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Metabolism and Excretion Rates of Parent and Hydroxy-PAHs in Urine Collected after Consumption of Traditionally Smoked Salmon for Native American Volunteers

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Abstract

Few studies have been published on the excretion rates of parent polycyclic aromatic hydrocarbons (PAHs) and hydroxy-polycyclic aromatic hydrocarbons (OH-PAHs) following oral exposure. This study investigated metabolism and excretion rates of 4 parent PAHs and 10 OH-PAHs after the consumption of smoked salmon. Nine members of the Confederated Tribes of the Umatilla Indian Reservation consumed 50 g of traditionally smoked salmon with breakfast and five urine samples were collected during the following 24 hours. The concentrations of OH-PAHs increased from 43.9 μg/g creatinine for 2-OH-Nap to 349 ng/g creatinine for 1-OH-Pyr, 3

to 6 hr post-consumption. Despite volunteers following a restricted diet, there appeared to be a secondary source of naphthalene and fluorene, which led to excretion efficiencies greater than 100%. For the parent PAHs that were detected in urine, the excretion efficiencies ranged from 13% for phenanthrene (and its metabolite) to 240% for naphthalene (and its metabolites). The half-lives for PAHs ranged from 1.4 hr for retene to 3.3 hr for pyrene. The half-lives for OH-PAHs were higher and ranged from 1.7 hr for 9-OH-fluorene to 7.0 hr for 3-OH-fluorene. The concentrations of most parent PAHs, and their metabolites, returned to the background levels 24 hr post-consumption.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are common organic pollutants (Usenko et al., 2007; Usenko et al., 2010). They are formed during incomplete combustion of any carbon-based matter, such as wood (Li et al., 2011), coal (Simoneit et al., 2007), meat (barbequing, charcoaling, grilling) (Akpambang et al., 2009; Alomirah et al., 2011), and others (Baek et al., 1991). Humans are exposed to PAHs mainly through ingestion or inhalation (Motorykin et al., 2015; Suzuki and Yoshinaga, 2007; Wang et al., 2012; Zhang et al., 2014), but dermal exposure is also possible (McClean et al., 2004). Once PAHs are inside the human body, they are metabolized by the family of CYP-450 enzymes to more water-soluble hydroxy-PAHs (OH-PAHs) and excreted via urine (Guo et al., 2013; Jacob and Seidel, 2002; Ramesh et al., 2004). Some portion of unmetabolized PAHs are also excreted via urine (Campo et al., 2007), however the main route of excretion is feces (Bouchard and Viau, 1998; Ramesh et al., 2004), especially for the higher molecular weight PAHs.

PAHs and OH-PAHs pose a threat to human health because some are toxic, carcinogenic (Flowers et al., 2002; Pufulete et al., 2004), and/or mutagenic (Bostrom et al., 2002). The United

States Environmental Protection Agency (U.S. EPA) priority pollutant list includes 16 PAHs and some of these PAHs have been classified as mutagens and animal carcinogens (U.S. EPA, 1992). The World Health Organization also ranked some of the PAHs as possible or probable human carcinogens (WHO, 1998). Some hydroxy-PAHs are more toxic than the parent PAHs and can bind to DNA causing genetic mutations and tumor growth (Wang et al., 2009).

Limited numbers of animal and human studies have been conducted to investigate the fate of orally ingested PAHs. Laurent et al.(2001) studied the concentration of benzo(a)pyrene and phenanthrene in pigs after oral exposure to spiked milk. The peak concentration in blood occurred at 6 hr and 5 hr for benzo(a)pyrene and phenanthrene, respectively. The elimination of phenanthrene, pyrene, and benzo(a)pyrene via milk, urine, and feces in lactating goats was studied by Grova et al. (2002). This study showed that 40.4%, 11.4%, and 6.3% of the total amount of phenanthrene, pyrene, and benzo(a)pyrene was excreted via urine, respectively. Buckley and Lioy (1992) investigated the excretion kinetics of 1-OH-pyrene after oral exposure to benzo(a)pyrene. The estimated half-life of 1-OH-pyrene was 4.4 hr (with a range from 3.1 to 5.9 hr). Zhang et al. (2014) studied dietary and inhalation exposure to PAHs in a Beijing population and found the ingested amount of phenanthrene and pyrene were positively correlated ($p<0.01$) with urinary levels of 2-OH-phenanthrene and 1-OH-pyrene, respectively. Li et al. (2012) studied the excretion rates and half-lives of 10 PAH metabolites after oral ingestion of barbequed chicken. They reported that the half-lives ranged from 2.5 hr for 2-OH-naphthalene to 6.1 hr for 3-OH-flurene. Additionally, the maximum levels of urinary 1-OH-pyrene after oral exposure were 8 times higher than those of heavy smokers (over 20 cigarettes per day) and were similar to urinary levels observed in coke oven workers or graphite electrode plant workers.

These studies indicate the importance of dietary exposure to PAHs and the need for more research on the elimination kinetics of PAHs.

The tradition of smoking game and fish to preserve food is common in many Native American communities. There are different ways to smoke game or fish, including a traditional tipi (Figure S1). The fish fillets are hung approximately 2 m above the fire in the tipi and, as the smoke rises from the fire, the meat is cooked. Depending on the wood type and temperature of the fire, it may take 24 to 48 hr to completely smoke and cook the fish. Gas and particulate phase PAHs are emitted from the fire and deposit onto the surface of the fish. Due to the long cooking time, the PAH concentration sorbed by the fish can become elevated. A recent study showed that the concentration in traditionally smoked salmon was 40-430 times higher than commercially smoked salmon, and was in the range from 2000 µg/kg to 6000 µg/kg (Forsberg et al., 2012). Another study by Duedahl-Olesen et al. (2010) showed that smoking increased the total PAH load in fish meat from 6 µg/kg to 32 µg/kg, however the skin contained the highest PAH concentration of 392 µg/kg (a 65 fold increase compared to raw fish). Because smoked salmon is a traditional food for Native Americans in the Pacific Northwest, it is important to understand the absorption, metabolism and excretion of PAHs in this population.

In this study, nine non-smoking members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) consumed 50 g of traditionally smoked salmon and provided 5 urine samples over a 24 hour period, for analysis of PAH and OH-PAH. The objective of this study was to investigate the metabolism and excretion rates of PAHs and OH-PAHs in members of this Native American community. To the best of our knowledge, this is the first study to investigate the metabolism of PAHs in a Native American community after the consumption of traditionally smoked food.

2. Experimental section

2.1 Reagents and materials

All PAH standards, including naphthalene (Nap), acenaphtylene (Acy), acenaphthene (Ace), fluorene (Flo), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), retene (Ret), benz(a)anthracene (BaA), chrysene (Chr), triphenylene (TriPh), benzo(b)fluoranthene (BbFlt), benzo(k)fluoranthene (BkFlt), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (I(1,2,3-cd)Pyr), dibenz(a,h)anthracene (BahA), benzo(ghi)perylene (BghiPer), and all and OH-PAH standards, including 1-hydroxynaphthalene (1-OH-Nap), 2-hydroxynaphthalene (2-OH-Nap), 2,3-dihydroxynaphthalene (2,3-OH-Nap), 1,3-dihydroxynaphthalene (1,3-OH-Nap), 1,5-dihydroxynaphthalene (1,5-OH-Nap), 1,6-dihydroxynaphthalene (1,6-OH-Nap), 2,7-dihydroxynaphthalene (2,7-OH-Nap), 2,6-dihydroxynaphthalene (2,6-OH-Nap), 9-hydroxyfluorene (9-OH-Flo), 3-hydroxyfluorene (3-OH-Flo), 2-hydroxyfluorene (2-OH-Flo), 1-hydroxy-9-fluorenone (1-OH-Flon), 2-hydroxy-9-fluorenone (2-OH-Flon), 2-hydroxyanthraquinone (2-OH-AntQn), 4-hydroxyphenanthrene (4-OH-Phen), 3-hydroxyphenanthrene (3-OH-Phen), 2-hydroxyphenanthrene (2-OH-Phen), 1-hydroxyphenanthrene (1-OH-Phen), 3-hydroxyfluoranthene (3-OH-Flt), 1-hydroxypyrene (1-OH-Pyr), 2-hydroxybenz(a)anthracene (2-OH-BaA), 3-hydroxybenzo(c)phenanthrene (3-OH-BcPhen), 10-hydroxybenzo(a)pyrene (10-OH-BaP), 12-hydroxybenzo(a)pyrene (12-OH-BaP), 7-hydroxybenzo(a)pyrene (7-OH-BaP), 9-hydroxybenzo(a)pyrene (9-OH-BaP), 3-hydroxybenzo(a)pyrene (3-OH-BaP), 4-hydroxychrysene (4-OH-Chr), 6-hydroxychrysene (6-OH-Chr), 3-hydroxychrysene (3-OH-Chr), 2-hydroxychrysene (2-OH-Chr), 1-hydroxychrysene (1-OH-Chr), 2,6-hydroxyanthraquinone (2,6-OH-AntQn), 11-hydroxybenzo(b)fluoranthene (11-OH-BbFlt) (Table S1) were purchased from AccuStandards, Inc. (New Haven, CT), Sigma-

Aldrich (Milwaukee, WI), MRI Global (Kansas City, MO), VWR international, Inc (Radnor, PA), or TCI America (Portland, OR). The isotopically labeled PAH standards, including [$^{2}H_{10}$]-fluorene, [$^{2}H_{10}$]-phenanthrene, [$^{2}H_{10}$]-pyrene, [$^{2}H_{12}$]-triphenylene, [$^{2}H_{12}$]-benzo(a)pyrene, [$^{2}H_{12}$]-benzo(ghi)perylene, and isotopically labeled OH-PAH standards, including 1-hydroxy[$^{2}H_{7}$]naphthalene, 2-hydroxy[$^{2}H_{9}$]fluorene, 4-Hydroxy[$^{13}C_{6}$]phenanthrene, 1-hydroxy[$^{13}C_{6}$]pyrene, 1-hydroxy[$^{13}C_{6}$]benz(a)anthracene, 3-hydroxy[$^{13}C_{6}$]benzo(c)phenanthrene, 3-hydroxy[$^{13}C_{6}$]chrysene, (Table S1) were purchased from Cambridge Isotope Laboratories (Andover, MA), Santa Cruz Biotechnology Inc. (Santa Cruz, CA), MRI Global (Kansas City, MO), or C/D/N isotopes Inc. (Pointe-Claire, Quebec, Canada). The SPE cartridges, including Bond Elut Plexa (60 mg, 3 mL), Bond Elut C18 (100 mg, 3 mL), and Bond Elut Si (500 mg, 3mL), were purchased from Agilent Technologies (New Castle, DE). All solvents (methanol, hexane (Hex), ethyl acetate (EA), acetonitrile (ACN), and dichloromethane (DCM); all optima grade) and 20 ml clear glass vials were purchased from Thermo Fisher Scientific (Santa Clara, CA). Glass urine collection cups were purchased from VWR International (San Francisco, CA). Acetate buffer (pH=5.5, APHA) was purchased from Ricca Chemical Company (Arlington, TX) and β-glucuronidase/arylsulfatase was purchased from Roche Diagnostics Corporation (Indianapolis, IN). Toluene (>=99.9%) and MTBSTFA (>97%) were purchased from Sigma-Aldrich (Milwaukee, WI). The GC amber vials (1.5 mL) and inserts (300 μl) were purchased from VWR International (San Francisco, CA). Standard Reference Materials (SRMs) were kindly provided by National Institute for Standards and Technology (NIST) (Gaithersburg, MD). Portable coolers (5 L) and freeze packs were purchased through Amazon (Seattle, WA).

2.2 Selection of Study Participants

The study was approved by the Institutional Review Board of Oregon State University, the CTUIR Health Commission, and Portland Area Indian Health Board.

The volunteers were non-smoking Native American adults over the age of 18, with no known occupational PAH exposure. There were total of 9 participants (2 males and 7 females). This sample size is similar to other non-occupational exposure studies, including the study by Li et al. (2012)

2.3 Preparation of Smoked Salmon

Freshly caught spring Chinook salmon from the Columbia River was purchased from a commercial Native American fisherman near Celilo, Oregon and smoked in a traditional tipi three days prior to the start of the experiment. The fish was stored in a refrigerator at 5 °C. Two fish fillets were homogenized (skin was removed) and 50±1 g of salmon was weighed for each volunteer to consume with breakfast on the first day of observation.

2.4 Fish Consumption and Collection of Urine Samples

Three days before the experiment in June 2014, the participants were invited to an informational session where the purpose of the study, and their responsibilities as the participants, were explained. Upon signing consent forms, each participant was given a list of foods to avoid that are known to contain PAHs, including fried, broiled, charcoaled, roasted or toasted foods. Participants were asked to avoid consumption of these foods for the 24-hours prior to the study, as well as for the duration of the observation period. On the day of the study, each participant was given a urine collection kit and a survey to evaluate alternative exposures to PAHs. Participants also received complimentary lunch and dinner with approved foods. All participants and their samples were assigned a unique code to keep their information confidential.

The first urine sample was collected before the consumption of the smoked salmon (8:00 am) and was used to measure background PAH and OH-PAH concentrations. Urine was collected into provided 250 ml plastic cup using mid-stream collection technique, and an aliquot was transferred to a 60 ml amber glass urine collection container. After the initial urine sample was collected, the participants ate breakfast that included the pre-weighed 50g of traditionally smoked salmon along with other approved (non-smoked) food items. Four additional urine samples were collected at approximately 3, 6, 12, and 24 hr (8:00 am next day) after the smoked salmon consumption. The participants stored their urine samples in a cloth cooler containing two ice packs at approximately +5 °C. The coolers were transported to the Oregon State University on the same day the last urine sample was collected. An aliquot (10 ml) of each sample was transferred to a clear 20 ml glass vial for creatinine measurements and the remaining sample was stored at -70°C until analysis. All samples were extracted for PAH and OH-PAH analysis within 2 weeks of sample collection.

2.5 Analytical Method

The analytical method developed for the determination of PAH and OH-PAH in urine is shown in Figure S1 and described in Motorykin et al. (2015). Briefly, a 3 ml aliquot of urine was taken and mixed with 5 mL of acetate buffer (pH=5.5). Fifteen labeled surrogates were spiked into the mixture for quantitation and 10 μl of β-glucuronidase/arylsulfatase was added. The mixture was incubated overnight (37 °C, 16-17 hours) to hydrolyze OH-PAHs. Bond Elut Plexa and Bond Elut C18 solid phase extraction cartridges were used, in series, to extract PAHs and OH-PAHs from the urine matrix. Bond Elute Silica cartridges were then used to fractionate the extract. The extract was loaded onto the silica column and eluted with 5 ml of 5% EA in Hex (the PAH fraction), and with 5 ml of 20% of EA in Hex (the OH-PAH fraction). The two

fractions were concentrated separately to ~20µl under a gentle stream of nitrogen in an amber GC vial. Internal standards (10 µL, 1 mg/L) and 20 µl of toluene were spiked to both fractions. The OH-PAH fraction was solvent-exchanged to acetonitrile (addition of 100 µl and evaporation to ~20 µl) and 30 µl of MTBSTFA was added to it. The OH-PAH fraction was then incubated at 65 °C for 25 minutes and both fractions were analyzed separately by GC-MS (Motorykin et al., 2015). All urine samples were extracted and analyzed in triplicate. The estimated method detection limits in urine was calculated based on response factors according to the US EPA method 8280A (U.S. EPA, 1996) and are shown in Table S1. Creatinine was measured in the urine samples at PeaceHealth Labs (Springfield, OR) using a colorimetric method (Husdan and Rapoport, 1968).

An aliquot of the homogenized smoked salmon (same fillet that participants ate) was analyzed for PAHs according to Forsberg et al. (2012). Briefly, 1g of fish was spiked with isotopically labeled surrogates and extracted with acetone, ethyl acetate, and isooctane (2:2:1; v/v/v), followed by dispersive SPE cleanup (2012). The internal standard was then added to the extract and analyzed by GC-MS using an Agilent 5975B GC−MS with electron impact ionization (70 eV) and a DB-5MS column (30 m length, 0.25 µm film thickness, 0.25 mm I.D.).

2.6 Excretion Efficiency of PAHs in Urine

The PAH excretion efficiency in urine was calculated using Equation 1 for each participant, and individual PAH isomer, measured in the smoked salmon:

$$EE^{j}_{PAHi} = \frac{C\,mean^{j}_{PAHi} * TDC}{M^{fish}_{PAHi}} * 100\% \qquad (1)$$

where, EE^{j}_{PAHi} is the i^{th} PAH + OH-PAH excretion efficiency in urine for participant *j* (%), $Cmean^{j}_{PAHi}$ is the mean excreted concentration of i^{th} PAH + OH-PAH for participant *j* measured

205 at 3, 6, 12, and 24 h minus the concentration measured at 0 h (ng/g creatinine), TDC is the total
206 daily creatinine excretion rate (g creatinine/day), and M^{fish}_{PAHi} is the total amount of i^{th} PAH
207 consumed from the smoked salmon (ng/day). Creatinine is excreted from the human body in
208 urine at a fairly constant rate per day and is dependent on the sex of the individual (1.642 g/day
209 for men and 1.041g/day for women, on average (James et al., 1988)). Because the total daily
210 volume of urine excreted was not measured in this study, we used these creatinine excretion rates
211 in our calculation.

212 *2.7 Pharmacokinetic Model*

213 To calculate the PAH and OH-PAH excretion rate constants and half-lives, the creatinine
214 adjusted PAH and OH-PAH concentrations were used. For every time point, starting at 3 hr post
215 smoked salmon consumption, the means of the individual PAH and OH-PAH concentrations for
216 all 9 participants were calculated and the following nonlinear mixed effects model (Equation 2)
217 was used to the estimate first order excretion kinetics (Bartell, 2012; Li et al., 2012):

218 $$C_i = C_{0i} + a_i * e^{-k_i(t_i - 3)} \quad (2)$$

219 Where C_i is the metabolite *i* concentration at time *t* (mg/g creatinine), C_{0i} is the
220 background concentration of the metabolite *i* (mg/g creatinine), a_i is the initial increase in the
221 concentration of metabolite (mg/g creatinine), t_i is the time of sample collection (hr), and k_i is the
222 first-order elimination rate constant (hrs^{-1}). After estimating k_i, the half-life of each metabolite
223 was estimated using Equation 3 (Li et al., 2012):

224 $$t_{1/2i} = \frac{\ln(2)}{k_i} \quad (3)$$

225 **3. Results and Discussion**

226 *3.1 Creatinine, PAH and OH-PAH Concentrations in Urine and Smoked Salmon*

The mean creatinine concentration for all samples was 115±66 mg/dL, with a range of 16 mg/dL (sample P-2 6 hr) to 249 mg/dL (sample P-5 24 hr, Figure S2). For most participants (except P-3), the creatinine concentrations were highest in the morning, decreased after 3 hr, and then increased again after 6 hr (P-3, P-4, P-5, P-6, and P-8), 12 hr (P-7 and P-9), or 24 hr (P-1 and P-2).

The urinary PAH and OH-PAH concentrations were creatinine adjusted and plotted against time (Figures 1, S3, and S4). Table S3 shows the median, highest and lowest PAH and OH-PAH concentrations for all participants and Figure 2 shows the comparison of these concentrations to the concentrations we measured in urine for our previous study on the inhalation of PAH from $PM_{2.5}$ during fish smoking (Motorykin et al., 2015) and the 2008 National Health and Nutrition Examination Survey (NHANES) values (Centers for Disease Control and Prevention (CDC), 2013). The mean OH-PAH pre-exposure concentrations ranged from 6.9 µg/g creatinine for 2-OH-Nap to 56 ng/g creatinine for 2-OH-Phen, and were in the range of the 25^{th} to 75^{th} percentile of NHANES concentrations. The highest post-consumption OH-PAH concentrations ranged from 43.9 µg/g creatinine for 2-OH-Nap to 349 ng/g creatinine for 1-OH-Pyr. The concentrations of most OH-PAHs were highest three hours post smoked salmon consumption. However, some participants had peak concentrations at six and nine hours post-consumption (Fig 1, S3 and S4). With the exception of 1-OH-Pyr, the median urinary OH-PAH concentrations were higher in this study compared to the NHANES concentrations. The maximum concentrations of OH-PAHs were also higher in this study compared to the NHANES concentrations, except for 1-OH-Nap, 3-OH-Flu and 1-OH-Pyr. However, it should be noted that the NHANES database contains metabolite concentrations for the general U.S. population (with and without previous exposure) and our two exposure studies had controlled exposure to PAHs.

We also compared the results of the concentrations measured in this study with the general population of several Asian countries, reported by Guo et al.(2013). With the exception of 4-OH-Phen and 1-OH-Pyr, the mean concentrations were similar. The mean concentration of 4-OH-Phen was constantly higher, and the mean concentration of 1-OH-Pyr was constantly lower, in this study, compared to Guo et al. (2013).

We compared the results from this study to the results of our previous study, where the inhalation exposure to the smoke while smoking salmon was investigated (Motorykin et al., 2015) (Figure 2). The route of exposure was different between these two studies (inhalation vs ingestion), but both involved a traditional activity (smoking salmon and eating smoked salmon). However, the mean pre-exposure concentrations for the two studies were similar for all metabolites, except for 4-OH-Phen. The median urinary concentrations were similar for the two studies for 2-OH-Nap, 4-OH-Phen, 3-OH-Flu, and 9-OH-Flu. However, the inhalation exposure resulted in higher median concentrations for other metabolites, including 1-OH-Nap, 3-OH-Phen, 1-OH-Phen, 2-OH-Phen, and 1-OH-Pyr. The maximum concentrations, however, were higher for ingestion exposure for most of metabolites, including 1-OH-Nap, 2-OH-Nap, 4-OH-Phen, 1-OH-Phen, 2-OH-Phen, 9-OH-Flu, and 2-OH-Flu. The 3-OH-Phen, 3-OH-Flu, and 1-OH-Pyr maximum urinary concentrations were higher for the inhalation exposure.

Table S4 shows the PAH concentrations measured in the smoked salmon consumed in this study. In the smoked salmon, 1,5-dimethylnaphthalene had the lowest concentration (3 ng/g) , while phenanthrene had the highest concentration (102 ng/g). Figure 3 shows the normalized PAH profiles (the concentrations of all individual PAHs divided by the individual PAH with the highest concentration) for the smoked salmon and urine (sum of PAH + OH-PAH concentrations for the individual PAH isomers). In the smoked salmon, phenanthrene had the

highest concentration, followed by naphthalene (74%), fluorene (42%), pyrene (29%), fluoranthene (24%), anthracene (21%), and benzo[ghi]perylene (8%). The PAH profile in urine was different from the smoked salmon in that naphthalene and its metabolites had the highest concentration, followed by fluorene and its metabolites (23%), phenanthrene and its metabolites (10%), pyrene and its metabolite (3%) and fluoranthene and its metabolite (2%). Anthracene and benzo[ghi]perylene were not detected in urine and no standards were available for metabolites of these two PAHs.

3.2 Excretion Efficiency of PAHs in Urine

Figure 4 shows the excretion efficiency of parent+hydroxy PAHs in urine for each of the nine participants, calculated using Equation 1. The excretion efficiencies for PAH+OH-PAHs were 240±198%, 111±92%, 13±14%, 14±33% and 22±23% for naphthalene (and its metabolites), fluorene (and its metabolites), phenanthrene (and its metabolites), fluoranthene (and its metabolite), and pyrene (and its metabolite), respectively. Excretion efficiencies higher than 100% (for naphthalene and fluorene) suggest an additional source of exposure to these PAHs other than from the smoked salmon and not fully accounted for at 0 h. This could be from inhalation and/or exposure through consumer products. The excretion efficiencies lower than 100% suggest that not all of the OH-PAH metabolites were measured (due to the lack of commercially available standards) and/or excretion via feces. The urinary excretion efficiency was inversely correlated with the log octanol-water partition coefficient (K_{ow}) of the parent PAH (p-value=0.011), suggesting that feces becomes the major route of PAH excretion for PAHs with log K_{ow} greater than 4.5. The parent PAHs, naphthalene, fluorene, phenanthrene, fluoranthene, and pyrene made up 0%, 14%, 42%, 100%, and 56%, respectively, of the total PAH+OH-PAH concentration measured in urine.

3.3 The Ratio of the OH-PAH to PAH Concentrations

The ratio of the OH-PAH to parent PAH concentration in urine was used to better understand the efficiency of PAH metabolism. Figure S5 shows the ratio of the sum of all OH-phenanthrene concentrations to the phenanthrene concentration for all participants. A ratio greater than one indicated that the OH-PAH concentration was greater than the parent PAH concentration, and an increasing ratio indicated that the rate of excretion of metabolites was higher than the parent PAHs. Most of the participants (6 out of 9) had an increased ratio 3 to 12 hr post-consumption, where the excretion of hydroxy-PAHs was higher than parent PAHs (and the ratio was greater than one). After the peak in the ration, there was slower excretion of OH-PAHs compared to parent PAHs. For 3 participants (P-1, P-2, P-9), no peak was observed and the ratio was lower, or close to 1, during the 12 hrs post-consumption. This may be due to differences in hydration among the participants. Participants P-1, P-2, and P-9 had the lowest creatinine concentration during the day (average 45 mg/dl vs average 133 mg/dl for all other participants), indicating that they drank more fluids than the other participants.

Figure S5 also shows the ratio of 1-OH-pyrene to pyrene for six participants (the other three participants did not have concentrations above the limit of quantitation). The ratio peaked at 3-6 hr post-consumption for all participants. For P-1, P-2, and P-9, the ratio was always less than one, even at its peak concentration, and for P-3, P-4, and P-5, the ratio was greater than one at its peak concentration. This also indicated faster elimination of parent PAHs compared to OH-PAHs for P-1, P-2, and P-9.

3.4 Pharmacokinetics and Half-Life Estimates.

Table 1 lists the modeled pharmacokinetics parameters for 5 parent PAHs and 10 OH-PAH and Figure 5 shows the first order elimination curves. The modeled background

concentrations were similar to the measured pre-exposure background concentrations (Table 1), and ranged from 5.3 µg/g creatinine for 2-OH-Nap to 41 ng/g creatinine for 1-OH-Pyr. The smallest and largest percent difference between the modeled and measured background concentrations was 0.2% for 3-OH-Phen and 66.6% for 4-OH-Phen, respectively.

The median half-lives for the OH-PAHs ranged from 1.7 hr for 9-OH-Flu to 7.0 hr for 3-OH-Flu, and were in the same range as the study by Li et al. (2012) (Table 1). The half-lives were statistically significantly lower in this study, compared to the Li et al study (2012), for 3-OH-Phen (3.6 hr vs 4.1 hr), 1-OH-Phen (3.1 hr vs 5.1 hr) and 9-OH-Flu (1.7 hr vs 3.1 hr). The median half-lives for the parent PAHs ranged from 1.4 hr for retene and 3.3 hr for pyrene. The median half-life for phenanthrene was 2.2 hr, compared to 2.6 hr for 3-OH-Phen, 2.9 hr for 4-OH-Phen, 3.1 hr for 1-OH-Phen, and 3.7 hr for 2-OH-Phen. The median half-life for pyrene was 3.3 hr, compared to 4.4 hr for 1-OH-Pyr. These excretion half-lives suggest that parent PAHs are excreted more rapidly than OH-PAHs from the human body. In addition, most of the PAH and OH-PAH concentrations in urine returned to the background concentrations 24-hours post smoked salmon consumption.

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342

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Tables

Table 1. Measured and modeled parameters for the elimination kinetics of PAHs and OH-PAHs.

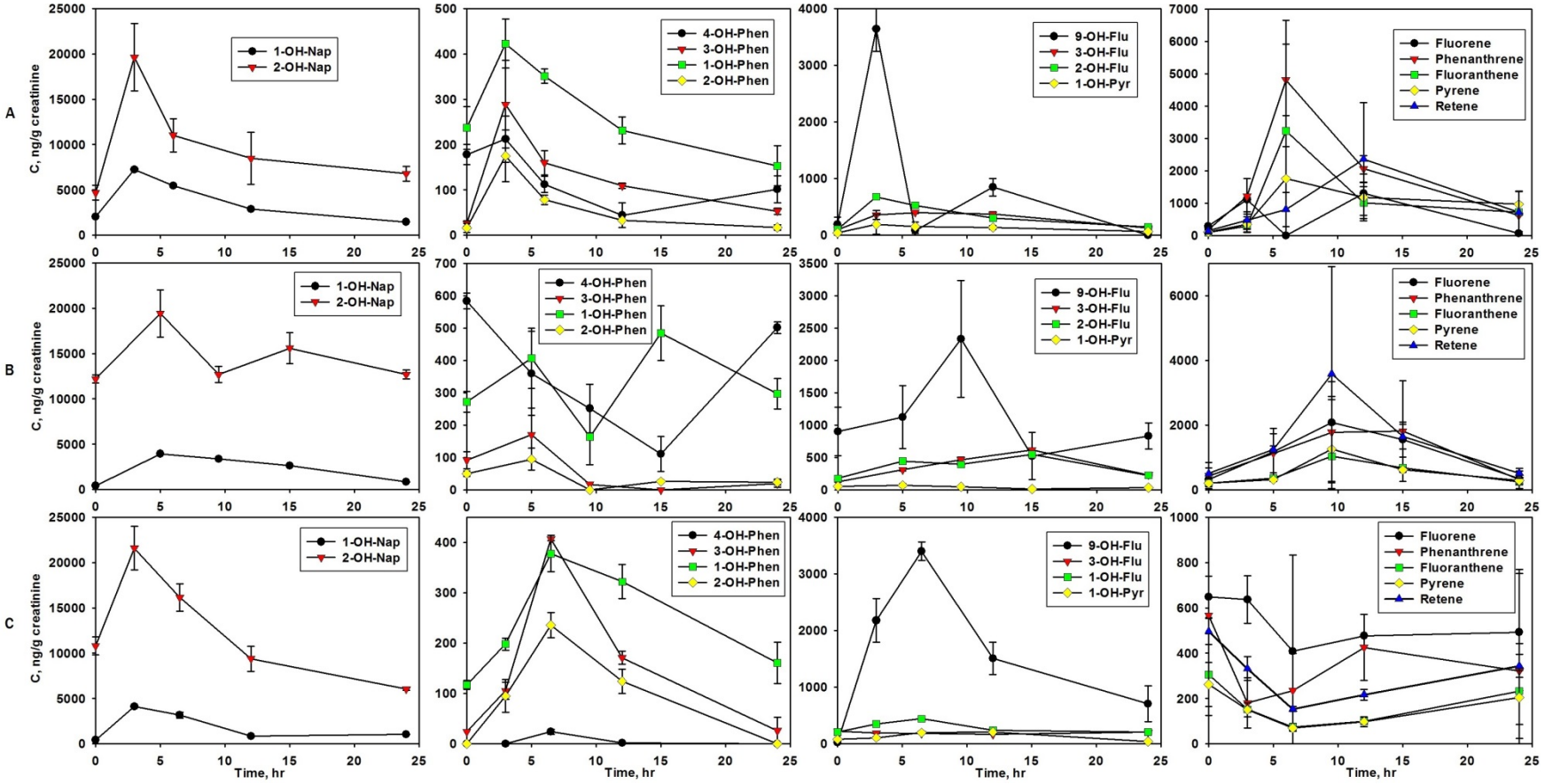
* - statistically different (p-value<0.05), NM – not measured.

		Modeled parameters			Li et al.(2012)
	Mean measured background level, ng/g creatinine	Mean background level, C_0, ng/g creatinine	Mean elimination rate constant (±SE), *k*, 1/hr	Median half-life (95% CI), hr	Median half-life (95% CI), hr
1-Hydroxynaphthalene	761±745	1011±172	0.201±0.020	3.4 (3.1−3.8)	4.3 (3.3−6.2)
2-Hydroxynaphthalene	6932±4751	5294±807	0.288±0.060	2.4 (2.0−3.0)	2.5 (2.0−3.4)
4-Hydroxyphenanthrene	277±194	93±2	0.237±0.012	2.9 (2.8−3.1)	3.5 (2.7−4.8)
3-Hydroxyphenanthrene	68±45	68±11	0.269±0.033	2.6 (2.3−2.9)	4.1 (3.3−5.6)*
1-Hydroxyphenanthrene	208±91	179±9	0.224±0.023	3.1 (2.8−3.4)	5.1 (4.3−6.1)*
2-Hydroxyphenanthrene	56±39	50±15	0.186±0.044	3.7 (3.0−4.9)	3.9 (3.4−4.6)
9-Hydroxyfluorene	602±690	775±4	0.408±0.001	1.7 (1.7−1.7)	3.1 (2.6−3.8)*
3-Hydroxyfluorene	146±70	115±12	0.099±0.011	7.0 (6.3−7.9)	6.1 (4.9−8.1)
2-Hydroxyfluorene	167±48	191±47	0.264±0.081	2.6 (2.0−3.8)	2.9 (2.3−4.0)
1-Hydroxypyrene	67±16	49±8	0.158±0.027	4.4 (3.7−5.3)	3.9 (3.0−5.7)
Phenanthrene	863±486	548±2	0.309	2.2	NM
Fluoranthene	149±87	321	0.248	2.8	NM
Pyrene	161±58	175	0.211	3.3	NM
Retene	267±178	112	0.499	1.4	NM

455 **Figures**

456 Figure 1. Mean creatinine adjusted PAH and OH-PAH concentrations over the 24 hr time period (N=3, the error bars represent

457 standard deviation). The consumption of fish occurred at time zero. A) Participant-1, B) Participant-2, C) Participant-3.



458

Figure 2. Boxplots of urinary OH-PAH concentrations for this study, the CTUIR inhalation study (Motorykin et al., 2015) and for NHANES values from 2007-2008 survey years (Centers for Disease Control and Prevention (CDC), 2013). The grey rectangle represents the first and third quartile (25th and 75th percentile), the line inside is the median, and whiskers are minimum and maximum values. The black diamond is pre-exposure concentration for the ingestion and inhalation studies.

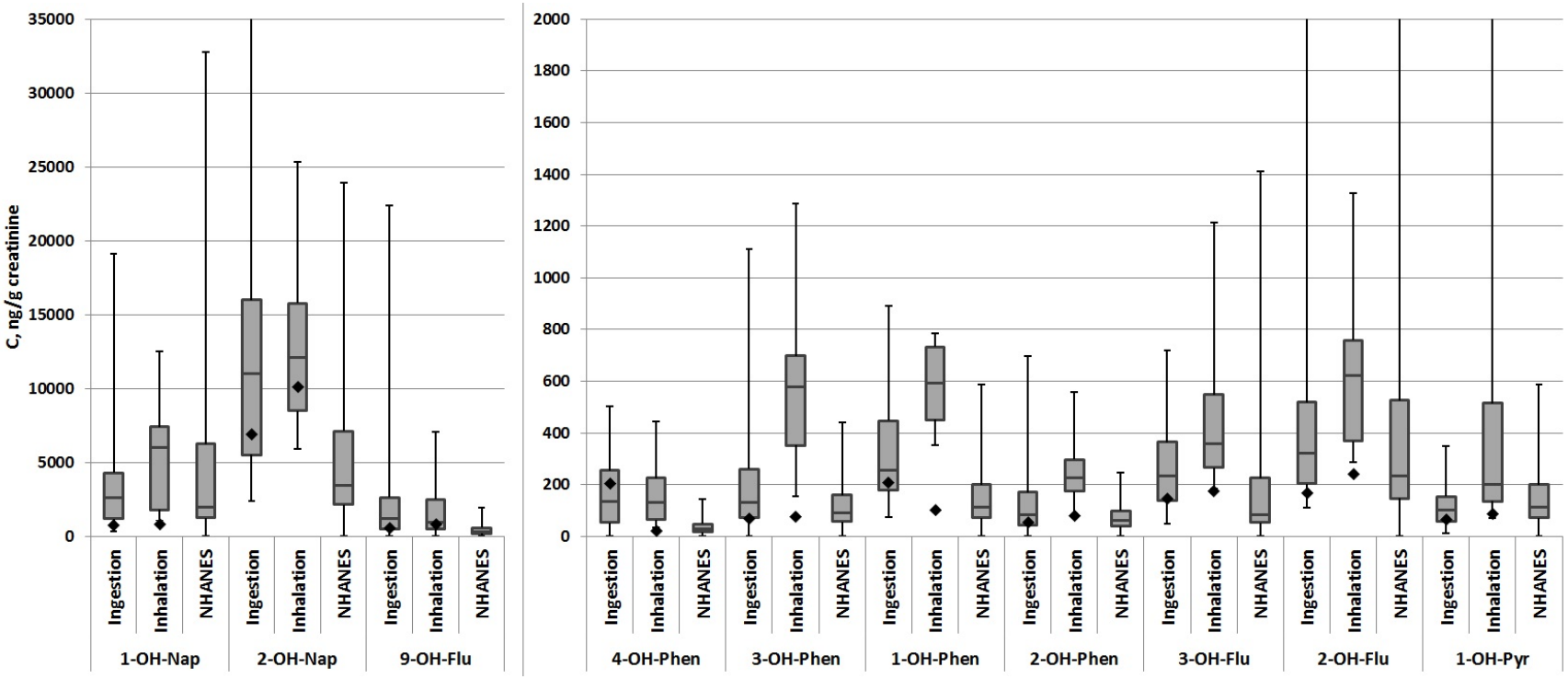
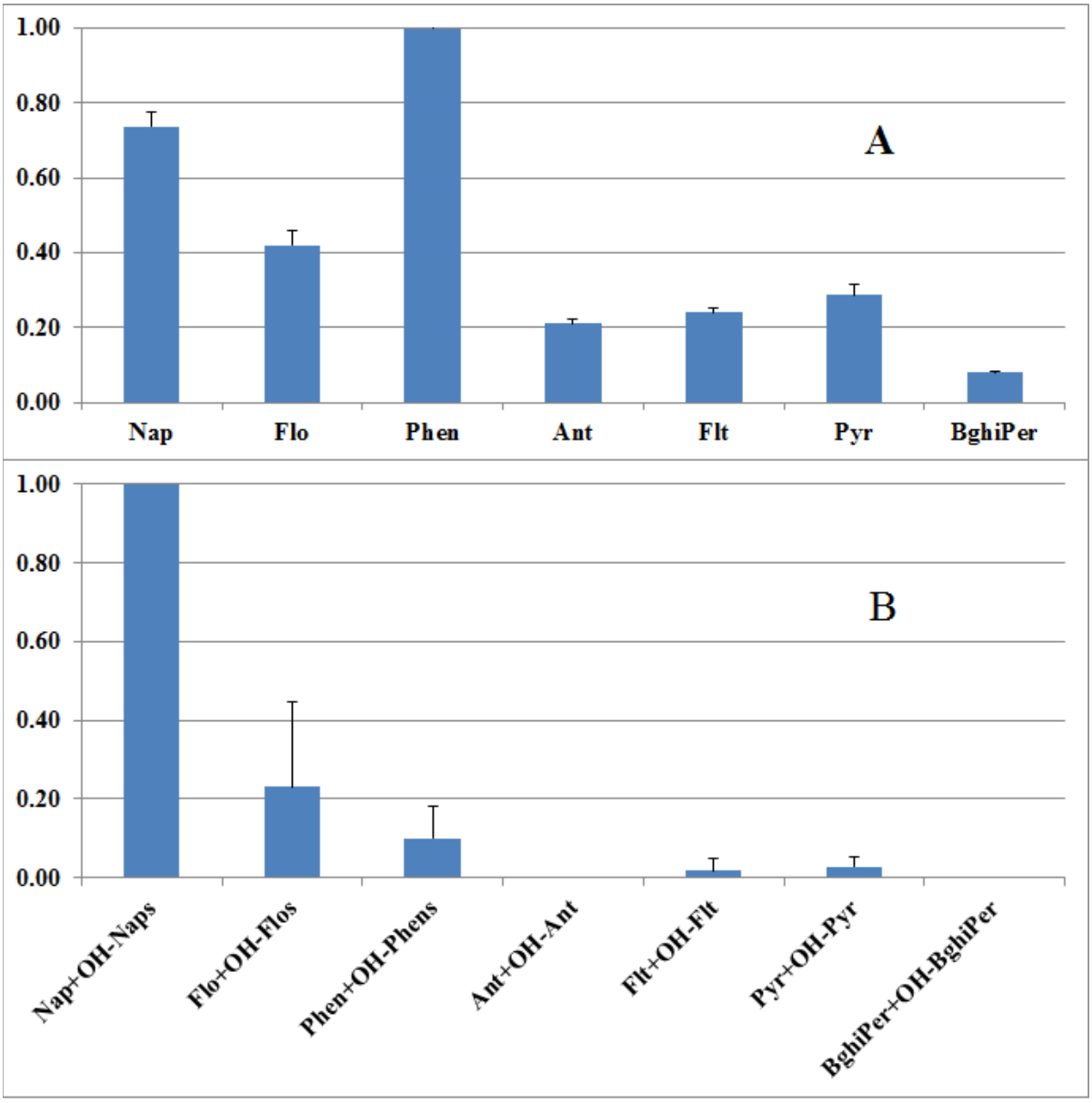
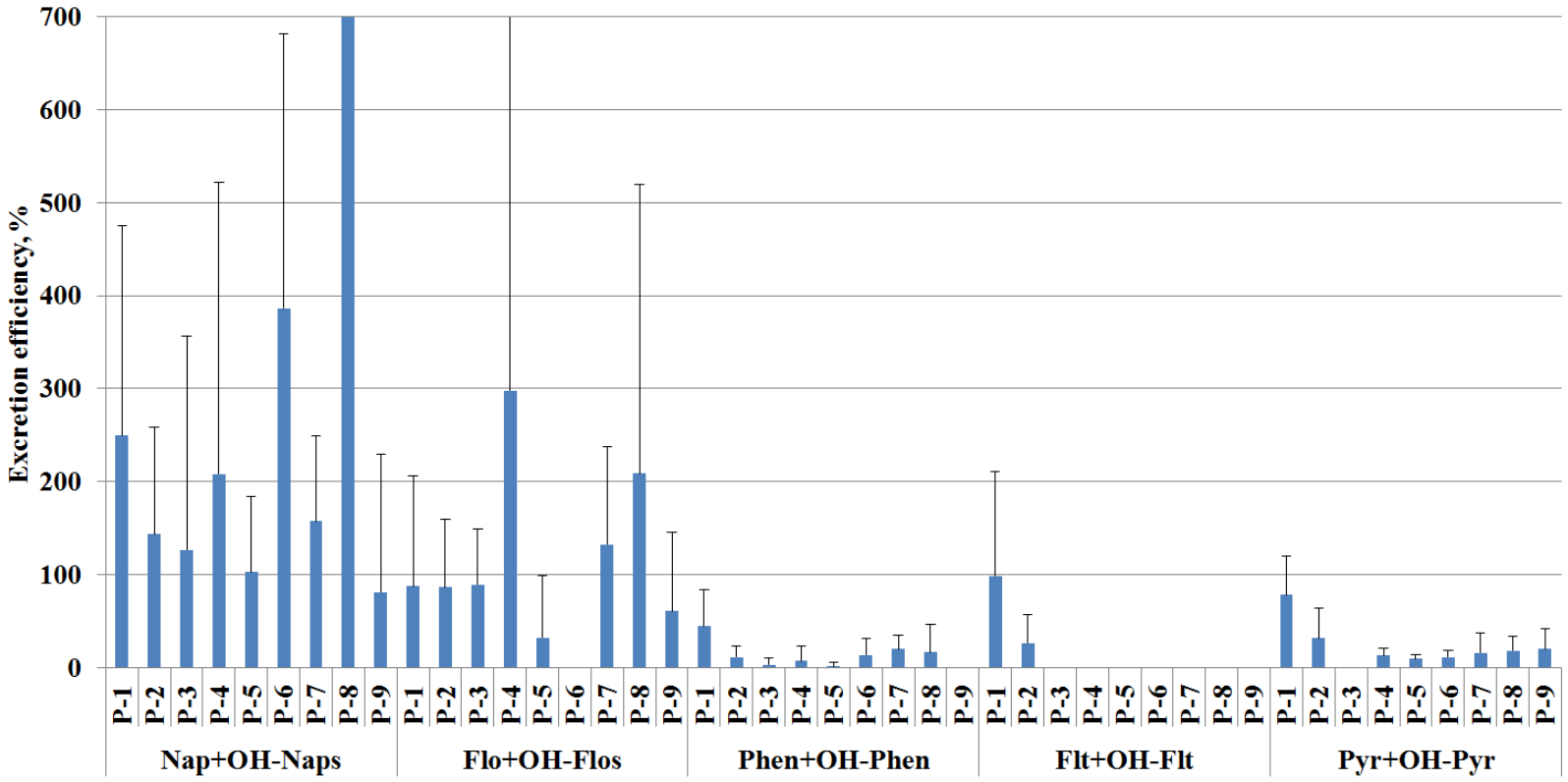


Figure 3. Normalized PAH profile in smoked salmon (A) and normalized PAH + OH-PAH profile in urine (B).



469 Figure 4. Excretion efficiencies of PAH+OH-PAHs in urine calculated using Equation 1. Error bars represent the standard deviation.



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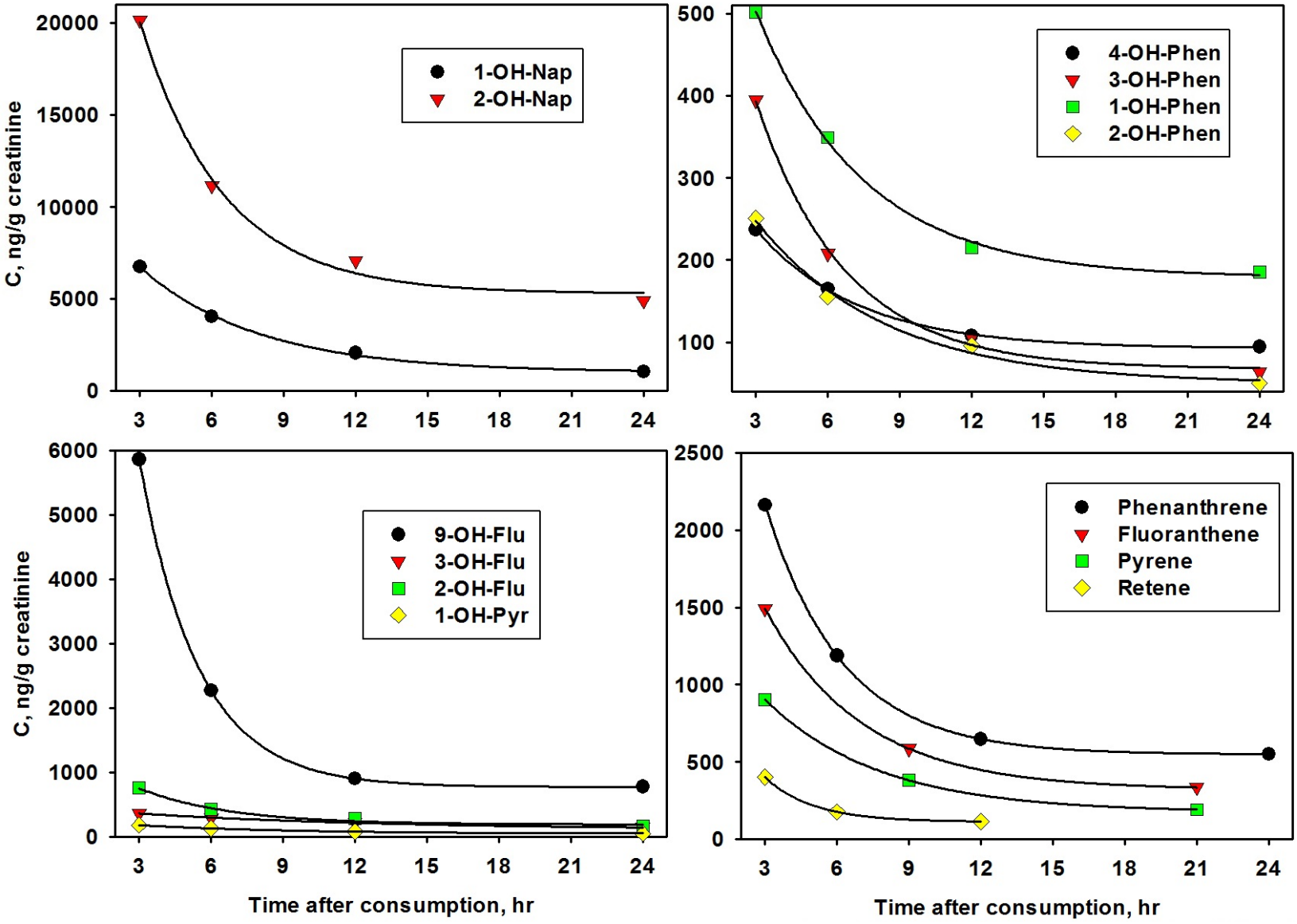
471 Figure 5. Urinary PAH and OH-PAH first order elimination curves. Fish consumption occurred at time zero.



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