**Bioaccessibility of Nitro- and Oxy-PAHs in Fuel Soot Assessed by an *in vitro*
Digestive Model with Absorptive Sink**

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ABSTRACT

Ingestion of soot present in soil or other environmental particles is expected to be an important route of exposure to nitro and oxygenated derivatives of polycyclic aromatic hydrocarbons (PAHs). We measured the apparent bioaccessibility (B_{app}) of native concentrations of 1-nitropyrene (1N-PYR), 9-fluorenone (9FLO), anthracene-9,10-dione (ATQ), benzo[*a*]anthracene-7,12-dione (BaAQ), and benzanthrone (BZO) in a composite fuel soot sample using a previously-developed *in vitro* human gastrointestinal model that includes silicone sheet as a third-phase absorptive sink. Along with B_{app} , we determined the 24-h sheet-digestive fluid partition coefficient ($K_{s,24h}$), the soot residue-fluid distribution ratio of the labile sorbed fraction after digestion ($K_{r,lab}$), and the maximum possible (limiting) bioaccessibility, B_{lim} . The B_{app} of PAH derivatives was positively affected by the presence of the sheet due to mass-action removal of the sorbed compounds. In all cases B_{app} increased with imposition of fed conditions. The enhancement of B_{app} under fed conditions is due to increasingly favorable mass transfer of target compounds from soot to fluid (increasing bile acid concentration, or adding food lipids) or transfer from fluid to sheet (by raising small intestinal pH). Food lipids may also enhance B_{app} by mobilizing contaminants from nonlabile to labile states of the soot. Compared to the parent PAH, the derivatives had larger $K_{r,lab}$, despite having lower partition coefficients to various hydrophobic reference phases including silicone sheet. The B_{lim} of the derivatives under the default conditions of the model ranged from 65.5% to 34.4%, in the order, 1N-PYR > ATQ > 9FLO > BZO > BaAQ, with no significant correlation with hydrophobic parameters, nor consistent relationship with B_{lim} of the parent PAH. Consistent with earlier experiments on a wider range of PAHs, the results suggest that a major determinant of bioaccessibility is the distribution of chemical between nonlabile and labile states in the original solid.

Keywords: PAH derivatives; bioaccessibility; digestive model; soot.

Capsule: *In vitro* bioaccessibility of native-borne PAH derivatives in soot and how it changes with inclusion of absorptive sink and digestive fluid chemistry

1. Introduction

Nitro- and ketone- or quinone-substituted polycyclic aromatic hydrocarbons (nitro-PAHs and oxy-PAHs, respectively) are formed along with PAHs during the combustion of fossil fuels and biomass materials, and a substantial portion of these derivatives remains associated with the particulate phase (Bamford and Baker, 2003; Walgraeve et al., 2010). Shen et al. (2012) determined the emission factors of 28 PAHs, four oxy-PAHs, and nine nitro-PAHs in both the gas and particulate phase from residential combustion of 27 wood fuels, and found that oxy-PAHs and nitro-PAHs showed relatively greater tendency than parent PAHs to be present in the particulate phase. The derivatives may also originate from secondary reactions of PAHs in the gas phase or particle-bound phase with sunlight-generated free radicals in the atmosphere (Ringuelet et al., 2012; Zimmermann et al., 2013). For example, 2-nitropyrene is formed in the atmosphere by gas-phase reaction of hydroxyl radical with pyrene in the presence of NO_x (Bamford and Baker, 2003; Arey et al., 1986). These gas phase-generated PAH derivatives may also partition to the particulate phase during aging in the atmosphere.

Nitro- and oxy-PAH derivatives emitted to or formed in the atmosphere distribute ubiquitously in the terrestrial environment following wet and dry deposition. Oxy-PAHs in atmospheric particulate matter (Albinet et al., 2007), outdoor dust (Wei et al., 2015), and surface soil (Arp et al., 2014) are often found at levels comparable to the parent PAHs, while nitro-PAHs are usually found at levels 1–2 orders of magnitude lower (Albinet et al., 2007; Niederer et al., 1998; Wei et al., 2015). Like PAHs, the levels of these PAH derivatives are often related to the soot content in environmental particles (Wei et al., 2015). In the terrestrial environment, PAH derivatives are believed to be more mobile than their corresponding parent PAHs due to their polarity, and often exhibit greater direct toxicity (Lundstedt et al., 2007; Wang et al., 2011). Oxy-PAHs were found to induce similar or elevated DNA damage in medaka fish embryos compared to their parent PAHs (Dasgupta et al., 2014). In addition, the minimum mutagenic culture concentration of 1-nitropyrene was found to be 0.54 mg/L to human B-lymphoblastoid cells, whereas no mutagenic effect was observed for pyrene even at 100 mg/L (Durant et al., 1996). In fact, 1-Nitropyrene is

identified as a Group 2A (probable) human carcinogen by the International Agency for Research on Cancer ([IARC, 2015](#)).

Environmental particles can be ingested via hand-to-mouth transfer and, once in the digestive tract, can pose adverse health impacts when the associated contaminants mobilized from these particles are transferred through the intestinal epithelium to the circulatory system ([Ruby et al., 2012](#)). Using a fugacity-based multimedia chemical fate model, [Jones-Otazo et al. \(2005\)](#), found that inadvertent ingestion of household dust was the main pathway of exposure to polybrominated diphenyl ethers (PBDE) for both children and adults. Serum PBDE levels were found to correlated significantly with PBDE levels in house dust and/or handwipes ([Stapleton et al., 2012](#); [Whitehead et al., 2015](#)). [Gevao et al. \(2007\)](#), found that household dust ingestion contributed about 42% of nondietary intake of PAHs in children.

The gastrointestinal bioaccessibility of PAHs in environmental particles using *in vitro* digestive models has been extensively studied for soil and dust ([Collins et al., 2015](#); [Harris et al., 2013](#)), and to a lesser extent soot ([Gouliarmou et al., 2013](#); [Zhang et al., 2015a](#); [2015b](#)), but to our knowledge no study has appeared on the bioaccessibility of PAH derivatives in environmental particles, despite their widespread occurrence and high levels. The behavior of a given PAH derivative in the digestive tract cannot be assumed to be the same as the parent PAH due to differences in polarity, solubility, and possibly formation mechanism. Some researchers have suggested that a fraction of PAHs may be physically trapped in the soot matrix as a result of gas phase co-condensation with nascent particles during or just after combustion, and that the entrapped PAHs are highly resistant to extraction under vigorous conditions ([Jonker and Koelmans, 2002](#); [Pignatello, 2013](#)). The same might be true for PAH derivatives formed during the combustion process. However, it is possible that PAH derivatives formed by secondary gas-phase or surface reactions may be distributed among more available sites as a consequence. Therefore, research on the bioaccessibility of soot-borne PAH derivatives is imperative.

Addition of a third-phase absorptive sink at the small intestinal stage is now widely adopted by the research community for *in vitro* gastrointestinal models ([Collins et al, 2013](#); [2015](#); [Zhang et al., 2015a](#);

2015b). The sink is intended to perform a similar function as the intestinal epithelium *in vivo*—to remove contaminants from the digestive fluid, thereby establishing a thermodynamic concentration gradient between the particles and the digestive fluid that promotes mass transfer from the particles into the fluid (reduce residue sorption), and thus to increase the determined *in vitro* bioaccessibility and better predict the *in vivo* values (James et al., 2011; Li et al., 2015a). Sink materials like Tenax beads and activated carbon/silicone mixture have been observed to increase the bioaccessibility of PAHs and flame retardants in environmental particles (Collins et al., 2013; Fang and Stapleton, 2014; Li et al., 2015a). However, these studies did not directly test the ability of the sink materials to promote desorption of contaminants from the particles, which is essential in evaluating the performance of the sink.

Therefore, the first objective of this study is to assess the performance of silicone sheet as a sink material in promoting mass transfer of soot-borne PAH derivatives by determining the partitioning of target compounds to silicone sheet. We showed before with soot-borne PAHs that this function is well accomplished by the silicone sheet (Zhang et al., 2015a). The second objective is to estimate the residue sorbed fraction in the absence and presence of silicone sheet by characterizing the partitioning between soot digestion residue and digestive fluid, in order to determine the influence on bioaccessibility measurement and to estimate the maximum possible (limiting) bioaccessibility. The third objective is to test the influence of changes in fluid chemistry within the variability of human digestive tract expected upon food consumption on bioaccessibility of PAH derivatives. The results of the derivatives are compared with those of the corresponding parent PAHs in parallel experiments. This is the first study that concerns the bioaccessibility of PAH derivatives within digestive tract. It should be noted that PAH derivatives in this study refers only to the single nitro-PAH and four oxy-PAHs that are present natively at readily quantifiable concentrations in the soot composite of interest, and does not include other nitro- and oxy-PAHs, nor other types of derivatives like alkyl PAHs.

2. Experimental section

2.1 Materials and chemicals

The fuel soot was a composite sample collected from local commercial boilers and provided by a cleaning company in Connecticut. The composite was mixed thoroughly and then filtered through a 75 μ m mesh sieve. The fuel soot contained by weight 14.5% C, 14.2% organic C, 36.4% O, 13.9% S, 2.36% H, 0.66% N determined using an elemental analyzer; 11.8% Fe determined by ICP-MS; and 33.5% ash determined by combustion at 900 °C for 4 h. The relative percentage of carbon functional groups was analyzed by solid-state ¹³C-NMR spectroscopy (Avance III, Bruker) using the cross-polarization/magic angle spinning (CP/MAS) technique, after treatment with HCl to remove mineral components (Mitchell and Simpson et al., 2013). Dichloromethane extraction (5.0 g with 100 mL for 24 h, followed by filtration and rotary-evaporation of solvent at 27 °C) was performed to determine whether the soot contained significant levels of unburned fuel components or derivatives that could potentially influence bioaccessibility; the dichloromethane-extractable fraction was only 0.49% by weight. The surface area, total porosity, pH, and sulfate content were provided previously (Zhang et al., 2015b).

Five native PAH derivatives, 1-nitropyrene (1N-PYR), 9-fluorenone (9FLO), anthracene-9,10-dione (ATQ), benzo[a]anthracene-7,12-dione (BaAQ), and benzanthrone (BZO) (Table S1), were tested. The four corresponding parent PAHs tested for comparison are pyrene (PYR), fluorene (FLO), anthracene (ANT), and benzo[a]anthracene (BaA) (Table S1). See Supplementary Information (SI-1) for sources of chemicals, standards, enzymes, bile acids, lipid (soybean oil), and silicone sheet.

2.2 Digestive model

The *in vitro* digestive model sequentially simulated the salivary, gastric, and small intestinal stages. The silicone sheet was added at the small intestinal stage, where most absorption of nutrients, pharmaceuticals, and contaminants occurs. The details of the model were reported previously (Zhang et al., 2015a; 2015b) and are provided in SI-1. The default conditions were: 37 °C; 50 mg soot; gastric pH, 1.21; small intestinal pH, 6.82; combined salivary, gastric, and small intestinal fluid volume (“digestive fluid”), 32.5 mL; and

addition of silicone sheet (2.0 g) and bile acid (8.62 g/L) at the small intestinal stage. The default silicone mass in the bioaccessibility tests was 2.0 g based on preliminary absorptive efficiency experiments, which showed 80.5–90.0% absorption of target PAH derivatives in the digestive fluid during a 24-hour incubation period (SI-1, Figure S1). It was also shown in our previous paper that further increase of silicone sheet to 4.0 g and 6.0 g does not increase bioaccessibility of PAHs except for the highest molecular weight ones (Zhang et al., 2015a). Digestive conditions were varied to mimic the changes in fluid chemistry expected to occur with food ingestion (Zhang et al., 2015b), including bile acid concentration, small intestinal pH, and lipid concentration (SI-1).

After simulated digestion, the silicone sheet was withdrawn and the digestive fluid was separated from the solid pellet (“digestion residue”) by centrifugation (3000 rpm, 20 min), which removed all visible particles from the supernatant phase. The masses of target compounds in the sheet (Q_s), digestive fluid (Q_f), and the solid digestion residue (Q_r) were extracted and quantified; their sum is referred to in this paper as the total extractable concentration. The apparent bioaccessibility (B_{app} , %) is defined as the combined fractions of target compound in the digestive fluid and silicone sheet after simulated digestion:

$$B_{app} = \frac{Q_f + Q_s}{Q_f + Q_s + Q_r} \times 100\% \quad (1)$$

The partition coefficient (K_s , mL/g) of target compounds between silicone sheet and digestive fluid was determined with different amounts of standards (50–400 ng) after 24 h of incubation under default conditions at 37 °C. A 24-h equilibration period was deemed sufficient based on the time needed to reach apparent equilibrium in the case of the eleven PAHs (~4 h) (Zhang et al., 2015a). The concentrations in the sheet (c_s , µg/g) and fluid (c_f , µg/mL) were linearly regressed and the slope was taken as the 24 h partition coefficient ($K_{s,24\text{ h}}$).

2.3 Sample extraction, cleanup, and analysis

PAH derivatives in silicone sheet and digestive fluid were extracted by acetone and *n*-hexane, respectively.

The soot digestion residue was extracted by *n*-hexane/acetone mixture (1:1, v/v) using a microwave-accelerated system (CEM Mars Xpress). The extracts were concentrated and purified by a silica/alumina column. Internal standards were spiked before analysis with a gas chromatograph coupled with a mass selective detector (GC/MSD, Agilent 7890A/5975) in negative chemical ionization mode. Details of the extraction, cleanup, and analysis can be found in **SI-1**. The detection limits and method recoveries of the PAH derivatives and the corresponding parent PAHs for digestive fluid, silicone sheet, and soot residue are provided in **Tables S2** and **S3**. The addition of soybean oil did not affect the extraction efficiency of the target compounds from digestive fluid (**Table S4**). The software SPSS 13.0 (SPSS Inc.) was used for statistical analysis at a significance level of 0.05.

2.4 Governing equations

The soot contaminants may exist in a continuum of states from instantly desorbable to completely entrapped. The practice of dividing sorbed contaminants into sharply-defined compartments, such as “rapidly desorbing”, “slowly desorbing”, and “sequestered”, is widely practiced, however (Ortega-Calvo et al., 2015). We therefore postulate for simplicity that PAH derivatives can be assigned to either a nonlabile state (C_{NON} , $\mu\text{g/g}$) or a labile state (C_{LAB} , $\mu\text{g/g}$) in the original fuel soot (Zhang et al., 2015a). The assigned quantities are assumed to be specific to a given set of digestive conditions of time, temperature, and fluid chemistry. Molecules in the nonlabile state are those that resist desorption due to tortuous or sterically-hindered diffusion paths, even entrapment in closed pores. Molecules in the labile state are those that are capable of desorbing into the digestive fluid—i.e., are exchangeable—but may or may not do so for thermodynamic reasons (Cornelissen et al., 2005). The portion of soot contaminants that remains with the solid after digestion is referred as residue sorbed fraction ($B_{\text{r,lab}}$, %). It does not appear in the fluid or sheet phases at the time of measurement; thus it does not contribute to the apparent bioaccessibility (B_{app}). In order to determine $B_{\text{r,lab}}$ and characterize its impact on B_{app} for each compound, we estimated the distribution ratio between digestion residue and digestive fluid ($K_{\text{r,lab}}$, mL/g) under the default digestion conditions. It is to be understood that $K_{\text{r,lab}}$ is time dependent, and therefore not a true partition coefficient.

Different masses of fuel soot, 25 mg, 50 mg, 75 mg, and 100 mg, were digested without silicone sheet and the measured fluid-phase concentration (c_f) and soot mass (m_r , g) were used to estimate c_{LAB} and $K_{r,lab}$ by regression of the following equation:

$$c_f = \frac{c_{LAB}}{\frac{V_f}{m_r} + K_{r,lab}} \quad (2)$$

where V_f (mL) is the digestive fluid volume, 32.5 mL. Silicone sheet was excluded here to obtain higher thus more accurate c_f . The value of $B_{r,lab}$ is calculated by substituting $K_{r,lab}$ into eq 3:

$$B_{r,lab} = \frac{K_{r,lab} c_f m_r}{Q_r + Q_s + Q_f} \times 100\% \quad (3)$$

The maximum possible (limiting) bioaccessibility (B_{lim} , %) is given by (Zhang et al., 2015a):

$$B_{lim} = B_{app} + B_{r,lab} \quad (4)$$

It may also be calculated according to eq. 5:

$$B_{lim} = \frac{c_{LAB}}{Q_r + Q_s + Q_f} \times 100\% \quad (5)$$

3. Results and Discussion

3.1 Concentrations of PAH derivatives in the fuel soot

The concentrations of the four oxy-PAHs monitored in the fuel soot were found to be: ATQ (3.55 ± 0.12 $\mu\text{g/g}$), BZO (2.22 ± 0.09 $\mu\text{g/g}$), 9FO (1.73 ± 0.06 $\mu\text{g/g}$), and BaAQ (0.395 ± 0.02 $\mu\text{g/g}$) (Table S5). These oxy-PAHs have been detected in other combustion sources such as diesel soot (Cho et al., 2004; Sidhu et al., 2005), but at different concentrations than in the fuel soot. Of the twelve nitro-PAHs in our reference standard, only one (1N-PYR) was found in the fuel soot at a concentration (0.215 ± 0.004 $\mu\text{g/g}$) high enough to be useful for accurately monitoring changes in the digestion tests in this study. 1N-PYR is

thought to result mainly from combustion processes (Bamford and Baker, 2003), whereas many other nitro-PAHs found in atmospheric particles at higher concentrations result primarily from gas-phase photo-initiated reactions of PAHs (Bamford and Baker, 2003; Arey et al., 1986), a pathway not open to the boiler soot sample. The concentration of 1N-PYR was about one order of magnitude lower than the concentrations of the oxy-PAHs, similar to the relative values reported for other environmental particles (Li et al., 2015b; Niederer et al., 1998; Shen et al., 2012; Wei et al., 2015).

3.2 Partitioning of PAH derivatives between silicone sheet and digestive fluid

The silicone sheet is thought to act as a passive absorbent that promotes desorption by steepening the soot-fluid concentration gradient, simulating the net effect of the intestinal epithelium. The 24 h silicone sheet-digestive fluid partition coefficients ($K_{s,24\text{ h}}$) of the PAH derivatives and their corresponding parent PAH are shown in **Figure 1A** (also listed in **Table S6**). First, we note that the $K_{s,24\text{ h}}$ in each case is much less than expected if the fluid phase were pure water due to favorable partitioning into bile acid micelles. This makes sense if we compare $K_{s,24\text{ h}}$ with coefficients that represent partitioning from pure water to a hydrophobic liquid phase (*n*-octanol-water; K_{ow}) or a hydrophobic solid phase (polyoxymethylene-water; K_{POM-w}): $K_{s,24\text{ h}}$ is less than K_{ow} by 1.26–2.64 log units (**Table S1**), and is less than K_{POM-w} by 0.97–3.22 log units (Josefsson et al., 2015). Second, we note that the PAH derivatives are less hydrophobic than the corresponding parent PAH on the same basis: K_{ow} of each oxy-PAH is less than K_{ow} of its parent PAH by 0.58–1.24 log units (**Table S1**) (the K_{ow} of 1N-PYR and PYR are the same), and K_{POM-w} of oxy-PAH is generally less than K_{POM-w} of PAHs by 0.5–1.0 log units (Josefsson et al., 2015). This reflects the influence of substituent polarity on partitioning between water and a hydrophobic phase. Third, we find that $K_{s,24\text{ h}}$ of the PAH derivative is smaller than $K_{s,24\text{ h}}$ of the parent PAH by 0.02–0.43 log units. This indicates that substituent polarity disfavors partitioning into the sheet relative to the bile micelles. The observed negative correlation between $\log K_{s,24\text{ h}}$ and $\log K_{ow}$ for the eleven PAHs (Zhang et al., 2015a) was not apparent for the derivatives, possibly due to the smaller data set size and spread.

Figure 1. Measured 24-h silicone sheet-digestive fluid partition coefficients (log $K_{s,24h}$) (A) and the estimated soot digestion residue-digestive fluid partition coefficients (log $K_{r,lab}$) for individual PAH derivatives (white bars) and the corresponding parent PAHs (grey bars).

3.3 Apparent bioaccessibility of the PAH derivatives

The B_{app} of PAH derivatives with or without the silicone sheet under otherwise default conditions are compared in **Figure 2**. The sum of the five PAH derivatives in the digestive fluid decreased from 6.04 $\mu\text{g/L}$ without sheet to 1.32 $\mu\text{g/L}$ with sheet. PAHs derivatives in the 2.0 g of silicone sheet account for 74.8–87.1% of the total in the fluid and sheet phases. The sharp decrease in fluid-phase concentration as a result of including the sheet is expected to promote desorption of target compounds from the soot. Consistent with this expectation, B_{app} increased significantly for all target PAH derivatives ($p < 0.05$) when sheet was present. The percent increase is greater for the higher molecular weight PAH derivatives: the B_{app} values of BaAQ and BZO almost doubled, whereas the B_{app} of 9FO increased only by 17.9%. The B_{app} with sheet follows the order, $\text{ATQ} > 9\text{FO} > \text{BZO} > 1\text{N-PYR} > \text{BaAQ}$, showing no correlation with molecular weight, water solubility, or K_{ow} . By contrast, a negative correlation of B_{app} with log K_{ow} is often observed for PAHs in soil and soot (Gouliarmou et al., 2013; Tao et al, 2010; Zhang et al., 2015a).

Figure 2. Comparison of the apparent bioaccessibility (B_{app}) of individual PAH derivatives with and without addition of 2.0 g of silicone sheet. For those with silicone sheet, the contributions to B_{app} of the silicone sheet (cross-hatching) and digestive fluid are depicted separately. The default B_{app} of the corresponding parent PAHs is shown as grey circles. Mean and standard deviation of triplicates are shown.

Figure 2 also depicts the B_{app} of the corresponding parent PAHs (grey circles), except for BZO, under default conditions. Comparing derivatives with parent, no clear trend is seen. The B_{app} of 1N-PYR, 9FO, and BaAQ are each less than the B_{app} of the parent PAH, while the B_{app} of ATQ is more than double the B_{app} of the parent PAH. The value of B_{app} in the presence of absorptive sink for hydrophobic contaminants in a solid is determined mainly by two factors: *i*) the labile-nonlabile distribution in the original solid, which is governed by the nature of the solid—i.e., how the PAH derivatives were formed and subsequently condensed with the solid during or after pyrolysis, and the chemistry of interaction between the digestive fluid and the solid matrix; and *ii*) the partitioning behavior of the labile fraction among the sink, fluid, and digestion residue phases, which is governed by molecular properties and soot carbon content. In previous studies, the *in vitro* digestive bioaccessibility of hydrophobic compounds in soil or dust has been found to correlate positively (Tao et al., 2009), negatively (Tao et al., 2010; Weston and Mayer 1998), or not at all (Collins et al., 2013; Fang and Stapleton, 2014) with K_{ow} and/or the organic carbon content. These disparate trends might be explained after realizing that an increase in hydrophobicity simultaneously favors, retention by organic matter in the test material (Pignatello, 2009),

partitioning to bile acid micelles (Wiedmann and Kamel, 2002), and retention by the third-phase sink material, if applied (Josefsson et al., 2015; Gouliarmou et al., 2013), leading to a complex result if the effect is not uniform for these three phases. While substituent polarity clearly influences partitioning behavior through its effects on solubility, hydrophobicity, and possibly specific binding forces with the solid, it is less clear how polarity could influence the original labile/nonlabile distribution.

3.4 Sorption to the soot residue

The soot residue-digestive fluid distribution ratio ($K_{r,lab}$) of labile state PAH derivatives in comparison with parent PAHs are shown in **Figure 1B**. $K_{r,lab}$ is calculated by regression with eq. 2 and the results of the regression are provided in **Table S7**. Recall that $K_{r,lab}$ is not an equilibrium parameter due to the short contact time of the digestive model. Log $K_{r,lab}$ of the derivatives is positively correlated with log K_{ow} ($p < 0.05$), reflecting the effects of increasing hydrophobicity (Zhang et al., 2015a). However, $K_{r,lab}$ of PAH derivatives exceeds $K_{r,lab}$ of the corresponding parent PAH by 0.32–0.70 log units, despite their lower K_{ow} (by 0.58–1.24 log units; **Table S1**) and lower K_{POM-w} (by 0.5–1.0 log units) (Josefsson et al., 2015). This finding suggests that the labile-state PAH derivatives interact with the soot residue more strongly than their hydrophobicity based on K_{ow} would suggest.

In an attempt to further test this hypothesis for a selected derivative, 1N-PYR, we constructed the soot-digestive fluid sorption distribution curves for the spiked model compounds, 1-nitronaphthalene, 2-nitronaphthalene, and naphthalene, to the soot residue under the default conditions (**SI-5** and **Figure S2**). The measured distribution ratio in this time-limited experiment was assumed to represent a hypothetical $K_{r,lab}$ for these model compounds had they been originally present in the soot. The measured log $K_{r,lab}$ followed the order, 2-nitronaphthalene (2.75) > 1-nitronaphthalene (2.43) > naphthalene (2.00), which is the reverse order of their log K_{ow} values (**SI-5**). Thus, substitution of a nitro group enhances sorption to the soot residue, consistent with the log $K_{r,lab}$ measured for the native compounds, 1N-PYR (2.95) > PYR (2.26). The enhanced sorption may be due to π - π electron donor-acceptor interactions between nitro aromatic compounds and polyaromatic surfaces in the soot, which has been shown

previously for chars and graphite (Pignatello, 2013). The solid-state CP/MAS NMR spectrum (**Figure S3**) shows that apolar aromatic C accounts for a large portion (probably more than half) of the total C in the fuel soot. Further discussion accompanied by plausible explanations for substituent effects are given in **SI-5**. Additional research would be required to identify and resolve the contributions of specific driving forces for sorption of the derivatives by the soot.

The $B_{r,lab}$ of each PAH derivative under default digestion conditions without and with the addition of 2.0 g of silicone sheet was calculated according to eq. 3 and is compared with the corresponding values for the parent PAH in **Figure 3**. The $B_{r,lab}$ of PAH derivatives decreased significantly ($p < 0.05$) from 23.5–39.0% in the absence to 2.17–13.7% in the presence of silicone sheet. This proves that the sheet facilitates desorption of the residue sorbed compounds from the soot. However, the $B_{r,lab}$ of each PAH derivative was significantly greater than the $B_{r,lab}$ of its corresponding parent PAH ($p < 0.05$), whether or not sheet was present. This is due to the generally larger $K_{r,lab}$ and smaller $K_{s,24\text{ h}}$ of the derivative compared to the parent (**Figure 1**).

The limiting bioaccessibility (B_{lim}) of the PAH derivatives calculated according to eq 5 ranged from 65.5%–34.4 %, following the order: 1N-PYR > ATQ > 9FLO > BZO > BaAQ (**Figure 3**). These values are similar to B_{lim} obtained by adding $B_{r,lab}$ to B_{app} (eq.4) in the absence or presence of silicone sheet. The B_{app} in the presence of silicone sheet approaches that of B_{lim} , indicating 2.0 g of silicone sheet is sufficient for the extraction of PAH derivatives from 50 mg of soot under default conditions. The B_{lim} is not well correlated with molecular weight, water solubility, or K_{ow} . Moreover, there is no consistent relationship between B_{lim} of the derivatives and B_{lim} of the corresponding parent PAH. These findings suggest that B_{lim} may depend to a large degree on the labile-nonlabile distribution of the compound in the soot and that sorption affinity is of secondary importance.

Figure 3. Estimated maximum possible (limiting) bioaccessibility (B_{lim}), the experimentally determined apparent bioaccessibility (B_{app}) and the estimated labile sorbed fraction ($B_{r,lab}$) remaining on the soot digestion residue without and with the addition of silicone sheet for PAH derivatives and the corresponding parent PAHs. $B_{r,lab}$ and B_{lim} were calculated according to eq. 2 and eq. 5, respectively. Mean and standard deviation of triplicates for B_{app} and $B_{r,lab}$ are shown.

3.5 Effect of fluid chemistry

The effects of changes in digestive fluid chemistry on B_{app} of the five PAH derivatives are revealed in **Figure 4**. We tested changes in physiological conditions that are experienced by humans expected with food intake and that are previously found (Zhang et al., 2015b) to significantly increase B_{app} for PAHs, including bile acid concentration (C_{bile} , 2.0–10 g/L), small intestinal pH (pH 5.84–7.15), and addition of lipid (soybean oil; 4.88–19.5 g/L). These changes in fluid chemistry affect B_{app} by modifying c_{LAB} , K_s , and $K_{r,lab}$. Variation in ionic strength, increase in gastric pH, and increased concentration of protein and carbohydrate (by adding nonlipid milk) had been found to have no effect on B_{app} (Zhang et al., 2015b).

Figure 4. The effect of bile acid concentration (C_{bile}) (**A**), small intestinal pH (**B**), and soybean oil (lipid) concentration (**C**) on the apparent bioaccessibility (B_{app}) of individual PAH derivatives in the fuel soot under otherwise default conditions (columns, left coordinate) and the determined 4-h sheet-fluid partition coefficients ($K_{s,4h}$) (circles, right coordinate) after simulated digestion. The contributions to B_{app} of the silicone sheet (cross-hatching) and digestive fluid are depicted separately. Increases in B_{app} (total bar height, from left compared to the right bar) that are statistically significant ($p < 0.05$) are marked with “*”. Mean and standard deviation of triplicates are shown.

314 The bile acids in the small intestine generally increase from 4–6 mM under fast condition to 10–20 mM
315 after intake of food (Rautureau et al., 1981), thus 2.0–10 g/L were tested for influence of C_{bile} on B_{app} . For
316 all the PAH derivatives except 9FO, B_{app} increased significantly ($p < 0.05$) when C_{bile} was elevated from
317 the fast condition level (2.0 g/L) to the fed condition range (5.0–10 g/L) (Figure 4A). The B_{app} of ATQ,
318 BaAQ and BZO leveled off within the fed condition range of C_{bile} . The plateau was higher for the higher
319 molecular weight, more hydrophobic compounds, as found for PAHs. An increase in C_{bile} drives
320 contaminant to the fluid phase due to micellization and its subsequent effects on contaminant
321 solubilization, which reduces both K_s and $K_{r,\text{lab}}$, and thus causes B_{app} to plateau (Zhang et al., 2015b).
322 Derivatives with higher $K_{r,\text{lab}}$ require a higher C_{bile} to reach the plateau. This is evident from a comparison
323 of the derivatives with the corresponding parent PAH: the plateau of B_{app} occurred at consistently lower
324 values of C_{bile} for the parent PAH (Figure S4) because the $K_{r,\text{lab}}$ of the PAH is lower than that of the
325 derivative in each case (Figure 1B).

326 Small intestinal pH varies among individuals and generally ranges from 4.0 to 8.0, depending on position
327 of the small intestine and diet status (Charman et al., 1997). The B_{app} values of the PAH derivatives at pH
328 5.84 were only about half of those at circumneutral pH (pH 6.89 and 7.15) (Figure 4B). A rise in pH
329 promotes micelle disaggregation due to the increasing charge of bile acid molecules, a situation that
330 disfavors partitioning into micelles and favors partitioning into the sheet. For PAHs, the rise in pH also led
331 to an increase in C_{LAB} and a decrease in $K_{r,\text{lab}}$ (Zhang et al., 2015b). The overall effect of an increase in pH,
332 therefore, is an increase in B_{app} .

333 The B_{app} of the derivatives was unaffected by addition of 4.88 g/L lipid, but increased by 29.5–86.0% at
334 the 9.76 g/L level, and by 16.1–123% at the 19.5 g/L level, relative to the default condition (Figure 4C).
335 The tested lipid concentrations correspond to 50%, 100%, and 200%, respectively, of the mean daily
336 ingested level for children of 2–5 years old in the U.S (Guyton and Hall, 2006; Smithers et al., 2000;
337 Troiano et al., 2000). At the highest rate of lipid addition (19.5 g/L), free-floating lipid was observed in the
338 vial. This may explain the smaller increase in B_{app} from 9.46 g/L to 19.5 g/L lipid than from 4.88 g/L to

9.76 g/L lipid. The B_{app} of the derivatives varies in a similar pattern as with PAHs (Zhang et al., 2015b). Lipid addition results in formation of mixed lipid-bile acid micelles (Dulfer et al., 1996), which favors partitioning of PAH derivatives to the digestive fluid, and thus a decrease in both K_s and $K_{r,lab}$. It is noted that for all the derivatives except 1N-PYR, B_{app} corresponding to the two higher lipid levels exceeded the value of B_{lim} estimated by eq 5 under default conditions where no lipid was present (Figure 5). This indicates that lipids may also be able to promote mass transfer of molecules from the nonlabile to the labile state (i.e., increase in c_{LAB}), probably in a similar fashion as an organic solvent (Tanaka et al., 2015). In short, the net effect of decreases in both K_s and $K_{r,lab}$ and an increase in c_{LAB} is an increase in B_{app} .

Figure 5. Comparison between the maximum possible (limiting) bioaccessibility (B_{lim}) estimated according to eq. 5 under default condition and the apparent bioaccessibility (B_{app}) at lipid concentrations of 9.76 g/L and 19.5 g/L for PAH derivatives. Mean and standard deviation of triplicates for B_{app} are shown.

4. Conclusions

Many PAH derivatives found in soot particles are of concern for their toxicity. Since hand-to-mouth ingestion of environmental particles is an important route of exposure to their entrained contaminants, especially in children, it is important to assess the gastrointestinal bioaccessibility of these contaminants in realistic *in vitro* models when *in vivo* assessments are not possible. A major conclusion that can be drawn from this study is that bioaccessibility of the target nitro-PAH and oxy-PAH derivatives determined in our model responds to digestive conditions in a similar manner as observed previously for the eleven PAH series (Zhang et al., 2015a; 2015b): bioaccessibility increases after inclusion of a third-phase absorptive

sink—more so with increasing compound hydrophobicity—mainly by promoting desorption of the labile residue sorbed chemicals; and bioaccessibility increases with fed conditions, including increases in bile acid concentration, small intestinal pH, and lipid concentration within the normal range of the physiological variability experienced by humans. The agreement in behavior between the derivatives and the PAH series with respect to these factors lends consistency to the digestive model. The increase in bioaccessibility with pH suggests that bioaccessibility in the small intestine will increase as the ingested particles move along the pH gradient from the normally acidic stomach end to the normally neutral (pH 4.0–8.0) environment further into the small intestine (Charman et al., 1997). The increase in bioaccessibility with bile acid and lipid concentrations underscores the generally positive effects of food consumption on bioaccessibility. The observed sharp increase in bioaccessibility with lipid addition of both PAH and PAH derivatives suggests that exposure to chemicals present in environmental particles (soot particles, at least) may be much greater when ingested with food that is high in fat. Since the labile fraction within the particles increases with lipid addition, both among the derivatives and the PAHs (Zhang et al., 2015b), it is possible that lipids extract contaminants present in nonlabile states in a similar manner as would an organic solvent.

We also find that, like the eleven PAH series, the limiting bioaccessibility (B_{lim}) of the derivatives under default conditions falls within the range 34.4–65.5% of the total extractable concentration, and is apparently not well correlated with molecular properties such as molecular weight, water solubility, and K_{ow} . This suggests that bioaccessibility may be more dependent on the nature of the tested soot and the distribution of contaminant between nonlabile and labile states in the original sample, than on molecular properties. The finding that bioaccessibility is substantially under 100% should be considered in risk assessment models. Still, it should be emphasized that, although it is not the focus of this study, the effectiveness of the proposed model should be verified by an *in vivo* test. Only those *in vitro* bioaccessibility that could be calibrated by *in vivo* relative bioavailability could be applied to risk assessment models. However, the modest over-estimation by assuming 100% bioavailability of the

analytical concentration (up to 3-fold) seems small and inconsequential when viewed against other, much larger uncertainties inherent in risk assessment models.

Compared to the parent hydrocarbons, the more polar derivatives have greater affinity for the soot digestion residue, but lower affinity for the silicone. The former finding is interesting and warrants further study of its underlying causes. The latter finding is important as a practical matter and indicates the need for more efficient polymer materials to serve as the sink in digestive models, also for the purpose of better predicting the *in vivo* bioaccessibility value.

Lastly, an important unresolved question is the effect of aging of soot particles in the soil or sediment environment. Aging can lead to transfer of soot-borne contaminants to other soil particles and can affect the surface and pore properties of the soot particles, potentially causing a redistribution of contaminants within the soot itself. Different bioaccessibility patterns may result. The issue of aging will be addressed in a future paper.

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