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The bioavailability of polycyclic aromatic hydrocarbons from different dose media after single and sub-chronic exposure in juvenile swine

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HIGHLIGHTS

- Juvenile swine were used as a model for human exposure to PAHs.
- The effect on bioavailability of repeated exposure to PAHs was studied.
- Swine were exposed to PAHs in a reference material, soil, food, and corn oil.
- No significant change in PAH bioavailability was noted following repeated exposure.

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ABSTRACT

Humans are constantly exposed to contaminants in the environment, which may lead to changes in physiological processes by altering enzyme activities that could affect bioavailability. However, bioavailability estimates are typically made from a single exposure to an animal model, which may lead to overestimating bioavailability. This study uses juvenile swine to model human exposure to benzo[a]pyrene (BaP) and anthracene in certified reference material (CRM), spiked soil, spiked food, or spiked corn oil after one and seven days of dosing. Area under the curve (AUC) was calculated after one and seven days of exposure for both BaP and anthracene for each exposure media. Whereas there were significant differences in AUC between different media, there were no significant changes in AUC after sub-chronic exposure to BaP or anthracene. Average BaP bioavailability for CRM, spiked soil, spiked food and corn oil was 71%, 0.72%, 0.03% and 0.97% respectively. Average anthracene bioavailability was 1.7% and 43% for corn oil and CRM respectively. Anthracene was not detected above background in swine exposed to spiked food and spiked soil. Thus, this study indicates that exposure media impacts bioavailability, but there is no statistical evidence that sub-chronic exposure affects systemic exposure.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a family of compounds that are common environmental contaminants (CCME, 2008). PAHs are produced through incomplete combustion of organic compounds and are present in fuel oil, and as such, are released into the environment from fires and fuel processing, and humans can be exposed to them through a number of sources (Garcia-Falcon and Simal-Gandara, 2005; Rey-Salgueiro et al., 2008, 2009). Although PAHs are ubiquitous, they are commonly found in soil due to their physicochemical properties (Semple et al., 2003). PAHs are highly lipophilic substances, and the high molecular weight compounds are not particularly volatile, thus they will preferentially stay in soil.

Some PAHs are known or suspected animal carcinogens, and as such, have very conservative environmental guidelines for oral exposure (CCME, 2008). These guidelines are derived with the assumption that the absorption from the guideline media is the same as the exposure media used to derive the guideline; however, PAHs have been shown to have a different bioavailability than that of the original media (Koganti et al., 1998). The definition of bioavailability is the fraction of a compound that reaches systemic circulation following exposure, and bioavailability is calculated by dividing the total amount of compound that reached circulation by the dose without correction for dose media. Bioavailability of parent PAHs from animal dosing studies is commonly reported between 80 and 100% (Ramesh et al., 2004).

Abbreviations: BaP, benzo[a]pyrene; AUC, area under the curve; CRM, certified reference material.

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PAH uptake after an oral exposure is complicated, with the lipid content of the diet (Stavric and Klassen, 1994), presence of bile (Laher and Barrowman, 1987; Rahman et al., 1986), and chylomicrons (Grubbs and Moon, 1973; Laher et al., 1984) all potentially having an effect on the absorption of PAHs from the gastrointestinal tract. The presence of lipids have been shown to increase bioavailability of PAHs (Stavric and Klassen, 1994), and PAHs appear to be absorbed concurrently with lipids (Laher et al., 1984; Laurent et al., 2001); however, changing the amount of lipids does not appear to affect bioavailability (Laher et al., 1984). The presence of bile in the intestinal tract has been shown to significantly increase bioavailability of PAHs, particularly for more lipophilic compounds (Rahman et al., 1986). Finally, chylomicrons can lead to the transport of PAHs in the lymphatic system, effectively bypassing the first pass effect (Busbee et al., 1990; Grubbs and Moon, 1973; Laher et al., 1984); however, other studies have shown that PAH transport through the lymphatic system accounts for only a small fraction of what is absorbed into the organism (Laher et al., 1984; Laher and Barrowman, 1987).

When calculated using blood AUC values, bioavailability of PAHs is typically calculated from a single exposure to a compound (Hecht et al., 1979; Withey et al., 1991; Moir et al., 1998; Cavret et al., 2003); however humans are more likely to be exposed repeatedly to a mixture of multiple compounds in the environment, including background exposure to PAHs in food (Yebra-Pimentel et al, 2013). Although these compounds do not often cause a noticeable physiological effect, they still may induce changes in the biochemical processes of the body. A common change induced by repeated contaminant exposure is the induction of enzyme activity. Mono-oxygenase enzymes, such as CYP 1A1, CYP 1A2, CYP 1B1, and the CYP 2B, 2C and 3A families are critical to the phase I metabolism and excretion of PAHs and other lipophilic compounds, converting them into more hydrophilic analogs, that can be excreted more readily (Ramesh et al., 2004). If there is an increase in enzyme activity, more of the compound will be metabolized and excreted. Since bioavailability of orally dosed PAHs is influenced by rapid uptake and metabolism in the liver (first pass effect), an increase in enzyme activity in the liver will potentially decrease bioavailability.

The metabolic products of PAHs cause the toxicological effects associated with PAH exposure (Ramesh et al., 2004) but where these metabolites are produced is a critical determinant of their toxicity; in essence, toxicity occurs in the organ where metabolites are formed, not from metabolites in the systematic circulation (Uno et al., 2004; Galvan et al., 2005). Studies conducted with both CYP 1A1 knockout mice (which therefore have limited free PAH metabolites in systemic circulation) and mice with low affinity AhR characteristics demonstrated that the parent compound is activated at the site of toxic action rather than metabolites formed elsewhere causing toxicity at the site of toxic action (Uno et al., 2004; Galvan et al., 2005). Metabolites produced in the liver were determined to contribute little to peripheral adduct formation as AhR activation of enzymes predominantly happens in the liver, and mice with low affinity AhR characteristics demonstrated greater peripheral PAH toxicity (Galvan et al., 2005). Similarly, Uno et al. (2004) measured multiple toxic endpoints in CYP 1A1 knockout mice exposed to BaP (DNA adducts, body and organ weight, bone marrow cell counts, etc.), and observed significantly higher toxicity in CYP 1A1 knockout compared to CYP 1A1 wild-type mice. This was attributed to lower circulating parent BaP from CYP 1A1 activation in the wild-type mice clearing the compound in the liver (Uno et al., 2004). For these reasons, in this paper we tracked only parent PAH bioavailability.

Although correlations can be made from in vitro systems, bioavailability is best measured using an in vivo model as a substitute for human exposure. There are four models that are commonly used to predict human exposure; rodents (Withey et al., 1991; Moir et al., 1998; Ramesh et al., 2001), swine (Laurent et al., 2002; Roos et al., 2002), monkeys (Freeman et al., 1995; Akabane et al., 2010), and humans (Hecht et al., 1979; Viau et al., 2002). Although humans and monkeys are the most accurate models for human exposure, monetary and ethical restraints typically exclude their use in bioavailability studies.

Rodents are commonly used as models for human exposure, as they are well defined as a model, rather inexpensive, and easy to handle. However, the gastrointestinal system and nutritional requirements of a rodent are very different from humans. Rodents have different energy expenditures and food intakes than humans in comparison to body size, and the intestinal morphology and gastric microbiota of the rodent also differs from that of humans (Patterson et al., 2008). Therefore, from a physiological perspective, rodents are not the best model for oral exposure in humans. Swine have emerged as a promising model for human oral exposure to contaminants due to similarity in gastrointestinal anatomy and physiology, down to the cellular level, and as such, have been commonly used as models for a variety of compounds (Roos et al., 2002; Casteel et al., 2006; Budinsky et al., 2008). Additionally, swine have similar nutritional requirements to humans, ensuring that compounds will be absorbed in similar quantities, and at a similar rate (Patterson et al., 2008).

Studies of the effect of sub-chronic exposure on bioavailability are commonly done in association with new drug testing (Andersson et al., 1990; Ferruzzi et al., 2009), and, less commonly, in toxicology studies (Schultz and Shangraw, 2006). No known studies are available for the effect of sub-chronic exposure on PAH bioavailability, although literature is available demonstrating enzymatic increases in response to PAH exposure (Roos et al., 2002, 2004; Harrigan et al., 2006). This study intends to assess the influence of sub-chronic PAH exposure on PAH bioavailability estimates in the swine model, and to characterize the time course of PAH concentrations in swine serum and tissues following oral exposure.

2. Materials and methods

2.1. Swine

Female Landrace cross pigs were obtained from the Prairie Swine Centre (Saskatoon, SK), and were housed at the Animal Care Unit of the Western College of Veterinary Medicine (University of Saskatchewan, Saskatoon, SK). Swine were allowed to acclimate for 7 days prior to exposure in the facility and were maintained on water and standard grower ration ad libitum. Swine were divided into 2 groups; one to assess effects of sub-chronic exposure on PAH bioavailability from different media ($n = 24$) and one to characterize the time course of PAH uptake into tissues ($n = 21$). 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).

2.2. Sub-chronic exposure study

Swine were divided into 4 groups ($n = 6$) and exposed orally to PAHs in one of four media, daily, for 7 days. Exposure media included artificial soil, food (dough ball), corn oil, and a certified reference material (CRM soil). Daily doses of each media were administered in a dough ball composed of feed, oats, flour, and molasses. Certified reference material (CRM 140-100, Resource Technology Corporation Lot 010572) was not modified before being given to swine and each pig was given 5 g of CRM or artificial soil. The CRM soil is a natural clay soil collected from a contaminated site in the United States and contained 15 individual PAHs (Supplementary Table 1). Artificial soil was made according to Environment Canada guidelines (2007) and was spiked with benzo[a]pyrene (BaP) and anthracene according to Reid et al. (1998). To simulate exposure to PAHs from food only, swine received dough balls spiked with neat BaP and anthracene. The soil and neat compounds were added to the dough ball with the flour. To simulate bioavailability from a lipophilic media, BaP and anthracene were dissolved in corn oil (Safeway store brand), which was then put into a gel capsule (0.5 ml, gelatin pharmaceutical capsules) and hidden in a dough ball for daily