Science of the Total Environment 473–474 (2014) 147–154

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

In vivo measurement, in vitro estimation and fugacity prediction of PAH bioavailability in post-remediated creosote-contaminated soil

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HIGHLIGHTS

- Low PAH bioavailability in remediated creosote soil limited further biodegradation.
- However residual PAHs were highly bioavailable when assessed using an in vivo model.
- In vitro PAH bioaccessibility underestimated in vivo PAH bioavailability.
- Fugacity modelling underestimated in vivo PAH bioavailability.
- These results impact exposure and risk calculations for incidental soil ingestion.

ARTICLE INFO

Article history:
Received 22 July 2013
Received in revised form 3 October 2013
Accepted 6 December 2013
Available online 22 December 2013

Keywords:
Benzo[a]pyrene
Bioaccessibility
Bioavailability
Bioremediation
Fugacity
Polycyclic aromatic hydrocarbons

ABSTRACT

In this study, PAH bioavailability was assessed in creosote-contaminated soil following bioremediation in order to determine potential human health exposure to residual PAHs from incidental soil ingestion. Following 1000 days of enhanced natural attenuation (ENA), a residual PAH concentration of 871 ± 8 mg kg^{-1} (∑16 USEPA priority PAHs in the <250 μm soil particle size fraction) was present in the soil. However, when bioavailability was assessed to elucidate potential human exposure using an in vivo mouse model, the upper-bound estimates of PAH absolute bioavailability were in excess of 65% irrespective of the molecular weight of the PAH. These results indicate that a significant proportion of the residual PAH fraction following ENA may be available for absorption following soil ingestion. In contrast, when PAH bioavailability was estimated/predicted using an in vitro surrogate assay (FOREhST assay) and fugacity modelling, PAH bioavailability was up to 2000 times lower compared to measured in vivo values depending on the methodology used.

1. Introduction

Polycyclic aromatic hydrocarbon (PAH) contamination of urban soil is widespread due to a variety of anthropogenic activities. PAHs may be released to the environment during the processing of coal (up to 1.5 g kg^{-1}) and petroleum products, through emissions from power plants, incinerators, aluminium, steel, iron and phosphorus production, foundries, sinter and bitumen processes, vehicles and from wood preservation activities (Environment Australia, 2002; Juhasz and Naidu, 2000; Wilson and Jones, 1993). The historical practice of manufactured gas production has also resulted in PAH-contaminated sites in major towns and cities. As PAHs exhibit toxic, mutagenic, teratogenic and carcinogenic properties (ATSDR, 1995) there is concern regarding the presence of these compounds in the environment due to their potential impact on human health.

In order to reduce the concentration of PAHs in soil and reduce the human health risk associated with contaminated soil exposure, a number of remediation technologies are available. Bioremediation strategies, including enhanced natural attenuation (ENA), are readily available and relatively low cost remediation techniques for the treatment of PAH-contaminated soil. However, bioremediation may be limited by the time required to reach acceptable endpoints or may fail to achieve remediation targets due to microbial bioavailability or biodegradability limitations (i.e. recalcitrance of high molecular weight PAHs). In a previous study (Juhasz et al., 2005a,b), the aforementioned bioremediation limitations were highlighted following pilot scale treatment of creosote-contaminated soil (7767 ± 1286 mg kg^{-1}) using an ENA strategy. Although 82–99% and 33–81% of three- and four-ring PAHs were removed over the 182 day treatment period respectively, high residual concentrations of biodegradable PAHs such as pyrene and fluoranthene remained in the soil as a result of sequestration

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http://dx.doi.org/10.1016/j.scitotenv.2013.12.031

processes which limited microbial bioavailability. In contrast, five- and six-ring PAHs were generally recalcitrant to microbial degradation as a result of factors including unfavourable Gibbs free energy, minimal transport of these compounds across the cell wall and/or microbial bioavailability constraints.

Although microbial bioavailability and/or biodegradability limitations resulted in a residual PAH fraction which restricted further biodegradation, the residual PAH fraction was bioavailable to other ecological receptors (Juhasz et al., 2010). The residual PAH concentration in the post-ENA creosote soil was able to exhibit toxicological effects on earthworms as a result of the different PAH release and uptake mechanisms of *Eisenia fetida* compared to bacteria. Presumably the mode of toxic action on *E. fetida* was via narcosis, affecting the fluidity and functioning of cell membranes (Sverdrup et al., 2002). While the toxicological effect of residual PAHs towards a variety of ecological receptors has been reported (Brown et al., 2004; Eom et al., 2007; Parrish et al., 2006; Smith et al., 2006), there is a paucity of data on the potential exposure of humans to residual PAHs in soil post-bioremediation.

When assessing risk to humans associated with the incidental ingestion of contaminated soil and dust, a major unknown is quantifying the dose that has been solubilised in the gastrointestinal tract and absorbed into the systemic circulation (i.e. the bioavailable fraction). Determination of PAH bioavailability is challenging due to the complexity of in vivo PAH metabolism, distribution and excretion. Following ingestion, PAHs that are absorbed across the intestinal epithelium may enter hepatic portal circulation and be transported to the liver (first pass effect) where PAH metabolism may occur as a result of cytochrome P450 monooxygenase activity (Ramesh et al., 2004; Shimada, 2006). From hepatic portal circulation, metabolised and non-metabolised PAHs may enter the systemic circulation via hepatic veins whereas a proportion of metabolised PAHs may be excreted in the faeces via the bile (Ramesh et al., 2004). Non-absorbed PAHs in the gastrointestinal tract are also excreted via the faeces. Assessment of PAH bioavailability is complicated by the fact that PAHs may be absorbed from the gastrointestinal tract and transformed in the hepatic portal system but may not reach the systemic circulation (due to biliary excretion) which by definition is the bioavailable fraction. Conversely, transformed PAHs may enter the systemic circulation, however, this may not be taken into consideration for PAH bioavailability estimations if only the concentration of the parent compound is determined.

A number of approaches including blood, urine and faecal analysis have been utilised for the assessment of PAH bioavailability (Grøn et al., 2007; James et al., 2011; Pu et al., 2004; Reeves et al., 2001; Stroo et al., 2000; Van Schooten et al., 1997), however, all these approaches have certain limitations. Assessment of PAHs in the systemic circulation may underestimate PAH bioavailability due to the absorption of transformation products following PAH metabolism in the liver as a result of first pass effects (Van Schooten et al., 1997). Furthermore, PAHs may be rapidly cleared from the blood compartment of animal models (Kotin et al., 1959) which may result in inaccurate determination of area under the PAH–blood time curve. PAH transformation products (e.g. OH-PAHs) may be determined in urine as an estimation of PAH bioavailability. This approach has been utilised as a marker of PAH exposure (Campo et al., 2011; Li et al., 2010), however, may underestimate PAH bioavailability due to the lack of quantification of non-metabolised PAHs, the complexity of PAH metabolite quantification and the faecal excretion of transformed PAHs. In contrast, estimating PAH bioavailability by the determination of PAHs excreted in the faeces (i.e. the non-bioavailable fraction) may represent an upper-bound measure of PAH bioavailability as this approach does not consider other loss mechanisms such as biliary excretion of metabolised PAHs which have been absorbed but haven't reached the systemic circulation (Stroo et al., 2000; Van Schooten et al., 1997) and microbial metabolism within the intestinal lumen (Cavret and Feidt, 2005). In addition, van Schooten et al. (1997) highlighted analytical detection limitations associated with administering single PAH-contaminated soil doses when determining PAH bioavailability. Administering multiple PAH doses may overcome analytical issue but may influence bioavailability determinations as a result of the induction of cytochrome P450 enzymes which may enhance PAH metabolism if a measurement method assessing metabolites was employed.

Although in vivo studies utilising animal models are an appropriate method for determination of PAH bioavailability in contaminated soil for inclusion in human health exposure assessment, the time required for in vivo studies and the expense of animal trials preclude their use as routine relative bioavailability assessment tools. As a result, rapid, cost effective in vitro methods simulating human gastrointestinal conditions have been developed in order to estimate contaminant bioavailability. These assays determine PAH concentrations that are solubilised following gastrointestinal extraction (i.e. the bioaccessible fraction) and are therefore potentially available for absorption into the systemic circulation. Studies to date have demonstrated that PAH bioaccessibility in contaminated soil is variable (0.1–89%) and is dependent on the physicochemical properties of the PAH and soil in addition to soil–PAH residence time (ageing) (Cave et al., 2010; Grøn et al., 2007; Hack and Selenka, 1996; Pu et al., 2004; Van de Wiele et al., 2004). PAH bioavailability may also be predicted using fugacity modelling (James et al., 2011) although limited information is available regarding this approach for predicting in vivo data. While in vitro assays and fugacity modelling have the potential to overcome the time and cost limitations of in vivo studies, limited information is available regarding the validity of estimating or predicting PAH bioavailability using these approaches which are a prerequisite to their use as surrogate bioavailability methods.

The objective of this study was to assess PAH bioavailability in creosote-contaminated soil following ENA to determine whether residual PAHs are bioavailable following incidental soil ingestion. Initially, PAH bioavailability was measured using an in vivo mouse model. In addition, PAH bioavailability was estimated/predicted using bioaccessibility (the FOREhST in vitro assay) and fugacity approaches to ascertain the difference in data derived from these methods. It was hypothesised that the bioavailability constraints that resulted in a residual PAH fraction following biodegradation may not be present during in vivo assessment due to the difference in PAH release and uptake mechanisms of bacteria compared to the mouse model.

2. Materials and methods

2.1. Creosote-contaminated soil

Previous studies investigated the efficacy of natural attenuation, enhanced natural attenuation (ENA) and bioaugmentation strategies for the remediation of PAHs in creosote-contaminated soil (7767 ± 1286 mg kg^{-1}) (Juhasz et al., 2005b). Soil from ENA biopile was recovered after approximately 1000 days of treatment, air dried and sieved to recover the <250 μm soil particle size fraction. This particle size fraction was used for bioaccessibility and bioavailability studies as this has been identified as the upper limit of the particle size fraction likely to adhere to children's hands and is therefore available for incidental ingestion (USEPA, 2006). PAH concentrations in the <2 mm and <250 μm soil particle size fractions are shown in Table 1.

2.2. Assessment of PAH bioavailability

In vivo PAH bioavailability studies were conducted with male Balb/c mice weighing 22–24 g. Animal care was compliant with the Standard Operating Procedures of the Veterinary Services Division, Institute of Medical and Veterinary Science and the Guidelines for the Care and Use of Laboratory Animals (NRC, 1996). Animals were housed in metabolic cages with 12/12 light/dark cycles with water supplied ad libitum.

PAH bioavailability was assessed by administering material to mice following incorporation into feed. Crushed animal feed (Specialty Feeds, Glen Forrest, Australia) was mixed with creosote-contaminated soil and water to create a dough consistency. The bioavailability