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Comparison of oral bioavailability of benzo[*a*]pyrene in soils using rat and swine and the implications for human health risk assessment

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ABSTRACT

Background: There are many uncertainties concerning variations in benzo[*a*]pyrene (B[*a*]P) soil guidelines protecting human health based on carcinogenic data obtained in animal studies. Although swine is recognised as being much more representative of the human child in terms of body size, gut physiology and genetic profile the rat/mice model is commonly used in practice.
Objectives: We compare B[*a*]P bioavailability using a rat model to that estimated in a swine model, to investigate the correlation between these two animal models. This may help reduce uncertainty in applying bioavailability to human health risk assessment.
Methods: Twelve spiked soil samples and a spiked silica sand (reference material) were dosed to rats in parallel with a swine study. B[*a*]P bioavailability was estimated by the area under the plasma B[*a*]P concentration-time curve (AUC) and faecal excretion as well in the rats. Direct comparison between the two animal models was made for: firstly, relative bioavailability (RB) using AUC assay; and secondly, the two assays in the rat model.
Results: Both AUC and faecal excretion assays showed linear dose-response for the reference material. However, absolute bioavailability was significantly higher when using faecal excretion assay ($p < 0.001$). In aged soils faecal excretion estimated based on solvent extraction was not accurate due to the form of non-extractable fraction through ageing. A significant correlation existed between the two models using RB for soil samples ($RB_{rat} = 0.26RB_{swine} + 17.3$, $R^2 = 0.70$, $p < 0.001$), despite the regression slope coefficient revealing that the rat model would underestimate RB by about one quarter compared to using swine.
Conclusions: In the comparison employed in this study, an interspecies difference of four in RB using AUC assay was identified between the rat and swine models regarding pharmacokinetic differences, which supported the body weight scaling method recommended by US EPA. Future research should focus on the carcinogenic competency (pharmacodynamics) used in experiment animals and humans.

1. Introduction

Benzo[*a*]pyrene (B[*a*]P), a high molecular weight polycyclic aromatic hydrocarbon (PAH), is known as a probable human carcinogen based on increased occurrence of lung, dermal and gastro-intestinal tumours appearing in laboratory animals exposed to B[*a*]P (US EPA, 1994). Along with other PAHs, B[*a*]P mainly forms as a result of incomplete combustion of organic substances with both natural and anthropogenic origins (FAO/WHO, 1991). It commonly occurs at current and disused industrial sites, such as coal gasification and coke production plants, aluminium, iron and steel foundries, and creosote and asphalt production works (Zhang et al., 2009). Although commonly found as PAH mixtures, B[*a*]P has often been used to indicate the risk of PAHs (Bostrom et al., 2002; CCME, 2010; FAO/WHO, 2006; HPA, 2010; MfE, 2011; Schneider et al., 2002).

Given the lack of human epidemiological studies, the current soil guidelines for B[*a*]P and PAHs in Australia and many other countries are based on carcinogenicity in rodent (Brune et al., 1981; Culp et al., 1998; Neal and Rigdon, 1967). Typically, a benchmark dose (BMD) that gives rise to a 10% response (BMD_{10}) derived from fitting of dose-response data is used as a point of departure (PoD). For B[*a*]P, a lower confidence limit of BMD_{10} ($BMDL_{10}$) of 0.1 mg/kg body weight per day was used to calculate the risk of PAHs in food (MfE, 2011). From this critical toxicological value in animal studies large safety factors were applied to address uncertainties in extrapolating them to humans (Safety, 2014). More detailed information about the uncertainties

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associated with extrapolation has been documented in Dong et al. (2015). Briefly, a margin of exposure (MoE) approach of 1/10,000 was applied in Europe (HPA, 2010), in which a modifying factor of 10 was employed to account for the interspecies differences between mice and humans. The US EPA used the same default factor accounting for the interspecies differences but also recommends using a body weight (bw) scaling factor and a rounded uncertainty factor of 3 when considering the results of different animal models (US EPA, 2011). An interspecies uncertainty factor of 5 was adopted in a study developing soil guideline in Australia, where a guideline value of 5 mg/kg for B[*a*]P was derived (Fitzgerald et al., 2004). This value is very close to the current national soil guideline (4 mg/kg) for residential land use in Australia (NEPC, 2013).

Besides the uncertainty over interspecies differences, exposure from ingestion of contaminated soil does not delineate between the fraction that subsequently absorbs (bioavailable fraction) and the total concentration. Such an approach is likely to result in overestimation of risk and as a consequence remediation of sites that could potentially be safe. In the latest National Environmental Protection Measure of Australia, using site-specific oral bioavailability data of contaminants has been encouraged when available (NEPC, 2013). Bioavailability is defined as an internal estimation of the actual uptake or absorption of contaminants that enters the body (internal dose), and therefore provides a better estimation of the risk. Significantly reduced bioavailability of some PAH(s) in soil has been reported using animal models including goat and rat in comparison to dose in solution (Goon et al., 1990, 1991) or oil feed (Ounnas et al., 2009; Pu et al., 2004; Van Schooten et al., 1997). However, there is considerable uncertainty regarding the utilisation of oil as a reference material in these studies given its lack of relevance to environmental exposure, and therefore the implication of these results being used in modifying current soil guidelines. Also, compared to rodents, swine are preferred for human health risk assessment as they share many similar traits to humans, such as body weight, anatomy, genetics and physiology (Ng et al., 2013; Walters and Prather, 2012). However, conduct swine study is much more expensive compared to using rat. As a consequence, to date only a handful of animal studies have used swine to estimate PAH bioavailability in soils (Duan et al., 2014; James et al., 2011, 2016; Peters et al., 2015).

The limited number of swine studies and the lack of data illustrating interspecies extrapolation prompted us to carry out a comparative study using both rats and swine. The swine study result was published earlier with the focus on the effects of soil properties and ageing on B[*a*]P bioavailability (Duan et al., 2014). In this paper, we present a parallel rat study, in which B[*a*]P bioavailability was calculated using two different assays: plasma *versus* faeces. The major objectives of this study are: 1) to investigate if consistent bioavailability results could be found using the rat model instead of the more expensive swine model; 2) to compare the bioavailability results obtained from the two assays in the rat model. Finally, we discuss implications for human health risk assessment of bioavailability data from the rat and swine models.

2. Materials and methods

2.1. Soils

Eight soils varying in soil properties including organic matter (TOC: 0.72 ~ 7.5%; DOC: 8.5 ~ 108.4 mg/L), clay content (5.6% ~ 30.9%), pH, EC, CEC (and clay mineralogy), and texture, *etc*., were employed in this study. Detailed soil properties are presented in Table 1.

The soils were spiked at a B[*a*]P concentration of 50 mg/kg on a dry weight basis as described in the swine study. Briefly, following pre-treatment of soils, an appropriate portion of the sample was spiked with 1% (*v*/*w*) B[*a*]P stock solution (5000 mg/L) prepared in a mix-solvent (toluene: acetone = 1:1, *v*/*v*). Additional 1% (*v*/*w*) acetone was used to rinse the glass storage vial three times to ensure complete transfer of the mass. Spiked samples were left in a fume hood for 24 h to allow the solvent to evaporate. Following this, each sample was homogenised again before being stored for ageing. Homogeneity of the spiked samples and the spike recovery were carefully examined by checking the concentrations of B[*a*]P in subsamples.

An exhaustive solvent extraction method, modified from US EPA method 3550, using a mixed solvent including a water-miscible solvent-acetone and a water-immiscible solvent-dichloromethane (DCM/Ace) at 1:1 ratio (*v*/*v*) was used to measure the sample concentrations. The extraction was facilitated by sonication in a water basin (40 kHz, 15 min twice) and was repeated three times for each sample. Specifically, 1.5 g soil or sand was mixed with 3 g anhydrous sodium sulphate using a stainless spatula and extracted three times with 10 mL of the mixed solvent extractant each time. The solvent extract was separated following centrifugation. Samples were vortexed in between extraction to maximum mixing. The combined extract was evaporated under gentle nitrogen gas flow, following which 5 mL acetonitrile was added to uptake the sample and about 2 mL aliquant was filtered through a 0.45 μm PTFE syringe and stored in an amber HPLC vial for analysis. Spike recovery in sand was >99% (99.7 ± 0.5%, n = 5) and in soil ranged from 85.2 ± 0.3% to 92.6 ± 4.8% (n = 3) using four contrasting soil samples (Duan et al., 2014).

After spiking, the soils were stored in glass jars and deionised water added to bring the moisture content to 60% of the specific water-holding capacity for each sample. Following this, samples were kept in darkness at room temperature (22 ± 3 °C) over the ageing period (90 days).

2.2. The experiment design

The aged soil samples were air-dried overnight and pulverised before being dosed to rats and swine at the same time. A single dose was given to each group of animals in triplicate. In total there were 12 sets of data used in the rat and swine model comparison, including

Table 1
Properties of the soils used in this study.
Modified from Duan et al. (2014).

Soil ID	TOC (%)	DOC (mg/L)	pH_w	EC (μS/cm)	CEC ($cmol_c$/kg)	Partical size fraction (%)			Surface area (m^2/g)	Mesopore volume (cm^3/g)	Pore size (Å)	PF[a] <6 nm (%)	FPAC[b] (%)
						Sand	Silt	Clay					
MTA	7.5	103.2	5.1	87	6.37	61.9	16.8	21.2	51.7	0.097	76.2	24.4	5.1
I	5.06	108.4	5.1	69.1	7.91	68.1	21.2	10.7	6.02	0.012	81.4	24.7	14.4
BDA	3.27	95.5	6	75.5	38.7	53.0	16.1	30.9	4.01	0.008	81.3	22.8	7.3
GTA	2.88	80.4	6.3	144	31.8	49.4	26.9	23.7	7.12	0.013	71.1	23.9	6.3
TXA	0.96	21.8	6.2	36.9	6.4	80.6	11.2	8.1	15.0	0.020	54.2	39.9	17.6
N	1.71	47.7	7.1	402	9.44	87.6	6.7	5.7	7.9	0.008	39.4	51.0	20.1
GIA	0.78	18.2	5.9	53.6	4.73	78.1	16.2	5.6	9.91	0.012	49.0	46.7	27.9
GIB	0.72	8.5	7.8	192	11.1	65.2	14.3	20.5	35.3	0.041	46.2	49.9	48.3

[a] Pore volume proportion for average pore width <6 nm.
[b] FPAC (fine particle associated carbon), estimated by (silt + clay) / TOC.