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Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils?

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ABSTRACT

Bioaccessibility tests can be used to improve contaminated land risk assessments. For organic pollutants a 'sink' is required within these tests to better mimic their desorption under the physiological conditions prevailing in the intestinal tract, where a steep diffusion gradient for the removal of organic pollutants from the soil matrix would exist. This is currently ignored in most PBET systems. By combining the CEPBET bioaccessibility test with an infinite sink, the removal of PAH from spiked solutions was monitored. Less than 10% of spiked PAH remained in the stomach media after 1 h, 10% by 4 h in the small intestine compartment and c.15% after 16 h in the colon. The addition of the infinite sink increased bioaccessibility estimates for field soils by a factor of 1.2–2.8, confirming its importance for robust PBET tests. TOC or BC were not the only factors controlling desorption of the PAH from the soils.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental organic pollutants, a number of which are known carcinogens. They can arise from point sources such as petroleum installations and old gasworks or from more diffuse sources as a consequence of the continuous deposition of airborne pyrogenic particles. Soils are the major environmental sink for PAHs and it has been estimated that over 90% of the U.K. PAH burden resides in soil (Wild and Jones, 1995), while the past land use at legacy sources may result in levels above guidance limits preventing its reuse.

Total pollutant concentration is frequently used in the assessment of risk posed by contaminated land to human health. However, it has been widely established that such an approach may significantly overestimate the amount of pollutant available for uptake by biota, including humans. Such an overestimation of risk can result in significant additional remediation costs and reduce the sustainability of land use for development. This issue is especially important for PAHs as they are usually emitted into soils where they are readily sorbed within domains that have been shown to reduce substantially their bioavailability, like soot (Jonker and Koelmans, 2002) or coal tar (Khalil et al., 2006). A number of chemo-mimetic methods have thus been developed and used to determine the various bioavailability parameters for worms (Gomez-Eyles et al., 2011; Jonker et al., 2007), plants (Gomez-Eyles et al., 2010) and microbes (Reid et al., 2000; Stokes et al., 2005) in contaminated soils (Reichenberg and Mayer, 2006).

For human exposure to PAH contaminated soils direct ingestion as a result of hand-to-mouth activity is frequently the most significant pathway (Jeffries and Martin, 2008). To address this several *in vitro* physiologically-based extraction tests (PBETs) have been proposed for metal (Ruby et al., 1996; Wragg et al., 2011) and organic pollutants (Cave et al., 2010; Van de Wiele et al., 2004). *In-vitro* alternatives are a cheaper pre-cursor of bioavailability measures, but they are only able to give information on the bioaccessible fraction, i.e. that which is released from the soil matrix into the gut fluid (Oomen et al., 2002).

In recent work we developed a colon extended physiologically-based extraction test (CEPBET) (Tilston et al., 2011), this enhanced the bioaccessibility of PAHs compared to a two compartment, i.e. stomach and small intestine, model. This was proposed to arise in part from the enhanced 'sink' capacity of the carbohydrate rich colon medium (James et al., 2011). In a recent study, a better correlation was found between a PBET and bioavailability to juvenile

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swine when a sink in the form of a C18 solid phase extraction membrane was included within the PBET (James et al., 2011). The addition of a lipid like sink provides a closer mimic of PAH bioaccessibility to humans as the gastrointestinal tract (GIT) provides a considerable sorptive sink for PAHs due to its large surface area (300 m^2), lipophilic nature, active absorption mechanisms and long contact time with the gut fluid.

In recent work it has been reported that a diffusive sink made of a composite of silicone and activated carbon can act as a contaminant trap, which enhances the desorption of PAH from soils and enables the quantification of the PAH fraction within these soils that is resistant to microbial degradation (Mayer et al., 2011). The aim of the current study is to combine the CEPBET bioaccessibility test with the contaminant trap method in order to maintain a full diffusion gradient for the desorption process. Due to the high affinity of PAHs for activated carbon (Zimmerman et al., 2004) the contaminant trap will maintain low PAH concentrations in the gut fluid, simulating PAH absorption by the GIT and potentially enable more PAH desorption from the soils into the gut fluid. This will provide a measure of the maximum mobilized fraction of PAHs under simulated digestive conditions, enabling the refinement of current contaminated land risk assessments whilst still providing a conservative measure of human exposure due to the high PAH sorption capacity of the contaminant trap. The research was divided into two phases: 1) determining the PAH elimination kinetics from the solutions of the 3 gut compartments of CEPBET induced by the contaminant trap, and 2) determining the impact of the contaminant trap addition to CEPBET on four field soils contaminated with PAH.

2. Materials and methods

2.1. Contaminant traps

Contaminant traps were prepared according to (Mayer et al., 2011). Activated carbon, 'activated charcoal, untreated powder 100–400 mesh' from Sigma–Aldrich (Vallensbæk Strand, Denmark) was added to the PDMS pre-polymer (Dow Corning SILASTIC 9161 RTV Silicone rubber) in the ratio 1:10 (mass: mass) and then stirred until it reached a homogenous grey colour. The curing agent (Dow Corning SILASTIC 9162 RTV catalyst containing ethyl silicate) was added (16%, mass/mass) and the mixture was again thoroughly stirred. 50 g of this mixture was transferred to the bottom of 500 ml glass jars and the "contaminant trap" composite was then cured at room temperature for 2–3 weeks.

2.2. CEPBET

The configuration of the CEPBET system has been described in detail elsewhere (Tilston et al., 2011). The gastro-intestinal extraction test comprised of three compartments, specifically the stomach (ST), small intestine (SIN) and colon (COL) which are incubated for 1, 4 and 16 h respectively. Details of the configuration of the CEPBET and composition of these three media are in the supporting information (SI) (Fig. S1, Table S1). Incubations were performed in 500 ml capacity glass jars, placed in a shaking water bath at 37 °C. The medium (100 ml) was pre-warmed for 30 min and either a PAH solution or soil (1 g d wt.) added. Incubation vessels were then sealed with Teflon-lined screw-caps for the duration of the incubation.

2.2.1. Spiking and sampling procedure

The ST, SIN and COL media were spiked individually with 1 ml of 100 μg ml^{-1} 16 EPA PAH standard (Sigma Aldrich, UK) in 1:1 (v:v) acetone:hexane. The ST, SIN and COL media (100 ml) and spike were shaken vigorously by hand and then placed in the shaking water bath (37 °C, 120 rpm). A 5 ml sample was taken at 1 h for ST; 1, 2 and 4 h for SIN and 1, 2, 8 and 16 h for COL. Samples were then shaken with 5 ml acetone:hexane (5:4) containing fluorobiphenyl and d-terphenyl as surrogate standards for 1 h at 225 rpm. The hexane layer (1 ml) was then placed in GC vials. Recoveries of the surrogates ranged from 75 to 91%, 71–89%, 51–80% in ST, SIN and COL respectively and data were adjusted accordingly.

2.3. Soils

The soils used in the study were collected from a number of contaminated sites within the UK. The soils were air-dried and passed through a 2 mm sieve and stored in air tight containers prior to experiments. To determine the total amount of PAHs in the soil five replicate 5 g portions of soil were agitated in 10 ml of 1:1 (v:v) acetone:hexane containing fluorobiphenyl and terphenyl as surrogate standards for 2 h on an orbital shaker at 250 rpm. After extraction the samples were passed through a column containing 2 g of a 1:1 deactivated silica and dry sodium sulphate mixture for clean-up, before transferring to gas chromatography vials for GC–MS analysis (LOD = 0.05 mg kg^{-1}). This method was adapted from a mechanical shaking method previously reported to give better recoveries than a Soxhlet extraction (Song et al., 2002). Samples were then evaporated to dryness under a stream of nitrogen and resuspended in 1 ml hexane containing deuterated internal standards for quantification by GC–MS.

TOC and BC were determined using established methods (Agarwal and Bucheli, 2011), the procedure is as follows (1) removal of inorganic carbonates from the dried (60 °C) and ball ground sample (10 mg or less) via in situ acidification (25 μl of 1 M HCl) in silver capsules; (2) removal of non-pyrogenic organic carbon in a programmable tube furnace at 375 °C under controlled air flow, and (3) quantification of residual carbon as black carbon using a CHN elemental analyser (Carlo Erba model 1106, combustion temperature 1030 °C, oxygen boost time 28 s). Total organic carbon was determined in a similar way but without stage two of the BC method. All data was determined in triplicates.

2.3.1. Soil incubations

For the soil incubations CEPBET was used in the sequential mode. Exposure to ST and SIN compartments was achieved by exposing the test substrate to the ST compartment for 1 h before converting the ST medium to SIN medium by addition of bile salts and pancreatin with elevation of the pH from 2.5 to 7.0. The bottles were then resealed and incubation continued for a further 4 h. The transition between SIN and COL compartments was effected by physical transfer: the test substrate was recovered by centrifugation (3000 *g*, 10 min), added to a bottle of pre-warmed colon medium and incubated for a further 16 h. Each incubation was replicated three times and trap and no-trap treatments were undertaken in parallel.

Media were extracted by liquid:liquid extraction with 20 ml acetone:hexane (1:1) by placing on a rock and roll shaker for 1 h. The solvent layer was removed following phase separation and cleaned using dispersive SPE (Superclean-PSA, Supelco) before analysis by GC–MS. The residual soil was extracted as described for the soils above.

Analytical grade inorganic salts (Fisher Scientific, Loughborough, U.K.) were used throughout and all other reagents were obtained from Sigma–Aldrich (Gillingham, U.K.).

2.4. Gas chromatography

A Thermo Trace GC Ultra system equipped with a Thermo TR-5MS capillary column (30 m × 250 μm × 0.25 μm) operating with helium as a carrier gas, coupled to a Thermo ITQ 1100 mass spectrometer (MS) connected through a heated transfer line (300 °C). The GC injector (220 °C) was operated in a pulsed splitless mode, 1 μl aliquots were injected using an autosampler, and the GC oven was programmed to hold 60 °C for 3 min then ramped by 15 °C min^{-1}–290 °C, and held for 10 min. The MS was operated with the ion source at 220 °C and a damping flow of 0.3 ml min^{-1}.

2.5. Statistical analysis and data presentation

All data analyses were tested for normality (Kolmogorov–Smirnov) and transformed where necessary using either the natural logarithm or the square root of the initial value. Analyses of variance and regression analyses were performed using MINITAB (release 16, MINITAB, State College, U.S.A.) and means were separated according to Tukey's honestly significant difference test ($p < 0.050$) for the analysis of the bioaccessibility of the real soils and the impact of TOC and BC on this bioaccessibility.

Data are presented as the ratio of residual concentrations in soils after incubation with and without contaminant trap. Where these ratios are close to 1 there is little impact of the contaminant trap on the measured matrix concentration. Bioaccessibility values for the non-trap treatments were calculated using Eqn. (1). For the CEPBET with the contaminant traps Eqn. (2) was used to calculate bioaccessibility, since the accessible pollutants absorbed by the traps cannot be recovered and quantified.

$$\text{Bioaccessibility} = A/C_{\text{total}} \times 100\% \quad (1)$$

$$\text{Bioaccessibility} = (C_{\text{total}} - B)/C_{\text{total}} \times 100\% \quad (2)$$

C_{total} = total pollutant in soil (μg).
A = pollutant in gut medium non-trap treatment (μg).
B = pollutant in residual soil trap treatment (μg).

3. Results and discussion

3.1. Trap sorption kinetics – spiked solutions

There was very rapid and complete sorption into the contaminant traps for all PAH in ST compartment with less than 10% of the spike