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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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.

Key words: Benzo[a]pyrene, oral bioavailability, interspecies extrapolation, rat, swine, soil

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 (U.S. 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 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 (U.S.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; Goon et al. 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; James et al. 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.

Materials and methods

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.

Insert 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 ºC) over the ageing period (90 days).

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 eight soil samples after 90 days of ageing (D90) and four soil samples selected due to contrasting soil properties dosed at 50 days of ageing (D50) as well to test the effect of ageing.

Before testing bioavailability in soils, we performed a dose-response study using silica sand (Sigma-Aldrich Pty Ltd, Sydney, Australia) as a reference material in both the rat and swine models, with the silica sand spiked as described for soils.

Rat bioavailability assay

This study was approved by the Animal Ethics Committee of the South Australian Health and Medical Research Institute (SAHMRI) (AEC approval number 47/12). Animal care and surgical procedures complied with both the Standard Operating Procedures of the Veterinary Services Division, Institute of Medical and Veterinary Science and the Australian code of practice for the care and use of animals for scientific purposes (NHMRC 2013). Prior to being used in experiment, Male Sprague-Dawley rats (300 ± 20 g, from Animal Resource Centre, WA, Australia) were acclimatised for about one week to reach 350 ± 50 g body weight (bw). They were housed in plastic boxes in groups of two in a room at 22 ± 3ºC, 50% humidity, and a 12/12 h light/dark cycle, with standard rodent lab feed (Specialty Feeds, Glen Forrest, Australia) and water provided *ad libitum*. Prior to treatment the animals were housed individually and fasted for 16 h. Constrain to food access was maintained until 2 h post dosing.

In the experiment, soil/sand sample was suspended in a food thickener paste (at 8%, Karicare food thickener, mainly containing maltodextrin, starch from maize, carob, bean gum) and administered as slurry by gavage using a 14G animal feeding needle (Able Scientific, Australia). The dose rate was 2 g/kg bw at 0.25 g soil/mL and 8 mL/kg bw. Equivalent dose (100 µg/kg bw) of B[a]P was administered by intravenous (IV) injection through the tail vein at an injection volume of 2 mL/kg bw in an ethanol : fresh clean rat plasma at a ratio of 1: 4 (v/v) modified from previous studies (Pu et al. 2004; Weyand and Bevan 1986).

The dose remaining in the syringe and gavage needle was rinsed three times with water, ethanol and water again into the dose storage tube and estimated by determining the mass dry weight using a filter paper. On average, 8.9 ± 1.7% (n = 18) of the dose was un-dosed for sand and for soils this ranged from 7.0 ± 0.2% to 12.5 ± 1.8% (n = 3), on average at 8.4 ± 1.4%. These adjustments were made in rats in order to compare BA with that in swine where dosing was complete.

Serial blood samples (~0.25 mL) were collected from tail veins in heparinised tubes at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8 and 24 h following oral administration of the spiked soil or sand. For IV dosing, additional samples at 5 min and 10 min were collected. An indwelling IV catheter was used for the first 4 h of blood collection while the remaining time points of samples were collected by tail vein bleeding using needle sticks. Background samples were taken from control rats in the same batch. Plasma was separated immediately by centrifugation at 1037 g for 15 min and about 0.12 mL aliquot of sample was taken and stored in an amber glass vial (4 mL) with PTFE-lined cap at -20°C until extraction.

Extraction of B[a]P from plasma was carried out as described in the swine study (Duan et al. 2014) with a slight modification, wherein 1.5 mL hexane instead of three times the sample volume was added to each vial and subjected to sonication (40 kHz, 5 mins) twice. Spike recovery in clean plasma at three concentrations (0.25, 1.25 and 6.25 µg/L) indicated that average spike recovery ranged from 84.5% to 91.3% with a standard deviation of < 10%.

Rat faeces samples were collected for each individual in the first 12 h post-oral dosing or IV injection and then every 24 h until after 72 h. Before extraction faeces samples were stored at -20°C. A preliminary study showed after 72 h post-dosing further excretion was < 5% for both soil and sand (Supplemental Material Figure S-1). All rats were sacrificed by cervical dislocation by the end of the 72 h sampling period.

Faecal excretion of B[a]P was estimated by the DCM/Ace extraction method used for soil extraction. The only difference was homogenisation with anhydrous sodium sulphate (about

three times the volume of the faeces) was carried out in a blender after thawing the faeces from -20˚C to room temperature.

In total, 18 rats were used for the dose-responses relationship of B[a]P in the reference material (silica sand coated with B[a]P). Initially, eight rats in four groups of two were given doses at 20 μg/kg bw, 40 μg/kg bw, 60 μg/kg bw and 100 μg/kg bw in sand. This was repeated at the end of the study, with two each at the two lower doses and three each at the higher doses subjected to larger variability. One group of rats (n = 3) was used for the IV dose to calculate the absolute bioavailability. Twelve groups of rats (n = 3) were used to test soil samples aged for different times.

Quantification of B[a]P was carried out using an Agilent 1100 Series HPLC system coupled with a diode array detector (HPLC-DAD) at a wavelength of 267 nm for soil and faeces samples, and a fluorescence detector (HPLC-FLD), with an excitation wave length at 297 nm and emission wavelength at 405 nm, for the plasma samples. An Eclipse PAH reverse-phase C18 column (1.8 μm particle size, 4.6 μm inner diameter and 50 mm length) coupled with an XDB-C18 guard column was used for analysis. The column was maintained at 25 °C on both sides using a column heater. Isocratic elution was performed at a flow rate of 1.0 mL/min using the mobile phase of acetonitrile: water = 90:10. Each sample run time was 5 min with a 1 min post run before injecting the next sample. Needles were rinsed after each sample. The retention time for B[a]P was 3.6 min.

Bioavailability of B[a]P

Two types of bioavailability measurements are frequently used in pollutant biota investigations and risk assessment studies; namely, absolute bioavailability (AB) and relative bioavailability (RB). AB is defined as the fraction of a dosed amount reaching the systemic circulation after oral ingestion, while RB is the comparative bioavailability of a specific chemical for different exposure media given by the same route (Ng et al. 2013). Most frequently, the time course absorption by the area under the plasma concentration-time curve (AUC) is used to estimate bioavailability. AB is typically calculated by the AUC of a dose from oral ingestion compared with that from an IV injection (Equation 1), while the RB of a chemical is compared in the environmental material (e.g. soil) to a standard reference material. In this study, silica sand served as the reference material and RB was calculated using Equation 2:

$$AB = \frac{AUC_{oral}/dose_{oral}}{AUC_{IV}/dose_{IV}}$$ **Equation 1**

$$RB = \frac{AUC_{soil}/dose_{soil}}{AUC_{sand}/dose_{sand}}$$ **Equation 2**

AUC for IV injection (AUC_{IV}) was estimated by a one compartment exponential model:

$$C_t = b + C_0 \times e^{-kt}$$

Where C_t is the concentration of B[a]P in the plasma at time *t*, C_0 is the concentration of B[a]P in the plasma immediately following IV administration (t = 0), *b* is the background concentration, and *k* is the first-order elimination rate constant. AUC equals the integration of $C_0 \times e^{-kt}$, which is C_0/k.

AUC for oral doses (AUC_{sand} and AUC_{soil}) was estimated by a mathematical model based on gamma distribution ($g(t;\alpha,\beta) = 1$) previously described in (Duan et al. 2014):

$$C_t = b + a \times g(\mathrm{t}, \alpha, \beta)$$

Where C_t is the concentration of B[a]P in the plasma at time *t*, *b* is background concentration and AUC equals *a* as integration of $g(t;\alpha,\beta) = 1$.

Integration of AUC terminates when C_t fell to ± 10 % of the back ground concentration (b).

Bioavailability was also calculated based on faecal excretion (BA) as shown in Equation 3, given this portion was not bioavailable (Juhasz et al. 2014).

$$BA = \frac{dosed\ amount - excreted\ amount}{dosed\ amount}$$ **Equation 3**

In this study the dosed amount was 100 µg B[a]P/kg bw for all soils and the faecal excretion of B[a]P was the amount of B[a]P in faeces estimated by DCM/Ace extraction.

The bioavailability between the two animal models was compared using the relative bioavailability (Equation 2). As an absolute value, BA calculated from rat faecal excretion (Equation 3) was compared with AB calculated from AUC.

Implications of RB in soil guideline derivation

RB could be used to adjust exposure of soil-borne contaminants. The cancer risk (CR) as shown in Equation 4 is associated with a maximum daily intake (DI) or could be referred to as a RfD and the Cancer Slope Factor (CSF) for the contaminant(s) (U.S. EPA 2007):

$$CR = DI \times CSF \qquad \textbf{Equation 4}$$

Both the RfD and CSF were derived from critical toxicity study based on animal studies. RB as a measure of internal dose compared to the reference material can be used to adjust RfD. Therefore, a modified soil guideline value (*S*) could be estimated as follows:

$$\boldsymbol{S = \frac{DI \times \omega \times bw}{daily\ soil\ consumption \times RB}} \qquad \textbf{Equation 5}$$

In which ω is allocation from soil contributing to all pathways and *bw* is body weight. It should be noted that for different animals, the CSF may differ depending on the dose-effect responses. For PAHs, however, a lack of interspecies studies means that this is not well understood.

Results

Dose-response for reference material using different bioassays in the rat model

Time-course B[a]P plasma concentration profile of IV and oral doses in sand

Figure 1 illustrates the plasma B[a]P concentration-time profile following IV and oral dosing. After IV injection the plasma B[a]P concentration indicated an exponential decline over time, decreasing rapidly within two hours to < 2 µg/L followed by a slow decrease and reaching a background of 0.09 ± 0.01 µg/L after 6 h (Figure 1a). Following oral dosing with sand, the plasma B[a]P concentration revealed a biphasic process including an initially rapid increase, reaching a maximum concentration within 1 h, then rapidly decreasing within 2 h, finally reaching a range ± 10% of the background concentration after 6 h (Figure 1b).

Insert Figure 1

Faecal excretion of B[a]P following IV injection and oral doses in sand

A small portion (0.2 ± 0.1%) of the dose was found in faeces following IV injection (Table 2). The negligible amount of B[a]P excreted in faeces followed by IV dose suggests that partition from blood to organ and excretion through bile was negligible at the study dosage of 100

μg/kg bw. This confirms that the excreted fraction of B[a]P following oral dosing in the present study did not go through hepatic circulation, which infers that this fraction is not bioavailable. As shown in Table 1, a significant amount (14.7 ± 4.8%) of the dosed B[a]P was excreted in faeces following oral dosing with sand at the dose rate ranging from 20 μg/kg bw to 100 μg/kg bw.

Insert Table 2

AUC of B[a]P following IV injection and oral doses in sand

The AUC (responses in the rat plasma) was found to increase linearly with the B[a]P dosage in sand, with AUC = 0.033 dose − 0.50; R^2 = 0.98, $p < 0.001$ (Table 2). AB was consistent over the dose range between 20 μg/kg bw to 100 μg/kg bw, averaged at 15.1 ± 5.1%. Similarly, response in faecal excretion of B[a]P was consistent over the dose range, averaged at 14.7 ± 4.8%. As only a small portion (0.2 ± 0.1%) of the IV dose was detected in faeces at the dosage of 100 μg/kg bw, BA of B[a]P in sand would be 85.3% on average. This value is significantly (~ 6 times) higher than AB calculated using AUC ($p < 0.001$), suggesting contrasting results would be derived when using different assays in the animal study.

Bioavailability of B[a]P in soils- rat compared to swine

Table 2 summarises the bioavailability results using the rat model, including: the relative bioavailability estimated by rat (RB_{rat}); the bioavailability (BA) calculated by the rat faecal excretion; the relative bioavailability of B[a]P in swine (RB_{swine}); and B[a]P extractability estimated by two solvent extraction methods using DCM/Ace and BuOH, which showed significant correlation with RB_{swine}.

Insert Table 2

It is apparent that extractability of B[a]P in soils after ageing decreased dramatically and ranged from 12.2 % to 62.2 % for DCM/Ace extraction and 9.7 % to 58.1 % for BuOH extraction, respectively. Faecal excretion of B[a]P following oral dosing of soils was

generally low, which resulted in high BA for all soils (> 88 %). Both RB_{rat} and RB_{swine} were < 100%, with RB_{rat} significantly lower than RB_{swine} ($p < 0.001$).

The rat faecal excretion assay

Faecal excretion of B[a]P following oral dosing of all aged soils (from 0.7 ~ 10.6 %) was even lower than B[a]P excreted following oral dosing of sand (averaged at 14.7 ± 4.8%, Table 1). This suggests that the direct calculation of BA using equation 3 would result in higher absorption from aged soils than from sand. This is mainly due to the formation of a non-extractable fraction during ageing, which is evidenced by the decrease in extractability after ageing (DCM/Ace). In fact, a parallel study using ^{14}C-B[a]P in four contrasting soils showed significant decrease in B[a]P extractability over a 160-day period using the exhaustive DCM/Ace extraction method (extractability < 50 %). However, a complete sample oxidation method indicated more than 77% ^{14}C-radioactivity was still present in the soils (Duan et al. 2015).

Our results indicate that bioavailability (BA) using the faecal excretion assay significantly overestimates the B[a]P bioavailability (RB_{rat}) using AUC.

The AUC assay

Comparison of RB_{rat} and RB_{swine} showed a strongly significant correlation between the two animal models ($RB_{rat} = 0.26\ RB_{swine} + 17.3$, n = 12, $R^2 = 0.70$, $p < 0.001$, Figure 2), despite the large variance among the individuals within each group.

Insert Figure 2

The effects of ageing on the correlation of RB between the two animal models was observed by estimating the correlations (R^2) of four contrasting soils at D50 and D90. The correlations (R^2) between RB_{rat} and RB_{swine} decreased dramatically after ageing, dropping from 0.95 at D50 to 0.62 at D90, respectively (Figure 3). Additionally the slope coefficient of the correlation decreased slightly after longer ageing time, from 0.40 at D50 to 0.26 at D90, suggesting that the decrease in RB over ageing was more dramatic in the swine model compared to that in the rat model. In other words, the swine model is more sensitive to the change in RB in regard to ageing. It is also worth noting that the effect of ageing on RB was

not significant in rats while at least for one highly clayey soil, BDA, in swine the ageing effect was significant (Table 2).

Similar to that in the swine model, the influence of simple soil properties was not significant in RB_{rat} (Supplemental Material, Figure S-2). Nevertheless, the strong significant relationships between the two complex soil properties identified in the swine study and RB_{swine} – namely: 1) fine particle associated organic carbon (FPAC) defined as (Silt + Clay)/TOC; and 2) proportion of < 6 nm pore size with two outlier soils excluded – was found significant only for one (FPAC) in rats (Supplemental Material, Figure S-3). Also, significant correlation between B[a]P extractability using DCM/Ace and BuOH and RB_{swine} was not found for rats (Supplemental Material, Figure S-4). This is mainly due to the lower RB in the rat model which consequently reduced the difference amongst samples. However, it is difficult to further improve the accuracy of RB/AUC in rat as it was limited by the small volume of blood sample that could be drawn from each individual over the required sampling period.

Discussion

During the last ten years there has been a significant shift towards using chemical bioavailability in contaminated soils to estimate the risks posed to human health. A tiered approach was used. Where total concentration is exceeded, conventional extraction (*in vitro*) methods mimicking bioavailability processes may be applied to modify the guideline value. However, the challenge has been to validate these methods against an *in vivo* animal model where rodents have been frequently used. This is particularly the case where inter-species extrapolation to human/large safety factor for relevant uncertainties is applied to protect human daily exposure. Human and rodent are quite different in terms of body size, gut physiology (anatomy) and genetic profile which potentially influences the metabolic rate relevant to certain enzyme activities. Swine has been recognised as a better model for human for the same reason mentioned above. Comparison of bioavailability data from the rodent model and swine model is likely to reduce any uncertainty in the interspecies extrapolation to human.

Bioavailability of an ingested compound has been described as consisting of three processes (Oomen et al. 2006): 1) release from the dose matrix; 2) transport across the intestinal

384 epithelium; and 3) reaching systemic circulation without being metabolised as shown in
385 Equation 4.

386 $$F = F_b \times F_a \times F_h$$ **Equation 6**

387 where F is the bioavailable fraction of the oral dose; F_b is the fraction of an external dose that
388 could be released from soil (referred to as bioaccessibility); F_a is the fraction of F_b that could
389 be transported across the intestinal epithelium; and F_h is the unmetabolised fraction of F_a that
390 finally reaches systemic circulation.

391

392 Several bioassays have been used in bioavailability studies, and besides blood/plasma
393 concentration and excretion in faeces, the most frequently used bioassay was excretion of
394 metabolites in urine. However, due to the large variability in metabolism rate among
395 individuals as well as the unstable nature of PAH metabolites, an accurate dose-responses
396 relationship which can be used for comparison of bioavailability based on PAH metabolites
397 has not yet been established, especially at low doses relevant to daily exposure. For long-term
398 studies tissue concentration may be used. However, not many such experiments have been
399 carried out for organic contaminants.

400 In the present study, the plasma B[a]P concentration-time profile was based on the parental
401 compound (unmetabolised), and the interspecies comparison between rat and swine models
402 was made using a relative bioavailability data compared to the same reference material. The
403 time-course plasma B[a]P concentration observed in our study is most similar to a previous
404 rat study ((Foth et al. 1988) where the published data was reviewed and figure was redrawn in
405 Crowell et al. (2011) and similar low doses of B[a]P were dosed in peanut oil. However, in
406 another rat study where a higher dose at 100 mg/kg bw was given, two peaks in the blood
407 concentration occurred, the first peak at around 2 h being much smaller than the second peak
408 at around 8 h post-dosing. It was suggested that the second peak relates to hepatic circulation
409 through bile excretion at high doses. This highlighted the importance of measuring
410 bioavailability at an environmentally relevant concentration and thus different studies' results
411 may not be appropriate, depending on the dose range used especially if the dose-responses
412 curve was significantly nonlinear. In the dose range (20 ~ 100 μg B[a]P/kg bw) the effect of
413 hepatic circulation was not obvious (no clear second peak) and the dose-response (AUC) was
414 almost linear (Figure 4). A linear dose-response correlation was found in the swine study at a
415 similar dose range as well (Figure 4).

416

417 Insert Figure 4

418 It is notable that the ratio of AUC in rat was approximately 4 times higher than that in swine
419 for sand over the dosing range (Figure 4). Meanwhile the correlation between RB_{rat} and
420 RB_{swine} (Figure 2) showed RB_{rat} is about a quarter of RB_{swine}. The plasma B[a]P profile in rats
421 is similar to that observed in the swine model despite the actual concentration being much
422 lower in swine and the peaking concentration occurring slightly earlier in rats, at 0.80 ± 0.29 h
423 in rat and at 0.99 ± 0.15 h in swine, respectively. The rapid absorption and removal of B[a]P
424 in plasma is consistent with the highly lipophilic nature of B[a]P (log Kow ~ 6.1) and the
425 rapid biotransformation. A peaking concentration of B[a]P in blood (serum) at 2 h post-dosing
426 was observed in another swine study using PAH contaminated soils (James et al. 2011). The
427 slight difference may be due to the swine being fed a small serving (5g) of dough, instead of
428 the full meal provided in our swine study (Duan et al. 2014). In another swine study where
429 ^{14}C labelled B[a]P was dosed in milk to pigs and total radioactivity in blood was measured
430 over time, a peaking radioactivity at 6 h following oral dosing was observed (Laurent et al.
431 2001). Employing a radiolabelled compound is a good approach for estimating total
432 absorption including the metabolised fractions, however, this was not possible for our swine
433 study due to the high cost of handling radioactive waste. With the linear dose-response
434 relationship using the parent compound (B[a]P) observed in both the two animal models, we
435 think it is prudent to use AUC of the parental compound to represent absorption within each
436 animal model and RB can be compared between the two models.

437

438 The presence of a slightly faster peaking concentration of B[a]P in plasma is most likely due
439 to the higher fundamental metabolic rate in the smaller animal (Kleiber 1947) and possibly
440 has been influenced by the different food constituents dosed along with soil/sand. The lower
441 B[a]P concentration in plasma in swine may either be due to a lower absorption including
442 partitioning from gastrointestinal organ to blood or higher metabolic rate specific for
443 biotransformation of the parent compound. Actually, partition from organ to blood has been
444 reported to be half in humans compared to that in rats (Crowell et al. 2011), and this may
445 probably apply to swine when compared with rat.

446 Correlation between the RB_{rat} and RB_{swine} ($RB_{rat} = 0.26RB_{swine} + 17.3$, $R^2 = 0.70$, $p < 0.001$)
447 suggested bioavailability may be underestimated if RB derived from the rat model was used
448 for soil guideline derivation directly. However, the reality is an interspecies difference
449 uncertainty factor is already incorporated in the guideline derivation. The US EPA. (2011) set
450 up a default adjusting factor of 10 for the deviation of an equivalent dose for human (RfD_H)

from an animal study while a body weight scaling method using $bw^{3/4}$ which was recommended when extrapolating data from different animal models and a rounded uncertainty factor of 3 accounting for pharmacodynamics differences. The body weight scaling factor was approximately 3-fold from rat (0.35 kg) to swine (32 kg) and 1.2-fold from swine to human (70 kg). Our comparative study showed a good consistency in the RB in the aged soils between the two animal models and the difference between rat and swine was about 4 which is close to the body weight scaling method. Further studies may be required to investigate the carcinogenic competency (pharmacodynamics) of contaminants for the reference material. A freshly spiked silica sand was used in both rat and swine. It is recommended in the future that analyses link the toxicity of this material to that used by Culp et al. (1998).

It is difficult to remove the uncertainties in the interspecies extrapolation unless human epidemic data can be generated. However, our data from the rat and swine models supported the body weight scaling method which was recommended by the US EPA where uncertainty in the pharmacokinetic component is reduced. The difference in the carcinogenic competency between rat and swine will require a long-term carcinogenic analysis where carcinogenic endpoints can be determined. Alternatively, it would be advantageous if a conservative guideline for the plasma B[a]P assay can be recommended for screening exposure, just like the case of lead, where blood lead concentration was adopted.

Conclusion

Comparing RB of B[a]P between the rat and swine models in this study established a link between the two animal models for the first time. Although the results derived from the rat model were not as sensitive to the changes over ageing as well as to the influences of soil properties compared to that derived from the swine model, it accounts for about 70% of the variability in the swine study results. These findings have important implications for reducing uncertainties in the interspecies extrapolation from experiment animals to human with reference to human health risk assessment. Further research on the cancer competency of B[a]P for different animal models and the applicability for PAH mixtures is required.

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