# Measurement and modelling of the ingestion bioaccessibility of polyaromatic hydrocarbons in soils

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## HIGHLIGHTS

- In-vitro bioaccessibility of PAHs in soil was measured using FOREhST method.
- Infra-red spectra were used to provide proxy data on soil properties.
- Principle component analysis was used to summarise PAH properties.
- A Random Forest model of soil and PAH properties predicts the PAH bioaccessibility.

## ARTICLE INFO

## ABSTRACT

Total and human ingestion bioaccessible polycyclic aromatic hydrocarbon fractions of individual polycyclic aromatic hydrocarbons were determined (representative of a combination of the saliva, gastric and upper intestine compartments) on 26 soil samples from 3 gasworks sites and from a domestic garden. A Random Forest model using the Infra-red spectra of the soils and the PAH properties successfully predicted the bioaccessibility of PAHs in the soils. The Near Infra-red and Mid Infra-red diffuse reflection spectra of the soils were subjected to a mixture resolution algorithm. Comparison with spectra of known minerals tentatively identified carbonate, silica, clay and iron oxide components in the Mid Infra-red spectra. Multiple linear regression analysis suggested that three Mid Infra-red components were associated with the organic carbon. Principal Component Analysis of polycyclic aromatic hydrocarbon properties identified three components associated with the hydrophobicity, the aliphatic nature and the vapour phase partition coefficient of the polycyclic aromatic hydrocarbons.

## 1. Introduction

The increasing need for the redevelopment of contaminated land and concerns for human health associated with living in contaminated environments provides a driver for research into the estimation of human exposure to contaminants in soil. Ingestion of soils is considered to be an important exposure pathway for humans (Paustenbach, 2000). For risk assessments considering exposure by the ingestion pathway, it is not the total contaminant concentration that is important but the bioavailable fraction that enters the body. The bioavailability of a given substance may be formally defined as the fraction of an administered dose that reaches the central (blood) compartment from the gastrointestinal tract (Paustenbach, 2000). Bioavailability measurements, however, require in-vivo testing on humans or animal surrogates which can be expensive, time consuming and have difficulties associated with ethical approval. Considerable efforts have therefore been made to produce in-vitro tests (Denys et al., 2012; Drexler and Brattin, 2007) which mimic the physicochemical conditions found in the human gastrointestinal (GI) tract but can be carried out rapidly in commercial testing laboratories. These tests

measure the oral bioaccessibility of the contaminant, which is defined as the contaminant fraction of intake that is soluble in the human gastrointestinal system and is therefore available for absorption (Paustenbach, 2000). Bioaccessibility measurements should therefore provide a conservative estimate of bioavailability. For inorganic contaminants such as As, Cd and Pb a number of in-vitro bioaccessibility tests have been developed and, importantly, they have been validated against animal studies to show that they provide accurate predictions of in-vivo bioavailability (Denys et al., 2012; Drexler and Brattin, 2007). Organic contaminants are of equal concern, particularly polycyclic aromatic hydrocarbons (PAHs) which are ubiquitous environmental pollutants, and are key toxic substances for cancer risks in humans (Perera, 1997). Development of PAH bioaccessibility tests poses a number of difficulties. For the bioaccessibility test itself the extraction and analysis of the PAH from the complex simulated GI solutions is challenging. Validation of the in-vitro tests through animal models is also difficult since PAHs are metabolised to hydroxylated species making it difficult to carry out a complete mass balance of the PAH in the in-vivo endpoint. A recent comprehensive review of in-vitro methods used for PAH bioaccessibility has been published (Harris et al., 2013). To summarise developments; a number of in-vitro tests for PAHs have been reported. These have considered batch (Cave et al., 2010; DIN, 2000; Gron et al., 2007; Tao et al., 2010; Tilston et al., 2011) and dynamic systems (Van de Wiele et al., 2004), some mimic the stomach and upper intestine (Cave et al., 2010; DIN, 2000; Gron et al., 2007; Tao et al., 2010; Tilston et al., 2011) and others also include the colon compartment (Tilston et al., 2011; Van de Wiele et al., 2004). Some models include a food component (Cave et al., 2010; DIN, 2000; Gron et al., 2007) since lipids will have a large effect on the extraction of PAHs (Harris et al., 2013). Additional studies have also shown the importance of the inclusion of lipid sinks which mimic the stomach/intestinal wall and provide a fugacity gradient which potentially increases the bioaccessibility of PAHs (Collins et al., 2013; Gouliarmou and Mayer, 2012; Mayer et al., 2011). There have been successful comparisons between methods (Cave et al., 2010) but the in-vitro studies lack validation through in-vivo testing.

Cave et al. (2010) compared the Fed ORganic Estimation human Simulation Test (FOREhST) and the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) in-vitro procedure for 6 of the most carcinogenic PAHs in 11 gasworks soils to show that there was a good agreement between the two methods. This study also showed that the bioaccessibility of the PAH in the soil was dependent on the properties of the PAH and the soils, and the amount of total organic carbon (TOC) in the sample. The FOREhST method has not been validated against animal model so it cannot be claimed that it predicts in-vivo bioavailability in its present form. There are a number of factors, however, which suggest it is, at least, partially modelling the processes that solubilises PAHs from a contaminated soil in the human gastrointestinal tract. These are:-

 (i) The FOREhST method uses the physico-chemical conditions of the human stomach and upper intestine during ingestion of food as derived from the study carried out by the National Institute for Public Health and the Environment in the Netherlands (Versantvoort et al., 2004) and is therefore physiologically relevant;
(ii) The FOREhST method has been shown to correlate well with an independent in-vitro but not in-vivo validated model (SHIME) which has been used extensively in studies to assess bioavailability of organic compounds in foods (Cave et al., 2010).

With these limitations in mind, the aim of this study is to see if the bioaccessibility of PAHs as measured by the FOREhST method can be modelled and predicted using a combination of tabulated properties of PAH compounds and NIR spectra of soils as surrogates of soil properties. NIR was chosen as it is a rapid analysis technique which can be carried out with portable equipment at the soil sampling locations and can be used to predict a variety of soil properties (Viscarra Rossel et al., 2008). This could lead to rapid screening tests in the field environment. The modelling process may also provide some insights into the controlling factors which make PAHs available for absorption in the human gastrointestinal environment.

## 2. Materials and methods

### 2.1. Soils

26 soil samples from 3 gasworks sites of varying ages which had all produced gas in horizontal retorts and one representing a background soil were selected for the study. Eight samples were collected from a small former gasworks site (H GW) and closed in 1950 (surface soil samples 0–0.15 m), four samples from a very small former village gasworks (S GW) which closed in 1900 (0.2 to 0.3 m depth) and nine samples were taken from an early small gasworks (ES GW) which closed in 1860 (0.2 to 1.2 m depth). Five samples were also taken from a residential urban garden (UG) of a former gas employee's house where no gasworks related activities had taken place (0.1 to 0.5 m depth). The samples were freeze dried and sieved to <250 μm.

### 2.2. Bioaccessibility extractions and calculations

The FOREhST bioaccessibility extractions were carried out using the detailed procedure described in Cave et al. (2010). This is summarised in brief here. The FOREhST method is essentially a three stage static in-vitro bioaccessibility test intended to simulate the physico-chemical conditions in the fed state. The method is carried out at human body temperature (37° C) and utilises end-over-end rotation. The stages involved in the methodology are suggestive of the saliva, gastric and intestinal (duodenal and bile) phases of the human gastrointestinal system, with sample collection (by centrifugation) at the end of the extraction phase representative of small intestinal digestion. The measured bioaccessibility is therefore representative of a combination of the saliva, gastric and upper intestine compartments. Gastrointestinal fluid pH, ratios and transit times are all adjusted, compared to a fasted static model, to account for the physiological differences caused by the ingestion of food: saliva pH ($6.8 \pm 0.5$); gastric pH ($1.3 \pm 0.5$); small intestinal pH (duodenal pH $8.1 \pm 0.2$, bile pH $8.2 \pm 0.2$); GI fluid ratio for saliva: gastric: duodenal: bile (1:2:2:1), GI transit time (gastric 2 h, small intestine 2 h). For each contaminated soil under investigation, 0.3 g of contaminated material was extracted using the simulated saliva, gastric and intestinal (duodenal and bile) phases and a freeze dried rice porridge and vegetable oil to simulate fed conditions (pH values are checked and adjusted to the specified range after the soil, rice porridge and vegetable oil had been added). The method is designed to simulate the PAH bioaccessibility in a 4–6 year old child (Cave et al., 2010).