

1 Is received dose from ingested soil independent of soil PAH concentrations: animal model
2 results
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9 **Title Running Head**

10 PAH toxicokinetics in ingested soil

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19 **Abstract**

20 Human exposure to polycyclic aromatic hydrocarbons (PAHs) often occurs through the oral
21 route during hand to mouth transfer of contaminated soils. It is accepted that PAH bioavailability
22 from ingested soils will vary between soils, however, the nature of this variation is not well
23 characterized. Here, we used the juvenile swine model to link external exposure to internal
24 benzo[a]pyrene (BaP) and anthracene exposure following oral PAH ingestion of 27 different
25 real-world soils, soots, or spiked artificial soils. Internal exposure of BaP and anthracene,
26 represented by area under the plasma-time curve (AUC), did not correlate to soil concentration in
27 real-world soils. However, soil concentration correlated with internal exposure in spiked
28 artificial soil. Point of departure (POD) modeling identified soil PAH concentrations greater than
29 $1,900 \text{ mg kg}^{-1}$ as the concentration where internal exposures become proportional to external
30 doses. Alternatively, BaP and anthracene internal exposure below $1,900 \text{ mg kg}^{-1}$ averaged 21%
31 of external exposure but we could not detect a trend between internal and external exposure at
32 these low concentrations. Weak correlations between soil:simulated gastrointestinal fluid PAH
33 partitioning and AUC values indicate that desorption from soil does not play a large role in
34 influencing internal exposure of PAHs. We propose four PAH risk assessment options: (i)
35 assume 100% bioavailability, (ii) assume constant internal exposure below $1,900 \text{ mg kg}^{-1}$, (iii)
36 assume <100%, e.g. 21%, bioavailability below $1,900 \text{ mg kg}^{-1}$, or (iv) model internal exposure
37 through AUC versus soil characteristic relationships. In our opinion, our data best supports
38 option (ii) because we could not detect an increase in AUC with increasing soil concentrations
39 and our best efforts at (iv) do not robustly predict uptake of different PAHs.

40 **Keywords:** toxicokinetics, swine, PAHs, soil

41 **Introduction**

42 Human exposure to polycyclic aromatic hydrocarbons (PAHs) commonly occurs through
43 ingestion of impacted soil. The absorption and bioavailability of PAHs has been studied
44 extensively (see Ramesh et al. 2004 for a thorough review), and it is widely accepted that oral
45 bioavailability of PAHs can differ when present in different media. The observed bioavailability
46 of PAHs varies between different soil types (Stroo et al., 2000, Kadry et al., 1995, Juhasz et al.,
47 2014, James et al., 2011). These differences arise from contaminant weathering in soil, as well as
48 soil characteristics, which may include soil particle size or chemical partitioning (James et al.,
49 2011, Juhasz et al., 2014, Duan et al., 2014). However, rarely has a wide range of soils been fed
50 to a mammal and PAH bioavailability assessed. Here we fed 19 soils, 4 artificial soils and 4 soot
51 samples to juvenile swine. We used plasma concentrations of parent PAHs to calculate
52 bioavailability and elimination/absorption rates. Using a large data set is essential to
53 characterizing what occurs when mammals ingest PAHs.

54 Once ingested, PAHs transfer from the gastrointestinal tract to systemic circulation.
55 Transfer of PAHs into circulation occurs concurrent with lipids (Stavric and Klassen, 1994), and
56 it has been theorized that PAHs are transferred via chylomicron formation within enterocytes and
57 transferred into lymph, which would allow PAHs to bypass the liver and first pass elimination
58 (Hussain et al., 1996, Busbee et al., 1990). However, a study done in lymph and bile duct
59 cannulated rats determined that about 80% of absorbed PAHs transfer to circulation via hepatic
60 portal transport, rather than lymph (Laher et al., 1984). A more recent study confirms these
61 findings, concluding that approximately twice the PAHs entering into circulation cross through
62 hepatic portal transfer rather than through chylomicron formation and transport through the

63 lymphatic system (Kim et al., 2012). Thus, the majority of ingested PAHs enter the body via the
64 portal vein, to the liver and from there to systemic circulation

65 Rapid metabolism of PAHs can confound quantification of PAH uptake following oral
66 exposure. For example, the liver extensively metabolize PAHs (Ramesh et al., 2004). Analyzing
67 unlabeled metabolites in an organism is a very daunting task because PAHs are a family of
68 compounds, e.g. there are typically at least 9-16 PAHs of interest present in an impacted soil, and
69 each compound can convert into more than one metabolite (Ramesh et al., 2004). Using ¹⁴C
70 labeled compounds eliminates the complicated analysis necessary for unlabeled compounds;
71 however a number of factors make ¹⁴C analysis unfavorable for use in PAH bioavailability. First,
72 ¹⁴C labeled PAHs may overestimate risk to PAHs, as most absorbed PAHs metabolize to inert
73 metabolites that excrete quickly (Ramesh et al., 2004). Additionally, the use of ¹⁴C labeled
74 compounds is limited to spiked soil, and it would be difficult to compare results to those
75 obtained from naturally impacted soils.

76 Previously, systemic PAH metabolites were thought to be best estimate of PAH
77 bioavailability (Ramesh et al., 2004). These metabolites arise, in a large part, from liver mono-
78 oxygenase enzymes such as CYP 1A1, CYP 1A2, and CYP 1B1. It was initially assumed that
79 toxic metabolites form in the liver and transport in systemic circulation to cause peripheral
80 toxicity. However, animal studies using inbred mouse strains observed that circulating
81 metabolites do not cause of bone marrow and spleen toxicity (Legraverend et al., 1983, Uno et
82 al., 2004, Galvan et al., 2005). This is a reasonable observation, as toxic metabolites of PAHs
83 have epoxide groups present on the compound, and as such, are highly reactive and would not
84 travel far in circulation without reacting with epoxide hydrolase or a cellular component. These
85 results should not be taken to imply that the CYP family is unimportant for toxicity, but rather

86 that the first pass effect in which much of the ingested PAHs are metabolized as the portal vein
87 empties into the liver acts as a detoxification reaction. Thus, the assessment of parent PAHs in
88 the systematic circulation may be a better estimate of non-hepatic toxicity after oral exposure.

89 Animals, acting as surrogates for humans, are an excellent means to assess internal
90 exposure of PAHs. Swine have become a popular human exposure model, and have been
91 validated as a model for lead and arsenic (Casteel et al., 2006, Juhasz et al., 2008), as well as
92 gaining popularity for organic compounds like PAHs (Duan et al., 2014, James et al., 2011,
93 Peters et al., 2015). Swine are an alternative model to rodents due to the similarities between
94 swine and humans in gastrointestinal physiology and intestinal conformation, as well as the
95 cellular make-up of the organs (Patterson et al., 2008). Biochemically, swine AhR response to
96 agonists like PAHs manifests very similar in magnitude to that of humans (Lesca et al., 1994).

97 Assessing internal exposure of parent compounds in an animal that ingests contaminated
98 soils allows us to directly external to internal exposure of PAHs. It is widely assumed that
99 external and internal exposure will follow a linear trend. Bioavailability is the slope of internal
100 to external exposure. However, toxicologists, especially those concerned with mutagens, have
101 long recognized that their dose-response relationships are typically hockey-stick shaped. A
102 hockey-stick dose-response relationship comprises a linear and a sub-linear component, with
103 typically the sub-linear component occurring at lower doses. Thus, for example low doses of a
104 mutagen may cause no adverse effect until a break point is reached, at which point adverse
105 effects increase linearly with dose. Commonly, models such as a benchmark dose (BMD), or a
106 threshold dose (Td) model is used for such datasets(Gollapudi et al., 2013). Both Td and BMD
107 calculations utilize the entire dose-response data set and interpolate the data to derive a point of
108 departure where the response begins to differ significantly from the control. In other words, this

109 approach allows one to estimate two slopes in a biphasic relationship. It is exactly this type of
110 relationship, we observed in this study.

111 **Materials and Methods**

112 *Soils*

113 Artificial soil was prepared and spiked as in Peters et al. (2015). Soil spiked with BaP and
114 anthracene resulted in swine exposure of 1, 5, 10, and 20 mg kg-bw⁻¹ to each compound in 5 g of
115 soil. Soot was provided by the Meyer lab group at the Technical University of Denmark. More
116 detailed information on the soot, as well as swine bioavailability is available in Gouliarmou et al.
117 (2015). Here we only present data from BaP and anthracene from that data set. In short, a
118 composite soot sample collected from several wood-burning stoves in a small Danish town near
119 Roskilde was divided into two treatment groups, with one group of soot treated in contaminant
120 traps, while the other remained untreated. Treated and untreated soot were combined in different
121 ratios to create various PAH concentrations. Soot exposures were designated Soot 1, Soot 2, Soot
122 3, and Soot 4, and contained 100% untreated soot, 50% treated and 50% untreated soot, 17%
123 treated and 83% untreated soot, and 100% untreated soot respectively. Soils collected from PAH
124 impacted sites in the United Kingdom (n=12), Sweden (n=2), and Canada (n=5) were also fed to
125 swine for a total of 4 artificial soil, 19 real-world soil, and 4 soot exposures.

126 PAHs in the real-world soils were extracted by an ultrasonication method. Briefly, 5 ml
127 of 1:6 toluene:methanol solvent mix was added to 1 g of soil. The slurry was sonicated for 2
128 hours, centrifuged for 15 min at 3000 g, passed through a 0.45 µm filter, and stored at -20 °C
129 until analysis. Anthracene and BaP concentrations in the soot and soils are presented in Table 1.

130 *IV Dose*

131 The intravenous dose was prepared by completing a solvent transfer of a PAH calibration
132 standard containing 16 different PAHs (Supelco PAH Calibration Mix, 10 µg/ml in acetonitrile,
133 Sigma Aldrich) into glyceryl trioctanoate (Sigma Aldrich). Briefly, four 1 ml calibration
134 standards were combined and the acetonitrile was evaporated to near dryness under a stream of
135 high purity nitrogen gas, after which 10 ml of diethyl ether was added. Approximately half the
136 diethyl ether was evaporated under a stream of high purity nitrogen gas, 1 ml of glyceryl
137 trioctanoate added, and the remaining diethyl ether evaporated.

138 *Swine*

139 Female Landrace cross pigs (7-8 weeks in age) were obtained and housed at the Prairie
140 Swine Centre in Saskatoon, SK. Swine were housed in individual pens and allowed 7 days to
141 acclimate prior to exposure. During the acclimation period, staff trained swine to eat a dough ball
142 consisting of flour, molasses, pig chow, and vanilla. Swine were maintained on standard grower
143 ration at 4% body weight and given water ad libitum. Swine were divided into groups (n=6) and
144 exposed to PAHs by either IV or oral routes, as outlined below. Animals were monitored daily
145 during the exposure study by trained animal care staff, and were not observed to suffer ill effects
146 from exposure to PAHs. The study was reviewed and approved prior to initiation by the
147 University of Saskatchewan Animal Care and Ethics Committee (Animal Use Protocol Number:
148 20080153).

149 *Exposure Study*

150 In order to maximize the data generated by each pig, swine experienced multiple
151 exposures to PAHs in dose media. This was done by exposing swine to a single dose of PAHs,
152 either through oral (i.e. soil or soot) or IV exposure, generating a 48 hour plasma time course,

153 and allowing a 7 day washout period before subsequent exposure. Swine were dosed over a
154 period of 5 weeks, and euthanized at the end of the experiment.

155 Oral Exposure

156 Swine were given approximately 5 g of soil or 7 g of soot in a dough ball consisting of
157 flour, molasses, pig chow, and vanilla. The soil or soot was added to the dough ball along with
158 the addition of the flour. Swine were allowed to eat the dough ball passively, and generally
159 consumed it in less than a minute. If the dough ball was not consumed within 10 min, the swine
160 were restrained and the dough ball force fed to the pig.

161 IV Exposure

162 Swine were moved to a dedicated IV dose area and restrained with a hog snare and
163 handling board. A 1.5 inch, 20 gauge catheter was inserted into an ear vein following topical
164 application of lidocaine to numb the skin. The IV dose media containing PAHs (1 ml) was
165 injected through the catheter, and the catheter flushed with saline. The catheter was removed
166 immediately after the injection was completed, and pressure applied to the injection site until
167 bleeding had stopped. After bleeding ceased, the animals were returned to their individual pens.

168 *Blood Collection and Analysis*

169 Whole blood was collected from the jugular vein of four swine per treatment group at 0,
170 2, 4, 6, 8, 12, and 24 hours post-exposure into heparinized vacutainers. Sample collection was
171 limited to four swine per time point to minimize physical trauma to the animal caused by
172 restraint and blood collection. Blood was stored at 4°C until plasma separation by centrifugation
173 (1000 rpm for 15 min) and plasma stored at -20°C until extraction. Plasma was extracted by solid
174 phase extraction, as in James et al., (2011), and stored at -20°C until analysis.

175 *High Pressure Liquid Chromatography*

176 Plasma and soil extract was analyzed by high pressure liquid chromatography coupled
177 with fluorescence detection (HPLC-FD) using an Agilent 1260 Infinity system. A 10 μ L aliquot
178 of extract was injected on an Agilent PAH Pursuit column (3 μ m particle size, 100 mm length,
179 and 4.6 mm inner diameter) guarded by an Agilent MetaGuard 3 μ m C18 4.6 mm column. The
180 column was kept at 25°C during use by a column heater. Run time was set at 30 min and HPLC
181 grade water and acetonitrile (ACN) used as the solvents. The initial solvent gradient was 60:40
182 ACN:water, with a linear shift to 90:10 ACN:water between 0 min and 20 min. The 90:10
183 ACN:water gradient was maintained for 5 min, then the gradient was returned to 60:40
184 ACN:water for 5 min to re-equilibrate the column for the next sample. The Agilent 1260 system
185 was equipped with multisignal acquisition; therefore the excitation wavelength was set at 260
186 nm, while the 4 fluorescence detectors were set for emission wavelengths of 350 nm, 420 nm,
187 440 nm, and 500nm respectively.

188 *Quality Assurance and Control*

189 Plasma collected at the 0 hour time point was analyzed and used to correct the analytical
190 results from the plasma of the PAH exposed swine. Duplicates, blanks, and spikes were also
191 completed as part of the QAQC process. The average percent deviation of analytical duplicates
192 for swine samples was 18%. Average spike recovery from plasma during the solid phase
193 extraction process was 70%, and from the ultrasonication extraction process for soil was 94%.
194 The HPLC-FD was calibrated using dilutions of an external standard consisting of 10 μ g/mL of
195 each PAH and the calibration updated daily. Limits of detection for the HPLC-FD were 0.97
196 ng/mL for anthracene and 1.74 ng/mL for BaP. Plasma concentrations were corrected for
197 partitioning of PAHs into whole blood components, and the average recovery for anthracene and
198 BaP in plasma compared to whole blood were 42% and 43% respectively.

199 In order to determine if the washout period of 7 days was adequate to allow metabolic
200 processes to return to baseline levels between exposures, one group of swine was exposed to the
201 same soil in week 1 of exposure, as well as week 5, and calculated bioavailabilities were
202 compared. No statistically significant difference was observed between exposure weeks (data not
203 shown).

204 *Pharmacokinetic Parameter Calculations*

205 Area Under the Curve

206 Area under the curve (AUC) calculations were completed on the plasma concentration
207 time course for each compound in individual pigs. AUC calculations are assumed to represent
208 the total body exposure to a compound following an oral dose. AUC was calculated to the 24
209 hour time point with the MESS package (Ekstrom, 2012) in statistical program R (R Team,
210 2011) using the trapezoidal rule.

211 Absorption and Elimination Rates

212 Absorption (k_a) and elimination (k_{el}) rates were calculated for each compound in each
213 soil group as factors of the absorption and elimination slopes in the plasma concentration time
214 course. The absorption rate was calculated using the residual method, and the elimination rate
215 was calculated as the slope of the elimination phase of the log plasma concentration time course.
216 Both rates were calculated using PKSolver, an open-source Microsoft Excel add-in (Zhang et al.,
217 2010). Data for individual swine were pooled for each exposure group as blood samples were not
218 collected from each pig at all time points. Thus, standard error was not calculated for absorption
219 and elimination rate constants.

220 Bioavailability

221 Bioavailability for spiked artificial soil was calculated as the slope of the AUC vs soil
222 concentration relationship. Bioavailability for IV exposure was calculated by dividing the area
223 under the plasma time course by the total exposure (Equation 1).

$$224 \quad BA_{IV} = \frac{AUC_{IV}}{Dose_{IV}} \quad \text{Equation 1}$$

225 Absolute bioavailability of spiked soil was calculated by dividing spiked soil bioavailability by
226 BA_{IV} , as in Equation 2.

$$227 \quad BA_{abs} = \frac{BA_{soil}}{BA_{IV}} \quad \text{Equation 2}$$

228 *Point of Departure Calculations*

229 Threshold Effect Level

230 Threshold effect level values were calculated using a piecewise linear model. This model
231 defines a linear relationship for both the low and high part of the dose-response curve, as well as
232 an unknown knot point at the threshold dose. 95% upper and lower confidence intervals were
233 calculated for the threshold by bootstrap analysis. The 95% lower confidence interval is typically
234 reported as the point of departure. This model is available as part of the SiZer package
235 (Sonderregger, 2015) for the statistical software program R (R Team, 2011). The initial slope of
236 the line below the point of departure, along with 95% confidence intervals of the slope, is also
237 calculated with this model.

238 Benchmark Dose

239 Benchmark doses (BMD) are calculated by fitting models to the dose-response data and
240 using a predetermined response level, commonly 10% from background, to select a BMD. BMD
241 values were determined using the US EPA Benchmark Dose Software (BMDS) Version 2.5,
242 (<http://www.epa.gov/ncea/bmds/>). This software contains 30 different models that can be used to
243 calculate a BMD. The exponential model for continuous data was chosen for this data as it

244 provided the best fit. The lower bound 95% confidence limit on the BMD (BMDL) was also
245 calculated, and this value is typically reported as the point of departure. The exponential model
246 does not calculate a sub-linear slope for the data.

247 **Results and Discussion**

248 Swine anthracene and BaP AUC values following a single exposure to real world soils
249 did not demonstrate a correlation with soil concentration of PAHs (Figure 1, anthracene: $r^2=0.14$,
250 $p=0.54$; BaP: $r^2=0.13$, $p=0.56$). The highest soil concentration of anthracene and BaP (BGS 12)
251 was not included in the regression as it exhibited excessive leverage. Total soot and soil
252 anthracene doses ranged from 0.04 μg to 724 μg and averaged 66 (32) μg , while total BaP doses
253 ranged from 0.01 μg to 1450 μg and averaged 188 (64) μg . As no relationship was found
254 between real-world soils and internal exposure, average anthracene and BaP AUCs were
255 calculated (standard error (SE) in brackets). Anthracene AUCs ranged from 0.61 $\mu\text{g hr L}^{-1}$ to
256 14.4 $\mu\text{g hr L}^{-1}$ and averaged 3.6 (0.6) $\mu\text{g hr L}^{-1}$, while BaP AUCs ranged from 1.0 $\mu\text{g hr L}^{-1}$ to
257 7.2 $\mu\text{g hr L}^{-1}$ and averaged 2.6 (0.4) $\mu\text{g hr L}^{-1}$.

258 In contrast to real world soils, BaP and anthracene AUCs generated from swine exposed
259 to spiked artificial soil strongly correlated with soil concentration (Figure 1). Linear regressions
260 completed for both anthracene and BaP demonstrated AUC has a high dependence on soil
261 concentration (anthracene: $r^2=0.99$, $p=0.007$; BaP: $r^2=0.95$, $p=0.02$). The strong linear
262 relationship at high doses indicates that absorption in the swine model was not limited by
263 concentration of PAHs in soil. Bioavailability of anthracene and BaP was very low from spiked
264 artificial soil, and was found to be 0.7% and 0.5% respectively. Absolute bioavailability was also
265 determined, and calculated as 1.2% for anthracene and 0.7% for BaP.

266

267 Analysis of the biphasic external to internal exposure relationship

268 The shift from no relationship between AUC and dose in real-world soils to a strong
269 linear relationship between AUC and dose in spiked soils may occur because of biochemical
270 interactions between uptake and soil PAH concentration. PAHs are taken up concurrently with
271 lipids (Stavric and Klassen, 1994), and as such, may be conveyed to systemic circulation via
272 lipid transporters in the gastrointestinal tract. The dose swine were exposed to in the real-world
273 soil study may have been too low to actively compete for transfer via these transport proteins,
274 while the spiked artificial soil had a much higher PAH concentration, and therefore may have
275 had better success competing for transport. Alternatively, the linear dose-AUC relationship in
276 spiked soil exposed swine may result from passive diffusion playing a more active role as PAH
277 concentrations increased. Clearance mechanism saturation may occur in the body with higher
278 exposures, which would lead to proportional increases in internal exposure to PAHs. In contrast,
279 these clearance mechanisms may sufficiently clear PAHs before their entrance into systematic
280 circulation following real-world soil exposure.

281 Point of Departure (POD) modeling of AUC versus soil concentration indicated AUC did
282 not increase until soil concentration values greatly exceeded those typically seen in naturally
283 PAH-impacted soil. PODs calculated using the US EPA Bench Mark Dose Software (BMDS)
284 were 10,700 mg kg⁻¹ and 4,500 mg kg⁻¹ for anthracene and BaP respectively. Alternatively,
285 piecewise regression resulted in PODs of 7,500 mg kg⁻¹ for anthracene and 1,900 mg kg⁻¹ for
286 BaP. This analysis would suggest that below these concentrations, there is a limited link between
287 external dose and internal exposure. However, there are limitations associated with the data
288 generated from spiked artificial soil, namely weathering time. Soil collected from real-world
289 sites had experienced significant weathering time prior to collection, while the spiked artificial

290 soil had only a few weeks of weathering. Additionally, the gap between real-world and spiked
291 soil concentrations was very large, and as such, may skew the POD models.

292 Toxicokinetic parameters of PAHs ingested with soil

293 Like AUCs, anthracene absorption rate constants (k_a) calculated in swine exposed to real-
294 world or spiked artificial soils do not correlate to soil concentration, while BaP k_a values weakly
295 do (Figure 2, anthracene: $r^2=0.01$, $p=0.64$; BaP: $r^2=0.26$, $p=0.03$). Absorption rate constants
296 calculated for spiked artificial soils remained fairly constant, and the range of calculated values
297 did not differ greatly from those calculated for real-world soils. Average k_a values calculated for
298 combined artificial and real-world soils were $2.8 (0.7) \text{ hr}^{-1}$ and $3.7 (0.7) \text{ hr}^{-1}$ for anthracene and
299 BaP respectively.

300 Both anthracene and BaP k_a values calculated in swine compare to the range of available
301 literature PAH k_a values for rodents. Absorption rate constants reported in both rats and mice, for
302 benzo[a]anthracene, pyrene, and phenanthrene, range from 0.69 hr^{-1} to 18.8 hr^{-1} (Kadry et al.,
303 1995, Withey et al., 1991, Modica et al., 1983). The highest reported k_a of 18.8 hr^{-1} was found in
304 rats exposed to 4 mg kg^{-1} pyrene in a study consisting of a range of doses from 2 mg kg^{-1} to 15
305 mg kg^{-1} , and this value was much larger than the other reported k_a values for other doses from
306 the same study (Withey et al., 1991). If we exclude this value, the highest reported k_a value is 5.0
307 hr^{-1} , from Withey et al. (1991). Further, Kadry et al. (1995) reported similar k_a values for
308 phenanthrene between exposure media after oral exposure from neat compound, as well as
309 spiked clay and sand (0.69 to 1.4 hr^{-1}).

310 Like k_a , calculated elimination rate constants (k_{el}) for the artificial and real-world soils
311 did not show any correlation with soil compound concentration (data not shown). Therefore, k_{el}
312 values in orally exposed swine were averaged for real-world and artificial soils and the average

313 anthracene k_{el} was 0.54 (0.2) hr^{-1} , and the average BaP k_{el} was 1.4 (0.4) hr^{-1} . Elimination rate
314 constants calculated following IV exposure for both anthracene and BaP were found to be 5.3
315 hr^{-1} and 3.7 hr^{-1} respectively.

316 Calculated k_{el} values for both anthracene and BaP in orally exposed swine compare to
317 published rodent k_{el} values for oral exposure; however, the calculated k_{el} values for swine tended
318 to fall on the high end of the reported range. Published pyrene, benzo[a]anthracene,
319 phenanthrene, and BaP k_{el} values for both rats and mice range from 0.02 hr^{-1} to 1.3 hr^{-1} (Kadry et
320 al., 1995, Modica et al., 1983, Ramesh et al., 2001, Withey et al., 1991, Uno et al., 2004). Two of
321 these studies contain BaP k_{el} values, with widely variable results reported: 0.12 hr^{-1} by Ramesh
322 et al. (2001), and 1.3 hr^{-1} by Uno et al. (2004). These two studies were conducted in different
323 species (rats and mice respectively), which may account for the variability in reported k_{el} values.
324 As with oral exposure, swine k_{el} values after an IV exposure exceeded published values.
325 Elimination rate constants found in literature for IV exposure of pyrene and BaP range from
326 0.173 hr^{-1} to 3.5 hr^{-1} (Withey et al., 1991, Bouchard et al., 1998, Lipniak-Gawlik, 1998, Moir et
327 al., 1998). Moir et al. (1998) evaluated the kinetics of BaP in rats over a range of doses, and
328 reported k_{el} values ranging from 0.98 hr^{-1} to 2.85 hr^{-1} , the maximum of which is similar to the
329 BaP k_{el} for swine in this study. Additionally, Lipniak-Gawlik (1998) investigated the influence
330 of other PAHs on pyrene kinetics, and demonstrated that mixtures of PAHs may affect the
331 toxicokinetics of a compound.

332 Physiological explanation of low dose responses

333 Differences in IV and oral exposure elimination kinetics provide the clue to explain why
334 external exposure is not linked to internal exposure at low PAH concentrations. The differences
335 between IV and oral suggest that flip-flop kinetics is occurring. Flip-flop kinetics occur when the

336 gastrointestinal absorption rate is slower than the elimination rate of a compound (Yanez et al.,
337 2011). Flip-flop kinetics reduce systemic parent compound exposure as the compound
338 metabolizes at a faster rate than it is absorbed (Zhu et al., 2000). Work previously published by
339 Withey et al. (1991), and Viau et al. (1999) report that elimination kinetics of pyrene following
340 an IV and oral exposure do not differ significantly. However, both studies used a liquid carrier, a
341 saline/emulphor mix and glucose/emulphor mix respectively, for the oral exposure of pyrene. In
342 this study, the soil matrix may limit gastrointestinal absorption of PAHs, and therefore induce
343 flip-flop kinetics. This phenomenon may explain the lack of difference seen in AUC
344 measurements from various real-world soils as parent PAHs would be cleared very quickly
345 following absorption.

346 Anthracene AUCs weakly correlate to anthracene partitioning from soil in simulated
347 intestinal fluid ($r^2=0.18$, $p=0.13$, Figure 3). BaP bioavailability (AUC normalized to dose) also
348 correlates weakly with Forest fluid partition co-efficients (James et al., 2015). Both anthracene
349 and BaP demonstrate negative relationships with Forest fluid partitioning co-efficients – as the
350 partitioning co-efficient increases, AUC and bioavailability decrease. Partitioning co-efficients
351 represent the ratio of compound in soil to compound in fluid; therefore, partitioning co-efficients
352 increases signify a greater proportion of compound remaining in soil, rather than fluid. Thus,
353 increases in simulated GI fluid partitioning values indicate a stronger affinity of the compound,
354 whether anthracene or BaP, to the soil particles, and explains the negative relationship with
355 AUC.

356 Options for the risk assessment of contaminated soils

357 We propose four PAH risk assessment options: (i) assume 100% bioavailability, (ii)
358 assume constant internal exposure below $1,900 \text{ mg kg}^{-1}$, (iii) assume <100%, e.g. 21%,

359 bioavailability below $1,900 \text{ mg kg}^{-1}$, or (iv) model internal exposure through AUC versus soil
360 characteristic relationships. The most conservative, but least accurate, method of risk
361 assessment for PAH impacted sites assumes that 100% of ingested PAHs transfer into an
362 organism. As demonstrated in this study, as well as others, PAH bioavailability can vary widely
363 depending on dose media, and may lie below 1% in soil (James et al., 2015, Peters et al., 2015,
364 Ramesh et al., 2004). Therefore, this method may result in extremely elevated risk values for
365 impacted sites.

366 The second risk assessment option assumes humans absorb a constant amount of PAHs in
367 contaminated soil, irrespective of the contaminant level in these soils. The Incremental Lifetime
368 Cancer Risk (ILCR) is calculated by multiplying the external compound dose to the appropriate
369 cancer slope factor (CSF). Thus, if we assume that internal dose is not linked to external dose,
370 then using average BaP AUC value from our study, corrected for assumed adult and toddler soil
371 ingestion rates (CCME, 2006), the ILCR is 6.2×10^{-6} for adults and 1.6×10^{-6} for toddlers. In
372 contrast, if we assume 100% bioavailability and the average BaP concentration of our soils, the
373 ILCR is 2.3×10^{-5} for adults and 3.9×10^{-4} for toddlers. Although this approach is very simple, it
374 does not incorporate site-specific variations, and as such, may not accurately represent risk.

375 The third risk assessment option calculates bioavailability as the slope of the internal-
376 exposure dose curve at environmentally relevant soil concentrations. At these low
377 concentrations, this slope is often termed the sublinear portion because it is not significantly
378 different from zero. Piecewise regression of this sublinear portion indicates that bioavailability
379 estimates for environmentally relevant soil concentrations range from 1.6% to 21% for BaP and
380 0% to 21% for anthracene (Table 3). However, these approaches are highly sensitive to the
381 spiked soil doses, the limitations of which were discussed previously. Thus, our estimate of 21%

382 soil BaP concentration becoming bioavailable may be too inaccurate to use at a contaminated
383 site.

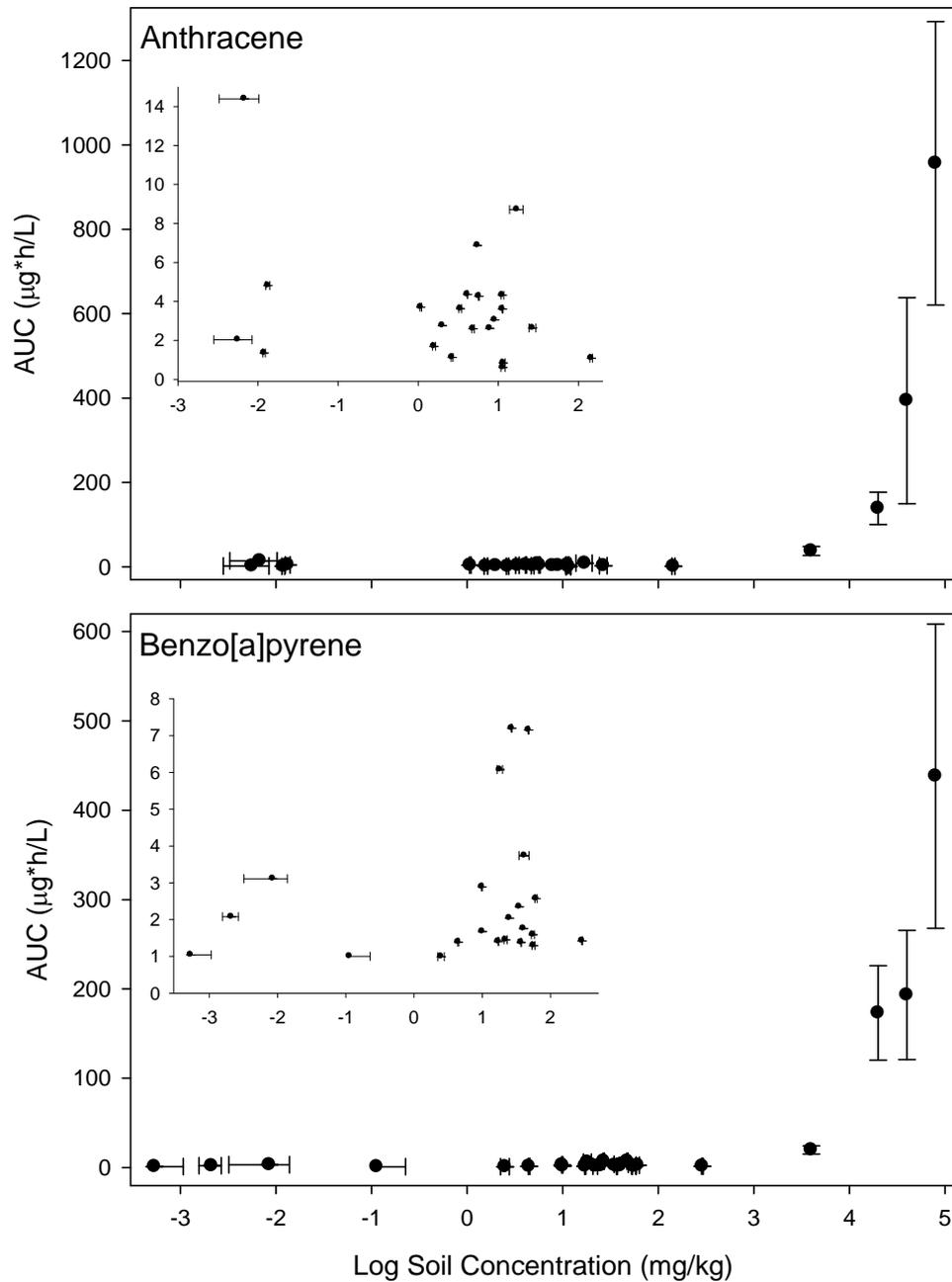
384 The fourth, and final, option is to use partitioning to estimate internal exposure of PAHs
385 to humans. For example, in Figure 3, there is a weak relationship between partitioning and
386 AUC. This approach can internal exposure estimates to site-specific soils but requires site-
387 specific data. Additionally, the observed correlations between partitioning co-efficients and
388 internal exposure in swine were very weak, and as such, may be inaccurate.

389 Synopsis

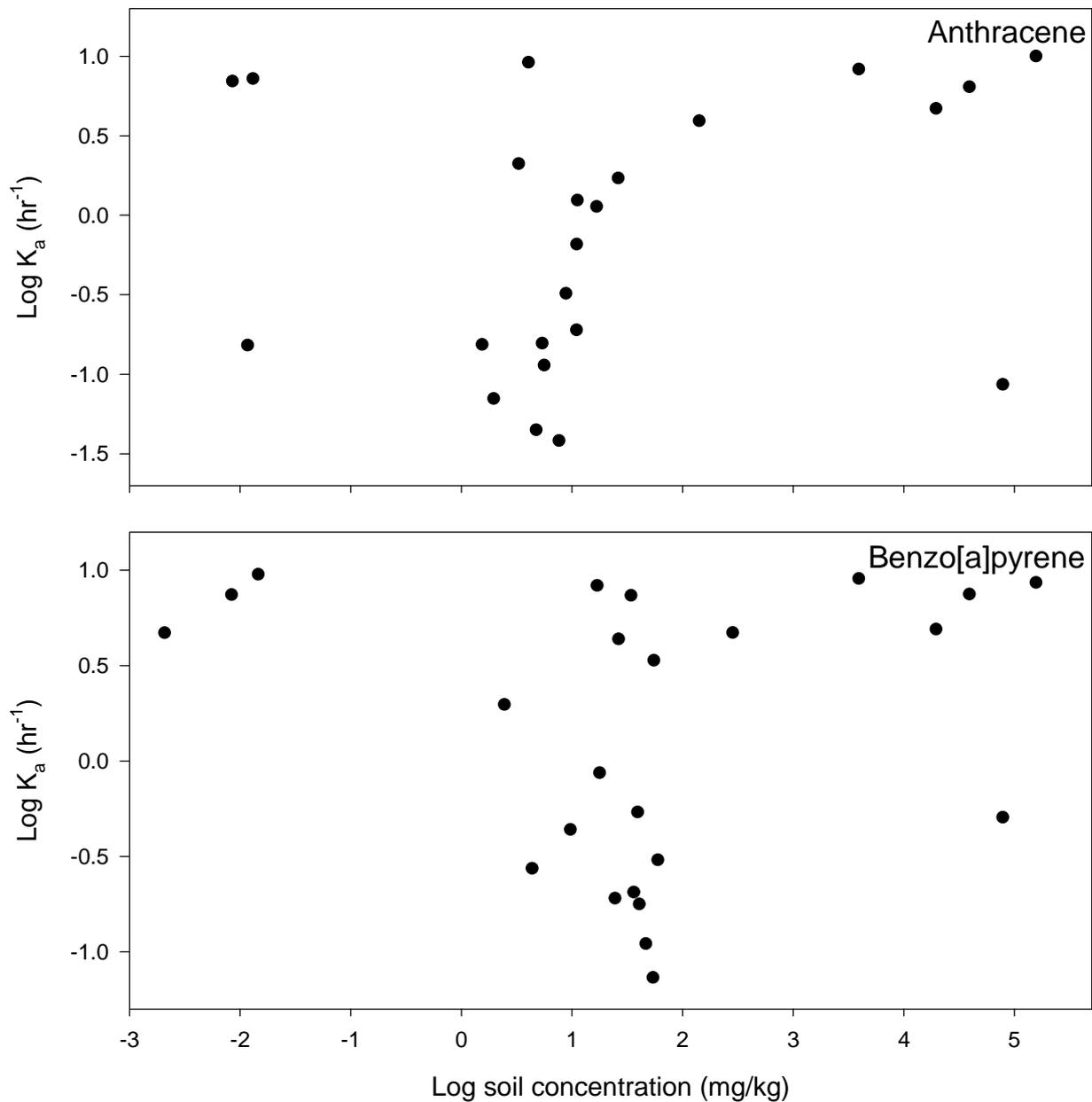
390 Analysis of swine anthracene and BaP toxicokinetics demonstrated PAH soil
391 concentration does not influence internal exposure of PAHs. This contradicts the common
392 assumption in risk assessment that risk relates linearly to the soil concentration, and therefore
393 external dose, of a compound. There appears to be a point of departure in soil concentrations
394 where internal exposure and external dose become related. Using two different point of departure
395 models indicated AUC and soil dose were only linked at soil concentrations much larger than
396 those typically seen in PAH impacted soils found in the environment. Thus, it may be reasoned
397 humans are exposed to a constant internal dose of PAHs, regardless of external dose. We
398 hypothesize this occurs because of limited absorption coupled with rapid elimination, leading to
399 a reduced amount of circulating compound. As this study measured parent PAHs in systemic
400 circulation as an indication of internal exposure, decreases in circulating compound would lead
401 to a decreased apparent internal exposure. However, our study design cannot speak to the risk of
402 exposure of the gastrointestinal lining, as PAH exposure to this tissue occurs during the
403 absorption phase, independent of systemic circulation.

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406 University of Saskatchewan's Animal Research Ethics Board, and adhered to the Canadian
407 Council on Animal Care guidelines for humane animal use.

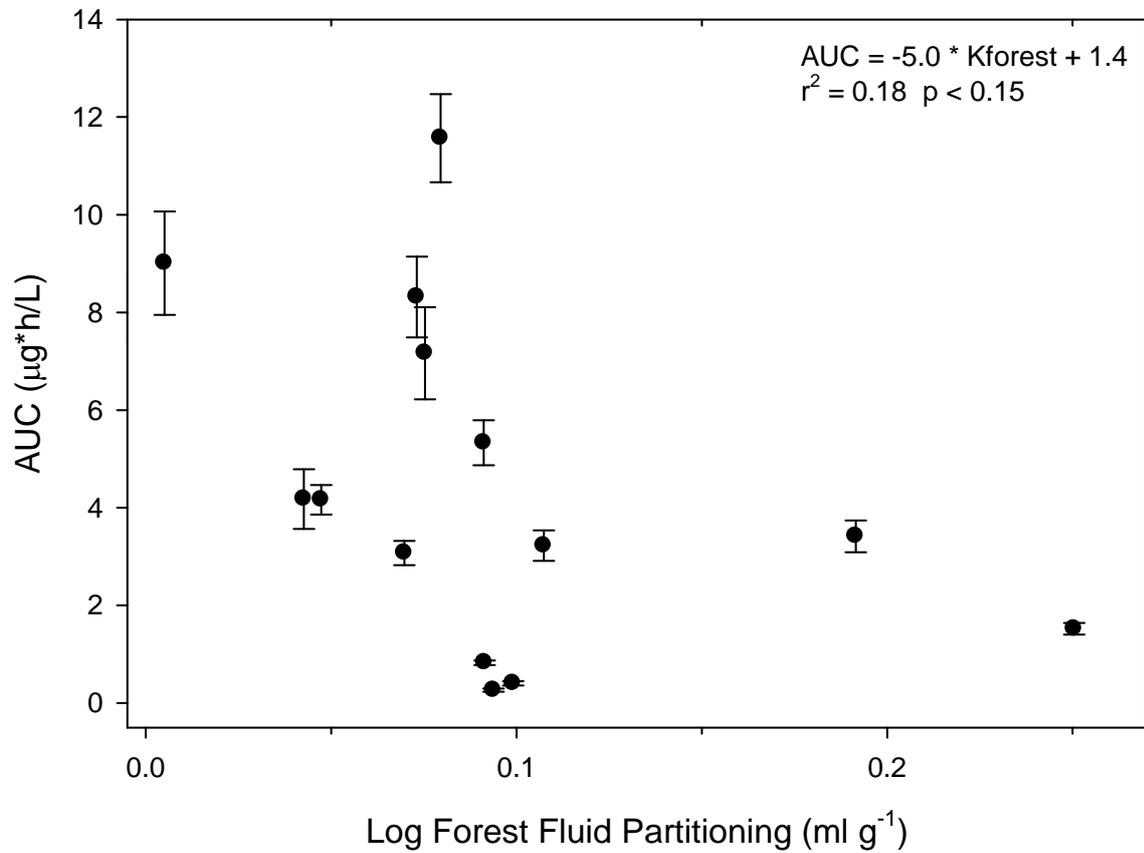


408
 409 Figure 1. The calculated AUC values for anthracene and benzo[a]pyrene in swine after a single
 410 exposure to spiked artificial and impacted real-world soil versus the concentration of anthracene
 411 and benzo[a]pyrene in soil. Error bars represent the standard error of the mean for both soil
 412 concentration (horizontal, n=3) and AUC (vertical, n=6). Linear regression for impacted real-
 413 world soils and soot (inset) did not demonstrate a correlation between AUC and soil
 414 concentration for either anthracene ($r^2=0.14$, $p=0.54$) or benzo[a]pyrene ($r^2=0.13$, $p=0.56$).
 415 However, linear regression for the spiked soils demonstrated a correlation between AUC and soil
 416 concentration for both anthracene ($r^2=0.99$, $p=0.007$) and benzo[a]pyrene ($r^2=0.95$, $p=0.02$).



417

418 Figure 2. Absorption rate constants for anthracene and benzo[a]pyrene in swine following a
 419 single exposure to one of 19 real-world soils or 4 spiked artificial soils versus soil concentration.
 420 A linear regression was completed for both benzo[a]pyrene and anthracene, and a significant
 421 correlation was not observed for either compound for real-world soils (anthracene: $r^2=0.014$,
 422 $p=0.64$; benzo[a]pyrene: $r^2=0.26$, $p=0.03$) or spiked soils (anthracene: $r^2=0.01$, $p=0.84$;
 423 benzo[a]pyrene: $r^2=0.02$, $p=0.81$).
 424



425

426 Figure 3. Anthracene AUC in swine (n=6) orally exposed to PAHs in real-world impacted soils

427 (n=14) versus log of simulated intestinal fluid partitioning co-efficient. Linear regression

428 demonstrated a weak correlation between variables ($r^2=0.18$, $p=0.13$).

429 Table 1. Measured soil concentrations of anthracene and benzo[a]pyrene in real-world soils
 430 given to swine. Standard error is in brackets.

| Soil | Anthracene (mg kg⁻¹) | Benzo[a]pyrene (mgkg⁻¹) |
|---------------|--|---|
| WP1 | 27 (2.4) | 18 (1.7) |
| GW5 | 1.1 (0.02) | 4.5 (0.08) |
| Soot 1 | 2 | 10 |
| Soot 2 | 5.5 | 25 |
| Soot 3 | 7.8 | 35 |
| Soot 4 | 9 | 40 |
| BGS 1 | 1.6 (0.07) | 2.5 (0.3) |
| BGS 2 | 11 (0.7) | 56 (2.6) |
| BGS 3 | 12 (0.6) | 55 (2.9) |
| BGS 4 | 11 (0.5) | 61 (2.4) |
| BGS 5 | 5.7 (0.1) | 27 (0.3) |
| BGS 6 | 4.8 (0.2) | 17 (0.2) |
| BGS 7 | 3.4 (0.1) | 9.9 (0.2) |
| BGS 8 | 4.1 (0.04) | 37 (0.4) |
| BGS 9 | 2.6 (0.07) | 22 (1.2) |
| BGS 10 | 17 (3.3) | 41 (8.9) |
| BGS 11 | 11 (0.1) | 48 (0.8) |
| BGS 12 | 144 (5.0) | 290 (5.8) |
| COT 1 | 0.008 (0.003) | 0.12 (0.1) |
| COT 2 | 0.009 (0.004) | 0.014 (0.0005) |
| COT 3 | 0 (0) | 0 (0) |
| COT 4 | 0.013 (0.0008) | 0.009 (0.005) |
| COT 5 | 0.012 (0.0004) | 0.002 (0.0006) |

432 Table 2. Pros and Cons of Risk Assessment Options for PAHs

| Option | Pros | Cons |
|--|--------------------------|---|
| 100% bioavailability | Simple, conservative | May overestimate risk |
| Constant AUC | Simple, likely realistic | Not site-specific |
| Constant (<100%) bioavailability | Simple | Highly dependent on spiked soil concentrations, may not be accurate |
| Soil:fluid partitioning | Site Specific | More complex, weak correlation, more labour intensive |

433

434 Table 3. Estimated bioavailability of BaP and anthracene from piecewise regression modeling

| Compound | Average Bioavailability (%) | Lower 95% Confidence Interval | Upper 95% Confidence Interval |
|-----------------------|--|--|--|
| Anthracene | 6.1 | 0 | 21 |
| Benzo[a]pyrene | 12 | 1.6 | 21 |

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