Is received dose from ingested soil independent of soil PAH concentrations: animal model results

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Title Running Head

PAH toxicokinetics in ingested soil

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Abstract

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

Keywords: toxicokinetics, swine, PAHs, soil

Introduction

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

Once ingested, PAHs transfer from the gastrointestinal tract to systemic circulation. Transfer of PAHs into circulation occurs concurrent with lipids (Stavric and Klassen, 1994), and it has been theorized that PAHs are transferred via chylomicron formation within enterocytes and transferred into lymph, which would allow PAHs to bypass the liver and first pass elimination (Hussain et al., 1996, Busbee et al., 1990). However, a study done in lymph and bile duct cannulated rats determined that about 80% of absorbed PAHs transfer to circulation via hepatic portal transport, rather than lymph (Laher et al., 1984). A more recent study confirms these findings, concluding that approximately twice the PAHs entering into circulation cross through 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 ^{14}C
70 labeled compounds eliminates the complicated analysis necessary for unlabeled compounds;
71 however a number of factors make ^{14}C analysis unfavorable for use in PAH bioavailability. First,
72 ^{14}C 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 ^{14}C 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

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

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

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

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

Materials and Methods

Soils

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

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

IV Dose

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

Swine

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

Exposure Study

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

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

Oral Exposure

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

IV Exposure

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

Blood Collection and Analysis

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

High Pressure Liquid Chromatography

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

Quality Assurance and Control

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

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

Pharmacokinetic Parameter Calculations

Area Under the Curve

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

Absorption and Elimination Rates

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

Bioavailability

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

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

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

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

Point of Departure Calculations

Threshold Effect Level

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

Benchmark Dose

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

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

Results and Discussion

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

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

Analysis of the biphasic external to internal exposure relationship

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

Point of Departure (POD) modeling of AUC versus soil concentration indicated AUC did not increase until soil concentration values greatly exceeded those typically seen in naturally PAH-impacted soil. PODs calculated using the US EPA Bench Mark Dose Software (BMDS) were 10,700 mg kg^{-1} and 4,500 mg kg^{-1} for anthracene and BaP respectively. Alternatively, piecewise regression resulted in PODs of 7,500 mg kg^{-1} for anthracene and 1,900 mg kg^{-1} for BaP. This analysis would suggest that below these concentrations, there is a limited link between external dose and internal exposure. However, there are limitations associated with the data generated from spiked artificial soil, namely weathering time. Soil collected from real-world 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) hr^{-1} and 3.7 (0.7) 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

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 constants calculated following IV exposure for both anthracene and BaP were found to be 5.3 hr^{-1} and 3.7 hr^{-1} respectively.

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

Physiological explanation of low dose responses

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

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

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

Options for the risk assessment of contaminated soils

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

359 bioavailability below 1,900 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 $6.2x10^{-6}$ for adults and $1.6x10^{-6}$ for toddlers. In
372 contrast, if we assume 100% bioavailability and the average BaP concentration of our soils, the
373 ILCR is $2.3x10^{-5}$ for adults and $3.9x10^{-4}$ 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%

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

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

Synopsis

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

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407 Council on Animal Care guidelines for humane animal use.

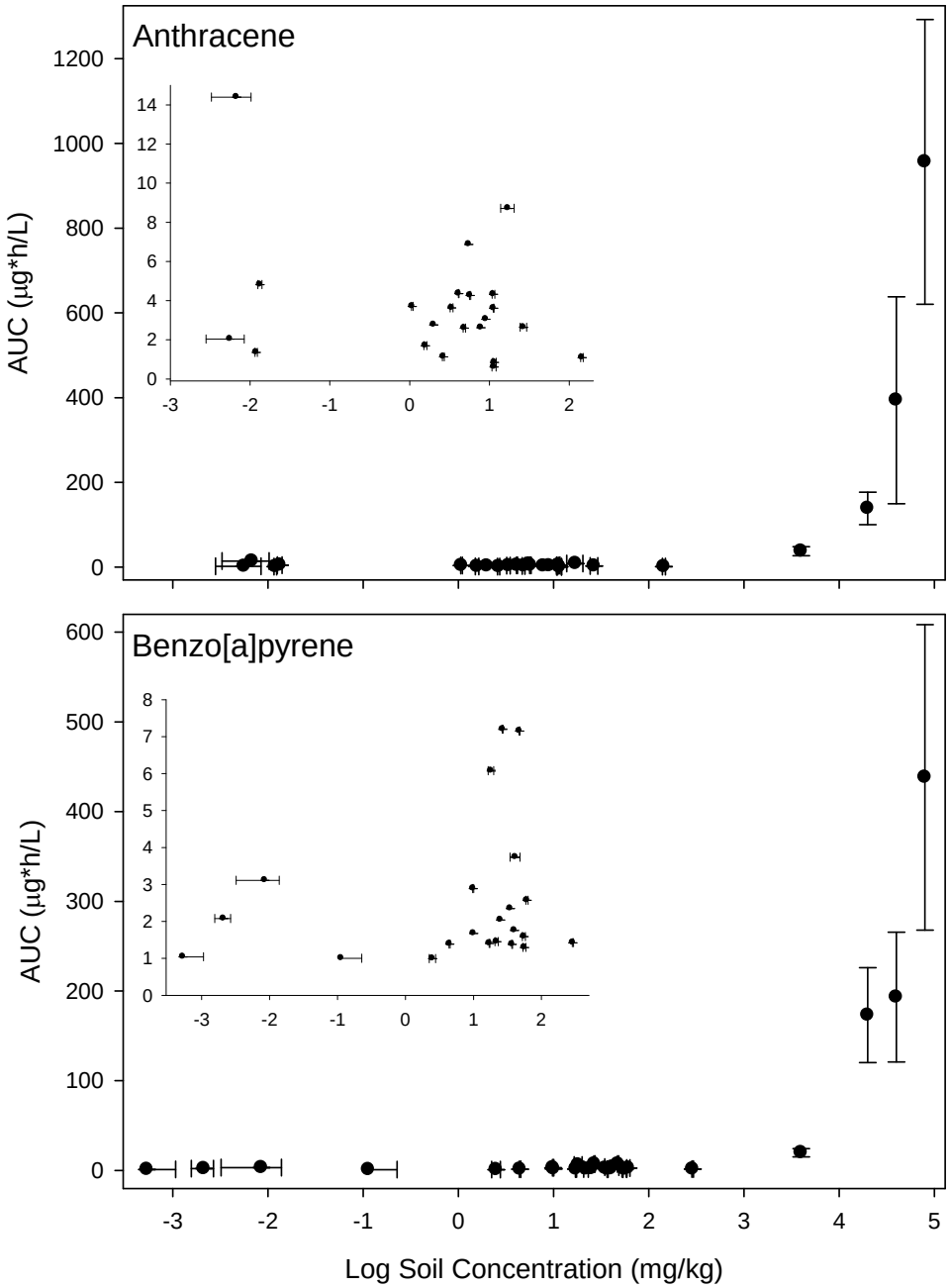

Figure 1. The calculated AUC values for anthracene and benzo[a]pyrene in swine after a single exposure to spiked artificial and impacted real-world soil versus the concentration of anthracene and benzo[a]pyrene in soil. Error bars represent the standard error of the mean for both soil concentration (horizontal,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

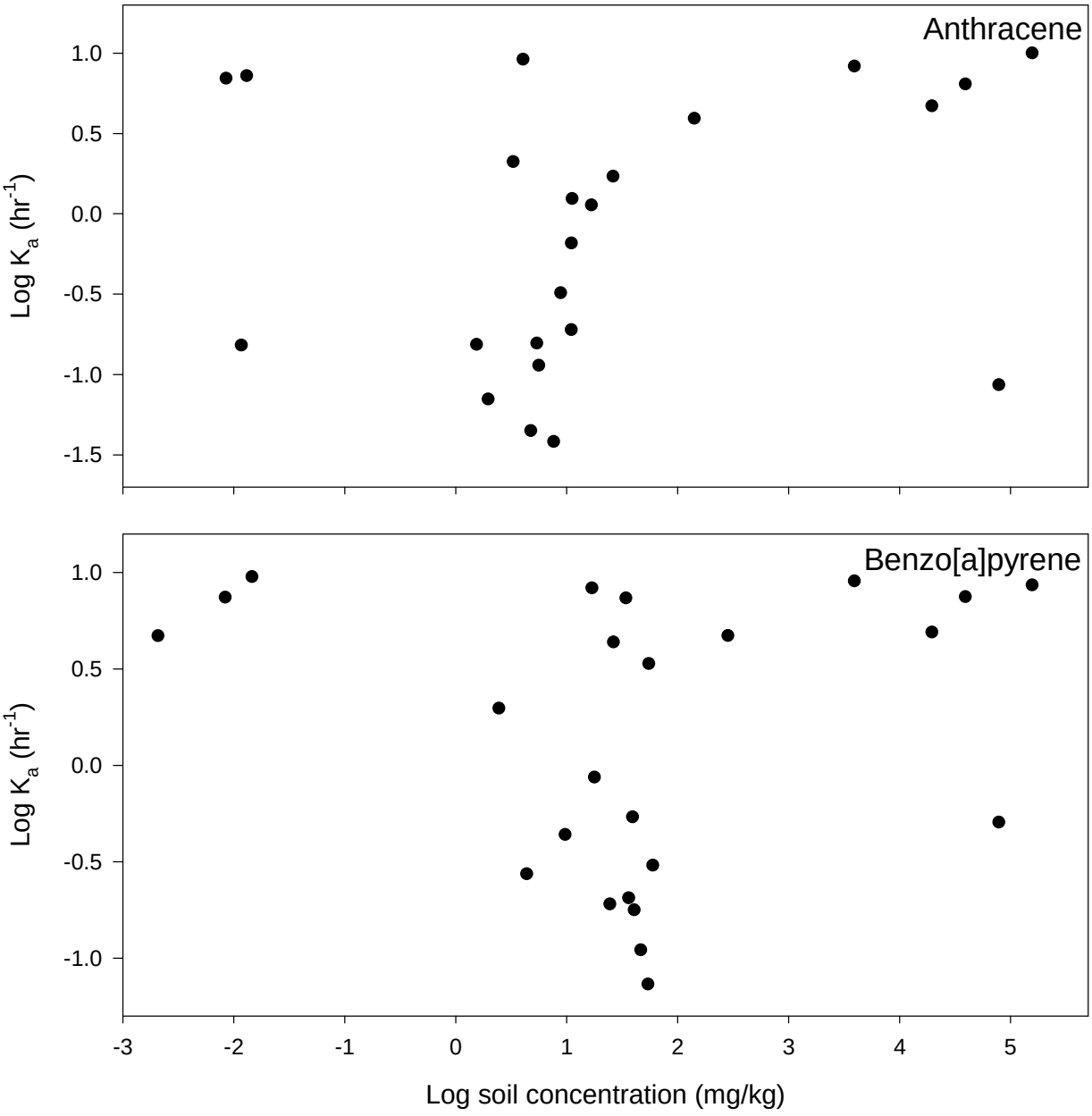

Figure 2. Absorption rate constants for anthracene and benzo[a]pyrene in swine following a single exposure to one of 19 real-world soils or 4 spiked artificial soils versus soil concentration. A linear regression was completed for both benzo[a]pyrene and anthracene, and a significant correlation was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

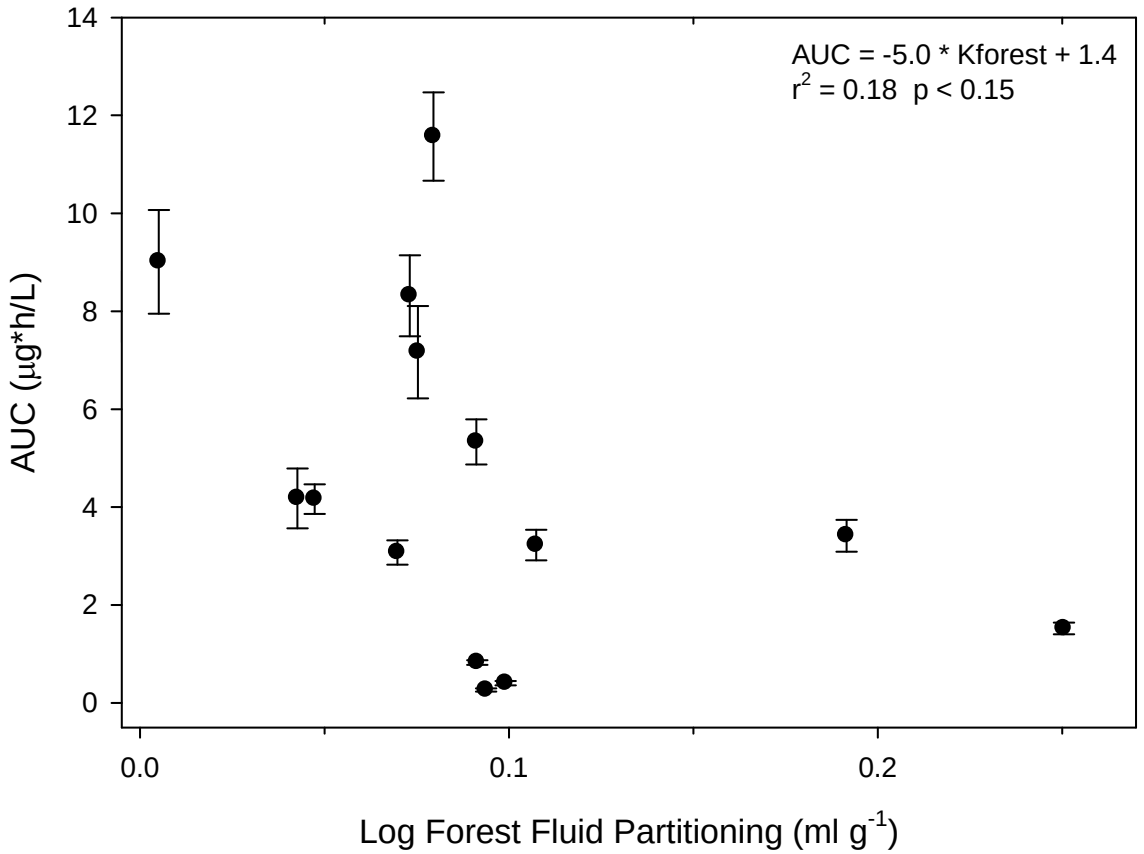

Figure 3. Anthracene AUC in swine (n=6) orally exposed to PAHs in real-world impacted soils (n=14) versus log of simulated intestinal fluid partitioning co-efficient. Linear regression demonstrated a weak correlation between variables (r^2=0.18, p=0.13).

Table 1. Measured soil concentrations of anthracene and benzo[a]pyrene in real-world soils 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)

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

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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