8.4 Methodology for Evaluating PAH Bioavailability

The methodology for evaluating PAH bioavailability is complicated because PAHs are a class of compounds rather than a single entity, so exposure is usually to a mixture. Cancer risk from ingestion of PAH contaminated soil is currently determined by the concentrations of seven specific potentially carcinogenic PAHs. Although not rigorously studied, bioavailability of these individual PAHs from soil is expected to vary, meaning that even in the simplest case—one PAH source introduced to soil with one set of characteristics at one time— there are at least seven PAH bioavailabilities to consider. How, then, can PAH bioavailability in soil be measured?

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Ideally, the bioavailability of each carcinogenic PAH in each soil sample would be determined and used with their RPFs to calculate the bioavailable BaP equivalent concentration. Until a rapid and inexpensive method to measure PAH bioavailability is developed, this approach is not feasible. To reduce the number of bioavailabilities to measure, an alternative approach would be to group the seven carcinogenic PAHs according to physicochemical properties thought to affect bioavailability, and then determine the RBA for a representative PAH from each group. The RBAs for the representative PAHs would be assumed to apply to other members of their group. This approach is also not yet feasible, because the physicochemical properties that determine PAH bioavailability from soil have not been determined with confidence. Yet another approach would be to reduce the number of bioavailability measurements to one, determining in some way a composite bioavailability for the seven carcinogenic PAHs. This could be done, for example, by using a reference material for comparison with the same PAH composition. However, the composite RBA would be valid only for a specific PAH mixture. Often, the composition of PAH mixtures varies within a site due to differing sources, uneven weathering, or other site conditions, and it is not clear how a single representative composite RBA value could be derived.

Lastly, an evaluation of PAHs present in soil could be used to determine which specific PAHs are driving risk at the site, and RBA assessment could be focused on the most important compounds. This approach would focus efforts on a limited number PAHs with the greatest impact on the risk assessment, and for near future may be the most practical approach (see Army Corps of Engineers site case study).

8.4.1 **In Vivo Methods**

Several studies have attempted to estimate the oral bioavailability of PAHs from soil using animal models. A summary of studies published through 2015 appears as supplementary information in a recent paper (Ruby et al. 2016) Table S1. Examples of additional recent studies include work by Juhasz et al. (2014); Peters, Wickstrom, and Siciliano (2015); Peters et al. (2016); and Roberts et al. (2016). Approaches to the measurement of bioavailability in vivo are influenced by the metabolism, distribution, and excretion of the chemical.

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As discussed in Methodology, the oral bioavailability of contaminants from soil typically is evaluated by measurement of the chemical and metabolites in blood, urine, feces, or tissues. For PAHs, there are challenges associated with the use of each of these endpoints, primarily due to the metabolism and excretion of PAHs (Ruby et al. 2016). PAHs that are absorbed from the GI tract are metabolized, and both parent compound and metabolites are excreted in urine (Ramesh et al. 2004). However, the percentage of a PAH dose appearing in urine is typically small—less than 10% and often less than 1% depending upon the PAH and the species (Ruby et al. 2016). Most absorbed PAH is returned into the gut through the bile. Some of the PAHs excreted in the bile are reabsorbed, leading to enterohepatic recirculation. PAHs excreted in the bile, as well as PAHs that are not absorbed, can undergo microbial metabolism in the large intestine. Products of this metabolism may be absorbed or eliminated in the feces (Ramesh et al. 2004). Ruby e t al. (2016) includes a figure (S1) illustrating the "absorption, distribution and elimination of PAHs in mammals."

PAH concentrations can be measured in blood over time following a PAH dose in either soil or food, and the area under the blood (or plasma or serum) concentration versus time curve (AUC) can be used to quantify the systemically absorbed dose. The principal limitation of this approach with respect to PAHs is that blood concentrations are generally low, and analytical sensitivity prevents accurate determination of AUCs except when the PAH dose is much higher than typical environmental exposures. This condition precludes RBA measurements at soil concentrations in the range at which risk-based decisions often must be made (Ruby et al. 2016).

Presystemic elimination is a potential confounder when assessing PAH absolute oral bioavailability. However, the bioavailability adjustment for PAHs in risk assessment is based upon relative bioavailability. As long as the fraction eliminated presystemically is the same for both sets of conditions (for example, when the PAH is administered in soil versus food), using blood as a measurement of endpoint should be satisfactory. The importance of equivalent clearance (including presystemic clearance) when measuring relative bioavailability is emphasized both in this chapter and the Methodology chapter.

PAHs typically have one or a few major metabolites, and concentrations of these compounds in urine can be used as a measurement endpoint (Woudneh et al. 2016). Since the fraction of dose excreted by this route is small, two problems arise:

1. As with blood measurements, limits in analytical sensitivity prevent estimating bioavailability except when doses are high relative to environmental exposures.
2. The reliability of estimating relative bioavailability based upon changes in a minor route of excretion is questionable (Ruby et al. 2016).

Because fecal excretion is the predominant route of elimination, concentrations of PAHs and their metabolites in feces can be high relative to their concentrations in urine. This effect is illustrated by a study of chrysene elimination in rats, which found a similar pattern of chrysene metabolites in both urine and feces, but the concentrations in feces were 100-fold higher (Grimmer et al. 1998). Vastly higher concentrations of PAH metabolites being eliminated in feces has the potential to contaminate urine samples with fecal material during separation. Animal studies use metabolism cages to separate urine and feces, which is generally functional but not completely effective. The presence of even very small amounts of fecal material in collected urine could result in substantial interference with urine concentration measurements.

Estimating nonabsorbed PAHs through fecal analysis is confounded by both the biliary excretion of PAHs and their intestinal metabolism (Ruby et al. 2016). Surgical placement of a bile cannula could be used to collect bile for measurement of PAH excretion by the dominant route, thus avoiding complications from enterohepatic recirculation and intestinal metabolism. This practice, however, interrupts the flow of bile acids to the intestine, and PAHs depend on intestinal bile acids for absorption. Thus, solving a problem in measuring the systemically absorbed dose would create another by artificially impairing PAH absorption.

Another option is to estimate the systemically absorbed dose by measuring tissue concentrations of the parent PAH or metabolite after dosing. As noted in Methodology, this measurement is preferably done after repeated doses to achieve steady state concentrations in blood and tissues. PAHs induce their own metabolism, however, and with repeated doses would be expected to alter their clearance in the body unless the doses were very low. Changes in clearance change the relationship between the systemically absorbed dose and tissue concentrations. Unless the clearance changes occur equally in all treatment groups, which is difficult to achieve experimentally, erroneous estimates of RBA will be obtained from the tissue concentrations.

Biomarkers have also been used in an attempt to assess bioavailability of PAHs from soil (Ruby et al. 2016; Peters, Wickstrom, and Siciliano 2016). Specifically, cytochrome P450 induction, the DNA adduct levels in tissues, and other biochemical changes associated with PAHs have been proposed as biomarkers. While these endpoints theoretically link changes in absorbed dose with biological effects, they do not fit the classical definition of bioavailability (measuring systemically absorbed dose), nor do they correlate well with toxicity. Although developmental work is underway to assess relative bioavailability in ways that better capture its implications for toxicity and risk, these approaches are not adequately developed for use in risk assessments.

8.4.2 **In Vitro Methods**

The development of an in vitro extraction method that predicts in vivo RBA measurements for PAHs across a wide range of PAH sources should, in theory, be feasible. No in vitro extraction has been shown to satisfy these criteria for PAHs, however, because of the complexity in mimicking the processes that affect how PAHs solubilize from soil and subsequently are absorbed in the gastrointestinal tract. Additionally, the metabolism and excretion issues in animal studies complicate the method development for PAHs and have made it challenging to determine which in vivo method is best suited for the comparison. Consequently, it is difficult to correlate in vitro extractions against in vivo models at environmentally relevant concentrations.

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Several physiologically based extraction tests (PBETs) address in vitro measurement of PAH bioaccessibility. Most of these tests are different permutations or extensions of early PBET methods developed for inorganic and organic contaminants (Ruby et al. 1996) (Rotard et al. 1995; Hack and Selenka 1996). Specific examples include the Unified Bioaccessibility

Method (UBM; ([BARGE 2016](#)); the Fed Organic Estimation Human Simulation Test (FOREhST; ([Cave et al. 2010](#)), and the Simulator of the Human Intestinal Microbial Ecosystem (SHIME; ([Van de Wiele et al. 2004](#)). These studies have clarified the parameters that affect dissolution of PAHs from soil under physiological conditions, including the amount and nature of organic matter in the soil, the effect of soil aging, PAH source and concentration dependence, differential behavior of various individual PAHs, and most recently, the effect of including a lipid sink in the extraction system.

Research has demonstrated that the simple, acid dissolution methods that have been investigated or validated as predictive of RBA for metal-contaminated soils are not appropriate for assessing the RBA of organics in soil. Factors other than pH are key in extracting organic chemicals from soil, and likely include the presence of lipids, bile acids, and a pH that is representative of the small intestine. Despite the many permutations of these methods, no single extraction has been shown to provide a consistently strong IVIVC across diverse soils. A comprehensive review of PBET study methods, parameters, and results is presented in the ([Ruby et al.](#)) study 2016; [Table S2](#). Table 8-2 below summarizes the range of conditions typically reported for PAH in vitro procedures.

Table 8-2. Examples of in vitro PBET parameters

(Source: Selected with permission from (Ruby, M.V., Y.W. Lowney, A.L. Bunge, S.M. Roberts, J.L. Gomez-Eyles, U. Ghosh, J. Kissel, P. Tomlinson, and C.A. Menzie. "Oral Bioavailability, Bioaccessibility, and Dermal Absorption of PAHs from Soil – State of the Science." Environmental Science & Technology 50, no. 5 ([2016](#)), Copyright 2016 American Chemical Society.)

PBET	Number of Compartments	Fed vs. Fasted	Gastric Compartment	Small Intestinal Compartment	Separation of Supernatant
Typical range of literature PBET parameters	1-3 (stomach, small intestine, and large intestine)	Both fasted and fed test systems in use; food sources in use are highly variable	pH: 1-2 time: 1-2h	pH: 6.5-8.5 time: 2-6h	Centrifugation or filtration
[Hack and Selenka (1996)](#)	2 (stomach and small intestine	Fed: whole milk powder	pH: 2.0 time: 2h	pH: 7.0 time: 6h	Centrifugation
UBM[a]	3 (mouth, stomach, small intestine)	Fasted	pH: 1.2 time: 1h	pH: 6.3 time: 4h	Centrifugation
FOREhST[b]	3 (mouth, stomach, duodenum)	Fed: infant food and sunflower oil	pH: 1.3 time: 2h	pH: 8.2 time: 2h	Centrifugation
SHIME[c]	3 (stomach, duodenum, colon)	Fed: supplemented infant formula	pH: 1.5 time: 2h	pH: 6.3 time: 5h	Centrifugation
Notes:					
[a] Unified BARGE (Bioaccessibility Research Group of Europe) Method ([BARGE 2016](#))					
[b] Fed Organic Estimation Human Simulation Test ([Cave et al. 2010](#))					
[c] Simulator of the Human Intestinal Microbial Ecosystem ([Van de Wiele et al. 2004](#))					

In vitro methods that are not physiologically based have been investigated to measure the bioaccessibility of PAHs. These methods include mild solvent extractions, such as butanol ([Kelsey, Kottler, and Alexander 1997](#)), Tenax extractions ([Cornelissen et al. 1998](#)), cyclodextrin extractions ([Reid et al. 2000](#)), and supercritical fluid extractions ([Hawthorne et al. 2002](#)), among others. These methods provide a measure of the "rapidly desorbing" PAH fraction in soils and sediments and have been used to determine the PAH fraction available for bioremediation ([Cornelissen et al. 1998](#)). These methods may also indicate the PAH fraction that would be released in the human gastrointestinal tract, and a recent study by [Duan et al. (2014)](#) found good IVIVC for two solvent extractions. Table 8-3 suggests in vitro methods that are not physiologically based may provide viable alternatives to PBETs. However, [Duan et al. (2014)](#) used laboratory-spiked soils, and RBA was calculated as a percentage of the total PAH spiked into the soil, not the concentration of PAHs in the soil measured by an exhaustive solvent extraction. These values can differ substantially ([Roberts et al. 2016](#)), and ultimately the RBA should be reported as a percentage of the total PAH concentration measured in a soil for these methods to be validated for use with field-contaminated soils.

Table 8-3. Examples of IVIVC studies for PAHs

Study	PAH Tested	Number of Soils Tested	In Vitro Method	In Vivo Model	IVIVC r^2 (n)
(Pu et al. 2004)	Phenanthrene	4 (lab-spiked at 2 doses)	3 compartment PBET (mouth, stomach, small intestine)	Rat	0.53 (8)
(Grøn et al. 2007)	Benzo(a)pyrene Dibenz(a,h)anthracene	7	3 compartment PBET (mouth, stomach, duodenum)	Mice (3 soils) Minipigs (4 soils)	0.81 (7)
(James et al. 2011)	Suite of 13 PAHs	8	2 compartment PBET (stomach, small intestine)	Swine	0.45 (8)
(Duan et al. 2014)	Benzo(a)pyrene	8	Two chemical extractions: DCM/Ace Butanol	Swine	DCM/Ace: 0.67(8) Butanol: 0.75
Notes: PBET: Physiologically Based Extraction Test DCM/Ace: Dichloromethane/Acetone 1:1 ultrasonication extraction					

8.4.3 **Validation** to Animal Models

Only a few peer-reviewed studies have attempted to validate in vitro extractions against in vivo models for PAHs; studies reporting correlations between in vitro bioaccessibility and in vivo RBA are summarized in Table 8-3 (see section 8.4.2). Other studies, however, have not been able to successfully predict in vivo bioavailability with in vitro tests for PAHs (Juhasz et al. 2014).

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One of the major obstacles for validating in vitro tests against in vivo studies is the lack of a definitive in vivo method for evaluating the RBA of PAHs. The research has shown that the in vitro methods for metals that rely on simple acid dissolution from soils are not appropriate for understanding the bioaccessibility of organic chemicals from soil. For example, the addition of a lipid sink to the OSU-IVG method developed for arsenic is required to obtain a correlation with an in vivo swine model (James et al. 2011). Other PBETs developed for organics already include additional phases (such as food, lipids, and bile) to enhance PAH dissolution.

Much more PAH dissolves in the small intestine relative to other gastrointestinal tract compartments. This dissolution is a function of the increased residence time in the small intestine, but is primarily due to the presence of bile salts in the small intestines, which enhance PAH solubilization from soils. Bile salts, in the presence of lipids and cholesterol form mixed micelles into which the PAHs can partition (Guyton and Hall 1996; Holman et al. 2002) and are then absorbed across the intestinal epithelium (Ruby et al. 2016). In rats, the absence of bile in the small intestine results in a 77% decrease in BaP absorption (Rahman, Barrowman, and Rahimtula 1986). Factors affecting the formation of micelles such as bile concentration, lipid concentrations, and pH of the small intestine are therefore critical components of PBETs for PAHs (Zhang et al. 2015).

Developments in PAH PBETs include the addition of membranes (such as Caco-2 cells), or membrane surrogates (such as ethyl vinyl acetate (EVA) thin films), to better simulate the passive uptake of PAHs across the intestinal epithelium (Vasiluk et al. 2007; Minhas et al. 2006). More recently, the use of infinite sinks (such as silicone rods) have been proposed to better mimic PAH uptake into systemic circulation, helping to maintain steep diffusion gradients from the soils to the digestive fluid, thereby enhancing PAH dissolution (Gouliarmou and Mayer 2012; Zhang et al. 2015). Although these types of membranes or sinks are defensible physiologically, whether their use can improve in vitro correlations with in vivo models is yet to be determined in PBETs developed for organics.