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In vitro prediction of polycyclic aromatic hydrocarbon bioavailability of 14 different incidentally ingested soils in juvenile swine☆

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HIGHLIGHTS

- FOREhST model was used to determine bioaccessibility of PAHs.
- Shaking method of FOREhST significantly affects PAH release from soil.
- PAHs interact with each other affecting PAH soil desorption.
- Including PAH interactions allows PAH bioaccessible predictions of bioavailability.

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ABSTRACT

Predicting mammalian bioavailability of PAH mixtures from *in vitro* bioaccessibility results has proven to be an elusive goal. In an attempt to improve *in vitro* predictions of PAH soil bioavailability we investigated how energetic input influences PAH bioaccessibility by using a high and low energetic shaking method. Co-inertia analysis (COIA), and Structural Equation Modeling (SEM) were also used to examine PAH-PAH interactions during ingestion. PAH bioaccessibility was determined from 14 historically contaminated soils using the fed organic estimation of the human simulation test (FOREhST) with inclusion of a silicone rod as a sorption sink and compared to bioavailability estimates from the juvenile swine model. Shaking method significantly affected PAH bioaccessibility in the FOREhST model, with PAH desorption from the high energy FOREhST almost an order of magnitude greater compared to the low energy FOREhST. PAH-PAH interactions significantly influenced PAH bioavailability and when these interactions were used in a linear model, the model predicted benzo(*a*)anthracene bioavailability with an slope of 1 and r^2 of 0.66 and for benzo(*a*)pyrene bioavailability has a slope of 1 and r^2 of 0.65. Lastly, to confirm the effects as determined by COIA and SEM, we spiked low levels of benzo(*a*)anthracene into historically contaminated soils, and observed a significant increase in benzo(*a*)pyrene bioaccessibility. By accounting for PAH interactions, and reducing the energetics of *in vitro* extractions, we were able to use bioaccessibility to predict bioavailability across 14 historically contaminated soils. Our work suggests that future work on PAH bioavailability and bioaccessibility should focus on the dynamics of how the matrix of PAHs present in the soil interact with mammalian systems. Such interactions should not only include the chemical interactions discussed here but also the interactions of PAH mixtures with mammalian uptake systems.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHS) are carcinogenic compounds produced from incomplete combustion of organic material. Due to their relatively low solubility and vapour pressure PAHs will accumulate in soil over time and humans are exposed to PAHs through the incidental ingestion of PAH contaminated soil. In the absence of a bioavailability estimate the default assumption for exposure assessment

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is that the relative bioavailability of soil PAHs is 100%, however a significant fraction of PAHs are strongly bound to soil constituents and are not released within the gastrointestinal tract (James et al., 2016; Juhasz et al., 2014a).

PAH bioavailability from soil can be estimated by monitoring uptake of PAHs into the bloodstream of a model organism, *e.g.* mice, swine or rats. Animals should, ethically, not be used for routine site assessments and thus, substantial effort has gone into developing *in vitro* bioaccessibility models to predict bioavailability. Current models for organic contaminants include Physiologically Based Extraction Test (PBET) (Gouliarmou et al., 2013; Li et al., 2015; Ruby et al., 2002), Colon-extended PBET (Tilston et al., 2011), Fed Organic Estimation human Simulation Test (FOREhST) (Cave et al., 2010; Juhasz et al., 2014a), Relative Bioaccessibility Leaching Procedure (RBALP) (James et al., 2011), as well as simulation of the human intestinal microbial ecosystem (SHIME) (Cave et al., 2010). To ensure that hydrophobic organic contaminant soil release is not limited to the compound solubility for the simulated intestinal fluids, a sorption sink such as C18 membranes (Hurdzan et al., 2008; James et al., 2011), tenax beads (Li et al., 2015), ethyl vinyl acetate thin films (Vasiluk et al., 2007), and silicone rods (Gouliarmou and Mayer, 2012) are incorporated into the models. These models can often predict the bioavailability of different PAHs within a soil (James et al., 2016), but typically are not successful in estimating bioavailability between soils. There are many ways to measure and define bioavailability (see James et al., 2016; Ruby et al., 2016; Ramesh et al. 2004), and here we define bioavailability as the fraction of parent PAHs that crosses the gastrointestinal epithelium and reaches systemic circulation. We measure and define bioaccessibility as the as the fraction of PAH that is solubilized in simulated intestinal fluid and partitions into silicone rods.

Juhasz et al. (2014b) noted that maximizing estimated bioaccessibility is not necessarily the most conservative measure of bioavailability (*i.e.*, bioaccessibility can be less than bioavailability). Bioaccessibility is dependent upon the desorption conditions within the *in vitro* model, *i.e.* shaking method, temperature, desorption media, and desorption time (Oomen et al., 2002; Reichenberg and Mayer, 2006). PAH release in *in vitro* models is linked to the activation energy of the desorption process (Enell et al., 2005) as well as organic matter composition (Crampon et al., 2014; Zhang et al., 2010). PAHs bind to either amorphous organic matter with non-competitive fast desorption kinetics or to carbonaceous geosorbents with competitive slow desorption kinetics (Cornelissen et al., 2005). A typical soil has both amorphous and carbonaceous geosorbents and regardless of carbon type, longer desorption times typically lead to greater desorption (Oomen et al., 2002). The RBALP model, which utilizes end-over-end rotation, can be coupled with a lipid sink and leads to high PAH release from soil (James et al., 2011). Under such conditions, PAH bioaccessibility closely tracks PAH soil concentration but not PAH bioavailability (James et al., 2011). *In vitro* models that use reduced energetic input, such as the TIM model (Van de Wiele et al., 2007), will result in lower PAH release and perhaps this release is linked more closely to bioavailability. Our rationale for this hypothesis is that many current generation *in vitro* models maximize bioaccessibility to provide a conservative estimate of bioavailability, but not necessarily providing the most accurate estimates. Our experience is that these *in vitro* approaches closely mirror chemical activity but not bioavailability. Hence, we modified the existing FOREhST model to reduce energetic inputs during extraction and compared this release to *in vivo* bioavailability results.

PAHs are present as mixtures and depending on the source of the PAHs, *e.g.* pyrogenic, petrogenic, *etc.*, the relative ratios of each PAH will change (Tobiszewski and Namiesnik, 2012). The nature of this PAH mixture is a major factor influencing PAH bioaccessibility/bioavailability (Juhasz et al., 2016). It is thought that these mixture effects occur because PAHs interact with other PAHs and influence their partitioning behavior. For example, phenanthrene solubility in various surfactants was enhanced in the presence of naphthalene yet reduced in the presence of pyrene (Chun et al., 2002). Benzo(*a*)pyrene concentrations in gut fluids increased in the presence of phenanthrene by 154% (Voparil et al., 2003). Given that PAHs interact with each other, understanding the interactions within an *in vitro* bioaccessibility model may better predict *in vivo* bioavailability.

Co-inertia analysis is statistical method developed to study the common structure of multiple sets of paired data (Thioulouse, 2011). Co-inertia analysis is a non-directional approach to identify individual variables within each matrix that influence the other corresponding matrix and is well suited to situations where the number of samples is low relative to the number of predictor variables. Here we use co-inertia to identify key PAHs in the bioaccessibility matrix that are influencing other PAHs in the bioavailability matrix. However, co-inertia analysis is largely an exploratory statistical approach, and thus we tested if these PAHs were significantly influencing bioavailability using structural equation modeling. Structural equation modeling (SEM) is well suited for assessing a hypothesis that links collinear variables in a causal network to predict a dependent variable (Lamb et al., 2011). Furthermore, unlike multiple regression approach, structural equation modeling explicitly accounts for collinearity and thus, allows one to estimate, not only the significance, but the strength of a relationship linking predictors (such as the bioaccessibility of single PAHs) to the bioavailability of a PAH.

Our goal here was to combine the concepts of bioaccessibility and bioavailability as outlined by Juhasz et al. (2014b) and Reichenberg and Mayer (2006), with explicit multivariate predictive approaches, to develop a numerical prediction of bioavailability based on a widely adopted bioaccessibility protocol. We then evaluated the robustness of this prediction by spiking PAHs into water, bile fluids or soil and confirming that the drivers identified by the multivariate approaches were indeed occurring in *in vitro* settings.

2. Materials and methods

2.1. Soils

A total of 14 PAH contaminated soils have been collected from the United Kingdom (n = 12) and Sweden (n = 2) as previously described by Cave et al. (2010) and James et al. (2011). Soil pH, organic carbon, and particle size were analyzed as previously described by Siciliano et al. (2009). PAH soil extraction was performed as previously described by James et al. (2016).

2.2. Sorptive sink

Silicone rods, poly(dimethylsiloxane) (PDMS), are chosen to act as a PAH sorption sink as they have established partitioning properties for PAHs and have been previously used for *in vitro* bioaccessibility testing (Gouliarmou et al., 2013; Juhasz et al., 2016). The silicone rod (Altec, Cornwall, United Kingdom) has a diameter of 2.87–3.13 mm with a density of 8.0 g m^{-3}. For all experimental uses, 1 m of silicone rod is used as previous research demonstrates the sorption capacity of silicone will not limit PAH solubility (Gouliarmou et al., 2013; Juhasz et al., 2016). To prepare the silicone rods for experimental use, the procedures of Gouliarmou and Mayer (2012) are followed, where the silicone was cleaned by soaking once overnight with ethylacetate, three times overnight with methanol, 3 times overnight with acetone, and 4 times overnight with Milli-Q water.

2.3. FOREhST

2.3.1. Shaking method/energetic input

To investigate the effects of energetic inputs two shaking methods were employed. The first is the standard high energy FOREhST where 125 mL glass bottles are rotated 30 rpm end-over-end inside of a water bath held at 37 °C. The second method uses a less aggressive

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