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Bioaccessibility of polycyclic aromatic hydrocarbons: relevance to toxicity and carcinogenesis

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Abstract

Introduction—Bioaccessibility is a growing area of research in the field of risk assessment. As polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants, they are the toxicants of focus to establish cancer risks in humans. Orally ingested PAHs also cause toxicity and even affect the pharmacokinetic behavior of some therapeutic agents. Toward this end, bioaccessibility is being used as a tool to assess the risk of PAHs via dietary exposures.

Areas covered—This review covers some *in vitro* bioaccessibility models for PAHs that have been used for the past one-and-a-half decade. This review also considers the factors that influence bioaccessibility and debates the merits and limitations of using a bioaccessibility concept for estimating risk from ingestion of PAH-contaminated soil and food. Finally, the authors discuss the implications of bioaccessibility for PAH-induced toxicity and cancers in the context of risk assessment.

Expert opinion—So far, much of the focus on PAH bioaccessibility is centered on soil as a preferential matrix. However, ingestion of PAHs through diet far exceeds the amount accidentally ingested through soil. Therefore, bioaccessibility could be exploited as a tool to assess the relative risk of various dietary ingredients tainted with PAHs. While bioaccessibility is a promising approach for assessing PAH risk arising from various types of contaminated soils, none of the models proposed appears to be valid. Bioaccessibility values, derived from *in vitro* studies, still require validation from *in vivo* studies.

Keywords

bioaccessibility; bioavailability; colon cancer; colon extended physiologically based extraction test; *in vitro* digestion; polycyclic aromatic hydrocarbons

1. Introduction

Most toxicants are released into the environment from a myriad of man-made and anthropogenic activities, and they eventually settle on soil. For a long time, the Soil Guidance Values based on the residue levels of toxicants have been regarded as the risk assessment criteria. However, the extent of soil taken up by humans differs widely and therefore runs the risk of being overestimated or underestimated. To avoid this pitfall, bioaccessibility testing has been proposed as one of the methods to refine risk assessment of land contaminated by the toxicants [1]. From a toxicological standpoint, bioaccessibility is defined as the fraction of the total amount of toxicant present in food or soil that is released during digestion. In other words, it is the fraction that after ingestion can be mobilized into the gut fluids [2]. Since this fraction reflects the maximum amount of a toxicant available for absorption in the gastrointestinal (GI) tract, quite naturally the factors that determine bioaccessibility are of interest to researchers.

Bioaccessibility is expressed as absolute bioaccessible concentration of a given toxicant in the matrix of choice (mg/kg soil or food) or as a percentage of the total concentration of the toxicant/family of toxicants in the matrix tested. Bioaccessibility tests are of two kinds: the first one uses chemical extraction tests and the second one employs GI analog tests that mimic the biochemical/physiological conditions existing in the human GI tract.

Bioaccessibility methods offer the following advantages [3] in that they: i) provide information on interactions between toxicants and matrix components; ii) reveal the effects of GI luminal factors (pH and digestive enzymes); iii) highlight the nature of matrix (food and/or soil); iv) reduce the dependence on whole animal studies; and v) serve as cost-effective screening tools for assessing the risk from toxicant exposure.

Given the present environmental and dietary contamination by polycyclic aromatic hydrocarbons (PAHs), bioaccessibility of these toxicants needs to be addressed in order to link the amount released during ingestion to the adverse health effects that ensue following toxicant exposure. During digestion, ingested PAHs or their metabolites are released into the lumen of the GI tract. The amount of PAHs released is considered the 'bioaccessible fraction'. Once the bioaccessible fraction is absorbed into the lymphatic system, the fraction that enters into the systemic circulation to elicit effects in target tissues is considered the 'bioavailable fraction' [4,5]. In other words, bioaccessibility is an estimate (experimentally) of the fraction of toxicant which is potentially bioavailable, whereas bioavailability is the fraction of toxicant which is systemically available in the body [6]. Unfortunately, the terms bioaccessibility and bioavailability are used in an interchangeable way in some literature reports and the nonconforming and operationally ill-defined usage confuses readers. Nonetheless, bioavailability incorporates bioaccessibility into its very nature and is the ultimate indicator of systemic exposure, which has a bearing on toxicity or carcinogenesis caused by ingested PAHs. Bioavailability can be understood as the product of two parameters, GI availability and hepatic availability, and represents the fraction of a toxicant that makes its way from the GI tract to the post-hepatic circulation. GI availability is the fraction of toxicant present in the GI tract that is absorbed by the enterocytes, which makes its way to the portal circulation plus that in the intestinal lymphatics, which in turn finds its

way to plasma by the thoracic duct. For PAHs, bioaccessibility is GI dissolution, but it cannot predict the GI availability by itself as permeability is not known from its determination. The dissolution of PAHs is a function of dose or exposure concentration [7]. The bioaccessible (absorbable) fraction is more toxic than the non-bioaccessible (non-absorbable) fraction because of the downstream formation of metabolic products, which are implicated in toxicity and cancer [8]. On the other hand, laboratory bioassays have shown that the non-bioaccessible parent PAH compounds were toxic to cells [9]. However, in everyday life, the PAH parent compounds were not known to cause any toxic effects in healthy non-smoking and non-occupationally exposed humans owing to low levels of exposure (3.7 µg/day, corresponding to 0.05 µg/kg body weight/day, assuming a person weighing 70 kg; [8]). This scenario changes in situations where dietary habits and occupational exposures change the exposure dynamics and consequent fate of PAHs [8]. Therefore, both bioaccessibility and bioavailability are linked to toxicity. The major processes involved in oral bioavailability are shown in Figure 1. How bioaccessibility and bioavailability concepts are integrated into the matrix association and eventual chemical fate of PAH toxicants in the body, resulting in target organ toxicities/cancer are schematically depicted in Figure 2.

2. Exposure matrices and PAH bioaccessibility

From a risk assessment standpoint, soil has been traditionally used as the preferred matrix for assessing the bioaccessibility of PAHs. The underlying premise is that soil is the readily accessible medium in which PAHs could be retained and sampled to monitor environmental levels of PAHs. From a human toxicity standpoint, of all age groups, children are more vulnerable to PAH exposure and therefore use of soil to assess bioaccessibility is justified.

Children living in old homes located in low-income neighborhoods or in close proximity to superfund sites or tar ponds [10] have more chances of getting exposed to many soil-bound PAHs through ingestion of contaminated soil, household dust and food. Soil is a poorly digested material because organic matter in the soil is recalcitrant to degradation by digestive enzymes [11]. Children have been reported to ingest between 50 and 200 mg soil/day [12].

While soil matrix contains organic matter that is recalcitrant to digestion and could influence the bioaccessibility of PAHs resident in that matrix, bioaccessibility of PAHs associated with several plant species are no different. Crops grown in contaminated soil and in the vicinity of oil fields and highways with a large volume of vehicular traffic carry significant contamination and affect the health of livestock [13]. Armstrong *et al.* [5] studied the bioaccessibility of PAHs derived from Arctic plants using the aryl hydrocarbon activity as an indicator of bioaccessibility. The bioaccessibility was found to vary with the plant species tested and to be influenced by the endogenous plant compounds within the diet [5]. While this study was designed for assessing the PAH bioaccessibility in small mammals, the concept may hold well in the case of humans too.

PAHs are not the only group of chemical contaminants that exist in the ingested food. In everyday life, humans ingest diverse classes of food contaminants, which include

agrochemicals (pesticides), veterinary drugs, chlorinated hydrocarbons (polychlorinated biphenyls [PCBs] and dioxins), heterocyclic amines, PAHs and heavy metals. Thus, there is a possibility of additive, synergistic, antagonistic or inhibitory interactions among the chemicals in the GI tract [14,15], which could have a bearing on the bioaccessibility of PAHs. Additionally, these interactions extend beyond the level of bioaccessibility and influence both bioavailability of reactive products of PAHs and eventually the molecular targets, which embrace Phase I, Phase II enzymes and efflux pumps (Phase III) in the intestines [16].

The matrix through which the PAH compound is ingested plays an important role by facilitating its release into the digestive fluid during transit of food through the GI tract. In other words, matrix influences the transport of PAH compound across the intestinal epithelium [17]. Additionally, the PAH compound-specific properties, namely molecular mass and lipophilicity, which are not matrix-dependent may also have a bearing on the bioaccessibility. If PAHs are ingested through food items, the constituents of food may compete with the resident PAH compound for transport across the intestinal epithelium [18].

Using artificial intestinal fluid, benzo[*a*]pyrene (BaP; a PAH compound) release from a variety of food items such as cellulose, starch, potato, rice flakes, bread, ovalbumin, spinach and dried fish were tested. Cellulose was found to release 76% of the bound BaP, while other carbohydrates and protein sources released ~ 50 – 55% BaP, followed by 37% for spinach and 7% for dried fish. When the absorption of BaP was measured in a rat model, triolein, soybean oil and cellulose absorbed 51, 39 and 30%, respectively [19]. These studies provide evidence that BaP release and absorption is dependent on coadministered food components (matrix).

There is lack of information on how BaP bioaccessibility is influenced by BaP uptake from different foods. Stavric and Klassen [20] reported that foods rich in fat and fiber reduce the uptake of BaP. Dietary fiber has been reported to affect the bioaccessibility of BaP, as it absorbs BaP [21]. The uptake of a mixture of PAHs from insoluble dietary fiber such as carboxymethyl cellulose, agar and young barley leaves were studied using artificial gastric and intestinal juices. These studies revealed that young barley leaves had a high sorption capacity for PAHs compared to carboxymethyl cellulose and agar both in artificial gastric and intestinal juices [21]. Similar results were obtained by Viau *et al.* [22] in Sprague-Dawley rats. Both the insoluble dietary fiber alpha-cell (bulk cellulose) and soluble fiber (pectin) were found to reduce the urinary excretion of 1-hydroxypyrene (a metabolite of pyrene) presumably due to low bioaccessibility of PAHs. Using a rat model, Kim *et al.* [23] have shown that green tea extract inhibits the intestinal absorption of BaP through the lymphatic route and enhances the excretion of absorbed BaP via the biliary route.

3. GI uptake processes for PAHs

For PAHs, dietary intake serves as a major route of exposure. Among the different dietary items of both plant and animal origin, dietary fat is important as this component possesses a hydrophobic domain that is not disrupted easily during lipolysis [24] and facilitates the transport of PAHs, as it serves as a carrier for these lipophilic toxicants. Typical Western

diet contain at least 80 g fat/day, while lipids contribute 42% of dietary energy in Western countries – 33% of it comes from dairy products [25]. Hence, the role of dietary fat cannot be overlooked. The products of fat digestion are monoglycerides, fatty acids, cholesterol and phospholipids, which are found in the intestinal lumen as micelles or aggregates. In the enterocytes, the monoglycerides and free fatty acids are converted back into triacylglycerols (TAGs). Triglycerides are formed via the monoglyceride (about 70%) and phosphatidic acid (about 50%) pathways. The lipids that were processed in the intestine accumulate in the vesicles of the endoplasmic reticulum taking shape as chylomicrons, which comprise 80 – 90% TAG, 10% phospholipids, 3% cholesterol and 2% protein [26].

Among the constituents of dietary lipids, TAGs are of extreme importance as this lipid subclass contributes to 90% of the total energy derived from dietary fat [27] and facilitates the gastric and intestinal absorption of ingested PAH compounds. A typical diet in the United States contains 70 – 100 gm of TAG [28] and surplus energy is stored as TAG in adipose tissue [29]. The sources of TAG are vegetable oils and animal fats, consumption of which account for 38% of total calories in the Western diet [30]. TAGs are involved in the absorption of dietary cholesterol [27] and quite naturally serve as a natural solvent for PAHs [31] during their passage from stomach to duodenum. By the interaction of PAH residues in diet with other dietary components, solubilization of PAHs in TAG occurs in the oral cavity and stomach. Serum TAG concentrations showed a positive correlation with BaP uptake [32] and this increased uptake results in TAG forming adducts with BaP and its metabolites [33]. By interacting with bile salts and phospholipids, the TAGs are emulsified. Subsequently, the TAGs are hydrolyzed into fatty acids and 2-monoacylglycerols that form mixed micelles.

To ensure efficient absorption, dietary fats are dispersed which is accomplished through bile salts. Bile salts allow formation of mixed micelles and lipid vacuoles in the small intestine [34]. The aqueous unstirred layer surrounding the intestinal membrane serves as a major barrier to absorption of lipids and/or lipophilic toxicants. The amphiphilic nature of bile salts promotes the diffusion of lipids through the unstirred layer [35]. Using a rat model, Rahman *et al.* [36] demonstrated that bile salt facilitated micellar solubilization, which drives the transport of PAHs phenanthrene, anthracene, 7,12-dimethylbenz(a)anthracene and BaP across the unstirred water layer to the enterocytes. Aside from toxicants, micelles could also solubilize lipid-rich ingredients of diet. Therefore, it is conceivable that the bioaccessibility of PAHs could be relatively low in fat-free or low-fat diets, consumed by vegetarians. This is not a cause for comfort either because regardless of whether a person is a vegetarian or a non-vegetarian, if a meal contains 35% of its calories in the form of a liquid vegetable oil [37], the matrix has the ability to solubilize PAHs and other dietary lipophilic toxicants in the mouth, stomach and small intestine.

The transport of lipids exhibits selectivity, as mentioned in the previous section. While some lipids are transported via the lymphatic system, others are transported via the portal vein. Fatty acids of smaller size (about 2 – 6 carbon atoms) and medium size (about 6 – 10 carbon atoms) are transported into the blood via portal vein; fatty acids of larger size (> 10 carbon atoms) are transported into the blood via lymph [38,39]. Lipid-soluble toxicants such as

PAHs have been reported to undergo absorption via the lymphatic route. The dietary TAGs serve as ideal vehicles for lymphatic absorption of PAHs [40].

The intestinal lymph containing chylomicrons is formed by enterocytes, where it is extracellularly secreted into interstitial space. From there, the chylomicrons are taken up by the surrounding mesenteric lymphatic system. Gershkovich and Hoffman [41] examined the uptake of BaP by chylomicrons in *ex vivo* conditions and compared the results with intestinal lymphatic bioavailability. A linear correlation between these two studies suggests that lymphatic absorption pathway plays an important role in the uptake of BaP by chylomicrons in enterocytes.

BaP is transported from chylomicrons to lipoproteins after entering systemic circulation. Studies conducted *in vitro* revealed that 70% of the BaP in blood was partitioned into plasma, out of which 95% was partitioned into plasma lipoproteins. Among the lipoproteins involved in the transport of toxicants, the apolipoprotein B (which is the primary apolipoprotein of chylomicrons and low-density lipoproteins) was found to be the main carrier for transport of BaP to target organs [42]. The rate of BaP transfer between chylomicrons and lipoproteins depend on the rate of chylomicrons clearance and metabolism [43]. *In vitro* studies also followed suit, which showed that elimination of BaP paralleled the removal of chylomicrons [44].

From the above-mentioned account, it is clear that chylomicrons are needed for lymphatic transport of PAHs. With an increased lipid burden, a prolonged transport and absorption of PAHs is expected. This assumption was challenged by the studies of Laher *et al.* [45], who reported similar bioaccessibility, bioavailability and lymphatic transport of BaP, when it was concomitantly ingested in two doses of dietary lipid. Their studies revealed that rat enterocytes adapt to varying doses of dietary fat and exposure regimens (single versus multiple exposures). As a result, a large flux of chylomicrons was not required for BaP transport, as BaP partitioning into lymph was independent of lymph fat.

Hydrocarbons that bind with TAGs are prone to lymphatic transport in enterocytes (via chylomicrons) and disposition in plasma post-absorption [46]. Additionally, accumulation of TAG in hepatocytes of rats fed with high-fat diet was greater than rats that were either food-restricted or served as controls. An inverse relationship between TAG content and BaP metabolism in hepatocytes was noted, which suggests that diet-induced intracellular TAG in hepatocytes may limit the amount of BaP available for metabolism [47].

4. Models to assess PAH bioaccessibility

To calculate the bioaccessibility of soil-borne toxicants, the *in vitro* physiologically based extraction test (PBET) was proposed by Ruby *et al.* [48]. This approach entails the use of artificial body fluids as part of a sequential extraction. The commonly employed body fluid is the human GI biofluid [49,50]. The rationale underlying this strategy is that it rapidly simulates human GI physiology to allow human exposure assessment. The utilitarian value of this approach is that it serves as a screening tool for soil samples to ascertain whether further testing is warranted, given the ingestion of soil-borne toxicants by children. Using

this method, the determined ranges for PAH bioaccessibility varied from 27 – 53% [51], 9 – 69% [52] to 15 – 63% [53].

Also, the mass-balance approach was used to calculate bioaccessibility of toxicants. Using this approach, the bioaccessibility was calculated from the ratio of the original sample's total toxicant mass to the recaptured toxicant fraction [54]. This approach was used for metals, which indicated that each metal toxicant's bioaccessible fraction was less than its metal content. This approach was not popular with PAHs.

The *in vitro* models developed to assess bioaccessibility are either static (simulated transit through the human digestive tract by sequential exposure of soil) or dynamic (mimic gradual transit of ingested toxicants) GI models. The various *in vitro* digestion models that are in vogue to study the bioaccessibility of toxicants were summarized by Oomen *et al.* [4] as follows:

1. The Simple Bioaccessibility Extraction Test (SBET) model
2. The German-Deutsches Institut für Normung (DIN 19738) model
3. The Netherlands-Rijks Instituut voor Volksgezondheid and Milieu (National Institute for Public Health and the Environment; RIVM) *in vitro* Digestion model
4. The Simulator of Human Intestinal Microbial Ecosystems (SHIME) model

The salient features of these models are given in Table 1. These models are proposed taking certain physiological parameters of the GI tract into consideration. A pH of 6.5 and a residence time of seconds to minutes in the oral cavity are presumed. The toxicant and the matrix are ingested through the esophagus into the stomach. A pH of 1 – 2 and 2 – 5 and residence time of 8 – 15 min and 0.5 – 3 h for fasting and fed conditions are assumed for the stomach. The contents from stomach are transported to the intestine, which consists of the duodenum, jejunum and ileum. The duodenum, jejunum and ileum possess a pH of 4 – 5.5, 5.5 – 7 and 7 – 7.5, respectively, while the residence times are 1.5 – 2, 5 – 7 and 15 – 60 h, respectively. The different digestion models tested illustrate that bioaccessibility is matrix-dependent and also chemical species (contained in the matrix)-dependent. Using this approach, certain soils showed bioaccessibility values lower for one heavy metal toxicant, whereas other soils tested indicated bioaccessibility values higher for the other heavy metal toxicants.

Other variables that influence the bioaccessibility values are low solid-to-liquid ratio in the GI compartments of some models, lack of conditions that simulate the GI tract with a meal, lack of physiological conditions such as the pH of gastric juice, the residence time of the matrix in the GI compartment, the duration of experiment and the like. [4]. For a compendium of the GI tract parameters and the consequence of their influence on the bioaccessibility, readers can refer to the publication by Oomen *et al.* [4].

The methods that incorporated lipid into the matrix for assessing PAH bioavailability are a modification of either the RIVM method representing a well-fed and fasting conditions or the SHIME method. The Fed Organic Estimation Human Simulation Test (FOREhST) developed by Cave *et al.* [55] simulates the physiochemical conditions of the GI tract similar

to that of the RIVM fed-state model. The RIVM study incorporated infant formula supplemented with vegetable oil to represent the food component, while the FOREhST method also used an infant food supplement with sunflower oil. While the FOREhST was a static *in vitro* bioaccessibility model, the SHIME model was a dynamic one. The FOREhST method yielded 20% higher bioaccessibility compared to the SHIME method.

The type of food component used in the above-mentioned methods was based on the following rationale. For example, the RIVM method used a food component to represent average diet for men and women (ages 19 – 65) in the Netherlands based on their mean intake of energy and nutrients [18]. On the other hand, the food component used in the FOREhST method were based on the nutrient composition of average diet of children (ages 4 – 6) in Britain [56].

Another modified version of the RIVM method used by Grøn *et al.* [57] simulates the digestive processes occurring in a fed child. The food component has an infant formula comprising protein, carbohydrates and fat (3%). On the other hand, the model of Vasiluk *et al.* [24] was conceived based on the fasting conditions in children. Skimmed milk powder (1.5% fat) was used in the food matrix. The method used by Yu *et al.* [58] incorporated freeze-dried powder from grass carp (2.1% fat-representing low fat condition) or large yellow croaker (> 9.6% fat-representing high fat condition) as the food component.

The human digestive tract model developed by Holman *et al.* [59,60] used the concept of diminished bioavailability of weathered petroleum residues and PAHs in soil as a function of solubilization of these compounds in the GI tract. The authors studied the fate of PAH compounds in fasted and fat digestion state using a synthetic upper small intestinal digestive fluid that contains mixed bile salts and intestinal lipids. While fasted state comprised mixed bile salts, the fat digestion state included mixed bile salts as well as intestinal lipids. The GI solubility of PAHs increased from the fasting phase to fat digestion phase. This model employed a stepwise solubilization system that involves absorption of bile salts to the soil surface, reacting with hydrocarbons to form micelles and desorption of these compounds from the soil and diffusing into lumen's fluid. Subsequently, the micelles penetrate the unstirred layer; hydrocarbons adsorb to the microvilli of enterocytes, diffuse across the cells and enter the blood and lymph circulation.

To extract contaminants of interest and assess bioaccessibility, synthetic digestive fluids with commercially available enzymes and surfactants have been used thus far. To mimic the *in vivo* situation, Weston and Maruya [61] have employed the digestive fluid of invertebrates to extract the contaminants including PAHs. While low-molecular mass PAHs could be extracted well using the digestive fluid, high-molecular mass PAHs registered reduced extraction efficiency. One handicap in using this approach is the likelihood of saturation of digestive fluid when toxicant-rich matrices (soil or sediment) are used. To overcome this weakness, matrix-to-digestive fluid ratio could be manipulated so that there are a sufficient number of liquids, such as surfactant micelles, available in the lumen of the GI tract to facilitate extraction [28]. While this approach seems ideal for assessing bioaccessibility and environmental bioavailability for aquatic animals, it may seldom occur in scenarios involving human exposure. Ingestion of a contaminated diet rich in high

molecular mass PAHs (PAHs containing four or more fused benzene rings) may be of an episodic occurrence as opposed to frequent intake of low-molecular mass PAHs (PAHs containing three or less fused benzene rings) by humans [62,63]. To explain the fate of ingested PAHs in such scenarios, *in vivo* studies are warranted using animal models to evaluate the integrity of *in vitro* models.

As the bioaccessible fraction of PAHs mobilized from the matrix during digestion could be ultimately absorbed by the GI tract, which will have a bearing on the bioavailability, additional techniques have been introduced to address this aspect. One of these techniques reported by Minhas *et al.* [64] employs Caco-2 human colorectal adenocarcinoma cells as an *in vitro* model of GI absorption and metabolism and employs ethylene vinyl acetate (EVA) thin film as a surrogate for intestinal membrane permeability. The PAH compound chrysene was used by Minhas *et al.* [64] to gain an understanding of bioaccessibility as this compound also enjoys concentrations and distribution similar to that of BaP, a prototypical PAH toxicant.

Since chrysene (a PAH compound) that partitioned into the GI fluid constitute the mobilized (bioaccessible) fraction, the concentration of chrysene in the EVA was considered as the bioavailable amount. In this experiment, the concentration of chrysene remained the same even when composition of GI fluids was altered. Even though the fugacity gradient between aqueous phase and EVA film remained the same, the Caco-2 showed a higher lipid-normalized fugacity than the EVA film. In other words, the uptake rate of chrysene was found to be similar in the EVA and Caco-2 components. These studies indicate that bioaccessibility of chrysene was controlled by the resistance of the non-stirred aqueous layer at the intestinal membrane–water interface.

This research group extended their studies to assess the bioavailability of BaP using BaP bioaccessibility from pristine soil and contaminated sediment [65]. In these studies, the Caco-2 cells and EVA were used as test systems to measure bioaccessibility. An inverse relationship between BaP bioaccessibility and percentage of organic matter was observed. The amount and quality of organic matter in soil/sediment, notwithstanding the bioaccessible fraction represented by the rate of desorption of BaP in Caco-2 cells, were twofold greater than that observed with the EVA thin film. Given the differences in results because of the test systems used, these studies reveal that measuring bioaccessibility as a surrogate of bioavailability may run the risk of underestimating the actual bioavailable fraction [65].

The foregoing narrative deals with PAH bioaccessibility using *in vitro* models designed based on the physiological conditions prevailing in non-ruminant mammals (represented by humans, who are monogastric). However, Jurjanz and Rychen [66] used an *in vitro* model to determine PAH bioaccessibility in ruminant mammals (represented by cows with four-chambered stomach). In this method, artificial ruminal liquid was used as the extraction medium; the nature and amount of lipid if any used was not clear. Since forage items (grass and dry fodder) and cow rations (cereals and oil seeds) are the sources of glycolipids and triglycerides, respectively [67], these ingredients may help in the extraction of ingested

PAHs by livestock and may need to be incorporated into the model to derive better estimates of PAH bioaccessibility from pasture soils.

5. Factors that influence PAH bioaccessibility

One of the factors that determine the bioaccessibility is the matrix with which the toxicant is associated. The nature of the matrix influences the fraction of toxicant that is released during its residence time or transit during the GI tract after ingestion [18]. Even though the matrix employed (soil or food) determines bioaccessibility, the fast or fed conditions also will have a bearing on bioaccessibility. The PAH bioaccessibility was found to be increased under well-fed state [2,18,59,60] compared to a fasting state [68].

Bioaccessibility of PAHs in food items have not received much attention. Yu *et al.* [58] reported that PAH bioaccessibility varied from 29 to 61% when animal-based foods (both terrestrial and aquatic) were used. Wang *et al.* [69] demonstrated PAH bioaccessibility values ranging from 24 to 31% when freshwater and marine fish were used. Both these research groups used the *in vitro* GI digestion model to assess bioavailability.

For orally ingested toxicants, such as PAHs, different dietary items such as fats and fibers [21,70,71] influence the bioaccessibility. Some of the bioaccessibility models do not factor a lipid matrix when they are proposed. Since PAHs are lipophilic, presence of lipids in the intestine alter the bioaccessibility of PAHs. The lipophilic PAHs which possess low water fugacity partition into the lipid sink of intestinal epithelial cells. The materials used as surrogates for lipid sinks are EVA [24] and C18 [68] membranes. Using a juvenile swine model, James *et al.* [68] have demonstrated a correlation between *in vivo* and *in vitro* studies when lipid sinks were used in *in vitro* digestors. Thus, for *in vitro* models to approximate human uptake processes, lipid sinks are indispensable.

The role of gut microflora in bioaccessibility of PAHs has not received much attention. Gut microbiota has been reported to regulate fat storage [72]. Additionally, in individuals with genetic susceptibility, gut microbes were suspected to cause Crohn's disease, a form of inflammatory bowel disease that shares etiology with colon cancer [73]. The PAH composition of ingested soil or diet influences their amenability for microbial breakdown. Using a GI tract simulator, van de Wiele *et al.* [74] have demonstrated the ability of human microbiota in colon to bioactivate the PAHs. While some microflora resident in the upper digestive tract may not be able to cleave dietary ingredients such as complex carbohydrates and larger proteins, microflora of the colon are capable of breaking these compounds [75]. PAHs with an increasing ring number have been shown to be recalcitrant to microbial degradation relative to PAHs with less number of rings [76]. Therefore, the bioaccessibility of high-molecular mass PAHs may be influenced by the microbial population. The bioactivation of PAHs by colon will have implications on human health, as the PAH metabolites could contribute to toxicity or cancers of the GI tract.

6. Advantages and limitations of bioaccessibility approach

In risk assessment, potency equivalent concentrations (PEC) or toxic equivalent factors are used. These values are estimates of compound-specific toxicity/potency relative to the

toxicity/potency of an index chemical pollutant [77]. Using this approach, the PEC for PAH residues in freshwater and marine fish collected from Hong Kong markets were found to be 0.53 and 1.43 ng/g, respectively. However, when bioaccessibility data was taken into consideration, the values dropped to 0.18 and 0.35 ng/g for freshwater and marine fishes, respectively [69]. When PAH mean dietary intakes from animal-based foods from Shanghai were computed, the values reached 848 ng/day; on the other hand, when bioaccessibility approach was used, the values dropped to 297 ng/day, suggesting that dietary intake from PAHs may have been overestimated [58]. These studies call our attention to the need for factoring bioaccessibility for health risk assessment of PAH intake through diet.

In spite of the advantages of bioaccessibility, bioaccessibility estimates sometimes fall short of accuracy. Siciliano *et al.* [78] reported the bioaccessibility of PAHs from brownfield sites to which humans are dermally exposed. When SHIME model was used to assess bioaccessibility, PAHs < 45 μ m size were found to be released to a lower extent (8%) in stomach and small intestine. Going by this data, the estimated incremental lifetime cancer risk was greater for the < 45 μ m fraction than the bulk fraction. When PAH bioaccessibility determined by the SHIME model was used, the cancer risk was found to be slightly lower for the < 45 μ m fraction [78], notwithstanding the fact that the concentrations of PAHs in soil were more in the < 45 μ m size fraction.

Additionally, due caution must be exercised when generalizing the bioaccessibility findings from various studies owing to differences in the matrix and methods employed. Most of the methods used for assessing bioaccessibility of PAHs were adopted from methods originally designed for a variety of soil-bound toxicants. These proposed bioaccessibility models have soil as an ingredient. The underlying idea is that constituents of the matrix along with bile salts aid in mobilizing hydrophobic toxicants through micellar formation and facilitate extraction of toxicants. The drawback in some of these methods is that there is no lipid component involved. For example, the method used by Tao *et al.* [79] for soil-bound PAH bioaccessibility was based on the method used for assessing the bioaccessibility of phytochemicals from fruits and vegetables [80]. Similarly, the method used by Khan *et al.* [51] was based on the method developed by Ruby *et al.* [48] for assessing the bioaccessibility of heavy metals from soil. The lack of lipid component in these models limits our ability to accurately assess the bioaccessibility.

Since environmental toxicants and dietary factors have been suspected of causing sporadic colon cancers, which contribute to 90% of the colon cancer cases [81], there is an immediate need for assessing the bioaccessibility of PAHs through different dietary ingredients and at different PAH concentrations/doses. The bioaccessibility of exposure matrix-bound toxicants has been studied with the GI extraction systems, which use the PBET system focusing mainly on stomach and small intestine. This approach has some limitations as absorption of nutrients and fermentation by-products of microbial activity [82,83] occur in the colon. The SHIME model developed by Molly *et al.* [84] was the only model until 2011 that factored colon microflora in assessing bioaccessibility. Given the fact that passage through colon accounts for 80% of the transit time through human GI tract, and considerable desorption of toxicants occur through colon, Tilston *et al.* [85] developed the colon extended PBET to assess bioaccessibility of soil-bound PAH. Basically, this model is similar to the

PBET system, except for addition of an 8-h colon compartment and carbohydrate-rich fed-state medium to mimic the physicochemical conditions of the human colon. Using this model, the bioaccessibility of soil-bound PAHs increased by 50% when laboratory soils are used and by a factor of 4 when field soils were used. While this model is tested with soilbound PAHs, its utility in assessing bioaccessibility with dietary items has not been tested yet. The contribution of dietary PAHs to colon tumor development makes this approach worth exploring the bioaccessibility.

Reservations were expressed on the validity and robustness of information derived from *in vitro* bioaccessibility methods because they match with *in vivo* bioavailability to a limited extent [86,87]. Additional limitations of accepting bioaccessibility assessment include: results depend on the assessment method used, matrix employed, toxicant tested and so on [88]. One more limitation of the simulation models is that they lack food components such as TAG that might solubilize PAH with bile more efficiently than bile itself.

7. Implications of bioaccessibility for PAH-induced carcinogenesis and toxicity

Several studies have shown that consumption of PAH-tainted dairy items, red meat (barbecued/grilled) and fatty foods (lard and pork fat) contribute to substantial intake of PAHs [13,89,90]. In fact, diet plays a major role in the human intake of PAHs compared to other routes of exposure. Therefore, evaluating the bioaccessibility of PAHs in various dietary items is of relevance to GI tract cancers and evokes interest from the viewpoint of health risk assessment.

The levels of PAH residues in dietary items and their intake [13,91] have evoked interest on the ability of these compounds to cause colorectal cancers. Even though isolated studies with colon cancer cell lines and animal models indicated that PAHs may contribute to tumor development in the GI tract [14], the field did not gather momentum until recently, when epidemiological studies revealed a close link between PAH levels in foods and incidence of colon cancers [92–95]. Studies conducted with transgenic animal models of sporadic colon cancer [96–98] also bolstered the hypothesis that dietary PAHs cause colon tumors.

The bioaccessibility of orally ingested PAHs is not only relevant to GI tract tumors but also relevant to a wide range of target organ toxicities and cancers [13,99,100]. Aside from colon cancer, PAHs also have been known to cause esophageal, lung and breast cancers. An association between ingestion of PAHs and esophageal cancer has been reported from Iran [101] and China [102]. The detection of greater levels of PAHs in lung tissue of smokers have contributed to the belief that PAHs in cigarette smoke are likely to contribute to the development of lung cancer in humans [103]. Epidemiological studies report an association between PAHDNA adducts and breast cancer incidence [104]. The above-cited reports of cancer causation by PAHs could not have been possible without the immune function being compromised [105].

Being lipophilic pollutants, the PAHs in the bloodstream of exposed people partition into adipose tissue, which serves as a storage depot for PAHs [106]. A positive correlation

between human plasma BaP levels and body mass index observed [107] demonstrate that obese individuals are at an increased risk due to PAH exposure. A commonality in the sequence of events presumed to occur in the early stages of atherogenesis and carcinogenesis (proliferative response of cells to inflammation, oxidative DNA damage in tissues, somatic mutations, etc.) lends credence to the assumption that dietary mutagens such as PAHs may contribute to atherosclerotic plaques [108] and accelerate abdominal aortic aneurysms [109]. In addition to people who consume barbecued meat and other fatty foods, occupational workers such as firefighters, coke industry workers, road pavers, petroleum industry workers, restaurant cooks and the like face fertility issues as PAHs have also been shown to cause infertility in men and women.

A correlation between urinary concentrations of PAH metabolites and increased idiopathic male infertility was reported [110]. Additionally, a significant association between PAH-enriched smoking and reduced fertility among female smokers [111] was noted. Coke oven workers exposed to PAHs showed impairments in neurobehavioral functions [112]. Additionally, prenatal exposure to PAHs has been reported to affect children's IQ and mental development [113].

In addition to causing some diseases and disorders mentioned above, PAHs can also affect the pharmacokinetic behavior of other drugs [114]. Intake of PAHs through cigarette smoke inhalation and ingestion of charcoal-grilled foods have been reported to influence the pharmacokinetic behavior of various drugs such as antipyrine, phenacetin (analgesic and antipyretic agents) theophylline (antiasthma agent), caffeine (ingredient of many beverages and neonatal apnea agent), clomipramine (tricyclic antidepressant), benzodiazepines (antipsychotic agents), thiothixene (antischizophrenic agent), hydrocodone (narcotic analgesic agent), propranolol (antihypertensive agent), irinotecan and erlotinib (anticancer agent), quinine (antimalarial agent), quinidine (antiarrhythmic agent), tizanidine (skeletal muscle blocker), amiodarone and ropivacaine (anesthetic agent).

The causative mechanism that underlie the increased clearance could be attributed to PAH induction of biotransformation enzymes, which are involved in the metabolism of these therapeutic agents [115]. The increased clearance of drugs in subjects exposed to PAHs may not be beneficial, resulting in larger doses, extended duration of treatment and hospital stay [116,117].

Compared to a healthy state, the altered drug disposition in disease state will have a significant impact on the efficacy of medications in clinical settings given the narrow therapeutic index of some drugs [118]. The consequences of such altered disposition and clearance in humans lead to reduced efficacy of drugs and exacerbate toxicity.

In summary, bioaccessibility measurements for PAHs are crucial to the protection of humans from exposure to PAH-contaminated soils and foods. We hope that once the limitations and uncertainties associated with bioaccessibility testing procedures are resolved by the research community, policy makers would embrace PAH bioaccessibility measurement to formulate appropriate regulatory strategies.

8. Expert opinion

The overarching goal of employing bioaccessibility is to assess the risk from PAHs via exposures such as accidental ingestion of soil, dermal contact with soil and inhalation of soil particles. The underlying premise is that soil-bound and food-borne PAHs can have reduced chemical activity if they are not released from the physical/chemical matrix of soil or food materials. To achieve this goal, information on the processes that occur in the human GI (chemical behavior and fate of PAH toxicant ingested through matrix of choice) is necessary. Also needed is the information on the chemical fate of PAH compound ingested under different nutritional/dietary regimes (feasting and fasting scenarios). Equally important is the fact that the models that are currently being developed reflect these situations.

While several models for bioaccessibility have been proposed, it is uncertain which one of the models furnishes bioaccessibility values that best mimic the human *in vivo* conditions. That the models are derived from parameters and processes of the human GI tract notwithstanding the *in vitro* bioaccessibility data has not been matched with the *in vivo* data yet. The limited data available for widely distributed PAHs restrict our confidence in determining *in vivo*–*in vitro* relationships because for risk assessment purposes, correlations between *in vivo* bioavailability and *in vitro* bioaccessibility for PAHs are necessary. For example, oral administration of labeled PAH compounds in animal models and measurement of leftover PAHs in GI tract at different time intervals or in portal blood (for PAHs that bypass lymphatic absorption) would provide an estimate of bioaccessibility. Some of the limitations in bioaccessibility assessment stem from not having adopted consistent strategies to correlate *in vivo* and *in vitro* data in diet or contaminated soils. Also, no standard (reference) food or soil samples have been employed to compare efficacy of various *in vitro* bioaccessibility methodologies and *in vivo* animal models. To alleviate this concern, interlaboratory comparisons of *in vitro*/*in vivo* bioaccessibility measurements are necessary. Until such a time, the utilitarian values of bioaccessibility in determining bioavailability from a toxicity/GI carcinogenesis standpoint remain to be addressed with caution.

Another drawback in using bioaccessibility is that this approach has been widely used for soil-bound PAHs. As the importance of dietary fats in promoting PAH-induced toxicity and GI tumors are recognized, PAH bioaccessibility through foods of plant and animal origins needs to be assessed. In fact, dietary fat is so important to bioaccessibility and bioavailability of lipophilic compounds, such as PAHs and chlorinated hydrocarbons, that bioaccessibility (if the matrix involved is food) cannot be determined without specifying the concentration of fat in the diet. Also, the type of lipid category deserves mention. A limitation of the proposed models is that they have not incorporated food components such as triglycerides that might solubilize PAH with bile more efficiently than bile itself. Triglycerides being the storage lipids may render a protective effect by sequestering high molecular mass PAHs without manifesting toxicity. However, in times of food deprivation or starvation, the mobilization of PAHs from fat stores and reaching concentrations that are toxic to humans cannot be ruled out. Therefore, without reference to the level and type of fat

in the diet, predictions on bioaccessibility of PAHs would be prone to errors and provide an inaccurate assessment of risk.

It is well known that PAHs in soil and foods seldom occur as a single compound but occur as mixtures. Therefore, bioaccessibility of individual PAH compound is expected to be different from that of a mixture owing to the variations in structural complexity, hydrophobicity and lipophilicity of PAHs. Additionally, the complexity of matrices (sandy, clay, muddy and loamy in the case of soil and fiber, fat and protein in the case of food items) present another challenge. All these factors must be reconciled in assessing the bioaccessibility of PAHs.

To summarize, none of the models proposed for bioaccessibility appear valid. Additionally, the biggest challenge in this area is to develop methods/techniques and algorithms that reflect bioaccessibility and bioavailability in humans with different dietary habits (vegetarians, non-vegetarians, calorie-rich, calorie-restricted regimes) and health statuses (healthy, disease-ridden, etc.). A great deal of research is expected to be conducted in the coming years in refining the existing models to incorporate the above-mentioned scenarios.

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Article highlights

- Bioaccessibility testing has the potential to provide a risk assessment of land contaminated by the toxicants.
- Contamination by PAHs is an important issue and the bioaccessibility of these toxicants needs to be addressed in order to better understand their adverse effects.
- Dietary intake serves as a major route of exposure to PAHs.
- A spectrum of diseases/disorders are caused by PAH, which can influence drug disposition.
- While there are several models for bioaccessibility, it is still uncertain which of these models produces bioaccessibility values that best mimic the human *in vivo* conditions.
- Values from bioaccessibility models require validation from future *in vivo* studies.

This box summarizes key points contained in the article.

Figure 1. Major processes in oral bioavailability of toxicants are illustrated

After ingestion, toxicants can be partially or totally released from the matrix (depending on their concentration and physicochemical properties) during digestion. The fraction of the toxicant that is mobilized from the matrix (soil/food) into the digestive juice (chyme) is defined as the bioaccessible fraction. This fraction represents the maximum amount of toxicant available for intestinal absorption. Subsequently, the toxicants that are bioaccessible can be absorbed, transported across the intestine and transferred into the blood stream. The toxicants then undergo the first-pass effect in which they are metabolized and excreted by the intestine or liver. Therefore, oral bioavailability of toxicants is the cumulative process of matrix ingestion, bioaccessibility, absorption and the first-pass effect.

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Figure 2. Fate of PAH toxicants in the body is shown

Oral intake of PAHs occurs through dietary items of both plant and animal origin. Of the principal dietary ingredients viz. carbohydrate, fat and protein, dietary fat in the form of triacylglycerols (TAGs) is the main driver involved in the absorption of PAHs. The PAHs are carried through chylomicrons and transported to liver, from where they are transported via lipoproteins in the blood. While the hydrophilic products are excreted through urine and feces, the lipophilic products are transported back to the intestine via enterohepatic circulation. The PAHs stored in adipose stores are released through TAG into milk during lactation. Under prolonged PAH exposure conditions, the PAH parent compounds and/or metabolites disrupt cellular homeostasis of target tissues causing toxicity or cancer.

Table 1

Schematic overview of different *in vitro* digestion models*.

Specific	SBET method (BGS), UK	DIN method (RUB), Germany	<i>In vitro</i> digestion model (RIV/M), Netherlands	SHIME method (LabMET/Vito), Belgium	TIM method (TNO) Nutrition, Netherlands
Input	1 g dry soil	2 g dry soil	0.6 g dry soil	10 g dry soil	10 g dry soil
General	Static stomach	Static GI	Static GI	Static GI	Static GI
Temperature	37°C	37°C	37°C	37°C	37°C
Mechanical treatment	End-over-end rotation, 30 ± 2 rpm	Agitator 200 rpm	End-over-end rotation, about 55 rpm	Mechanical stirring at 150 rpm	Peristaltic movements
Food components	No	Yes (whole milk powder 50 g/l) and no	No	Cream (18 g/l) and Nutrilon plus (15 g/l) in stomach compartment	no
Oral cavity	No	No	Yes	No	Yes
Saliva			9 ml		50 ml
Compartment volume of saliva			6.5		5
pH			5 min		5 min
Incubation time			Yes	Yes	Yes
Gastric compartment	Yes	Yes	13.5 ml	25 ml	250 ml
Volume of gastric juice	100 ml	100 ml	1.1	4	Initial gastric pH 5 decreasing to pH 3.5, 2.5 and 2 after 30, 60 and 90 min, respectively
pH	1.5	2			Gradual secretion time gastric content at 0.5 ml/min
Incubation time	1 h	2 h	2 h	3 h	Lipase, pepsin
Gastric secretion components		Pepsin, mucin	Pepsin, mucin bovine serum albumin (BSA)	Pepsin, mucin, cellobiose protease pepone, starch	Yes (three sections: duodenum, jejunum and ileum)
Intestinal compartment	No	Yes	Yes	Yes	3 × 70 ml
Volume of intestinal juice		100 ml	Duodenal juice: 27 ml bile juice: 9 ml	Pancreatic fluid: 15 ml	Duodenum: 6.5; jejunum: 6.8; ileum: 7.2
pH		pH intestinal mixture 7.5	Duodenal juice: 7.8; bile juice: 8; pH chyme mixture: 5.5	6.5	Duodenal secretion at 1 ml/min;
Incubation time		6 h	2 h	5 h	

Specific	SBET method (BGS), UK	DIN method (RUB), Germany	<i>In vitro</i> digestion model (RIVM), Netherlands	SHIME method (LabMET/Vito), Belgium	TIM method (TNO) Nutrition, Netherlands
Concentration of bile in chyme	0 g/l	4.5 g/l	0.9 g/l	1.5 g/l	total digestion time: 360 min
Origin of bile		Porcine	Bovine	Bovine	variable
Concentration of phosphate in chyme	0	1.0 mM	2.6 mM	None added	Porcine
Ionic strength of chyme		~ 0.15 M	0.14 M	Not determined	Variable
Intestinal secretion components		Trypsin, pancreatin	Pancreatin, lipase, BSA	Pancreatin	Not determined
Centrifugation filtration	No 0.45 – 6 m cellulose acetate disk filter	7000 g no	3000 g no	7000 g no	Pancreatin
Special treatment	pH filtrate within 0.5 pH units of the starting pH	Supernatant decanted pellet stirred in 30 ml water, recentrifuged and supernatant decanted again; decanted volumes combined	Determination of metal in starting material, that is, soil, chyme and pellet for mass balance		Hollow fiber membrane for determination of bioaccessibility
Destruction	1 ml concentration of HF to about 0.1 g soil, 24 h, 0.8 ml; concentration of HNO ₃ + 0.4 ml; HClO ₃ , 5 h at 100°C and 7 h at 190°C; taken up in 5% HNO ₃	0.4 g soil/pellet or 10 ml chyme in 6 ml HNO ₃ (65%) + 2 ml H ₂ O ₂ (30%) for 5 h at 200°C	3 g soil/ 6 ml milliQ, + 1 ml 65% HNO ₃ , microwave	Microwave HNO ₃ /HBF ₄ /H ₃ PO ₄ /HCl/HF	1 g soil or 30 – 50 g dialysate + 4 ml HNO ₃ (65%) + 12 ml HCl (25%) cooked for 2.5 h
Analytical method	ICPAES	AAS	ICPMS	ICPAES	ICPAES (Cd, Pb) and HAAS (As)

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* These models have been originally developed for soil-bound heavy metals. Over the years, these methods have been modified for assessing the bioavailability of other soil-bound contaminants such as PAHs and PCBs.

AAS: Atomic absorption spectrometry; HAAS: Hydride atomic absorption spectrometry; ICPAES: Inductively coupled plasma-atomic emission spectrometry; ICPMS: Inductively coupled plasma mass spectrometry.