

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

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21 **Background:** There are many uncertainties concerning variations in benzo[a]pyrene (B[a]P)  
22 soil guidelines protecting human health based on carcinogenic data obtained in animal studies.  
23 Although swine is recognised as being much more representative of the human child in terms  
24 of body size, gut physiology and genetic profile the rat/mice model is commonly used in  
25 practice.

26 **Objectives:** We compare B[a]P bioavailability using a rat model to that estimated in a swine  
27 model, to investigate the correlation between these two animal models. This may help reduce  
28 uncertainty in applying bioavailability to human health risk assessment.

29 **Methods:** Twelve spiked soil samples and a spiked silica sand (reference material) were  
30 dosed to rats in parallel with a swine study. B[a]P bioavailability was estimated by the area  
31 under the plasma B[a]P concentration-time curve (AUC) and faecal excretion as well in the  
32 rats. Direct comparison between the two animal models was made for: firstly, relative  
33 bioavailability (RB) using AUC assay; and secondly, the two assays in the rat model.

34 **Results:** Both AUC and faecal excretion assays showed linear dose-response for the reference  
35 material. However, absolute bioavailability was significantly higher when using faecal  
36 excretion assay ( $p < 0.001$ ). In aged soils faecal excretion estimated based on solvent  
37 extraction was not accurate due to the form of non-extractable fraction through ageing. A  
38 significant correlation existed between the two models using RB for soil samples ( $RB_{\text{rat}} =$   
39  $0.26RB_{\text{swine}} + 17.3$ ,  $R^2 = 0.70$ ,  $p < 0.001$ ), despite the regression slope coefficient revealing  
40 that the rat model would underestimate RB by about one quarter compared to using swine.

41 **Conclusions:** In the comparison employed in this study, an interspecies difference of four in  
42 RB using AUC assay was identified between the rat and swine models regarding  
43 pharmacokinetic differences, which supported the body weight scaling method recommended  
44 by US EPA. Future research should focus on the carcinogenic competency  
45 (pharmacodynamics) used in experiment animals and humans.

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

## 47 **Introduction**

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

58

59 Given the lack of human epidemiological studies, the current soil guidelines for B[a]P and  
60 PAHs in Australia and many other countries are based on carcinogenicity in rodent (Brune et  
61 al. 1981; Culp et al. 1998; Neal and Rigdon 1967). Typically, a benchmark dose (BMD) that  
62 gives rise to a 10% response ( $BMD_{10}$ ) derived from fitting of dose-response data is used as a  
63 point of departure (PoD). For B[a]P, a lower confidence limit of  $BMD_{10}$  ( $BMDL_{10}$ ) of 0.1  
64 mg/kg body weight per day was used to calculate the risk of PAHs in food (MfE 2011). From  
65 this critical toxicological value in animal studies large safety factors were applied to address  
66 uncertainties in extrapolating them to humans (Safety 2014). More detailed information about  
67 the uncertainties associated with extrapolation has been documented in Dong et al. (2015).  
68 Briefly, a margin of exposure (MoE) approach of 1/10,000 was applied in Europe (HPA  
69 2010), in which a modifying factor of 10 was employed to account for the interspecies  
70 differences between mice and humans. The US EPA used the same default factor accounting  
71 for the interspecies differences but also recommends using a body weight (bw) scaling factor  
72 and a rounded uncertainty factor of 3 when considering the results of different animal models  
73 (U.S.EPA 2011). An interspecies uncertainty factor of 5 was adopted in a study developing  
74 soil guideline in Australia, where a guideline value of 5 mg/kg for B[a]P was derived  
75 (Fitzgerald et al. 2004). This value is very close to the current national soil guideline (4 mg/kg)  
76 for residential land use in Australia (NEPC 2013).

77

78 Besides the uncertainty over interspecies differences, exposure from ingestion of  
79 contaminated soil does not delineate between the fraction that subsequently absorbs

80 (bioavailable fraction) and the total concentration. Such an approach is likely to result in  
81 overestimation of risk and as a consequence remediation of sites that could potentially be safe.  
82 In the latest National Environmental Protection Measure of Australia, using site-specific oral  
83 bioavailability data of contaminants has been encouraged when available (NEPC 2013).  
84 Bioavailability is defined as an internal estimation of the actual uptake or absorption of  
85 contaminants that enters the body (internal dose), and therefore provides a better estimation of  
86 the risk. Significantly reduced bioavailability of some PAH(s) in soil has been reported using  
87 animal models including goat and rat in comparison to dose in solution (Goon et al. 1990;  
88 Goon et al. 1991) or oil feed (Ounnas et al. 2009; Pu et al. 2004; Van Schooten et al. 1997).  
89 However, there is considerable uncertainty regarding the utilisation of oil as a reference  
90 material in these studies given its lack of relevance to environmental exposure, and therefore  
91 the implication of these results being used in modifying current soil guidelines. Also,  
92 compared to rodents, swine are preferred for human health risk assessment as they share many  
93 similar traits to humans, such as body weight, anatomy, genetics and physiology (Ng et al.  
94 2013; Walters and Prather 2012). However, conduct swine study is much more expensive  
95 compared to using rat. As a consequence, to date only a handful of animal studies have used  
96 swine to estimate PAH bioavailability in soils (Duan et al. 2014; James et al. 2011; James et  
97 al. 2016; Peters et al. 2015).

98

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

## 109 **Materials and methods**

### 110 **Soils**

111 Eight soils varying in soil properties including organic matter (TOC: 0.72 ~ 7.5%; DOC: 8.5  
112 ~ 108.4 mg/L), clay content (5.6% ~ 30.9%), pH, EC, CEC (and clay mineralogy), and  
113 texture, etc., were employed in this study. Detailed soil properties are presented in Table 1.

114 Insert Table 1

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

124 An exhaustive solvent extraction method, modified from US EPA method 3550, using a  
125 mixed solvent including a water-miscible solvent-acetone and a water-immiscible solvent-  
126 dichloromethane (DCM/Ace) at 1:1 ratio (v/v) was used to measure the sample  
127 concentrations. The extraction was facilitated by sonication in a water basin (40 kHz, 15 min  
128 twice) and was repeated three times for each sample. Specifically, 1.5 g soil or sand was  
129 mixed with 3 g anhydrous sodium sulphate using a stainless spatula and extracted three times  
130 with 10 mL of the mixed solvent extractant each time. The solvent extract was separated  
131 following centrifugation. Samples were vortexed in between extraction to maximum mixing.  
132 The combined extract was evaporated under gentle nitrogen gas flow, following which 5 mL  
133 acetonitrile was added to uptake the sample and about 2 mL aliquant was filtered through a  
134 0.45  $\mu\text{m}$  PTFE syringe and stored in an amber HPLC vial for analysis. Spike recovery in sand  
135 was > 99% ( $99.7 \pm 0.5\%$ ,  $n = 5$ ) and in soil ranged from  $85.2 \pm 0.3\%$  to  $92.6 \pm 4.8\%$  ( $n = 3$ )  
136 using four contrasting soil samples (Duan et al. 2014).

137 After spiking, the soils were stored in glass jars and deionised water added to bring the  
138 moisture content to 60% of the specific water-holding capacity for each sample. Following

139 this, samples were kept in darkness at room temperature ( $22 \pm 3$  °C) over the ageing period  
140 (90 days).

### 141 **The experiment design**

142 The aged soil samples were air-dried overnight and pulverised before being dosed to rats and  
143 swine at the same time. A single dose was given to each group of animals in triplicate. In total  
144 there were 12 sets of data used in the rat and swine model comparison, including eight soil  
145 samples after 90 days of ageing (D90) and four soil samples selected due to contrasting soil  
146 properties dosed at 50 days of ageing (D50) as well to test the effect of ageing.

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

### 150 **Rat bioavailability assay**

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

163

164 In the experiment, soil/sand sample was suspended in a food thickener paste (at 8%, Karicare  
165 food thickener, mainly containing maltodextrin, starch from maize, carob, bean gum) and  
166 administered as slurry by gavage using a 14G animal feeding needle (Able Scientific,  
167 Australia). The dose rate was 2 g/kg bw at 0.25 g soil/mL and 8 mL/kg bw. Equivalent dose  
168 ( $100 \mu\text{g/kg}$  bw) of B[a]P was administered by intravenous (IV) injection through the tail vein  
169 at an injection volume of 2 mL/kg bw in an ethanol : fresh clean rat plasma at a ratio of 1: 4  
170 (v/v) modified from previous studies (Pu et al. 2004; Weyand and Bevan 1986).

171

172 The dose remaining in the syringe and gavage needle was rinsed three times with water,  
173 ethanol and water again into the dose storage tube and estimated by determining the mass dry  
174 weight using a filter paper. On average,  $8.9 \pm 1.7\%$  ( $n = 18$ ) of the dose was un-dosed for  
175 sand and for soils this ranged from  $7.0 \pm 0.2\%$  to  $12.5 \pm 1.8\%$  ( $n = 3$ ), on average at  $8.4 \pm$   
176  $1.4\%$ . These adjustments were made in rats in order to compare BA with that in swine where  
177 dosing was complete.

178

179 Serial blood samples (~0.25 mL) were collected from tail veins in heparinised tubes at 0.25,  
180 0.5, 1, 1.5, 2, 4, 6, 8 and 24 h following oral administration of the spiked soil or sand. For IV  
181 dosing, additional samples at 5 min and 10 min were collected. An indwelling IV catheter was  
182 used for the first 4 h of blood collection while the remaining time points of samples were  
183 collected by tail vein bleeding using needle sticks. Background samples were taken from  
184 control rats in the same batch. Plasma was separated immediately by centrifugation at 1037 g  
185 for 15 min and about 0.12 mL aliquot of sample was taken and stored in an amber glass vial  
186 (4 mL) with PTFE-lined cap at  $-20^{\circ}\text{C}$  until extraction.

187

188 Extraction of B[a]P from plasma was carried out as described in the swine study (Duan et al.  
189 2014) with a slight modification, wherein 1.5 mL hexane instead of three times the sample  
190 volume was added to each vial and subjected to sonication (40 kHz, 5 mins) twice. Spike  
191 recovery in clean plasma at three concentrations (0.25, 1.25 and 6.25  $\mu\text{g/L}$ ) indicated that  
192 average spike recovery ranged from 84.5% to 91.3% with a standard deviation of  $< 10\%$ .

193

194 Rat faeces samples were collected for each individual in the first 12 h post-oral dosing or IV  
195 injection and then every 24 h until after 72 h. Before extraction faeces samples were stored at  
196  $-20^{\circ}\text{C}$ . A preliminary study showed after 72 h post-dosing further excretion was  $< 5\%$  for  
197 both soil and sand (Supplemental Material Figure S-1). All rats were sacrificed by cervical  
198 dislocation by the end of the 72 h sampling period.

199

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

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

204

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

212

213 Quantification of B[a]P was carried out using an Agilent 1100 Series HPLC system coupled  
214 with a diode array detector (HPLC-DAD) at a wavelength of 267 nm for soil and faeces  
215 samples, and a fluorescence detector (HPLC-FLD), with an excitation wave length at 297 nm  
216 and emission wavelength at 405 nm, for the plasma samples. An Eclipse PAH reverse-phase  
217 C18 column (1.8 µm particle size, 4.6 µm inner diameter and 50 mm length) coupled with an  
218 XDB-C18 guard column was used for analysis. The column was maintained at 25 °C on both  
219 sides using a column heater. Isocratic elution was performed at a flow rate of 1.0 mL/min  
220 using the mobile phase of acetonitrile: water = 90:10. Each sample run time was 5 min with a  
221 1 min post run before injecting the next sample. Needles were rinsed after each sample. The  
222 retention time for B[a]P was 3.6 min.

### 223 **Bioavailability of B[a]P**

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



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

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

237

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

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

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

243

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

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

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

248 Integration of AUC terminates when  $C_t$  fell to  $\pm 10\%$  of the back ground concentration ( $b$ ).

249

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

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

253

254 In this study the dosed amount was 100  $\mu\text{g}$  B[a]P/kg bw for all soils and the faecal excretion  
255 of B[a]P was the amount of B[a]P in faeces estimated by DCM/Ace extraction.

256

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

260

261 **Implications of RB in soil guideline derivation**

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

$$265 \quad CR = DI \times CSF \quad \text{Equation 4}$$

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

$$269 \quad S = \frac{DI \times \omega \times bw}{\text{daily soil consumption} \times RB} \quad \text{Equation 5}$$

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

## 274 **Results**

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

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

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

284

285 Insert Figure 1

286

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

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

291  $\mu\text{g}/\text{kg}$  bw. This confirms that the excreted fraction of B[a]P following oral dosing in the  
292 present study did not go through hepatic circulation, which infers that this fraction is not  
293 bioavailable. As shown in Table 1, a significant amount ( $14.7 \pm 4.8\%$ ) of the dosed B[a]P was  
294 excreted in faeces following oral dosing with sand at the dose rate ranging from  $20 \mu\text{g}/\text{kg}$  bw  
295 to  $100 \mu\text{g}/\text{kg}$  bw.

296

297 Insert Table 2

298

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

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

308

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

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

315

316 Insert Table 2

317

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

321 generally low, which resulted in high BA for all soils (> 88 %). Both  $RB_{\text{rat}}$  and  $RB_{\text{swine}}$  were  
322 < 100%, with  $RB_{\text{rat}}$  significantly lower than  $RB_{\text{swine}}$  ( $p < 0.001$ ).

323

### 324 *The rat faecal excretion assay*

325 Faecal excretion of B[a]P following oral dosing of all aged soils (from 0.7 ~ 10.6 %) was  
326 even lower than B[a]P excreted following oral dosing of sand (averaged at  $14.7 \pm 4.8\%$ , Table  
327 1). This suggests that the direct calculation of BA using equation 3 would result in higher  
328 absorption from aged soils than from sand. This is mainly due to the formation of a non-  
329 extractable fraction during ageing, which is evidenced by the decrease in extractability after  
330 ageing (DCM/Ace). In fact, a parallel study using  $^{14}\text{C}$ -B[a]P in four contrasting soils showed  
331 significant decrease in B[a]P extractability over a 160-day period using the exhaustive  
332 DCM/Ace extraction method (extractability < 50 %). However, a complete sample oxidation  
333 method indicated more than 77%  $^{14}\text{C}$ -radioactivity was still present in the soils (Duan et al.  
334 2015).

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

### 337 *The AUC assay*

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

341

342 Insert Figure 2

343

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

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

354

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

## 367 **Discussion**

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

381

382 Bioavailability of an ingested compound has been described as consisting of three processes  
383 (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.

$$386 \quad F = F_b \times F_a \times F_h \quad \text{Equation 6}$$

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  $RB_{\text{rat}}$  and  
420  $RB_{\text{swine}}$  (Figure 2) showed  $RB_{\text{rat}}$  is about a quarter of  $RB_{\text{swine}}$ . 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  $0.80 \pm 0.29$  h  
423 in rat and at  $0.99 \pm 0.15$  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 ( $\log K_{ow} \sim 6.1$ ) 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  $^{14}\text{C}$  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  $RB_{\text{rat}}$  and  $RB_{\text{swine}}$  ( $RB_{\text{rat}} = 0.26RB_{\text{swine}} + 17.3$ ,  $R^2 = 0.70$ ,  $p < 0.001$ )  
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 ( $RfD_H$ )

451 from an animal study while a body weight scaling method using  $bw^{3/4}$  which was  
452 recommended when extrapolating data from different animal models and a rounded  
453 uncertainty factor of 3 accounting for pharmacodynamics differences. The body weight  
454 scaling factor was approximately 3-fold from rat (0.35 kg) to swine (32 kg) and 1.2-fold from  
455 swine to human (70 kg). Our comparative study showed a good consistency in the RB in the  
456 aged soils between the two animal models and the difference between rat and swine was about  
457 4 which is close to the body weight scaling method. Further studies may be required to  
458 investigate the carcinogenic competency (pharmacodynamics) of contaminants for the  
459 reference material. A freshly spiked silica sand was used in both rat and swine. It is  
460 recommended in the future that analyses link the toxicity of this material to that used by Culp  
461 et al. (1998).

462

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

## 471 **Conclusion**

472

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

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482



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