rats, the absence of bile in the small intestine results in a
460 77% decrease in BaP absorption. Bile salts, in the presence of lipids and cholesterol, or dietary
461 lipids, form mixed micelles into which the PAHs can partition.[26,119] These mixed micelles can
462 then deliver the PAHs to the intestinal epithelium, where they can be absorbed. Factors affecting
463 the formation of micelles, such as bile concentration,[23,26,27,40,118] lipid concentration,[23,51] and pH
464 of the small intestine[51] are therefore important components in the development of PBET methods
465 for PAHs. The literature also identifies kinetic and PAH solubility constraints that can reduce the
466 amount of PAHs extracted by PBETs, and demonstrates how the addition of an infinite sink to
467 the small-intestinal phase (Table 3) enhances PAH dissolution from soil.[43,45,46,50] The addition of
468 an infinite sink could better mimic conditions of the gastrointestinal tract, where passive

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469 diffusion of PAHs across the intestinal epithelium and binding to other components within the
470 intestinal lumen are likely to maintain steep diffusion gradients, enhancing PAH dissolution from
471 soil. A study by James et al.[20] reports an improvement in the *in vitro* to *in vivo* correlation
472 (IVIVC) for PAHs from soil with the addition of a C18 membrane to the extraction fluid.
473 However, this is in comparison to results from a simple, buffered, acidic extraction system
474 developed for metals; it specifically excludes additions to mimic the intestinal environment and
475 the reported IVIVC is still poor. Therefore, whether the addition of an infinite sink improves the
476 correlation of *in vitro* data with *in vivo* models is yet to be determined.

477 **Validation of *In Vitro* Tests**

478 Validation of an *in vitro* test against RBA results from an animal model (i.e., an IVIVC) has been
479 attempted in five of the existing studies on the bioaccessibility of PAHs from soil.[16,17,20,21,31] Of
480 these five studies, Gron et al.[31] observed the best IVIVC ($r^2 = 0.81$), based on RBA values for
481 BaP in seven test soils. However, Gron et al. combined two sets of *in vivo* data, which were
482 based on different animal models (mouse [three soils] and mini pig [four soils]) and two different
483 biological endpoints (urinary excretion of 3-hydroxybenzo[*a*]pyrene in mouse urine and
484 unabsorbed BaP in mini-pig feces). Neither of these *in vivo* models or data sets was published in
485 the peer-reviewed literature; thus it is impossible to confirm the quality of the *in vivo* data that
486 serve as the basis of this IVIVC. Of the remaining four papers, only Pu et al.[16] provides sufficient
487 detail on the *in vivo* methods used (measurement of the AUC for PN in blood for eight spiked
488 soils) to allow for a critical assessment of the IVIVC. However, this *in vivo* study yielded RBA
489 values in excess of 100% for three of the eight soils dosed, most likely because of the low
490 absolute bioavailability measured for the corn-oil gavage reference dose (24%). The research by
491 Duan et al.[21] evaluated *in vivo* results against two *in vitro* methods that used simple chemical

492 extractions (butanol and cyclodextrin); however, the authors did not present RBA values or the
493 data from which they could be calculated, so an IVIVC cannot be developed from that study.
494 Finally, neither Stroo et al.[17] nor James et al.[20] observed strong correlations between their *in vivo*
495 and *in vitro* results. As a result, the development of a reliable IVIVC for cPAHs in soil is an
496 outstanding goal, and one that will require a set of RBA values from a range of soils that are
497 derived from a competent *in vivo* model.

498 Dermal Absorption of PAHs from Soil

499 Assessment of the dermal absorption of PAHs from soils is important to ensuring the accurate
500 evaluation of total systemic exposures from soil. However, if adjustments are made to account
501 for reduced RBA from ingestion of soil while default assumptions regarding dermal absorption
502 are not addressed, then the dermal pathway may inappropriately be perceived to drive overall
503 risk to humans. Many of the soil–chemical interactions that affect the absorption of PAHs from
504 ingested soil are likely to also influence the partitioning of chemicals from soil to skin, and hence
505 affect dermal absorption. To date, 12 studies have addressed the dermal absorption of BaP[11,53-
506 57,59,61-65] and five have addressed the dermal absorption of PN.[2,57,58,60,66] The key experimental
507 parameters, and their ranges, for these dermal absorption studies are summarized in Table S3,
508 and the test soils and the specific experimental conditions utilized in each study are detailed in
509 Tables S4 and S5, respectively. Due to space limitations, only a few of the important findings
510 from review of the dermal absorption studies are presented herein.

511 Given the wide variety of experimental conditions (e.g., PAH sources, species/skin sources,
512 *in vivo* versus *in vitro* studies, particle sizes used, soil aging times, exposure times, PAH
513 concentrations in test soils, and soil loadings; see Tables S4 and S5), it is difficult to compare

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514 across studies and draw conclusions from this body of literature. The effects of PAH source
515 material, soil particle size, and aging or weathering of PAHs in soil are discussed below.

516 **Effect of PAH Source Material**

517 Soils in five of the dermal absorption studies contained PAHs in a source material added to the
518 soil (petroleum crude or coal tar) or present in the contaminated soil sample as
519 lampblack.[54,56,61,63,66] The TOC varied among the soils and lampblack samples from less than
520 0.5% to more than 80%, although the proportion of TOC that is black carbon is not reported.
521 Soils in all of the other studies were spiked with BaP or PN in a solvent solution assumed to be
522 subsequently removed by evaporation. Given this limited data set and the variability in study
523 designs, the effect of PAH source material on dermal absorption is difficult to evaluate.

524 **Effect of Soil Particle Size**

525 Soil particle size affects skin adherence and chemical transfer to skin, as well as the soil's
526 capacity to sorb contaminants. Thus, experiments meant to provide dermal absorption
527 measurements for risk assessment should include only fine particles, on the order of <150 μm or
528 smaller, that would preferentially adhere to human skin.[71,120] For the studies included in this
529 review, soil particle sizes ranged from <100 to <710 μm (Table S4), with the majority of studies
530 focused on soil particles <150 μm. Of particular note is the study of Wester et al.,[55] which forms
531 the basis for the recommendation from EPA to assume a dermal absorption fraction of 13% for
532 PAHs in soil,[121] and which used a particle size fraction of 180 to 320 μm (fine to medium sand).

533 **Effect of Aging or Weathering of PAHs in Soil**

534 It is generally expected that aging or weathering of laboratory-contaminated soils would reduce
535 PAH absorption. While there is extensive literature on the effects of aging on uptake of PAHs

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536 from soil and benthic organisms,[69] experimental evidence related to dermal uptake in mammals
537 is very limited in the studies to date. For soils contaminated with coal tar, Roy and Singh[61]
538 observed no difference in BaP absorption from coal tar added to soils and aged 1 day compared
539 with soils aged for 45 days, and an approximately twofold reduction from soils aged 110 days.
540 Notably, the flux through skin after 110 days of aging was the same as that observed by Stroo et
541 al.[63] for two samples with similar BaP levels from an MGP site that had been closed for
542 approximately 50 years. In both of these studies, BaP would have been incorporated into the
543 PAH source material (either coal tar or lampblack), which may explain the minimal effects of
544 laboratory aging and environmental weathering in these studies. In the studies of Abdel-Rahman
545 et al.,[58,62] the effect of aging cannot be assessed because the freshly contaminated soil
546 experiments used as comparison samples appear to have contained residual solvent.

547 Research Needs

548 This detailed review of the literature indicates that much effort has been expended in assessing
549 the oral bioavailability and dermal absorption of PAHs from soil. An extensive body of literature
550 on the effect of PAH source materials and soil–PAH interactions is also available and facilitates
551 a theoretical understanding of the factors likely to control oral bioavailability and dermal
552 absorption of PAHs in humans. However, significant limitations still exist that hamper broad
553 application of bioavailability adjustments for cPAHs in risk assessment. Among these is a lack of
554 a) validated animal and *in vitro* models, and b) studies that demonstrate the influence of PAH
555 source material and soil chemistry, particularly in concentration ranges that are of significance
556 for remediation of contaminated sites.

557 Future research into the oral bioavailability of cPAHs should include a variety of soils that
558 reflect a range of PAH source materials and soil chemistries. It is particularly important to gain
559 an understanding of the role of different types of organic carbon, particularly black carbon, in
560 sequestering cPAHs and limiting their oral bioavailability. Measuring oral bioavailability
561 requires a validated measurement endpoint that reflects absorbed dose. It is possible that a viable
562 *in vivo* model based on urinary or fecal excretion, tissue concentration, or biomarkers could be
563 created; however, it should be demonstrated that the endpoint reflects absorbed dose as indicated
564 by the AUC. For risk assessment at contaminated sites, it is also important that the RBA values
565 are based on a comparison to absorption of PAHs from soil versus the diet, because dietary
566 exposures form the basis of the current regulatory toxicology of PAHs (i.e., the carcinogenic
567 potency of BaP). Ideally, the method will provide adequate sensitivity to allow for
568 characterization of absorption at environmentally relevant doses (in the range of 0.1 to 10 mg/kg
569 BaP in soil) so that PAH–soil interactions accurately reflect factors operating in the PAH
570 concentration range of relevance to remediation of contaminated sites. Finally, if a broad-scale
571 research effort were undertaken that included an adequate range of soils, an *in vitro* method
572 could be validated for a wide range of PAH source materials and types of contaminated sites.

573 With respect to dermal absorption of BaP, data gaps and limitations are apparent and should be
574 addressed in future research. Of particular concern are issues of soil particle size, effects of PAH
575 source materials and soil chemical characteristics, and chemical concentration in soil. Important
576 factors requiring further study are the use of environmentally relevant concentrations (i.e., $\leq$ 10
577 mg/kg BaP) and the fine-particle-size fraction of soils.[122,123] To avoid the complications of
578 animal skin and *in vivo* adjustment factors that cannot be independently tested, *in vitro* studies
579 using human skin are recommended. Investigations into the effect of BaP in different PAH

source materials compared with BaP in a solvent vehicle are especially needed. Nevertheless, given that EPA's default value for PAH absorption is derived from the Wester et al.[55] *in vivo* values, studies that are designed to critically evaluate the validity of the Wester et al. results would also be useful.

Such studies would form a basis to support broader application of bioavailability adjustments at PAH-contaminated sites, either by updating current default assumptions regarding oral or dermal absorption from soils, or by identifying key considerations and tools for assessing bioavailability on a site-specific basis.

Figure

Figure 1. PAH availability for oral or dermal absorption as a function of PAH source materials and soil chemistry.

Acknowledgments

This article is dedicated to the memory of Mike Ruby, a pioneer in the study of chemical bioavailability and a colleague, mentor, and friend to many. His human wisdom and compassion, as well as his scientific insights, are sorely missed.

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600 ## Supporting Information

601 The supporting information includes tables that provide details on the soils, experimental
602 conditions, and results for all of the oral bioavailability, PBET, and dermal absorption studies of
603 PAHs in soils that have been published to date, along with a figure detailing the absorption,
604 distribution, and elimination of PAH in mammals.

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Table 1. Sources of PAHs to Soils

Type of Source	PAH Source	Primary PAH-Bearing Materials
Natural	Forest fires	Soot, char
	Grass fires	Soot, char
	Volcanic eruptions	Soot, char
	Oil seeps	Weathered crude oil
	Sedimentary rock	Kerogen
Industrial	Manufactured gas plants	Coal tar, oil tar, pitch, coal, char, soot
	Coking operations	Coal tar, coal, coke, soot
	Aluminum production	Coal tar or petroleum pitch (making and disposing of anodes)
	Foundries	Coal tar pitch, creosote, fuel oil (used in making sand casts), soot
	Wood treating facilities	Creosote
	Refineries	Soot, various NAPLs (crude oil, fuel oil, diesel)
	Carbon black manufacture	Soot, oil tar
	Fuel spills and/or disposal	Various NAPLs (crude oil, fuel oil, waste oil, diesel)
Nonindustrial	Skeet	Coal tar pitch or bitumen (used as binder in targets)
	Asphalt sealants	Coal tar
	Landfills	Creosote (treated wood), soot, char
	Incinerators (industrial, municipal, hospital)	Soot
	Open burning	Soot, char
	Fire training	Soot, char
	Auto/truck emissions	Soot

Notes:

NAPL = nonaqueous-phase liquid

PAH = polycyclic aromatic hydrocarbon

Table 2. Summary of Key Parameters and Ranges Used in Oral Bioavailability Studies

PAH Sources	PAH Concentration in Soil	Soil Particle Size Tested	Number of Soils in Study
Coal tar, pitch, soot (lampblack), MGP wastes, creosote, petroleum products, industrial sites, soils spiked with PAHs	Range of 0.2 to 270 mg/kg BaP; most studies in the range of 20 to 120 mg/kg BaP Range of <1 to >4,000 mg/kg total PAHs, with few <10 mg/kg	Range of <45 to <6,400 µm; some studies conducted on bulk or pulverized soils	1 to 10; most studies included ≤ 4 soils, generally all from the same site
Test Species Used	**PAHs Assessed**	**Dose (µg/kg-day)**	**Dosing Medium**
Mouse Rat Mini pig Juvenile swine Goat	BaP Dibenzo[*a,h*]anthracene Benzo[*a*]anthracene Pyrene Phenanthrene Anthracene cPAHs EPA priority pollutant PAHs	Expressed as individual PAHs, cPAHs, or total PAHs; doses not always reported; generally in the range of 0.011 to 27 µg/kg-day BaP, 0.2 to 20,000 µg/kg-day total PAHs	Soil in mouse, rat, mini pig, juvenile swine, or goat feed; soil by gavage; soil on floor of cage
Reference Dose Medium[a]	**Endpoints Measured**		
PAHs in feed PAH source material in feed PAHs in aqueous solution by gavage PAHs in oil by gavage Clean soil spiked with PAHs in feed Clean soil in feed Clean soil or sand on floor of cage	AUC of BaP and metabolites in blood AUC of BaP in blood or plasma Urinary excretion of PAH metabolites Fecal excretion of parent PAH DNA adduct formation in tissues Parent PAHs in various tissues Liver enzyme induction		

Notes:
AUC = area under the curve
BaP = benzo[*a*]pyrene
EPA = U.S. Environmental Protection Agency
MGP = manufactured gas plant
PAH = polycyclic aromatic hydrocarbon; cPAH = carcinogenic PAH
[a]Reference dose medium refers to the dosing medium against which relative bioavailability is reported.

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Table 3. Key Parameters and Ranges Used in PBETs

PAH Concentrations in Soil	Soil Parameters Measured	Soil Particle Size Tested	Number of Compartments
Highly variable; range of 2 to 300 mg/kg BaP and <1 to 5,000 mg/kg total PAHs	pH Total organic carbon Black carbon Particle size distribution Surface area	Range of <45 µm to <4 mm; <250 µm is most common	1 to 3; gastric, small intestine, large intestine
Soil:Solution Ratio	**Fed versus Fasted**	**Gastric pH and Extraction Time**	**Gastric Components**
1:100 is most common; ratios of 1:10 to 1:250 have been used	Both fasted and fed test systems in use; food sources in use are highly variable	pH 1 to 2, generally unbuffered; 1 to 2 hours	Highly variable: −Pepsin −Mucine −Bovine serum albumin −Lipid sources −Various salts
Small Intestinal pH and Extraction Time	**Small Intestinal Composition**	**Colon Phase**	**Additional Sink in Small-Intestinal Phase**
pH 6.5 to 8.5, occasionally phosphate buffered; 2 to 6 hours	Variable: −Bile salts −Pancreatin −Lipase −Bovine serum albumen −Various salts	Uncommon (pH 6 to 7; 8 to 18 hours)	Used in more recent studies: caco-2 cells, EVA thin films, C18 disks, silicone rods, and silicone sheets

Notes:
EVA = ethylene vinyl acetate
PAH = polycyclic aromatic hydrocarbon
PBET = physiologically based extraction test

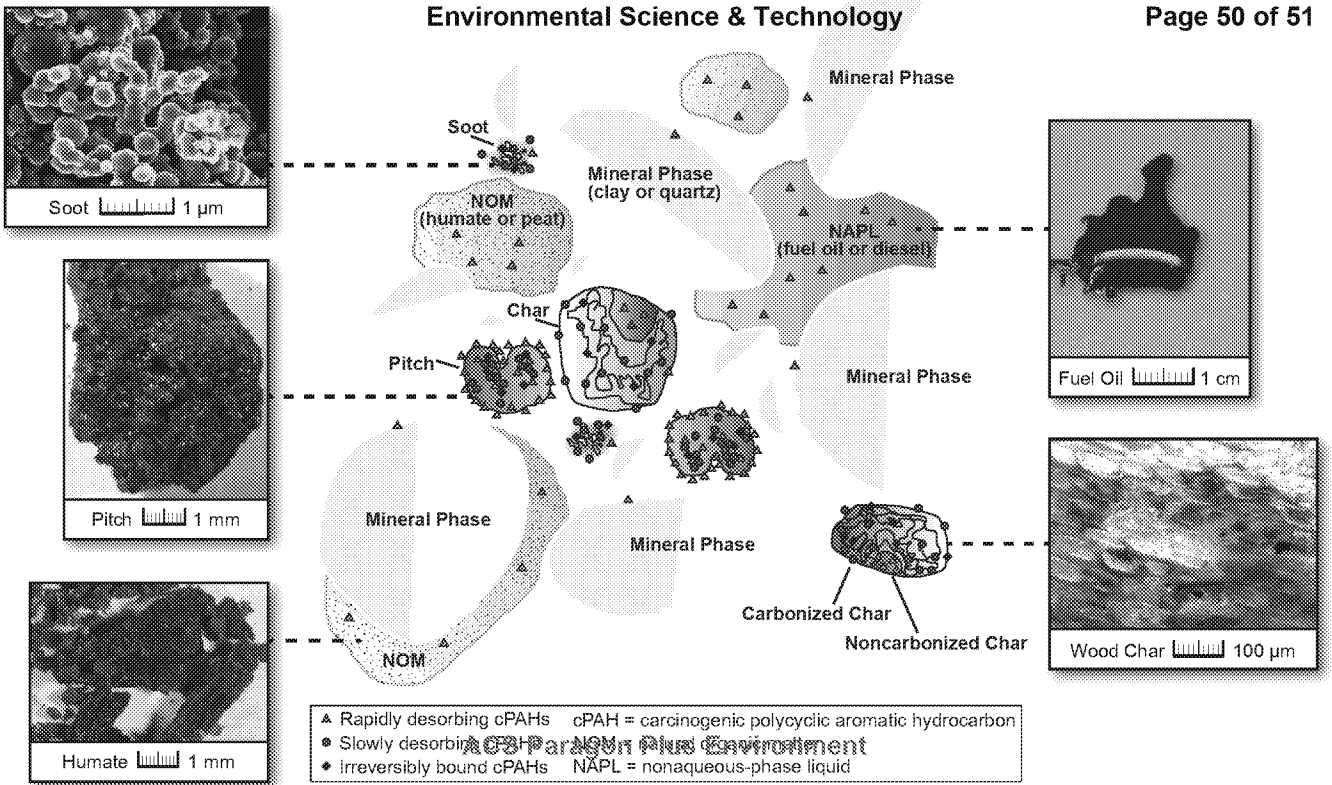

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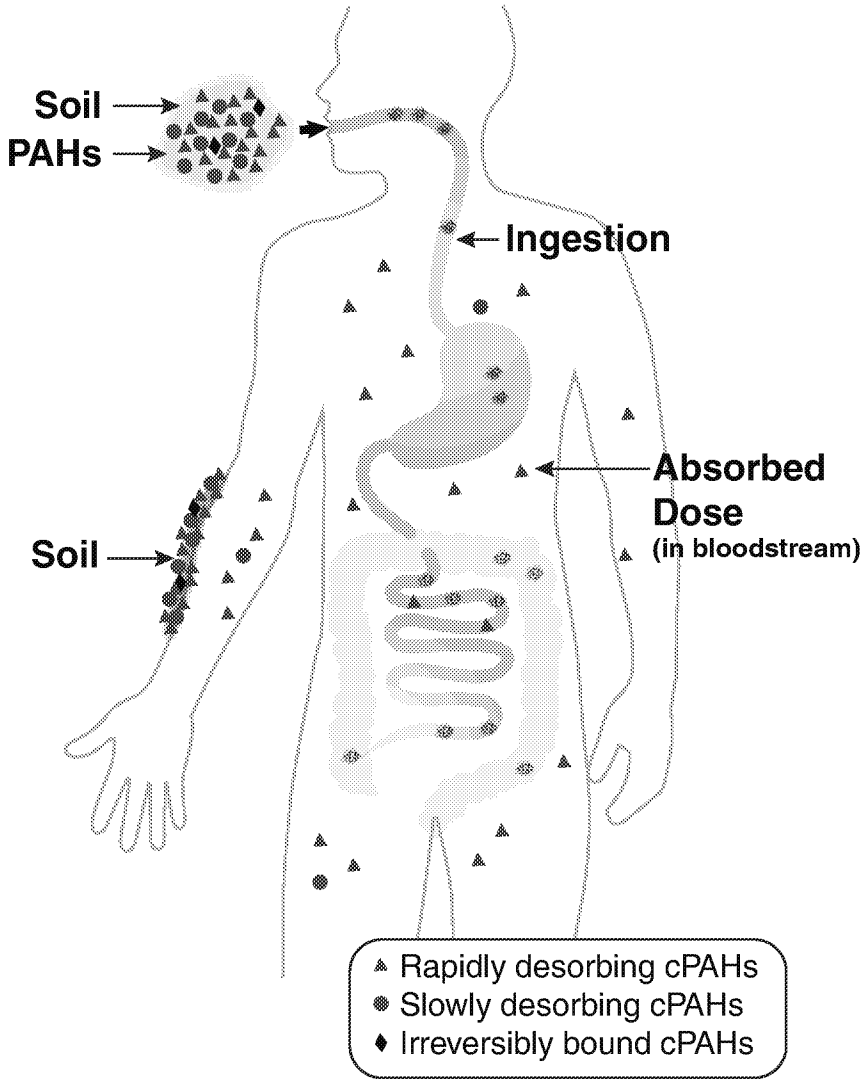
Figure 1. PAH availability for oral or dermal absorption as a function of PAH source materials and soil chemistry.


Soil
PAHs
Ingestion
Absorbed
Dose
(in bloodstream)
Soil
Rapidly desorbing cPAHs
Slowly desorbing cPAHs
Irreversibly bound cPAHs