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Pharmacokinetics of naphthalene, fluorene, phenanthrene, and pyrene in humans after single oral administration

다환방향족 탄화수소류의 인체 약동력학적 특성: 나프탈렌, 플루오렌, 페난트렌, 파이렌의 단회경구노출

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이 논문을 보건학석사 학위논문으로 제출함

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Abstract

Pharmacokinetics of naphthalene, fluorene, phenanthrene, and pyrene in humans after single oral administration

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Polycyclic aromatic hydrocarbons (PAHs), generated during incomplete combustion of organic matter, have health effects in multiple organs and can cause lung, skin, and bladder cancers in humans. After absorption via ingestion or inhalation, they are metabolized into hydroxy-PAHs (OH-PAHs) by enzymes at cyt-P450 and excreted in urine (mainly low-molecular-weight PAHs) or feces (mainly high-molecular-weight PAHs). Despite a large number of toxicological studies, information on PAHs absorption-distribution-metabolism-excretion (ADME) is very limited in humans. The purpose of this study is to understand the characteristics of absorption, distribution, metabolism, and excretion in the human body after single oral administration of PAHs, and to construct a pharmacokinetic model to explain this. A single dose deuterium-labeled naphthalene (d_8-Nap), fluorene (d_{10}-Flu), phenanthrene (d_{10}-Phe), and pyrene (d_{10}-Pyr) were administered at 0.02~0.04 mg/kg in 9 healthy adults (age: 33.7 ± 8.8 years). Then, serum and urine samples were collected at the designated time for 72 hours after single oral exposure. Parent compounds and ten metabolites were measured by HS-SPME-

GC-MS and LC-MS/MS, respectively. The excretion fraction in urine was calculated based on the time course of urinary excretion amount, and pharmacokinetic analysis was performed by non-compartment analysis, and a pharmacokinetic model composed of two compartments was constructed. As a result of non-compartmental analysis, naphthalene (Nap), fluorene (Flu), phenanthrene (Phe), and pyrene (Pyr) reached the highest concentration in serum within 0.31 h to 1.65 h after exposure quickly, and the half-live ($T_{1/2}$) in serum was confirmed to be within 2.76 h. $T_{1/2}$ and mean residence time (MRT) showed that the larger the molecular weight, the larger the tendency. The 72 h fractional urinary excretion (F_{ue}) of each parent compound was 0.020% for Nap, 0.001% for Flu, 0.011% for Phe, while Pyr was not detected in urine. F_{ue} of sum of urinary OH-PAHs was 1.68% for OHNap, 9.11% for OHFlu, 3.24% for OHPhe, and 11.23% for 1-OHPyr. Most of them excreted within 24 hours after exposure in the form of metabolites rather than the parent compound. Suggestively, PAH and OH-PAHs are excreted via other than urine (*e.g.* feces). In addition, a two-compartment pharmacokinetic model was constructed that described well the serum concentration and urinary excretion amount over time in the human body of naphthalene, fluorene, phenanthrene, and pyrene. This study provides information on the absorption, distribution, metabolism and excretion of naphthalene, fluorene, phenanthrene, and pyrene in the human body. It can be very useful in estimating the average daily intake (or oral-equivalent) amounts of PAHs among general populations.

Keyword: Polycyclic aromatic hydrocarbons (PAHs), Oral administration, Absorption-Distribution-Metabolism-Excretion (ADME), human pharmaco-kinetics, Fractional urinary excretion (F_{ue}), pharmacokinetic model

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Contents

List of Tables

List of Figures

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a chemical compound containing only carbon and hydrogen that is composed of multiple aromatic rings. They are widespread environmental contaminants formed by incomplete combustion of organic matter, such as wood (Li et al., 2011), coal (Simoneit et al., 2007), barbecued meat (Mottier et al., 2000), grilled meat (Kamal et al., 2018; Kim et al., 2021), smoked meat and fish (Alomirah et al., 2011; Wretling et al., 2010), tobacco smoke (Helen et al., 2012), engine exhaust (Tsai et al., 2004), occupational exposures (Stec et al., 2018; Unwin et al., 2006) and so on. It is easily exposed to the human body through several pathways in daily life. The acute or short-term health effects caused by reproductive, developmental, cardiovascular, nervous, and immune systems (Levine et al., 2015; Wells et al., 2010), furthermore skin irritation nausea, vomiting, diarrhea, and inflammation (Unwin et al., 2006; Kim et al., 2013). The chronic or long-term health effects caused by Lung, skin, and bladder cancers of humans (International Agency for Research in Cancer (IARC), 2006), Breast cancer (Shen et al., 2017; Lee et al., 2019), and stomach cancer (Phillips et al., 1999; Rengarajan et al., 2015).

Exposure can occur through ingestion, inhalation and dermal absorption; however, the main pathway to the human body is considered to be ingestion or inhalation (Falcó et al., 2003; Phillips., 1999). Human biomonitoring data from several studies have demonstrated that exposure of the general population to PAHs is widespread (CDC exposure report, 2019; Health canada report, 2017; Ministry of Food and Drug Safety, 2015; Murawski et al., 2020; National Institute of Environmental Research, 2018). When PAHs were exposure the body, most of them are converted into metabolites, in other words, hydroxyl-polycyclic aromatic hydrocarbons (OH-PAHs) by the CYT-P450 enzyme and excreted in the urine (Campo et al.,2006; Guo et al., 2013; Ramesh et al., 2004). In particular, in the case of high molecular weight PAHs, they are excreted mainly through feces (Bouchard and Viau, 1998; Schooten et al., 1997; Ramesh

et al., 2004).

Human exposure studies of some PAHs were conducted, and in this study, we were able to confirm the pharmacokinetics of urinary OH-PAHs and the elimination kinetics of PAHs and OH-PAHs. Viau et al. (1995) measured 1-hydroxypyrene (1-OHPyr) in urine after a single oral and dermal exposure of 500 μg pyrene (Pyr) to one adult male, respectively. Through this, it was confirmed that the half-lives of 1-OHPyr in urine were 12 h, and the average of the urinary cumulative excretion amount compared to the 48 h exposure amount was 3.67% for oral and 0.17% for dermal. Zhong et al. (2011) was investigated d_{10}-phenanthrene (d_{10}-Phe) metabolism in smokers after administration by inhalation in cigarette smoke or orally. Sixteen participants received 10 μg of d_{10}-Phe in a cigarette or orally and collected urine and serum before dosing to 48 h after exposure. Then, samples were analyzed for d_{10}-1,2,3,4-tetrahydro-phenanthrene (d_{10}-PheT), the major end product of the diol epoxide pathway, by gas chromatography-tandem mass spectrometry. Based on plasma area under the concentration-time curve ($AUC_{0-\infty}$) was 1.03 ± 0.32 and a large inter-individual variation in d_{10}-PheT formation was observed. Li et al. (2012) was the first study to estimate the excretion profile and half-live of 10 kinds of polycyclic aromatic hydrocarbon metabolites (OH-PAHs). Participants for 5 males and 4 females consumed and exposed barbecue chicken containing 25-44 μg naphthalene (Nap), 10-17 μg fluorene (Flu), 28-49 μg phenanthrene (Phe), and 12-22 μg pyrene (Pyr), respectively. All urine was collected from 15 h before to 60 h after exposure. PAHs were quantified from chicken samples and 10 OH-PAHs were quantified from urine samples using gas chromatography-mass spectrometry (GC-MS). Urinary OH-PAHs were excreted 58-79% within 12 h after exposure. The total excretion rates of Nap, Flu, and Phe metabolites and 1-OHPyr in 24 h urine were 182%, 60%, 11%, and 6.8%. The time at the maximum concentration (T_{max}) in the urine of the participants reached the maximum concentration within 8.5 h. Motorykin et al. (2015) was investigated the metabolism and excretion rates of Nap, Flu, Phe, and Pyr and 10 OH-PAHs after the consumption of 50 μg smoked salmon which is a traditional way of smoking, tipi to nine Native

American participants. urine was collected before exposure and at 3, 6, 12, and 24 hours after exposure. Urinary PAHs and OH-PAHs were excreted 240%, 111%, 13%, 22% for Nap, Flu, Phe, and Pyr, respectively. The urinary half-lives were 2.2 h for Phe and 3.3 h for Pyr. These previous studies have shown the importance of research on PAHs, but the reality is that there is still a knowledge gap of research on the pharmacokinetics of PAHs.

Human pharmacokinetic data derived from a controlled dosing study provides information on how the human body responds to environmental chemicals. Since general populations have been increasingly exposed to PAHs, it is essential to understand how it is absorbed, distributed, metabolized and excreted in the human body. To our knowledge, there is a shortage of pharmacokinetic data of PAHs. In this study, a single dose deuterium-labeled naphthalene (d_8-Nap), fluorene (d_{10}-Flu), phenanthrene (d_{10}-Phe), and pyrene (d_{10}-Pyr) were administered at 0.02~0.04 mg/kg in 9 healthy adults. Then, serum and urine samples were collected at the designated time for 72 hours after oral exposure. The objective of this study was to calculate urinary excretion fraction (F_{ue}), to confirm the PK parameters after single oral administration of d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr and to explain the compartmental pharmacokinetic model of Nap, Flu, Phe, and Pyr within the body. To our knowledge, this is the first human pharmacokinetic study to include the parent compounds and metabolites in urine and serum of naphthalene, fluorene, phenanthrene, and pyrene.

2. Materials and methods

2.1. Chemicals and reagents

In this study, d_8-naphthalene (d_8-Nap), d_{10}-fluorene (d_{10}-Flu), d_{10}-phenanthrene (d_{10}-Phe), d_{10}-pyrene (d_{10}-Pyr), 2-naphthol-d_7 (2-OHNap-d_7), 2-Hydroxy Fluorene-d_9 (2-OHFlu-d_9), Fluoren-3-ol-d_9 (3-OHFlu-d_9), 1-Phenanthrol-d_9 (1-OHPhe-d_9), 2-Phenanthrol-d_9 (2-OHPhe-d_9), 3-Phenanthrol-d_9 (3-OHPhe-d_9), 4-Phenanthrol-d_9 (4-OHPhe-d_9), 9-Phenanthrol-d_9 (9-OHPhe-d_9) and 1-hydroxypyrene-d_9 (1-OHPyr-d_9) were purchased from Toronto Research Chemicals, Inc. (Toronto, ON, Canada). 1-naphthol-d_7 (1-OHNap-d_7) was purchased from CDN Isotopes (Pointe-Claire, Quebec, Canada). NAPHTHALENE ($^{13}C_6$, 99%), FLUORENE ($^{13}C_6$, 99%), PHENANTHRENE ($^{13}C_6$, 99%), PYRENE ($^{13}C_3$, 99%), 1-Naphthol-$^{13}C_{10}$, 2-Hydroxy Fluorene-$^{13}C_6$, 3-Phenanthrol-$^{13}C_6$ and 1-Hydroxypyrene-$^{13}C_6$ were purchased from CIL (Andover, MA, USA) and used as an internal standard. Ethyl acetate was purchased from Burdick & Jackson (Muskegon, MI, USA) and hydrochloric acid was purchased from chemitop Inc. (Korea). Water and methanol (HPLC grade) were purchased from J. T. Baker (Phillipsburg, NJ, USA). β-glucuronidase/aryl sulfates, Ammonium hydroxide solution, and Sodium chloride (NaCl) were purchased from Sigma-Aldrich Laboratories, Inc. (St. Louis, MO, U.S.A.). NaCl is baked overnight before use. All SPME supplies were obtained from Supelco (Bellefonte, PA). Polydimethylsiloxane (PDMS) fiber (100μm) was used for sampling PAHs from urine headspace.

2.2. Study design and sample collection

Four healthy males and five healthy female volunteers were recruited for this study. They were nonsmokers and adults without occupational exposure to PAHs. A dose of 0.02, 0.04, 0.02, and 0.03 μg/kg-bw of d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr was orally single administered in a chocolate cookie, respectively. The criterion for establishing these exposures is the dose equivalent to the RfD provided by the United States Environmental Protection Agency (EPA), and for Phe where reference dose (RfD) does not exist, the structure is similar and the most conservative Nap value is borrowed. Deuterated Nap, Flu, Phe, and Pyr were used to avoid the potential effects of background concentrations. A dosing solution was prepared by dissolving d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr in 100% ethanol, and spiked to a chocolate cookie just before dosing. The study was conducted according to protocols approved by the Institutional Review Board of Seoul National University, Korea (IRB No. 2009/003-034). All participants were informed about the study design and provided written informed consent in advance of the experiment.

Blood samples were collected just before dosing (0 h) and at the time point of 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72 h after administration. Blood samples were taken into the serum-separating tube (Becton Dickinson, NJ, U.S.A.) using an intravenous cannula (Korea Vaccine, Seoul, Korea) by a nurse. After mixing thoroughly by repeatedly inverting the tube, samples were allowed to clot at room temperature for 30 min and centrifuged for 10 min at 4 ℃, 1300 g. Separated serum was transferred to self-standing screw tubes with a clear O-ring cap and stored at -70℃ until analysis. Likewise, urine samples were collected just before dosing (0 h) and at the time point of 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 h after administration. To determine cumulative excretion, all urine generated during the 72 hours, in addition to the designated point, was collected and volume was recorded. Urine samples were taken into a 1L polypropylene bottle and the volume of each urine sample was also measured. Collected urine was transferred to self-standing screw tubes with a clear O-ring cap and stored at -70℃ until analysis.

2.3. Analytical method

2.3.1. Parent compounds analysis

2.3.1.1. Sample preparation

Analysis of d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr in serum and urine were performed by following the method as previously described with some modifications (Waidyanatha et al., 2003). In brief, 1 ml of serum or urine was transferred to 2 ml crimp amber vials with 0.3 g NaCl. Samples were spiked with 1 µl of a PAH internal standard mixture in methanol to give a final concentration of Nap-$^{13}C_6$, Flu-$^{13}C_6$, Phe-$^{13}C_6$, and Pyr-$^{13}C_3$ at 1 µg/ml. Vials were immediately capped and transferred into an autosampler for gas chromatography-mass spectrometry detector (GC-MSD).

2.3.1.2. Headspace solid-phase micro-extraction

PAL COMBI-xt autosampler (CTC, Zwingen, Switzerland) was used in SPME mode to sample analytes from urine or serum headspace. Analytes were sampled from the headspace using a 100 µm PDMS solid-phase micro-extraction fiber (SPME) (Supelco, Milan, Italy). Pre-incubation temperature and time were 60 ℃, 3 min, and absorption temperature and time were 60 ℃, 30 min, respectively.

2.3.1.3. GC-MS analysis

Samples were analyzed by GC-MSD in electron ionization (EI) mode using Agilent Technologies 7890A coupled with Agilent Technologies 7975C inert XL MSD with Triple-Axis Detector. A DB-5MS column (30 m length, 0.25 μm film thickness, 0.25 mm I.D.) was used with helium as the carrier gas at a flow rate of 1.5 ml/min. A supelco Inlet liner (Ultra Inert, splitless, straight, 0.75 mm i.d., recommended for SPME injections) was used with an injector temperature of 270 ℃. The MS transfer-line and source temperatures were 280 ℃ and 150 ℃, respectively. The GC oven held 75 ℃ for 8 min and was ramped 20 ℃ /min to 270 ℃ where it was held for 10 min. So, the total runtime was 27.75 min, followed by column cleaning by post-run for 300 ℃, 10 min. The following ions were monitored for d_8-Nap (m/z 136), Nap-$^{13}C_6$ (m/z 134), d_{10}-Flu (m/z 176), Flu-$^{13}C_6$ (m/z 171), d_{10}-Phe (m/z 188), Phe-$^{13}C_6$ (m/z 184), d_{10}-Pyr (m/z 212), and Phe-$^{13}C_3$ (m/z 205). The retention times for d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr were 11.34, 14.88, 16.11, and 17.86 min, respectively. The parent compound properties are shown in the supplementary information (Table S1).

2.3.2. Metabolites analysis

2.3.2.1. Sample preparation

Analysis of 1-OHNap-d_7, 2-OHNap-d_7, 2-OHFlu-d_9, 3-OHFlu-d_9, 1-OHPhe-d_9, 2-OHPhe-d_9, 3-OHPhe-d_9, 4-OHPhe-d_9, 9-OHPhe-d_9, and 1-OHPyr-d_9 in urine was performed by following the method as previously described with some modifications (Park et al., 2015). In brief, after thawing at room temperature, an aliquot of 1 ml of urine was transferred into a 15 ml polypropylene tube, and 40 µl of an OH-PAH mixture (final concentration 0.40 ng/ml) was spiked as internal standard. The sample was buffered with 30 µl of 1 N HCl containing 10 µl of β-glucuronidase/aryl sulfates and digested at 36 ℃ for 16 h in an incubator.

Analysis of 1-OHNap-d_7, 2-OHNap-d_7, 2-OHFlu-d_9, 3-OHFlu-d_9, 1-OHPhe-d_9, 2-OHPhe-d_9, 3-OHPhe-d_9, 4-OHPhe-d_9, 9-OHPhe-d_9 and 1-OHPyr-d_9 in serum was performed by following the method. After thawing at room temperature, an aliquot of 0.2 ml of serum was transferred into a 15 ml polypropylene tube, and 40 µl of an OH-PAH mixture (final concentration 0.20 ng/ml) was spiked as internal standard. The sample was buffered with 30 µl of 1 N HCl containing 10 µl of β-glucuronidase/aryl sulfates and add 600 µl acetonitrile to precipitate the protein. After centrifugation at 8000 rpm, 10 min and 0.2 ml aliquot of serum sample was filtered. Transferred into an auto-sampler vial for high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis.

2.3.2.2. Clean-up solid phase extraction

Thereafter, the samples were extracted by clean-up solid phase extraction (SPE). The extraction was carried out after installing the prepared SPE cartridge (Strata-X 33 µm, 1 ml). The extraction was initiated into the condition of cartridges using 2 ml methanol followed by 2 ml of water. A 1 ml aliquot of urine sample was loading then, the cartridge was washed with 2 ml of water and 1 ml 50% methanol and drained completely before adding the elution solvent. Finally, the analytes were eluted with 3 ml of 0.1% Ammonium hydroxide in ethyl acetate. The eluted analytes were concentrated on near-dryness under a gentle nitrogen stream and then reconstituted with 100 µl of methanol, vortex mixed, and transferred into an auto-sampler vial for high performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) analysis.

2.3.2.3. HPLC-MS/MS analysis

An AB Sciex TQ 5500+ (ESI-MS/MS; AB SCIEX, Framingham, MA, USA), coupled AB SCIEX Exion LC system (Framingham, MA, USA) was used for identification and quantification of 10 kinds of deuterated-OH-PAHs. The chromatographic separation was performed by the Acquity UPLC BEH C18 column (1.7 µm, 2.1 x 100 mm; Kinetex, Phenomenex, USA) for 2-OHFlu-d_9, 3-OHFlu-d_9 and 1-OHPyr-d_9 and the new Kinetex F5 column (1.7 µm, 2.1 x 100 mm; Kinetex, Phenomenex, USA) for 1-OHNap-d_7, 2-OHNap-d_7, 1-OHPhe-d_9, 2-OHPhe-d_9, 3-OHPhe-d_9, 4-OHPhe-d_9 and 9-OHPhe-d_9. The sample injection volume was 2 µl and mobile phases were water (solvent A) and methanol (solvent B) at a flow rate of 300 µl/min. Target analytes were detected in the negative ion multiple reaction monitoring (MRM) mode. The source heater was kept at 400 ℃, and the nebulizer gas (ion source 1) and the heater gas (ion source 2) were set at 40, 60 psi, respectively. The target metabolite properties are shown in the supplementary information (Table S1).

2.4. Quality assurance, quality control and validation

Analysis method validation was performed by spiking three to five different concentrations of d_8-Nap, d_{10}-Flu, d_{10}-Phe, d_{10}-Pyr, 1-OHNap-d_7, 2-OHNap-d_7, 2-OHFlu-d_9, 3-OHFlu-d_9, 1-OHPhe-d_9, 2-OHPhe-d_9, 3-OHPhe-d_9, 4-OHPhe-d_9, 9-OHPhe-d_9, and 1-OHPyr-d_9 into urine and serum on two to three different days and evaluating intra- and inter-day precision and accuracy, ranging from 80 to 120% and within 15% RSD, respectively. For detailed results of these are shown in the supplementary information (Table S2, S3). A procedure blank (positive control), a spiked blank (negative control), and one or two matrix-spiked samples were analyzed for each set, following the same procedures as described above. For detailed results of these are shown in the supplementary information (Table S4). The procedure blank was prepared to use HPLC grade water instead of urine or serum sample. PAHs and OH-PAHs were not detected in any of the procedure blanks. The spiked blank was a sample that spiked the internal standard mixture of the concentration. The matrix-spiked samples were sampling that spiked analyte standard and internal standard at the concentrations we know. The calibration curve, ranging from 0.001 to 0.5 ng/ml for urine parent compounds, 0.01 to 5 ng/ml for serum parent compounds, 0.01 to 30 ng/ml for urine metabolites, and 0.05 to 30 ng/ml for serum metabolites, was plotted based on a logarithmic ratio of the peak area of PAHs or OH-PAHs to the peak area of the internal standard versus the logarithm of the PAHs or OH-PAHs concentration, whose regression coefficient (R) was >0.999 in urine and serum. The limits of quantitation (LOQ) were determined based on the lowest point where the logarithm form of the calibration curve, consisted of very low concentrations, became the straight line, and the LOQ of PAHs or OH-PAHs was 0.001 ng/ml for urine parent compounds, 0.01 ng/ml for serum parent compounds, ranging from 0.01 to 0.03 ng/ml for urine metabolites and ranging from 0.06 to 0.27 ng/ml for serum metabolites, respectively. For detailed results of these are shown in the supplementary information (Table S4). For chemicals that were detected no chromatogram, N.D. was used to replace the zero.

2.5. Non-compartmental analysis

Based on the time course of d_8-Nap, d_{10}-Flu, d_{10}-Phe and d_{10}-Pyr in serum, we estimated the descriptive kinetic parameter. That is to say, we implemented the non-compartmental analysis using WinNonlin (Pharsight, St. Louis, MO, U.S.A.). Several descriptive pharmacokinetic parameters, such as maximal concentration (C_{max}), peak time (T_{max}), terminal half-live ($T_{1/2}$), area under the curve (AUC), under the first-moment curve (AUMC), and mean residence time (MRT) were estimated.

Based on the time course of d_8-Nap, d_{10}-Flu, d_{10}-Phe, d_{10}-Pyr and their metabolites in urine, we estimated the elimination rate constant (K_u) and terminal half-live ($T_{1/2}$) by constructing the amount remaining to be excreted (ARE) plot or urinary excretion rate (Rate) plot (Boroujerdi, M., 2015; Gabrielsson, J., & Weiner, D., 2001). In addition, the fractional urinary excretion (F_{ue}) was calculated based on the amount of each parent compound and their metabolites excreted in urine over 72 h divided by orally administered dose. F_{ue}, in other words, the fraction of urinary excretion or urinary excretion fraction, refers to the ratio of the total amount excreted from the administrated dose to the urine as the excretion fraction in the urine.

2.6. Pharmacokinetic model

2.6.1. Construction model structure

A two-compartment model was constructed to describe the pharmaco-kinetic properties of Nap, Flu, Phe, and Pyr in the human body after a single oral administration (Figure 1). The rate of absorption, distribution, metabolism, and excretion was assumed to be first-order kinetics and the model includes pathways that are excreted in urine and other routes.

2.6.2. Model optimization and validation

The constructed two-compartment model was fitted into the time course in urine and serum from randomly picked six participants (three males and females, each) using Berkeley Madonna (version 8.3.9, University of California, Berkeley, CA, U.S.A.). The optimized model was validated by evaluating the model prediction using urine and serum data from the rest of the three participants.

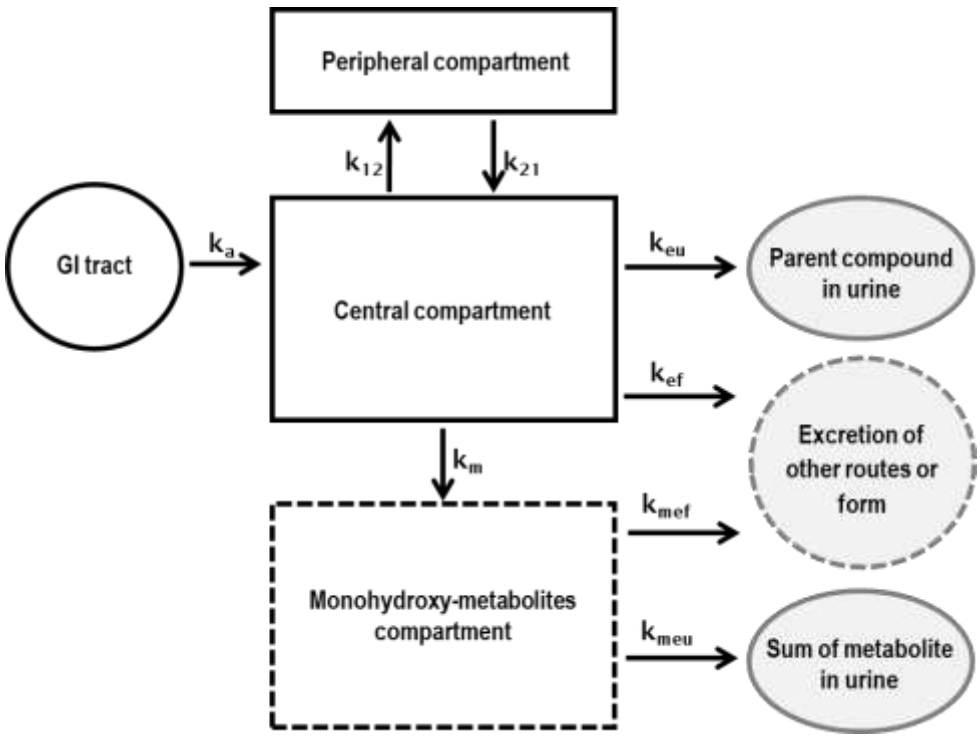

Figure 1. Structure of the two-compartment model for naphthalene, fluorene, phenanthrene, and Pyrene.

Abbreviation: GI tract, gastrointestinal tract; k_a, absorption in the gastrointestinal tract; k_{12}, distribution from the central compartment to the peripheral compartment; k_{21}, reabsorption from the peripheral compartment to the central compartment; k_{eu}, parent compound elimination with urine at central compartment; k_{ef}, parent compound elimination with other routes; k_{mef}, other forms except for parent compound elimination with other routes; k_m, metabolism from the central compartment to the monohydroxy-metabolites compartment; k_{meu}, a sum of metabolites elimination with urine at monohydroxy-metabolites compartment.

3. Results

3.1. Participant characteristics

Four healthy male and five healthy female volunteers participated in the study, and demographic characteristics are shown in Table 1. Their average age was 33.7 ± 8.8 years (35.5 ± 11.2 for males, 32.4 ± 7.4 for females) and BMI was 23.5 ± 3.7 kg/m^2 (23.0 ± 1.9 for males, 23.8 ± 4.9 for females). All participants are currently nonsmokers and four have smoked in the past (MT, MB, MC, FD). Two participants are usually exposed to second-hand smoke (FA, FD), and three participants are usually taking herbal medicine made from worn-out medicinal materials (FA, FB, FE). Participants were found to mainly use buses, taxis, or their own vehicles.

Table 1. Demographic characteristics of participants.

Participants	Age	Height (cm)	Body weight (kg)	BMI (kg/m^2)
MT	52	171	62	21.20
MB	31	175	67	21.88
MC	28	168	67	23.74
MD	30	171	74	25.31
FA	27	171	55	18.81
FB	40	165	57	20.94
FC	26	156	52	21.37
FD	28	157	75	30.43
FE	41	167	77	27.61

3.2. Pharmacokinetic characteristics of PAHs and OH-PAHs

The semi-logarithmic plot of the time course for d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr in Figure 2. After single oral administration, the serum concentration of deuterated-PAHs increased rapidly within 2 h, and deuterated-PAHs were observed in serum until 24 h After the peak time, PAHs concentration decreased in two phases, fast phase, and slow phase in order. However, care must be taken in interpretation as the concentration of posterior time points is close to the LOQ. The LOQs for Nap, Flu, Phe, and Pyr are 0.07, 0.06, 0.05, and 0.05 nM. On the other hand, the detection rate of nine metabolites excluding 2-OHFlu was less than 20%.

Cumulative urinary excretion of deuterated-PAHs and deuterated-OH-PAHs from each chemical is shown in Figure 3. The 72 h urinary excretion fraction (F_{ue}) was Nap 0.020%, Flu 0.001%, and Phe 0.011% for parent compounds, and Pyr was not detected. In the case of metabolites detected in urine, a sum of OH-PAHs was 1.68% for OHNap, 9.11% for OHFlu, 3.23% for OHPhe, and 11.23% for 1-OHPyr. The excreted amounts of parent compounds and OH-PAHs in 24 hours after oral administration reached 81% (Nap), 88% (Flu), 89% (Phe), and 95% (Pyr) of those in 72 hours. Most of the PAHs and OH-PAHs can be confirmed to be excreted within 24 hours in the form of metabolites rather than the parent compound. Suggestively, PAH and OH-PAHs are excreted via other than urine (*e.g.* feces). The values for F_{ue}s are shown in Table S5. The F_{ue}s of key metabolites of PAHs in urine were obtained in the present study, which could be useful in estimating the average daily intake (or oral-equivalent) amounts of PAHs among general populations.

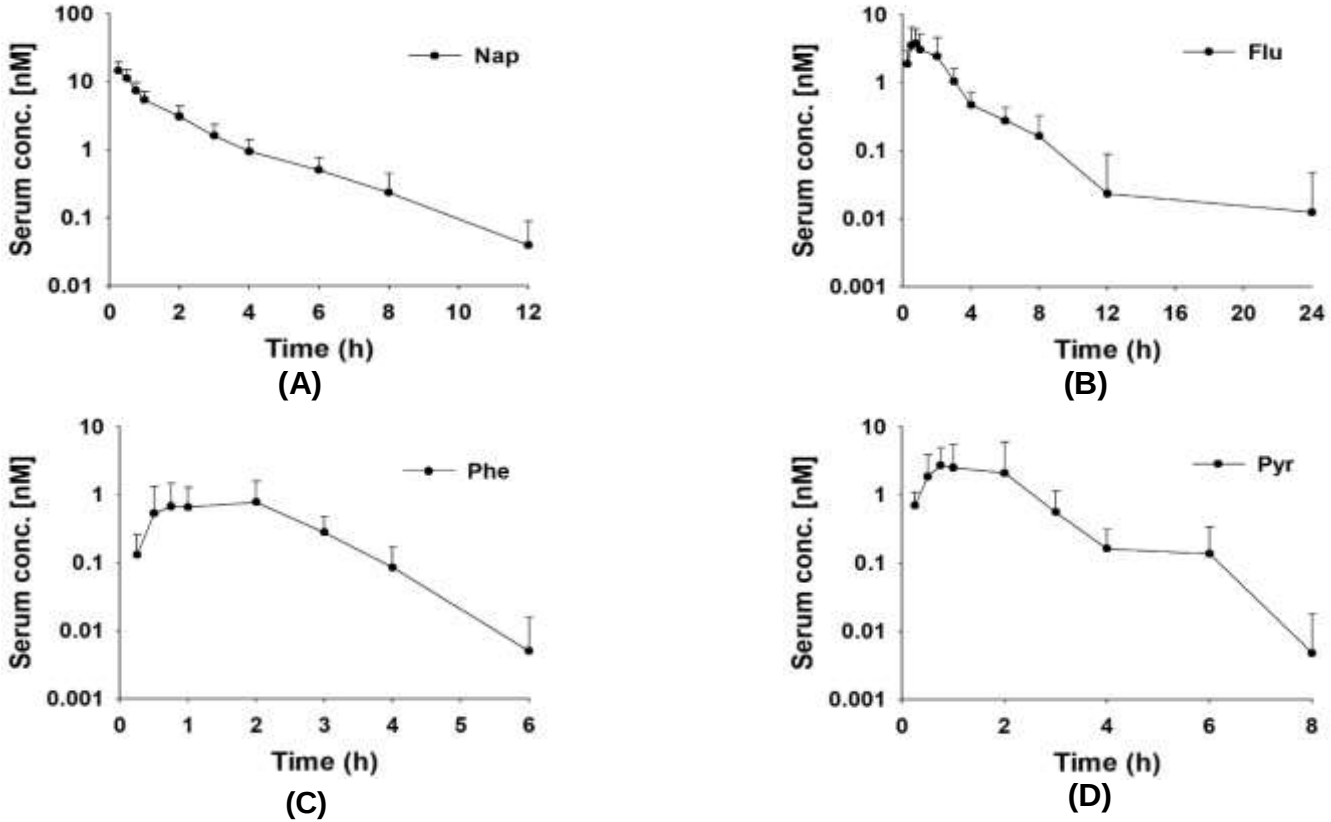

Figure 2. Time course of naphthalene (A), fluorene (B), phenanthrene (C), and pyrene (D) in serum. Each point and error bar represent the arithmetic mean and standard deviation of participants (n=9).

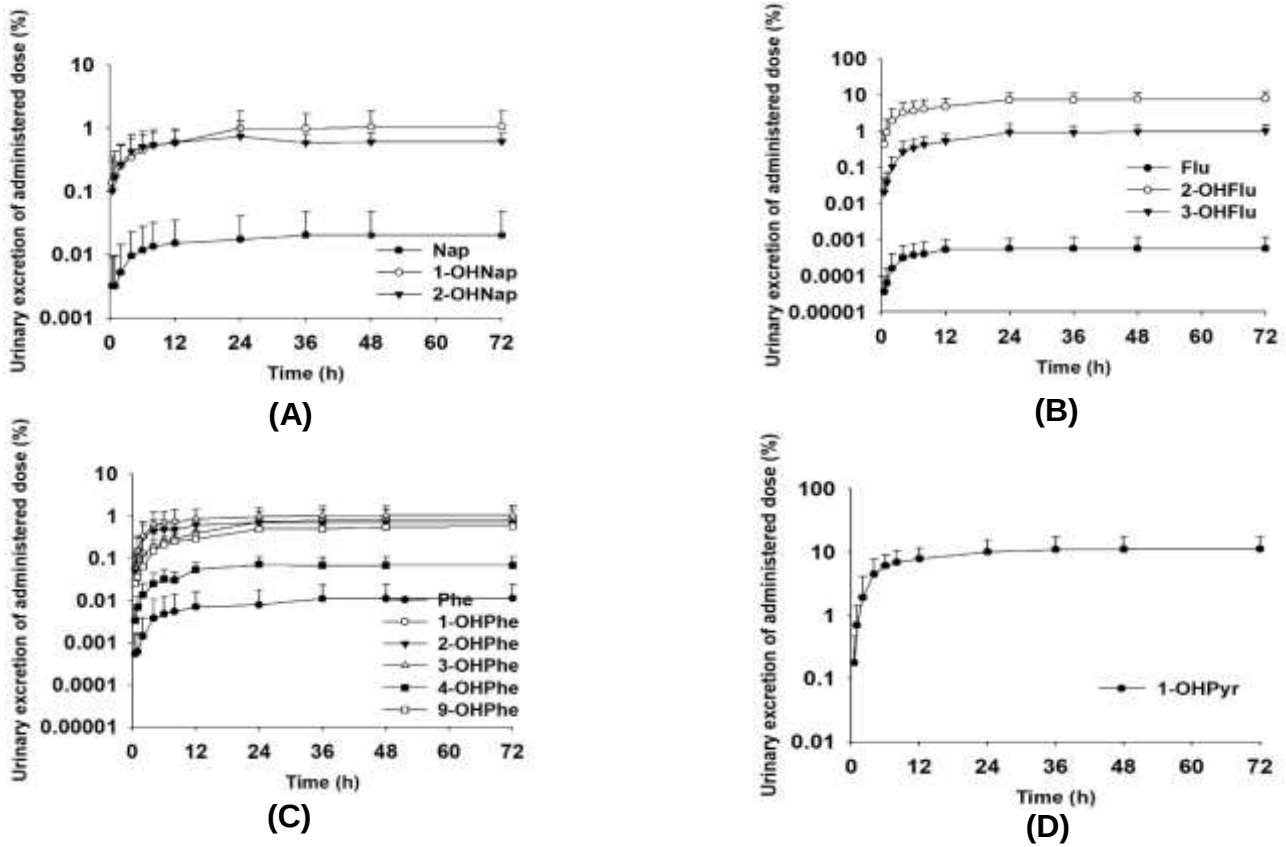

Figure 3. Urinary excretion amount of administrated dose naphthalene (A), fluorene (B), phenanthrene (C), and pyrene (D). Each point and error bar represent the arithmetic mean and standard deviation of participants (n=9).

3.3. Non-compartmental analysis of PAHs

Pharmacokinetic parameter estimates for d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr in serum from the non-compartmental analysis are presented in Table 2. While deuterated-OH-PAHs excluding 2-OHFlu-d_9 in serum had a detection rate of less than 20%, and data analysis wasn't implemented. The highest concentrations (C_{max}) were 14.06 nM for Nap, 3.24 nM for Flu, 0.85 nM for Phe and 2.29 nM for Pyr in serum. The highest concentration times (T_{max}) ranged between 0.31 and 1.65 h. The area under the time-serum concentration curve (AUC) ranged between 2.46 and 18.75 nMh and under the first-moment curve (AUMC) ranged between 5.66 and 37.96 nMh^2. half-live ($T_{1/2}$) in serum were 1.38 h for Nap, 2.09 h for Flu, 2.40 h for Phe and 2.76 h for Pyr and the mean resident time (MRT) were 2.00 h for Nap, 3.06 h for Flu, 2.98 h for Phe and 3.78 h for Pyr showed that the larger the molecular weight, the larger the tendency. The parameter estimates for d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr from non-compartmental analysis by sex and BMI are presented in Table S6 and Table S7.

The elimination rate constant (K_u) and the terminal half-live ($T_{1/2}$) of Nap, Flu, Phe, and Pyr in urine from the amount remaining to be excreted (ARE) plot. The terminal half-live ($T_{1/2}$) were 3.22 h for Nap, 6.19 h for Flu, 9.24 h for Phe, 10.35 h for 1-OHNap, 11.36 h for 2-OHNap, 12.38 h for 2-OHFlu, 12.16 h for 3-OHFlu, 10.19 h for 1-OHPhe, 9.50 h for 2-OHPhe, 8.15 h for 3-OHPhe, 6.54 h for 4-OHPhe, 13.59 h for 9-OHPhe, 8.25 h for 1-OHPyr, and Pyr was not detected. The results calculated by the ARE plot and the results calculated by the rate plot were not significantly different. The parameter estimates for d_8-Nap, d_{10}-Flu, d_{10}-Phe, and d_{10}-Pyr from non-compartmental analysis by sex and BMI are presented in Table S8.

Table 2. Parameter estimates naphthalene, fluorene, phenanthrene, and Pyrene in serum from the non-compartment analysis. Each value is presented as arithmetic mean ± standard deviation of participants (n=9).

Parameter	Nap	Flu	Phe	Pyr
Dose (mg)	1.30 ± 0.18	2.61 ± 0.36	1.30 ± 0.18	1.96 ± 0.27
C_{max} (nM)	14.06 ± 4.77	3.24 ± 2.28	0.85 ± 0.76	2.29 ± 3.44
T_{max} (h)	0.31 ± 0.11	1.65 ± 3.09	1.50 ± 0.60	0.89 ± 0.44
$T_{1/2}$ (h)	1.38 ± 0.38	2.09 ± 1.14	2.40 ± 2.41	2.76 ± 3.67
$AUC_{(0\rightarrow\infty)}$ (nM•h)	18.75 ± 5.45	9.78 ± 5.47	2.46 ± 2.07	7.17 ± 7.70
$AUMC_{(0\rightarrow\infty)}$ (nM•h^2)	37.96 ± 14.12	29.74 ± 22.63	5.66 ± 3.20	32.75 ± 55.83
$MRT_{(0\rightarrow\infty)}$ (h)	2.00 ± 0.40	3.06 ± 1.64	2.98 ± 2.20	3.78 ± 4.42

Dose, orally administrated dose; C_{max}, peak concentration; T_{max}, peak time; $T_{1/2}$, terminal half-live; $AUC_{(0\rightarrow\infty)}$, area under the time-serum concentration curve between 0 and ∞; $AUMC_{(0\rightarrow\infty)}$, under the first-moment curve between 0 and ∞; $MRT_{(0\rightarrow\infty)}$, mean residence time.

3.4. Pharmacokinetic modeling of PAHs and OH-PAHs

The two-compartment model for Nap, Flu, Phe, and Pyr were fitted to time-concentration data in serum and urine from randomly picked six participants, three males and three females, to avoid potential effects of sex. Pharmacokinetic parameters were manually adjusted to arrive at a visual best fit for the data. The LOQ of the parent compound in serum is 0.07, 0.06, 0.05, and 0.05 nM, when converted to molar concentrations of Nap, Flu, Phe, and Pyr, respectively. Therefore, values below LOQ reduced the weight when data fitting. As shown in Figure 4, the model showed a good prediction for the time course of PAHs and OH-PAHs in both serum and urine. Using the optimization two-compartment model, we determined the kinetic parameter for Nap, Flu, Phe, and Pyr after single oral administration, as provided in Table 3. The bioavailability was 1, assuming that oral administrated amounts were absorbed by the GI tract. The tendency of the GI tract to be absorbed into the central compartment (k_a) was inversely proportional to the molecular weight. The elimination amount of parent compound in urine (k_{eu}), the elimination amount of parent compound in other routes (k_{ef}), the elimination amount of metabolite in urine (k_{meu}), and the elimination amount of other forms except for parent compound in other routes (k_{mef}) tend to increase in this order, and volume of distribution of central compartment (V_1_F) of Nap, Flu, Phe, and Pyr was 190, 270, 4900 and 880 L, respectively. Half-live ($T_{1/2}$) of elimination was estimated by dividing 0.693 by k_{meu} (Boroujerdi, 2015).

The optimized two-compartment model was validated using time-concentration data in serum and urine from three other participants, one male and two females (Figure 5). The concentrations of Nap, Flu, Phe, and Pyr in serum and the cumulative amounts of PAHs and OH-PAHs in urine were well described by the predicted values from the model.

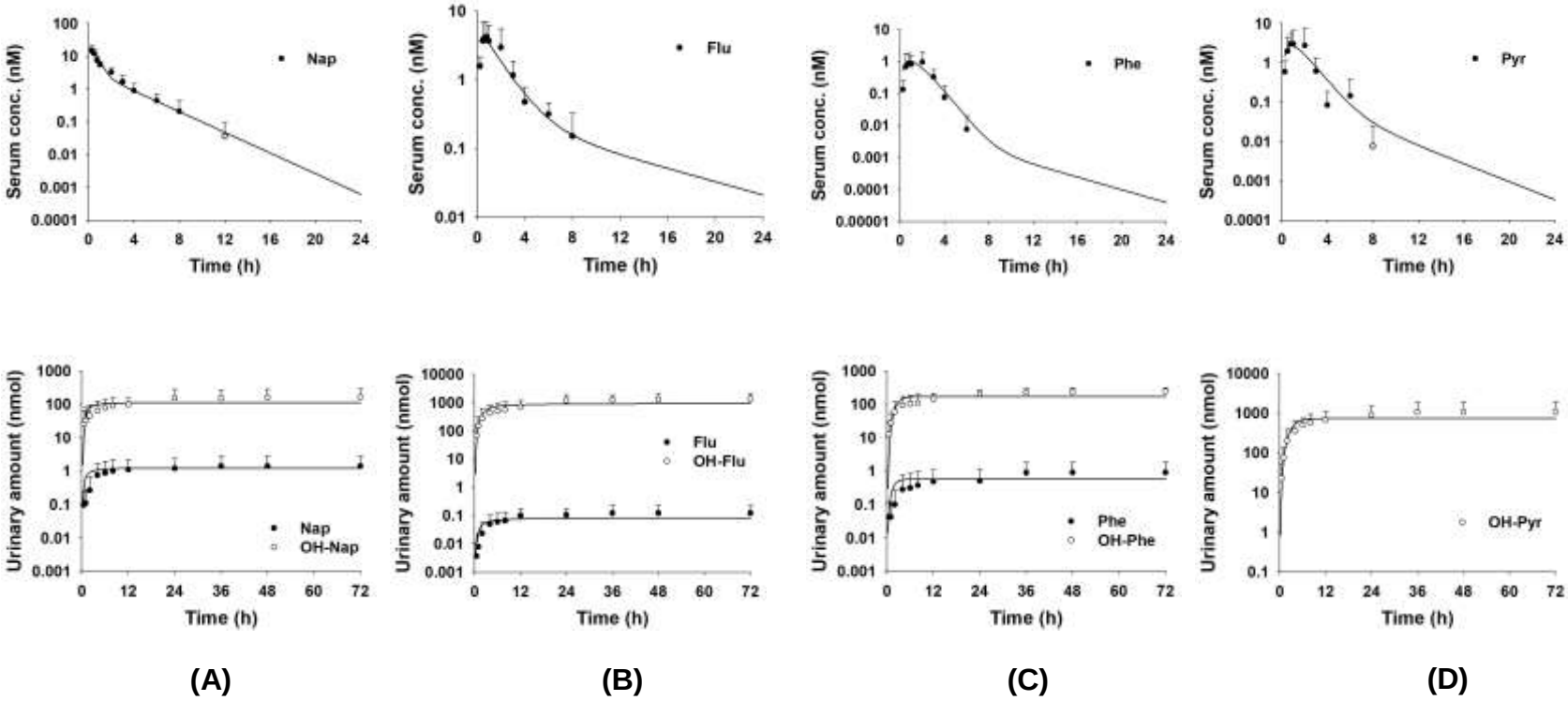

Figure 4. Model optimization of naphthalene (A), fluorene (B), phenanthrene (C), and pyrene (D) in serum and urine (n=6). Each point and error bar represents the arithmetic mean and standard deviation of randomly picked six participants. The dots indicate the measured values and the solid lines represent the optimized pharmacokinetic model in this study.

Table 3. Pharmacokinetic parameter estimates from the two-compartment model.

Parameter	Notes	Nap	Flu	Phe	Pyr
k_a (/h)	GI tract → central compartment	2.50	0.65	1.11	0.90
k_{12} (/h)	Central compartment → peripheral compartment	1.40	1.30	0.01	0.09
k_{21} (/h)	Peripheral compartment → central compartment	0.60	0.14	0.23	0.28
k_{eu} (/h)	Parent compound elimination with urine	0.00032	0.000028	0.00005	0
k_{ef} (/h)	Parent compound elimination with other routes	1.80	1.70	1.13	0.70
k_m (/h)	Central compartment → metabolites compartment	0.70	3.40	0.15	0.90
k_{meu} (/h)	Metabolites elimination with urine	0.13	0.12	0.11	0.10
k_{mef} (/h)	Other forms except for parent compound elimination with other routes	3.00	1.20	1.00	0.60
V_1–F (L)	Volume of distribution of central or metabolites compartment	190	270	4900	880
$T_{1/2}$ (h)	Half-live of elimination for metabolites elimination with urine	5.33	5.78	6.30	6.93

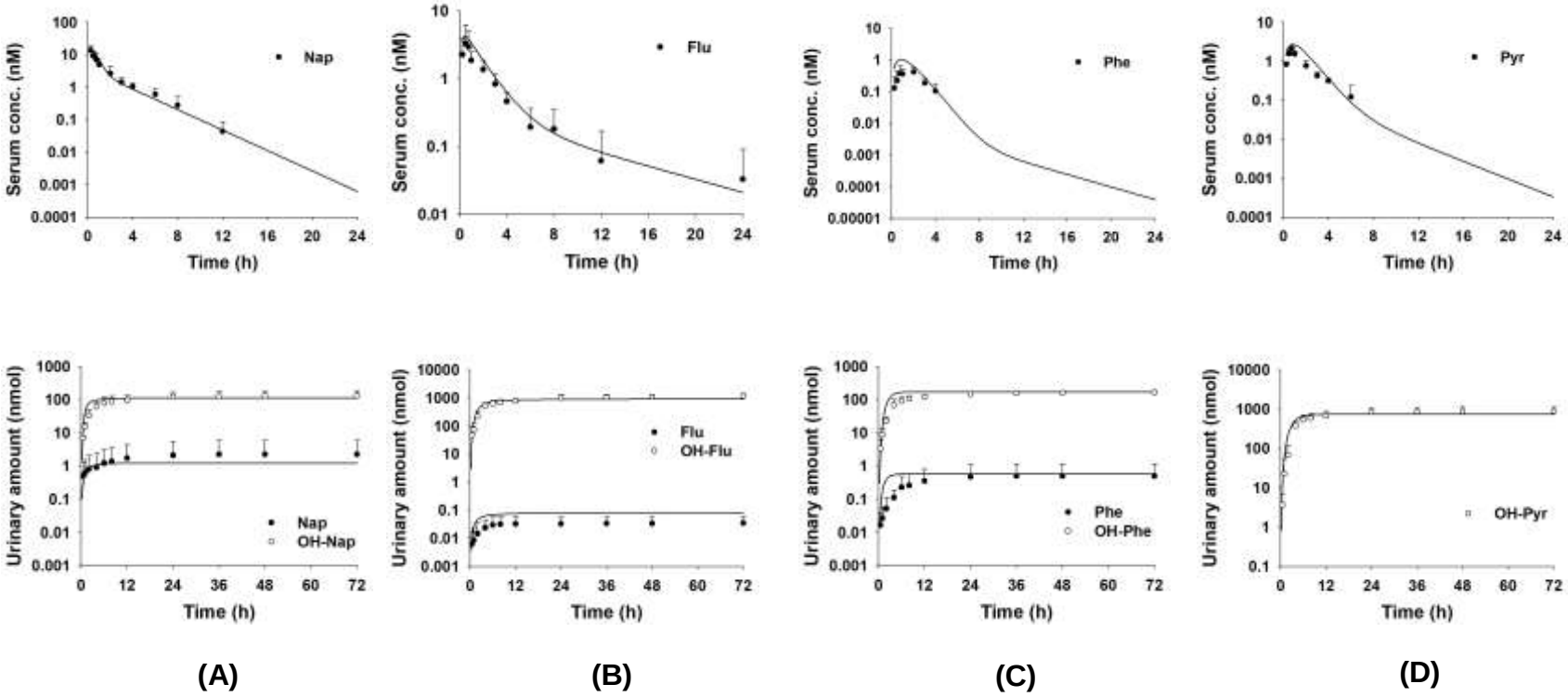

Figure 5. Model validation of naphthalene (A), fluorene (B), phenanthrene (C), and pyrene (D) in serum and urine (n=3). Each point and error bar represent the arithmetic mean and standard deviation of randomly picked three participants. The dots indicate the measured values and the solid lines represent the optimized pharmacokinetic model in this study.

4. Discussion

In this study, bolus doses of deuterium-labeled naphthalene (d_8-Nap), fluorene (d_{10}-Flu), phenanthrene (d_{10}-Phe), and pyrene (d_{10}-Pyr) were administered at 0.02 ~ 0.04 mg/kg in nine subjects. Then, parent compounds and their metabolites were monitored for 72 h to compute the urinary excretion fractions (F_{ue}), estimate the pharmacokinetic parameters of non-compartment, and compartment models explaining time-concentrations curves of serum and blood for Nap, Flu, Phe, and Pyr in humans.

As for the background levels of the PAHs representing daily life exposure, we monitored them with the unlabeled parents in the concurrent samples from the participants. The mean levels were 1.1 to 5516 times lower than the correspondingly labeled ones: the parents in blood - 5.59 nM (Nap), 0.77 nM (Flu), 0.02 nM (Phe), and 0.58 nM (Pyr); the parents in urine – 0.21 nmol (Nap), 0.02 nmol (Flu), 0.05 nmol (Phe), and 0.08 nmol (Pyr); the metabolites in urine – 1.06 nmol (1-OHNap), 8.57 nmol (2-OHNap), 0.13 nmol (2-OHFlu), 0.18 nmol (3-OHFlu), 0.06 nmol (1-OHPhe), 0.02 nmol (2-OHPhe), 0.04 nmol (3-OHPhe), 0.004 nmol (4-OHPhe), 0.03 nmol (9-OHPhe), and 0.03 nmol (1-OHPyr). Interestingly, the subject FC showed 7 to 28 times larger concentrations of the metabolites than the overall averages in urine samples after consumption of charcoal-grilled meats during the sample collection periods. Among them, the profiles of 2-OHNap, 2-OHFlu, 1-OHPhe, and 1-OHPyr reflected the exposure promptly, suggesting the selectiveness as exposure biomarkers of PAHs.

Figure 2 indicated quick absorption and short retention of each parent compound in serum. Nap reached the C_{max} at 0.3 h, and the others did around 0.9 ~ 1.7 h while mean resident times (MRTs) were 2 h for Nap (two-ringed structure), about 3 h for Flu and Phe (three-ring), about 4 h for Pyr (four-ring) (Table 2). These are consistent in previous studies using animal data and cheminformatic estimations (Geyer et al., 2002; Sarver et al., 1997). Noticeably,

there seem faster phase and slower phase of disposition, which suggested two-compartment modeling, and we successfully fitted the time course data of four PAHs into a PK model with same structure and different parameters (Figure 4, Table 3).

The classical PK model is composed of two-compartment for parent compound, which reflected metabolism in the central compartment and disposition of the parent compounds and their metabolites (*i.e.* hydroxylated ones) into urine and feces (Figure 1). After the preliminary analysis with various PK model structures (*e.g.* model without feces-excretion), we finalized with the present model showing better-fits for parents and metabolites in both serum and urine (Figure 4, Figure 5). Since we did not measure the other metabolites (*e.g.* dihydrodiols, tetrol, oxides, quinones, acids, -glucuronide, and -sulfate) and analytes in feces; however, it strongly suggests that absorbed PAHs are metabolized into mono-hydroxy PAHs with excretion via urine and feces. These are supported by the literature. Schooten et al. (1997), measured parent compound and their metabolites from blood, urine, and feces in rats after oral administration of PAHs in soil and oil, respectively; then, they detected parent compounds (Pyr) at 0.5% (via soil) or 0.2% (via oil) of dose in feces, 1-OHPyr at 5.1% (via soil) or 17.0 (via oil) in feces, 1-OHPyr at 0.2% (via soil) or 3.4 (via oil) in urine, and only low levels of the parent compounds were detected in the blood. Given that smaller molecules are excreted into urine easier, and larger molecules have more affinity to lipids (Li et al., 2012; Schooten et al.,1997), we could speculate that fecal excretion could be favored over urine for PAHs and OH-PAHs with higher molecular weight. Further studies are required to confirm the conjecture. Since we developed the PK models to describe ADME of the parent compounds and key metabolites used as exposure biomarkers, it is difficult to deal with all metabolites and excretion routes of PAHs in humans. Nevertheless, the present models provided more accurate biological half-times relative to previous studies using unlabeled chemicals or NCA.

Many biomarkers of exposure were used in risk assessment of environmental

chemicals with reverse-dosimetry via either urinary excretion fraction (F_{ue}) or PK modeling (Saravanabhavan et al., 2014; Zidek et al., 2017). Limited information has been available for the F_{ue}s of key metabolites of PAHs; however, we provide accurate measurements (Table S5, Figure 3). Interestingly, the F_{ue}s for 24 h (F_{ue} 24) were 81% (Nap, OHNap), 88% (Flu, OHFlu), 89% (Phe, OHPhe), and 95% (Pyr, OHPyr) while ones for 90% (2-OHNap), 89% (2-OHFlu), 87% (1-OHPhe), and 95% (1-OHPyr) relative to those for 72 h (F_{ue} 72), which indicate that considerable excretion of the parent compounds and their metabolites completed in about 24 h. Nonetheless, the present F_{ue}s were much smaller, which might be due to disposition via other routes other than urine. Noticeably, the present F_{ue}s are 1.35% for Nap and sum of OHNap, 8.02% for Flu and sum of OHFlu, 2.88% for Phe and sum of OHPhe, and 10.65 for OHPyr; in contrast, those are 182% for sum of OHNap, 60% for sum of OHFlu, 11% for sum of OHPhe, and 6.8% for OHPyr in Li et al. (2012) while those are 240% for Nap and sum of OHNap, 111% for Flu and sum of OHFlu, and 13% for Phe and sum of OHPhe, and 22% for OHPyr in Motorykin et al. (2015). We speculated that orally-exposed chemicals in the previous studies were not distinguished from background contributions from the other routes or sources of exposure such as diets (Bansal et al., 2015) or pesticides (Li et al., 2012). In addition, Pyr is lipophilic and its excretion appeared slow in the order as follows: Li et al. (2012), this study, and Motorykin et al. (2015), where Li et al. (2012) let the subjects ingest torn barbecue-chicken orally, Motorykin et al. (2015) did homogenized salmon orally. It would be reasonable to assume that the administered doses of substances in each experiment were different; however, we believe that our study design provided the most accurate results away from the background contributions.

The biological half-times ($T_{1/2}$) of urinary metabolites (*e.g.* OHNap, OHFlu, OHPhe, 1-OHPyr) ranged from 5.3 to 6.9 h in compartment models of the present study, which were comparable to the computation via ARE plot of urinary data (6.5 ~ 12.3 h; Table 4). Previously, Li et al. (2012) reported 2.9 to 6.1 h, and Motorykin et al. (2015) did 1.7 to 7.0 h for the analytes; the $T_{1/2}$s of 1-

OHPyr were 4.4 h (Buckley and Lioy et al., 1992), 12 h (Viau et al., 1995), and 5.7 h (Chien and Yeh et al., 2010). As mentioned earlier, deuterium-labelled chemicals should be distinguishable from those from backgrounds, and ours would be reliable.

There are some limitations in this study. All PK parameters reflect ADME after oral exposure only; therefore, those might be different in exposure via inhalation or dermal. Also, we measured parent compounds and their hydroxy metabolites in serum and urine; hence, our model would be useful in describing the ADME of our measurements in these sample media only. In fact, our model was developed for key PAHs and their metabolites used as exposure biomarkers. Although there are some inaccuracies about the fates of unmeasured metabolites, our models can provide usefulness for exposure simulation as well as reverse-dosimetry of PAHs biomarkers of exposure. Finally, our models came from adult volunteers. Thus, further studies should address the characteristics of PK in children, the elderly, or pregnant women and fetuses. Despite these limitations, this study established the unique human PK models of the key PAHs after oral administration without background contributions using repeated measurements for 72 h. Therefore, we explained their ADME with the first models for Nap, Flu, and Phe while we could provide a more accurate model for Pyr.

Table 4. Comparison of urinary metabolites half-lives ($T_{1/2}$) (h).

Chemicals	This study		Li et al. (2012)	Motorykin et al. (2015)
	NCA (ARE) [a]	Model	Model	Model
1-OHNap	9.4 ± 5.1	5.3	4.3 (3.3-6.2)	3.4 (3.1-3.8)
2-OHNap	9.5 ± 2.6		2.5 (2.0-3.4)	2.4 (2.0-3.0)
2-OHFlu	11.5 ± 3.8	5.8	2.9 (2.3-4.0)	2.6 (2.0-3.8)
3-OHFlu	12.1 ± 3.7		6.1 (4.9-8.1)	7.0 (6.3-7.9)
9-OHFlu	NM		3.1 (2.6-3.8)	1.7 (1.7-1.7)
1-OHPhe	9.5 ± 2.7	6.3	5.1 (4.3-6.1)	3.1 (2.8-3.4)
2-OHPhe	8.5 ± 2.9		3.9 (3.4-4.6)	3.7 (3.0-4.9)
3-OHPhe	6.7 ± 2.8		4.1 (3.3-5.6)	2.6 (2.3-2.9)
4-OHPhe	6.5 ± 2.2		3.5 (2.7-4.8)	2.9 (2.8-3.1)
9-OHPhe	12.3 ± 5.8		NM	NM
1-OHPyr	7.3 ± 1.6	6.9	3.9 (3.0-5.7)	4.4 (3.7-5.3)

NM: Not measured

a: Exclude FA data because of outlier

5. Conclusions

In the present study, we measured the time course data for naphthalene, fluorene, phenanthrene, and pyrene in human serum and urine, and constructed PK analyses. We characterized pharmacokinetic parameters through NCA and compartment modeling, which help to understand the ADME after single oral administration in humans. Despite the needs of further studies, our measurements and models should be useful in exposure assessment with biomonitoring especially for exposure simulation or dose-reconstruction, which could be applicable to estimating the average daily intake (or oral-equivalent) amounts of PAHs among general populations.

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Supplementary material

Pharmacokinetics of naphthalene, fluorene, phenanthrene, and pyrene in humans after single oral administration

Table S1. Properties of target parent compounds and metabolites.

Parent compounds				Target metabolites			
Name	Abbreviation	CAS #	MW (g/mol) [a]	Name	Abbreviation	CAS #	MW (g/mol) [a]
Naphthalene-d_8	Nap-d_8	1146-65-2	136.22	1-Naphthol-d_7	1-OHNap-d_7	124251-84-9	151.21
				2-Naphthol-d_7	2-OHNap-d_7	78832-54-9	151.21
Fluorene-d_{10}	Flu-d_{10}	81103-79-9	176.28	2-Hydroxy Fluorene-d_9	2-OHFlu-d_9	922510-18-7	191.27
				3-Hydroxy Fluorene-d_9	3-OHFlu-d_9	Unlabelled: 6344-67-8	191.27
Phenanthrene-d_{10}	Phe-d_{10}	1517-22-2	188.29	1-Phenanthrol-d_9	1-OHPhe-d_9	922510-23-4	203.28
				2-Phenanthrol-d_9	2-OHPhe-d_9	922510-19-8	203.28
				3-Phenanthrol-d_9	3-OHPhe-d_9	922510-20-1	203.28
				4-Phenanthrol-d_9	4-OHPhe-d_9	922510-21-2	203.28
				9-Phenanthrol-d_9	9-OHPhe-d_9	Unlabelled: 484-17-3	203.28
Pyrene-d_{10}	Pyr-d_{10}	1718-52-1	212.31	1-Hydroxy Pyrene-d_9	1-OHPyr-d_9	132603-37-3	227.30

a: molecular weight

Table S2. Intra-day precision and accuracy of PAHs and the metabolites.

Intra-day						
Chemical	Serum			Urine		
	Nominal concentration (ng/ml)	RSD (%) [a]	Accuracy (%)	Nominal concentration (ng/ml)	RSD (%) [a]	Accuracy (%)
Naphthalene-d_8	0.01	4.0	81	0.001	9.1	82
	0.05	4.5	83	0.003	6.2	82
	0.25	5.3	104	0.050	5.6	103
	2.50	10.6	100	0.100	5.5	99
Fluorene-d_{10}	0.01	9.6	120	0.001	10.6	84
	0.05	10.7	112	0.003	7.8	90
	0.25	4.7	96	0.050	6.4	102
	2.50	5.6	100	0.100	5.7	99
Phenanthrene-d_{10}	0.01	9.0	80	0.001	3.6	102
	0.05	7.8	112	0.003	10.2	90
	0.25	10.5	98	0.050	7.8	101
	2.50	3.7	100	0.100	7.7	100
Pyrene-d_{10}	0.01	7.1	118	0.001	6.0	88
	0.05	9.9	109	0.003	6.1	88
	0.25	5.7	97	0.050	4.1	102
	2.50	9.2	100	0.100	4.6	100
1-Naphthol-d_7	0.2	5.8	97	0.05	7.0	90
	1.0	3.0	113	0.10	7.5	104
	5.0	4.0	111	0.50	2.0	114
	-	-	-	1.00	2.0	112
	-	-	-	5.00	15.4	99
2-Naphthol-d_7	0.2	10.1	112	0.05	6.9	92
	1.0	1.4	109	0.10	7.9	101
	5.0	4.0	112	0.50	1.4	115
	-	-	-	1.00	2.9	116
	-	-	-	5.00	2.8	112
2-Hydroxy	0.2	6.0	110	0.05	6.1	84
Fluorene-d_9	1.0	5.8	99	0.10	3.5	90
	5.0	3.3	101	0.50	3.6	94
	-	-	-	1.00	3.3	94
	-	-	-	5.00	3.2	91
3-Hydroxy	0.2	2.8	112	0.05	5.4	101
Fluorene-d_9	1.0	4.8	108	0.10	5.8	96
	5.0	2.8	114	0.50	1.2	93
	-	-	-	1.00	4.3	97
	-	-	-	5.00	3.2	101

1-Phenanthrol-d_9	0.2	4.2	110	0.05	3.2	99
	1.0	1.7	99	0.10	2.2	102
	5.0	3.3	99	0.50	3.0	107
	-	-	-	1.00	1.2	108
	-	-	-	5.00	2.5	91
2-Phenanthrol-d_9	0.2	5.1	106	0.05	2.9	96
	1.0	2.4	98	0.10	4.8	99
	5.0	3.1	97	0.50	2.3	105
	-	-	-	1.00	1.4	106
	-	-	-	5.00	2.3	90
3-Phenanthrol-d_9	0.2	4.3	109	0.05	3.6	103
	1.0	5.0	101	0.10	2.1	107
	5.0	3.9	96	0.50	1.7	112
	-	-	-	1.00	1.2	113
	-	-	-	5.00	2.4	96
4-Phenanthrol-d_9	0.2	7.3	108	0.05	1.4	99
	1.0	2.4	104	0.10	2.5	104
	5.0	4.8	102	0.50	1.6	109
	-	-	-	1.00	2.2	109
	-	-	-	5.00	3.0	86
9-Phenanthrol-d_9	0.2	6.7	107	0.05	2.0	94
	1.0	7.0	100	0.10	2.1	94
	5.0	1.7	100	0.50	5.5	94
	-	-	-	1.00	2.7	105
	-	-	-	5.00	8.3	92
1-Hydroxy	0.2	2.0	101	0.05	5.0	86
Pyrene-d_9	1.0	10.8	106	0.10	3.5	95
	5.0	1.9	87	0.50	2.9	96
	-	-	-	1.00	3.0	98
	-	-	-	5.00	2.6	98

a: Relative standard deviation

Parent compounds in serum and urine were measured 4 concentration 5 times (N=20).

Metabolites in serum were measured 3 concentration 5 times (N=15).

Metabolites in urine were measured 5 concentration 5 times (N=25).

Table S3. Inter-day precision and accuracy of PAHs and the metabolites.

Inter-day						
Chemical	Serum			Urine		
	Nominal concentration (ng/ml)	RSD (%) [a]	Accuracy (%)	Nominal concentration (ng/ml)	RSD (%) [a]	Accuracy (%)
Naphthalene-d_8	0.01	13.3	86	0.001	13.0	87
	0.05	13.4	92	0.003	10.1	82
	0.25	8.5	104	0.050	7.2	102
	2.50	9.8	100	0.100	6.0	100
Fluorene-d_{10}	0.01	16.9	111	0.001	8.8	83
	0.05	9.6	104	0.003	7.1	90
	0.25	7.3	98	0.050	6.1	102
	2.50	6.8	100	0.100	5.7	100
Phenanthrene-d_{10}	0.01	19.8	96	0.001	4.2	95
	0.05	13.2	107	0.003	8.9	88
	0.25	12.9	101	0.050	6.7	100
	2.50	7.2	100	0.100	6.4	100
Pyrene-d_{10}	0.01	18.7	110	0.001	4.8	86
	0.05	9.0	106	0.003	6.2	86
	0.25	5.2	98	0.050	4.7	101
	2.50	8.6	100	0.100	5.0	100
1-Naphthol-d_7	0.2	8.1	98.0	0.05	4.7	85.7
	1.0	5.1	109.9	0.10	9.6	103.7
	5.0	5.1	106.9	0.50	2.7	103.8
	-	-	-	1.00	6.2	107.5
	-	-	-	5.00	9.2	98.4
2-Naphthol-d_7	0.2	9.7	111.5	0.05	7.9	100.4
	1.0	3.6	109.2	0.10	5.4	106.2
	5.0	4.7	110.6	0.50	2.1	115.5
	-	-	-	1.00	3.5	115.0
	-	-	-	5.00	3.1	109.2
2-Hydroxy Fluorene-d_9	0.2	5.9	105.8	0.05	4.5	91.1
	1.0	4.4	95.8	0.10	5.8	92.3
	5.0	4.2	98.2	0.50	4.0	90.3
	-	-	-	1.00	3.5	88.3
	-	-	-	5.00	3.2	88.9
3-Hydroxy Fluorene-d_9	0.2	4.8	108.1	0.05	7.3	104.3
	1.0	5.0	95.1	0.10	4.5	95.6
	5.0	3.2	98.8	0.50	2.3	103.6
	-	-	-	1.00	3.9	101.2
	-	-	-	5.00	2.7	102.6

1-Phenanthrol-d_9	0.2	4.3	106.0	0.05	2.9	96.1
	1.0	1.9	96.9	0.10	2.2	100.8
	5.0	3.3	96.7	0.50	2.6	101.6
	-	-	-	1.00	2.6	100.9
	-	-	-	5.00	2.5	87.9
2-Phenanthrol-d_9	0.2	4.3	108.4	0.05	2.3	96.3
	1.0	2.3	95.1	0.10	3.3	98.4
	5.0	4.1	94.2	0.50	2.3	101.0
	-	-	-	1.00	1.9	102.3
	-	-	-	5.00	1.8	87.9
3-Phenanthrol-d_9	0.2	4.6	107.6	0.05	3.5	98.3
	1.0	3.9	98.9	0.10	2.4	102.9
	5.0	5.6	95.2	0.50	1.5	104.6
	-	-	-	1.00	2.5	105.4
	-	-	-	5.00	2.5	91.6
4-Phenanthrol-d_9	0.2	7.1	104.5	0.05	3.5	96.6
	1.0	3.2	99.5	0.10	2.3	100.2
	5.0	4.4	97.4	0.50	1.9	102.0
	-	-	-	1.00	3.3	101.5
	-	-	-	5.00	2.5	84.0
9-Phenanthrol-d_9	0.2	6.4	102.5	0.05	5.1	95.7
	1.0	5.8	105.6	0.10	6.0	97.8
	5.0	2.2	96.0	0.50	3.6	95.9
	-	-	-	1.00	3.0	101.9
	-	-	-	5.00	5.5	94.3
1-Hydroxy Pyrene-d_9	0.2	2.4	98.5	0.05	2.8	95.6
	1.0	6.7	95.6	0.10	4.0	95.6
	5.0	3.1	87.2	0.50	2.5	91.0
	-	-	-	1.00	3.5	96.9
	-	-	-	5.00	2.0	98.0

a: Relative standard deviation

Parent compounds in serum and urine were measured 4 concentration 5 times (N=20)

Metabolites in serum were measured 3 concentration 5 times (N=15)

Metabolites in urine were measured 5 concentration 5 times (N=25)

Table S4. Results of quality assurance and quality control.

Chemicals	Serum					Urine				
	LOQ (ng/ml)	Calibration range (ng/ml)	QC			LOQ (ng/ml)	Calibration range (ng/ml)	QC		
			Nominal concentration (ng/ml)	RSD (%)	Accuracy (%)			Nominal concentration (ng/ml)	RSD (%)	Accuracy (%)
Naphthalene-d_8	0.01	0.01-5	0.05	8.3	100	0.001	0.001-0.5	0.005	14.7	92
			1.00	5.4	106			0.050	3.6	98
Fluorene-d_{10}	0.01	0.01-5	0.05	5.6	105	0.001	0.001-0.5	0.005	11.1	106
			1.00	3.3	103			0.050	5.9	100
Phenanthrene-d_{10}	0.01	0.01-5	0.05	6.6	101	0.001	0.001-0.5	0.005	7.6	106
			1.00	1.5	102			0.050	10.7	103
Pyrene-d_{10}	0.01	0.01-5	0.05	7.8	103	0.001	0.001-0.5	0.005	9.1	98
			1.00	4.5	101			0.050	4.3	99
1-Naphthol-d_7	0.22 [a]	0.05-30	0.50	4.1	102	0.02 [a]	0.01-30	0.05	6.8	107
			5.00	3.3	101			-	-	-
2-Naphthol-d_7	0.18 [a]	0.05-30	0.50	2.6	101	0.03 [a]	0.01-30	0.05	7.9	107
			5.00	2.4	105			-	-	-
2-Hydroxy Fluorene-d_9	0.27 [a]	0.05-30	0.50	8.0	106	0.01 [a]	0.01-30	0.05	6.6	102
			5.00	11.8	96			-	-	-
3-Hydroxy Fluorene-d_9	0.09 [a]	0.05-30	0.50	3.3	83	0.03 [a]	0.01-30	0.05	8.7	105
			5.00	7.3	86			-	-	-
1-Phenanthrol-d_9	0.11 [a]	0.05-30	0.50	4.6	94	0.03 [a]	0.01-30	0.05	3.8	100
			5.00	2.1	94			-	-	-

2-Phenanthrol-d_9	0.10 [a]	0.05-30	0.50	1.8	91	0.02 [a]	0.01-30	0.05	9.4	99
			5.00	1.9	87			-	-	-
3-Phenanthrol-d_9	0.06 [a]	0.05-30	0.50	1.9	93	0.03 [a]	0.01-30	0.05	3.4	111
			5.00	8.9	98			-	-	-
4-Phenanthrol-d_9	0.15 [a]	0.05-30	0.50	6.6	93	0.03 [a]	0.01-30	0.05	5.8	99
			5.00	3.2	93			-	-	-
9-Phenanthrol-d_9	0.14 [a]	0.05-30	0.50	6.9	101	0.02 [a]	0.01-30	0.05	7.8	109
			5.00	2.9	100			-	-	-
1-Hydroxy Pyrene-d_9	0.19 [a]	0.05-30	0.50	6.6	91	0.02 [a]	0.01-30	0.05	17.1	91
			5.00	5.2	93			-	-	-

a: LOD (ng/ml)

Table S5. Fractional urinary excretion (F_{ue}) of naphthalene, fluorene, phenanthrene, and pyrene (n=7). Each value is presented as arithmetic mean ± standard deviation of participants (n=7). Two participants (MT, FA) were excluded because urine collection was completed within 24 hours.

Chemicals	$F_{ue\ (0\text{-}24\ h)}$ (%)	$F_{ue\ (0\text{-}72\ h)}$ (%)
Nap	0.020 ± 0.026	0.020 ± 0.028
Flu	0.001 ± 0.001	0.001 ± 0.001
Phe	0.009 ± 0.011	0.011 ± 0.013
Pyr	ND	ND
1-OHNap	0.79 ± 0.41	1.06 ± 0.83
2-OHNap [a]	0.56 ± 0.21	0.62 ± 0.23
2-OHFlu [a]	7.21 ± 3.70	8.09 ± 3.94
3-OHFlu	0.82 ± 0.41	1.02 ± 0.48
1-OHPhe [a]	0.72 ± 0.55	0.83 ± 0.65
2-OHPhe	0.68 ± 0.29	0.71 ± 0.30
3-OHPhe	1.01 ± 0.71	1.05 ± 0.71
4-OHPhe	0.07 ± 0.04	0.07 ± 0.04
9-OHPhe	0.40 ± 0.19	0.57 ± 0.16
1-OHPyr [a]	10.65 ± 6.01	11.23 ± 6.59

a: Major biomarkers being measured in Korean National Environmental Health Survey (KoNEHS)

ND: Non-detected

Table S6. Parameter estimates from non-compartmental analysis by sex.

Parameter	Male (n=4)				Female (n=5)			
	Nap	Flu	Phe	Pyr	Nap	Flu	Phe	Pyr
Dose (mg)	1.34 ± 0.11	2.68 ± 0.23	1.34 ± 0.11	2.01 ± 0.17	1.28 ± 0.23	2.63 ± 0.48	1.31 ± 0.25	1.91 ± 0.34
C_{max} (nM)	17.21 ± 4.54	2.38 ± 2.22	1.12 ± 1.10	4.67 ± 5.19	11.53 ± 3.49	4.48 ± 2.26	0.63 ± 0.36	2.19 ± 0.17
T_{max} (h)	0.31 ± 0.13	3.03 ± 4.57	1.44 ± 0.66	1.06 ± 0.63	0.31 ± 0.11	0.56 ± 0.13	1.44 ± 0.66	0.75 ± 0.18
$T_{1/2}$ (h)	1.20 ± 0.53	1.61 ± 0.46	2.65 ± 2.71	1.56 ± 0.39	1.52 ± 0.15	1.86 ± 0.50	1.41 [a]	3.73 ± 4.92
$AUC_{(0\rightarrow\infty)}$ (nM•h)	22.55 ± 6.09	10.35 ± 7.08	2.75 ± 2.27	9.28 ± 11.52	15.71 ± 2.33	10.50 ± 4.44	1.30 [a]	5.49 ± 3.32
$AUMC_{(0\rightarrow\infty)}$ (nM•h^2)	43.20 ± 20.26	22.23 ± 11.57	6.42 ± 3.13	19.00 ± 21.68	33.76 ± 6.46	41.17 ± 29.97	2.63 [a]	42.86 ± 74.61
$MRT_{(0\rightarrow\infty)}$ (h)	1.82 ± 0.51	2.40 ± 0.50	3.22 ± 2.47	2.30 ± 0.41	2.15 ± 0.24	3.75 ± 2.38	2.02 [a]	4.96 ± 5.92

Table S7. Parameter estimates from non-compartmental analysis by BMI.

Parameter	Normal weight (n=5)				Overweight (n=1), Obesity (n=3)			
	Nap	Flu	Phe	Pyr	Nap	Flu	Phe	Pyr
Dose (mg)	1.18 ± 0.11	2.35 ± 0.21	1.18 ± 0.11	1.76 ± 0.16	1.47 ± 0.09	2.93 ± 0.17	1.47 ± 0.09	2.20 ± 0.13
C_{max} (nM)	13.28 ± 4.51	2.45 ± 2.24	0.99 ± 1.00	4.20 ± 4.59	15.03 ± 5.59	4.22 ± 0.21	0.67 ± 0.35	2.17 ± 0.59
T_{max} (h)	0.35 ± 0.14	2.48 ± 4.14	2.00 ± 0.00	1.00 ± 0.59	0.25 ± 0.00	0.63 ± 0.14	0.88 ± 0.14	0.75 ± 0.00
$T_{1/2}$ (h)	1.28 ± 0.49	2.47 ± 1.46	3.75 ± 4.13	3.68 ± 4.95	1.50 ± 0.20	1.61 ± 0.26	1.50 ± 0.29	1.62 ± 0.39
$AUC_{(0\rightarrow\infty)}$ (nM•h)	18.41 ± 5.74	10.79 ± 6.71	3.46 ± 3.28	10.13 ± 9.63	19.17 ± 5.89	8.53 ± 3.99	1.79 ± 1.24	3.48 ± 1.30
$AUMC_{(0\rightarrow\infty)}$ (nM•h^2)	37.09 ± 16.54	38.00 ± 28.14	8.59 ± 0.99	51.71 ± 71.84	39.04 ± 12.82	19.41 ± 7.30	3.71 ± 2.39	7.93 ± 3.51
$MRT_{(0\rightarrow\infty)}$ (h)	1.97 ± 0.55	3.62 ± 2.09	4.25 ± 3.74	4.94 ± 5.92	2.04 ± 0.13	2.36 ± 0.39	2.13 ± 0.19	2.32 ± 0.62

BMI follows Korean standards; Underweight: <18.5 kg/m^2, Normal weight: 18.5 ~ 22.9 kg/m^2, Overweight: 23.0 ~ 24.9 kg/m^2, Obesity: ≥25.0 kg/m^2

Table S8. Urinary half-lives from ARE plot analysis by sex and BMI.

Chemicals	Sex		BMI		Total (n=8)
	Male (n=4)	Female (n=4)	Normal weight (n=4)	Overweight (n=1), Obesity (n=3)	
Nap	6.05 ± 1.44	5.70 ± 2.17	6.49 ± 2.31	5.26 ± 0.70	5.87 ± 1.72
Flu	6.49 ± 3.68	7.04 ± 5.39	5.49 ± 1.82	8.05 ± 5.93	6.77 ± 4.28
Phe	11.67 ± 9.37	6.83 ± 2.05	6.17 ± 1.58	12.33 ± 8.94	9.25 ± 6.79
Pyr	ND	ND	ND	ND	ND
1-OHNap	8.17 ± 3.91	10.55 ± 6.49	6.42 ± 2.84	12.30 ± 5.48	9.36 ± 5.12
2-OHNap	8.37 ± 3.41	10.68 ± 0.87	8.71 ± 3.13	10.33 ± 2.10	9.52 ± 2.62
2-OHFlu	11.59 ± 5.71	11.31 ± 1.15	11.40 ± 5.65	11.49 ± 1.45	11.45 ± 3.82
3-OHFlu	10.54 ± 3.62	13.64 ± 3.49	10.92 ± 3.75	13.25 ± 3.75	12.09 ± 3.69
1-OHPhe	9.59 ± 3.46	9.38 ± 2.25	8.30 ± 3.03	10.67 ± 2.02	9.48 ± 2.70
2-OHPhe	7.43 ± 3.74	9.62 ± 1.42	8.16 ± 3.95	8.89 ± 1.81	8.52 ± 2.87
3-OHPhe	6.80 ± 4.19	6.50 ± 0.73	5.63 ± 2.15	7.66 ± 3.28	6.65 ± 2.79
4-OHPhe	6.61 ± 1.53	6.45 ± 3.05	5.58 ± 1.24	7.48 ± 2.78	6.53 ± 2.24
9-OHPhe	9.75 ± 7.42	14.91 ± 2.21	9.79 ± 5.85	14.87 ± 5.13	12.33 ± 5.77
1-OHPyr	6.66 ± 2.07	7.94 ± 0.87	7.14 ± 2.44	7.46 ± 0.31	7.30 ± 1.62

Exclude FA data because of outlier

BMI follows Korean standards; Underweight: <18.5 kg/m^2, Normal weight: 18.5 ~ 22.9 kg/m^2, Overweight: 23.0 ~ 24.9 kg/m^2, Obesity: ≥25.0 kg/m^2

국문초록

다환방향족 탄화수소류의 인체 약동력학적 특성: 나프탈렌, 플루오렌, 페난트렌, 파이렌의 단회경구노출

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다환방향족탄화수소류 (PAHs)는 유기물의 불완전연소로 생성되며 여러 장기에 건강영향을 미치며 인체에 노출될 경우 폐암, 피부암, 방광암, 유방암 그리고 위암을 일으키는 것으로 알려져 있다. 주로 섭취 또는 흡입을 통해 인체에 흡수되며 CYP-P450 효소에 의해 하이드록시기가 붙은 다환방향족탄화수소류 형태로 (OH-PAHs) 대사되고 저분자량의 다환방향족탄화수소류는 소변으로, 고분자량의 다환방향족탄화수소류는 대변으로 체내에서 빠져나간다. 수많은 독성연구에도 불구하고, 다환방향족탄화수소류의 인체 내 흡수-분포-대사-배설 (ADME)에 대한 정보는 부족하다. 본 연구의 목적은 다환방향족탄화수소류 4종의 단회 경구 투여 후 인체 내에서의 흡수, 분포, 대사 및 배설에 대한 특성을 파악하고, 약물동태학적 모델을 구축하여 이를 설명하는 것이다. 이를 위해 중수소치환된 나프탈렌, 플루오렌, 페난트렌, 파이렌을 건강한 성인남녀 9명 (나이: 33.7 ± 8.8세)에게 0.02~0.04 mg/kg으로 단회 경구 투여한 후, 노출 전부터

노출 후 72시간 동안 지정된 시점에서 소변과 혈액샘플을 수집하였다. 수집된 샘플은 헤드스페이스-고체상마이크로추출-기체크로마토그래피-질량분석기 (HS-SPME-GC-MS) 및 액체크로마토그래피-질량분석기/질량분석기 (LC-MS/MS)로 다환방향족 탄화수소류 원물질과 10종의 대사체 농도를 측정하였다. 시간에 따른 혈중 농도 및 누적소변배출량 프로파일을 바탕으로 소변 중 배설분율을 산출하고 약물동태학 분석을 수행하였으며, 두 구획으로 구성된 약물동태학 모델을 구축하였다. 비구획분석 (non-compartmental analysis) 결과 나프탈렌, 플루오렌, 페난트렌, 파이렌은 노출 후 신속하게 대개 0.31시간에서 1.65시간 사이에 혈중 최고농도에 도달하며, 혈중 반감기는 2.76시간 이내로 확인되었다. 반감기와 평균체류시간은 분자량이 증가함에 따라 증가하는 경향을 나타내었다. 또한 원물질보다 대사체의 형태로 노출 후 24시간 내 대부분 배설되는 것으로 보이며, 다환방향족탄화수소류는 소변이 아닌 다른 경로를 (예: 대변) 통해 배설됨을 추측할 수 있다. 또한 나프탈렌, 플루오렌, 페난트렌, 파이렌의 인체 내에서 시간에 따른 혈중 농도 및 누적 소변배설량 프로파일을 잘 예측하는 두 구획 약동력학 모델을 구축하였다. 본 연구는 인체 내에서 나프탈렌, 플루오렌, 페난트렌, 파이렌의 흡수, 분포, 대사 및 배설에 관한 정보를 제공하며, 소변 중 배설분율과 구축된 약물동태학적 모델은 국내외 나프탈렌, 플루오렌, 페난트렌, 파이렌의 바이오모니터링에 주로 활용되는 바이오마커의 일평균섭취량 (또는 섭취등가용량, Oral-equivalent dose)을 추정할 때 매우 중요하게 쓰일 수 있을 것이다.

주요어: 다환방향족탄화수소류, 흡수 · 분포 · 대사 · 배설, 경구투여, 인체 약물동태학, 소변 중 배설분율, 약물동태학 모델

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