Environment International 70 (2014) 192–202

Contents lists available at ScienceDirect

Environment International

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

Effects of ageing and soil properties on the oral bioavailability of benzo[a]pyrene using a swine model

Luchun Duan [a,b], Thavamani Palanisami [a,b], Yanju Liu [a], Zhaomin Dong [a], Megharaj Mallavarapu [a,b], Tim Kuchel [c], Kirk T. Semple [d], Ravi Naidu [a,b,*]

[a] Centre for Environmental Risk Assessment and Remediation (CERAR), University of South Australia, Mawson Lakes SA5095, Australia
[b] Co-operative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), University of South Australia, Mawson Lakes SA5095, Australia
[c] South Australia Health & Medical Research Institute (SAHMRI), Adelaide SA5000, Australia
[d] Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, United Kingdom

ARTICLE INFO

Article history:
Received 7 January 2014
Accepted 27 May 2014
Available online xxxx

Keywords:
Oral bioavailability
Benzo[a]pyrene
Swine model
Soil properties
Extractability
Ageing effect

ABSTRACT

Oral bioavailability of benzo[a]pyrene (B[a]P) was studied in a swine model using eight spiked soil samples after incubation for 50 and/or 90 days. Silica sand was used as a reference material and the relative bioavailability (RB) of B[a]P in soils was calculated as the quotient of the area under the plasma B[a]P curve (AUC) for soil and AUC for the silica sand. Significantly reduced RB was observed in all study soils after 90 days ageing, ranging from 22.1 ± 0.4% to 62.7 ± 10.1%, except for one very sandy soil (sand content 87.6%) where RB was unchanged (108.1 ± 8.0%). Apart from this, bioavailability decreased during ageing with the decrease (from day 50 to day 90) being only significant for a clayey soil containing expandable clay minerals. Statistical analyses of B[a]P RB at day 90 (eight soils) and soil properties showed no direct correlation between RB and specific soil properties such as total organic carbon (TOC) and clay content which were commonly linked to organic contaminant sequestration. However, strongly significant relationships ($p < 0.001$) were found between RB and the fine particle associated carbon (FPAC) defined as (Silt + Clay)/TOC, and between RB and the soil mesopore (<6 nm; $p < 0.001$) fraction, after two samples with high pH and high EC being excluded from the analyses. The bioaccessibility estimated by four *in vitro* extraction methods: dichloromethane/acetone sonication (DCM/Ace), butanol vortex (BuOH), hydroxypropyl-β-cyclodextrin extraction (HPCD) and Milli Q water leaching methods at different sampling time (1 day, 50 days and 90 days after spiking) also showed a decreasing trend. Significant correlations were found between B[a]P RB and DCM/Ace ($R^2 = 0.67$, $p < 0.05$) extractable fraction and BuOH ($R^2 = 0.75$, $p < 0.01$) extractable fraction.

1. Introduction

Remediation of contaminated sites is currently expected to cost approximately $5 to 8 billion in Australia (Australia, 1997; Powell, 1992), $1 trillion in the USA (Rao et al., 1996) and 0.5–1.5% of GDP per annum in Europe (Lanno et al., 2004). Despite much focus on contaminated site remediation, it is now recognized that the required regulatory levels may not be achieved for most contaminated sites, even after several decades of clean-up efforts using existing technologies (Doick et al., 2005; Oomen et al., 2006; Swindell and Reid, 2006). Regulations and decisions to remediate are often based on total contaminant concentrations in soil, sediments and groundwater. However, there is increasing evidence that many of the contaminants that persist in the environment are influenced greatly by soil properties and become progressively less available for uptake by organisms over time thereby becoming less toxic or susceptible to biodegradation and bioremediation by microorganisms (Alexander, 1995; Alexander, 2000; Beck and Jones, 1995; Boopathy, 2000; Ma et al., 2012; Schwarzenbach and Westall, 1981).

The extent to which a contaminant is bioavailable in soils depends on a variety of factors including the properties of both the contaminant and soil environment, as well as the exposure pathway (Bolan et al., 2006) which includes oral as well as dermal absorption and inhalation. Oral ingestion of contaminated soil is one of the most important exposure pathways of soil-bound contaminants for humans and accidental ingestion of large amounts of soil by children represents a worst-case scenario for acute exposures (CCME, 2010). Oral bioavailability may be limited by the degree of interaction with the solid soil matrix. However, soil particles that are ingested by mammals are exposed to gastric intestinal (GI) fluids that may release contaminants from soil to the mammalian gut. Little is known of the effects of GI fluids on the kinetics of the release of contaminants from the soil particles (Ruby et al., 1996; VARIABLE, 1999). However, it can be expected that the actual bioavailable fraction of a contaminant in the mammalian gut would be lower

* Corresponding author at: Centre for Environmental Risk Assessment and Remediation, Building X (Environmental Sciences Building), University of South Australia, Mawson Lakes SA 5095, Australia. Tel.: +61 8 8302 5041; fax: +61 8 8302 3124.
E-mail address: ravi.naidu@unisa.edu.au (R. Naidu).

http://dx.doi.org/10.1016/j.envint.2014.05.017
0160-4120/

than its total soil concentration. In the absence of such data, risk assessment of contaminated sites usually assumes 100% bioavailability of soil-bound chemicals. Such an approach would lead to an over-estimation of risks and, as a consequence, individual sites may require more intensive clean-up or a greater number of sites would require remediation (Naidu et al., 2013).

During the last two decades, much effort has been directed towards both *in vivo* and *in vitro* assessment of the oral bioavailability of inorganic contaminants, such as Pb and As in soils. However, there has only been a limited number of studies investigating the oral bioavailability of organic contaminants in different soil types (Cave et al., 2011; Wilson and Naidu, 2008a). In these studies, the focus has been on either a limited number of soils (Pu et al., 2004) or artificial soils (Saghir et al., 2007). Soil organic matter is one of the most important factors that controls organic contaminant interactions within soil and hence strongly influences their mobility and bioavailability (Nam et al., 1998; Semple et al., 2003). Adsorption of hydrophobic organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), onto soils has been found to be directly related to soil organic matter content, as well as to the hydrophobicity of the contaminant compounds (Nam et al., 1998), although other soil properties also influence adsorption (De Jonge et al., 2008; Duan and Naidu, 2013). Working with a number of soils, Pu et al. (2004) found an inverse relationship between oral bioavailability and organic matter and clay content. In a recent study, Duan and Naidu (2013) reported a strongly significant positive correlation between sorption of phenanthrene and total soil organic carbon, although inclusion of other soil properties, such as the soil texture index and dissolved organic carbon (DOC), increased the variance accounted for by the regression.

Hydrophobic compounds in soil, such as PAHs, tend to sorb strongly to the soil matrix which may influence oral bioavailability in human. The extent of physical interaction between hydrophobic organic compounds and soil, as estimated by various extraction methods, generally increases with ageing time (Bogan and Sullivan, 2003; Chung and Alexander, 2002; Hatzinger and Alexander, 1995). In the few studies that have used small laboratory animals such as mice, rats and miniature pigs, the results have generally suggested that only a fraction of the total amount of organic contaminants in soil are absorbed by a mammal's gastrointestinal tract (Pu et al., 2004; Wittsiepe et al., 2007). However, to date only a single *in vivo* study has been conducted to assess oral bioavailability of organic contaminants in soil using a juvenile swine model as a surrogate for exposure of a human child (Smith et al., 2012).

The present study was conducted to evaluate the effects of soil properties and soil-contaminant contact time of B[a]P in soil on the oral bioavailability of this carcinogenic PAH using a juvenile swine model. In contrast to studies with small laboratory mammals it is generally accepted that the GI tract of swine shares many similarities to the monogastric human digestive system and therefore provides a more appropriate estimation of oral bioavailability to humans, particularly children (Weis and LaVelle, 1991).

2. Materials and methods

2.1. Chemicals

Analytical grade chemicals including B[a]P, sodium sulphate, hydroxypropyl-β-cyclodextrin (HPCD) powder, 1-butanol (BuOH), dichloromethane (DCM), acetone (Ace) and *n*-hexane were obtained from Sigma-Aldrich Pty Ltd (Sydney, Australia). Silica sand was also purchased from the same suppliers.

2.2. Soils

A selection of eight soils (seven surface soils from 0 to 20 cm depth and one sub-surface soil from 20 to 40 cm), with varying chemical and mineralogical composition, was used in the present study. Following sampling in the field, bulk soil samples were dried using a fan-forced oven at 35 °C, crushed to pass through a 2-mm stainless steel sieve, homogenized and used for subsequent studies. Routine analyses of the soils were conducted, as described by Duan and Naidu (2013). Particular emphasis was placed on the organic matter content of soils. Specific surface area was calculated using the BET equation with mesopore size distribution determined by Barret–Joyber–Halenda (BJH) N_2 adsorption/desorption isotherms measured at −196 °C (Gemini 2380) (Luo et al., 2012). Pertinent properties of the soils are summarized in Table 1.

2.3. Spiking of B[a]P into soils and subsequent ageing

Soils were spiked with 50 mg/kg B[a]P on a dry weight basis. Each soil sample (0.6 kg dry weight basis, or 2 kg for the four soils used for the ageing study) was placed in a Teflon tray as a thin layer (~2 cm) in a fume hood and 1% (v/w) B[a]P stock solution (5000 mg/l) (toluene: acetone = 1:1) was added drop-wise. Complete transfer of B[a]P was made by rinsing the glass vial three times with additional 1% acetone. Following spiking, the soil samples were homogenized by thorough hand mixing and the solvent was allowed to evaporate over 24 h. Subsamples (n = 5) taken to determine the spike recovery showed extremely high recovery with relatively small SD (this is described later). The soils were distributed into separate glass jars of 3 l capacity (~0.6 kg/jar) and deionized water was sprayed onto the spiked soils to bring the moisture content to about 60% of soil water holding capacity. The jars containing the spiked soils were sealed between sampling times and then kept in darkness at room temperature (22 ± 3 °C) over the ageing period of this study (90 days). Subsamples of four of the soils were taken after 1, 50 and 90 days for soil extraction and swine feeding studies. For the other four soils, replicate subsamples were obtained for studies after ageing for 90 days. In addition to the soils, silica sand was used as a reference material (spiked in the same way one day before dosing) to deliver the same amount of B[a]P to the animals. Homogeneity in spiked soils is critical in this study. Following spiking, and at every time that subsamples were taken for dosing, triplicate samples were extracted by the exhaustive solvent (DCM/Ace), which has frequently been used to estimate the total amount of PAH in soil by other workers. Data obtained from extraction were shown as means ± SD. Homogeneity of subsamples is evidenced by the small standard deviation (SD) values.

2.4. The swine study

2.4.1. Animal care

The study was carried out with 42 gilts (Landrace Cross, obtained from PPPI Roseworthy Piggery, The University of Adelaide, South Australia) at approximately 30 to 35 kg live weight and 8–10 weeks of age. The animals were housed individually in pens in a well-ventilated room at ambient temperature (15–30 °C) at SAHMRI, PIRL (Gilles Plains, Adelaide, South Australia) and were fed twice daily with a commercial pig finisher diet (Lauke Mills, Daveyston, South Australia) (500 g per meal) and provided with water *ad libitum*. The experiment was conducted in accordance with the 'Code of Practice for the Care and Use of Animals for Scientific Purposes' (National Health and Medical Research Council: Canberra, 7th Edition, 2004) and was approved by the SA Pathology/CHN Animal Ethics Committee (Approval number: 47/12). For each trial, the animals were acclimatized for four days prior to surgery to insert a jugular vein catheter. Following surgery, the catheters were flushed twice daily with a heparinized (10 IU/ml) NaCl solution (9 g/l) under aseptic conditions to prevent obstruction by blood clots. The animals were fasted prior to surgery and all pigs were fed approximately one hour following surgery once they had recovered sufficiently from the effects of anesthesia. The day after surgery,