Supporting Information

Concentrations and Profiles of Urinary Polycyclic Aromatic Hydrocarbon Metabolites (OH-PAHs) in Several Asian Countries

Ying Guo[a], Kurunthachalam Senthilkumar[b], Husam Alomirah[c], Hyo-Bang Moon[d], Tu Binh Minh[e], Mustafa Ali Mohd[f], Haruhiko Nakata[g], and Kurunthachalam Kannan[a, h, *]

[a]Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Empire State Plaza, P.O. Box 509, Albany, New York 12201-0509, United States

[b]Department of Natural Sciences, Savannah State University, Savannah, Georgia 31404, United States

[c]Biotechnology Department, Kuwait Institute for Scientific Research, P.O. Box 24885, 13109 Safat, Kuwait

[d]Department of Marine Science and Convergence Technology, College of Science and Technology, Hanyang University, Ansan 426-791, South Korea

[e]UNIDO BAT/BEP Project Office, Pollution Control Department, Vietnam Environment Administration, Ba Dinh, Hanoi, Vietnam

[f] Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

[g]Graduate School of Science & Technology, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan

[h]International Joint Research Center for Persistent Toxic Substances, State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

*Corresponding author: K. Kannan
Wadsworth Center
Empire State Plaza, P.O. Box 509
Albany, NY 12201-0509
Tel: 1-518-474-0015
Fax: 1-518-473-2895

E-mail: kkannan@wadsworth.org

For submission to: Environmental Science and Technology

NO. OF PAGES: 10

NO. OF TABLES: 2

NO. OF FIGURES: 3

Sample Pretreatment

(1) Urine samples are removed from the freezer (–80°C) and thawed in a 4°C dark room overnight;

(2) At room temperature, urine samples are Vortex mixed for 30 seconds, and 2 mL of urine is transferred into a 15 mL glass tube using ejector variable volume micropipettor;

(3) Analytical standards are removed from the refrigerator and brought to room temperature. 80 μL of internal standards (16 ng for 1-NAP and 2-NAP, and 4 ng for others), 1 mL of ammonium acetate buffer (1 M, pH=5.5), and 50 μL of β-glucuronidase (200 units/mL) are added;

(4) Samples are gently mixed and then incubated overnight at 37°C (~16 h);

(5) Urine samples are diluted with 2 mL water; and then extracted with 5 mL organic solvent (80% pentane:20% toluene, v:v) by shaking 30 min;

(7) After centrifugation at 3600 x *g* for 20 min, the up layer is transferred into a 15 mL glass tube;

(8) Repeat the liquid-liquid extraction procedure one more time;

(9) The combined extraction is further purified with 1 mL $AgNO_3$ solution (1 M) by shaking 15 min. The solution is centrifuged 10 min at 3600 x *g* f and the up layer is transferred into a 15 mL glass tube;

(10) The collected fraction is concentrated under a gentle stream of nitrogen (first at 30 ^{o}C and then at 45 ^{o}C). The final solution is diluted to 0.4 mL by methanol for instrumental analysis.

Instrumental Analysis

An AB-Sciex 5500 triple quadrupole mass spectrometer (ESI-MS-MS; Applied Biosystems, Foster City, CA), equipped with an Agilent 1100 Series HPLC system (Agilent Technologies Inc., Santa Clara, CA), was used for analysis.

- Chromatographic separation is achieved using a Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 μm);
- The mobile phases are methanol (A) and methanol-water solution (2:3, v:v) (B) of 350 μL/min. The mobile phase gradient is as follows: 0.0-2.0 min, 95% B; 5.0-15 min, 45% B; 16.0-19.0 min, 20% B; 25.0-32.0 min, 95% B.
- The injection volume is 10 μL.
- Nitrogen is used as both curtain and collision gas.
- Target compounds are determined by multiple reaction monitoring (MRM) in the negative ionization mode.
- MS/MS parameters have been optimized for each target compound, by infusion of 20 ng/mL standard solution.
- The interface heater temperature is 500 ^{o}C and the IonSpray Voltage is -4500.

d_7-1-NAP and d_7-2-NAP were used as internal standards for quantification of 1-NAP and 2-NAP, ^{13}C-1-PYR, ^{13}C-6-CHRY, ^{13}C-3-BCP and ^{13}C-1-BAA are used as internal standards for quantification of 2-FLUO, 1-PYR, 1-CHRY and 6-CHRY, 3-BCP and 1-BAA, and ^{13}C-3-PHEN are used as internal standards for 2-FLUO, 2-PHEN, 3-PHEN, 4-PHEN and 9-PHEN, respectively.

The limit of quantification (LOQ) is determined based on the linear range of the calibration curve prepared at the concentration range of 0.025 to 20 ng/mL. Concentrations in samples which are at least the same as the lowest acceptable standard concentration are considered to be

94 valid. The LOQ (2 mL sample taken for extraction and 0.4 mL taken for instrumental analysis)

95 was 40 pg/mL for 1-NAP and 2-NAP and 10 pg/mL for other compounds.

96 The entire system from sample injection to data acquisition is computer-controlled with

97 Analyst 1.5 software Componemts for AB Sciex triple quad^{tm} 5500 system.

98

99

TABLE S1. Characters of Urine Samples from Asian Countries

	Age ≤10	10 < Age ≤ 40	Age > 40	Average
China (40/44) [a]	3/5	27/30	10/9	29±16
India (21/17) [b]	0/-	12/10	9/4	37±17
Japan (7/27)	0/1	7/20	0/6	32±10
Korea (18/22) [c]	0/0	12/18	6/4	35 ±11
Kuwait (15/23)	3/3	4/10	8/10	37±21
Malaysia (19/10)	0/0	16/8	3/2	30±9
Vietnam (13/10)	0/0	2/5	11/5	51±19

[a] (female/male); [b] ages of three samples from India are not clear; [c] information of 20 samples from Korea are not available

TABLE S2. The Results of Quality Assurance/Quality Control System (average values)

Compound	Method blank (n=10)	Blank spiked (n=8)	Matrix spiked (n=8)	Matrix Duplicate (n=8)	sample (n=308)
d7-1-NAP	40%	43%	37%	37%	34%
d7-2-NAP	26%	26%	38%	39%	32%
C^{13}-3-PHEN	65%	68%	66%	73%	68%
C^{13}-1-PYR	10%	17%	51%	48%	24%
C^{13}-6-CHRY	8%	14%	46%	55%	31%
C^{13}-3-BCP	62%	72%	59%	62%	62%
C^{13}-1-BAA	52%	57%	28%	28%	49%
2-NAP	0.039 ng/mL	109%	128%	19% (CV) [a]	~
1-NAP	0.028 ng/mL	105%	115%	21% (CV)	~
2-FLUO	< LOQ	89%	85%	18% (CV)	~
2-PHEN	0.010 ng/mL	NA [b]	NA [b]	19% (CV)	~
3-PHEN	0.009 ng/mL	92%	107%	20% (CV)	~
9-PHEN	< LOQ	NA [b]	NA [b]	19% (CV)	~
4-PHEN	< LOQ	70%	92%	18% (CV)	~
1-PYR	0.068 ng/mL	92%	109%	22% (CV)	~
1-CHRY	0.159 ng/mL	79%	137%	< LOQ	~
3-BCP	< LOQ	94%	98%	< LOQ	~
6-CHRY	0.045 ng/mL	69%	108%	< LOQ	~
1-BAA	0.021 ng/mL	87%	89%	< LOQ	~

[a] CV = coefficient variation

[b] 2-PHEN and 9-PHEN were not spiked into samples.

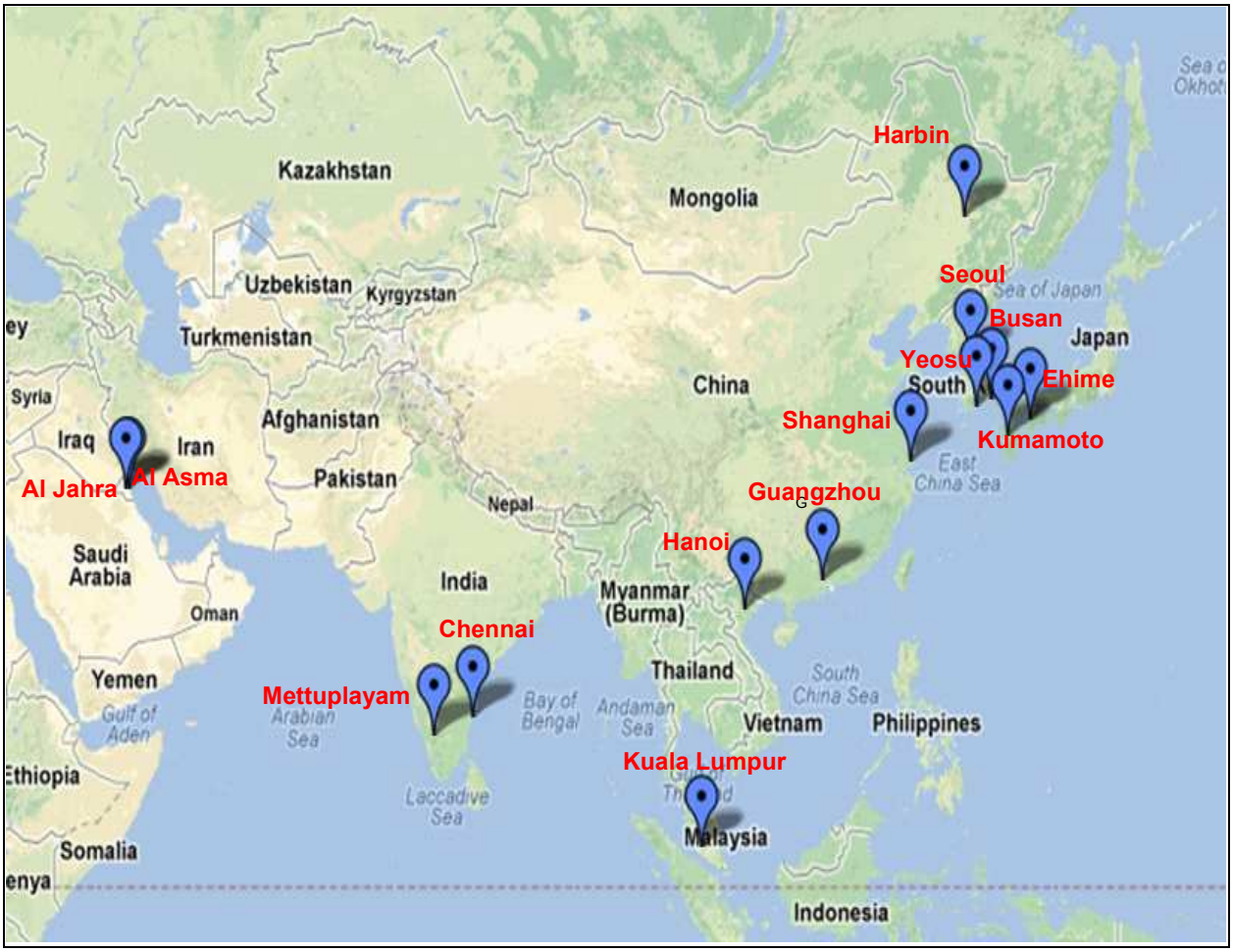

Figure S1. Urine sampling sites in Asian countries in the present study

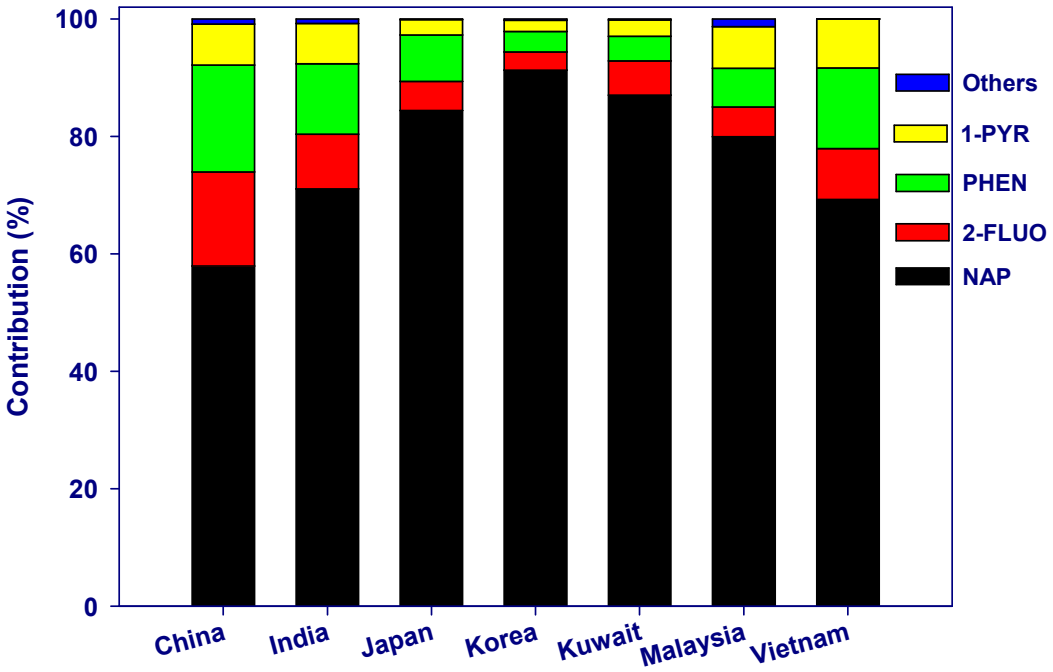

Figure S2. Concentration Contribution of OH-PAHs to Σ_{12}OH-PAHs in Asian Countries.

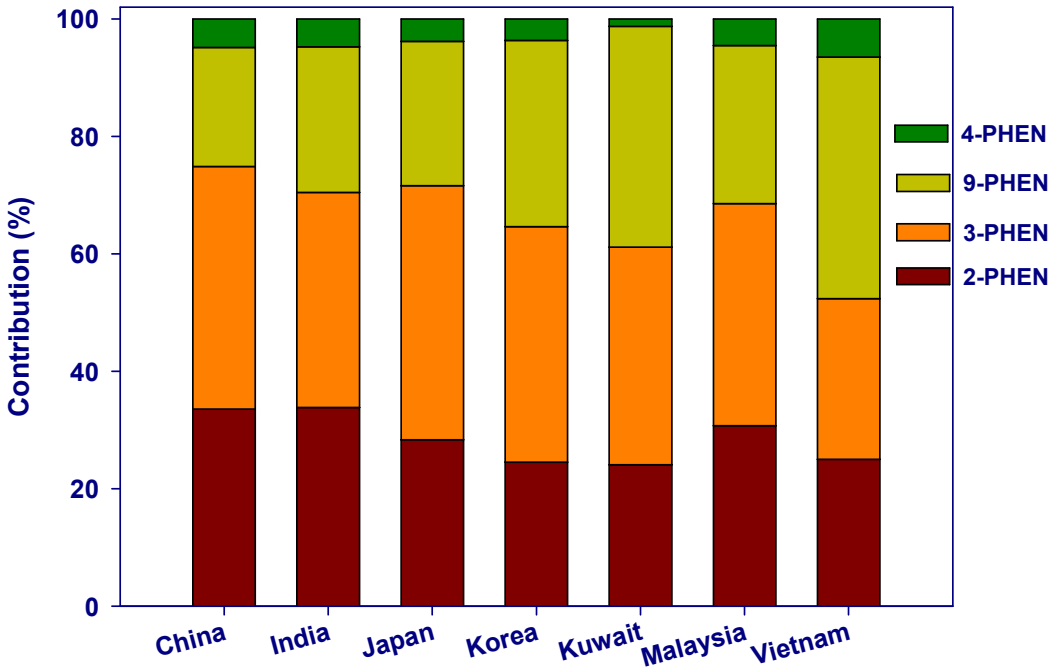

Figure S3. Concentration Contribution of Individual hydrxylic phenanthrene to PHEN in Asian Countries.