SUPPLEMENTARY INFORMATION

Urinary Polycyclic Aromatic Hydrocarbon Metabolites as Biomarkers to Woodsmoke Exposure – Results from a Controlled Exposure Study

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TITLE RUNNING HEAD: Urinary woodsmoke exposure biomarkers

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Table S1. Air pollutant concentrations obtained through the personal $PM_{2.5}$ samples collected by nine participants during the 2-h woodsmoke exposure period.

Air pollutant*	Unit	Mean	Min	Max	SD	CV
$PM_{2.5}$	μg/m^3	1515	840	2997	682	45%
Levoglucosan	μg/m^3	123	68	256	59	48%
Naphthalene	ng/m^3	36.9	32.4	47.7	4.8	13%
Acenaphthylene	ng/m^3	22.9	2.3	40.7	12.5	55%
Acenaphthene	ng/m^3	2.8	2.2	7.4	1.7	63%
Fluorene	ng/m^3	10.7	7.4	15.8	3.1	28%
Phenanthrene	ng/m^3	7.2	5.7	9.4	1.2	16%
Anthracene	ng/m^3	5.8	3.5	8.5	1.9	32%
Fluoranthene	ng/m^3	14.9	4.9	38.8	11.6	78%
Pyrene	ng/m^3	19.7	7.2	50.3	13.6	69%
Retene	ng/m^3	63.7	4.8	313.5	108.4	170%
Benz[a]anthracene	ng/m^3	81.4	43.1	173.2	41.9	52%
Chrysene	ng/m^3	36.5	19.9	77.9	18.8	52%
Benzo[b]fluoranthene	ng/m^3	33.3	19.0	65.8	14.6	44%
Benzo[k]fluoranthene	ng/m^3	37.3	20.2	86.4	23.7	64%
Benzo[e]pyrene	ng/m^3	20.7	12.5	41.9	9.5	46%
Benzo[a]pyrene	ng/m^3	46.6	28.6	92.5	20.8	45%
Perylene	ng/m^3	9.2	6.4	17.2	3.5	38%
Indeno[1,2,3-cd]pyrene	ng/m^3	7.7	4.6	15.0	3.5	45%
Dibenzanthracene	ng/m^3	12.0	8.3	19.8	3.8	31%
Benzo[ghi]perylene	ng/m^3	19.2	12.2	38.1	8.6	45%
Coronene	ng/m^3	7.1	4.9	12.2	2.6	36%

* $PM_{2.5}$-bound air pollutants concentrations. Small PAHs with low molecular weight, such as naphthalene and fluorene, exist primarily in the vapor phase. Assessment of exposure to small PAH based on their $PM_{2.5}$-bound concentrations would lead to underestimation.

Table S2. Pre (-24-0 h), post (0 h and later) and maximum urinary OH-PAH concentrations (creatinine adjusted, μg/g creatinine) from 9 volunteers exposed to woodsmoke (at 0-2 h). Data are presented as mean with standard deviation. Also given are the median and 75th percentile concentrations in the U.S. adult population (20 years of age and older).

Metabolites	Abbr.	Mean with SD concentrations (μg/g creatinine)					Time to peak, t_{max} (h)	Folds of increase (Max/pre)	US adult population[2]	
		-24-0 h (pre)	0-12 h	12-24 h	24-50 h	Max			Median	75th
1-OH-naphthalene[1]	1-NAP	1.96 ± 0.9	5.81 ± 2.29	2.36 ± 1.1	1.46 ± 0.7	7.71 ± 2.47	5.4 ± 2.6	4.5 ± 2.1	2.36	7.42
2-OH-naphthalene	2-NAP	1.20 ± 0.54	4.32 ± 1.88	1.70 ± 0.95	1.17 ± 0.38	6.12 ± 2.25	5.0 ± 2.5	7.2 ± 8.0	3.47	7.75
9-OH-fluorene	9-FLU	0.30 ± 0.10	0.79 ± 0.40	0.40 ± 0.12	0.28 ± 0.10	1.07 ± 0.52	5.8 ± 3.6	4.4 ± 4.0	0.31	0.62
3-OH-fluorene	3-FLU	0.07 ± 0.02	0.13 ± 0.04	0.10 ± 0.03	0.07 ± 0.02	0.15 ± 0.05	5.3 ± 2.9	2.6 ± 1.4	0.08	0.28
2-OH-fluorene	2-FLU	0.19 ± 0.05	0.35 ± 0.09	0.22 ± 0.05	0.19 ± 0.04	0.46 ± 0.15	4.6 ± 2.7	2.7 ± 1.4	0.23	0.63
3-OH-phenanthrene	3-PHE	0.09 ± 0.03	0.14 ± 0.04	0.11 ± 0.05	0.08 ± 0.03	0.18 ± 0.06	5.9 ± 3.0	2.4 ± 1.5	0.09	0.17
1-OH-phenanthrene	1-PHE	0.15 ± 0.05	0.19 ± 0.05	0.17 ± 0.06	0.13 ± 0.04	0.24 ± 0.07	7.7 ± 5.0	1.8 ± 1.1	0.14	0.23
2-OH-phenanthrene	2-PHE	0.06 ± 0.02	0.10 ± 0.04	0.07 ± 0.04	0.05 ± 0.02	0.14 ± 0.06	6.4 ± 3.6	2.5 ± 1.8	0.06	0.11
1-OH-pyrene	1-PYR	0.08 ± 0.04	0.12 ± 0.04	0.11 ± 0.06	0.08 ± 0.04	0.17 ± 0.08	8.3 ± 5.5	2.2 ± 0.9	0.11	0.19

1. 1-naphthol data from two subjects were excluded
2. Data from National Health and Nutritional Examination Survey (NHANES) 2007-2008

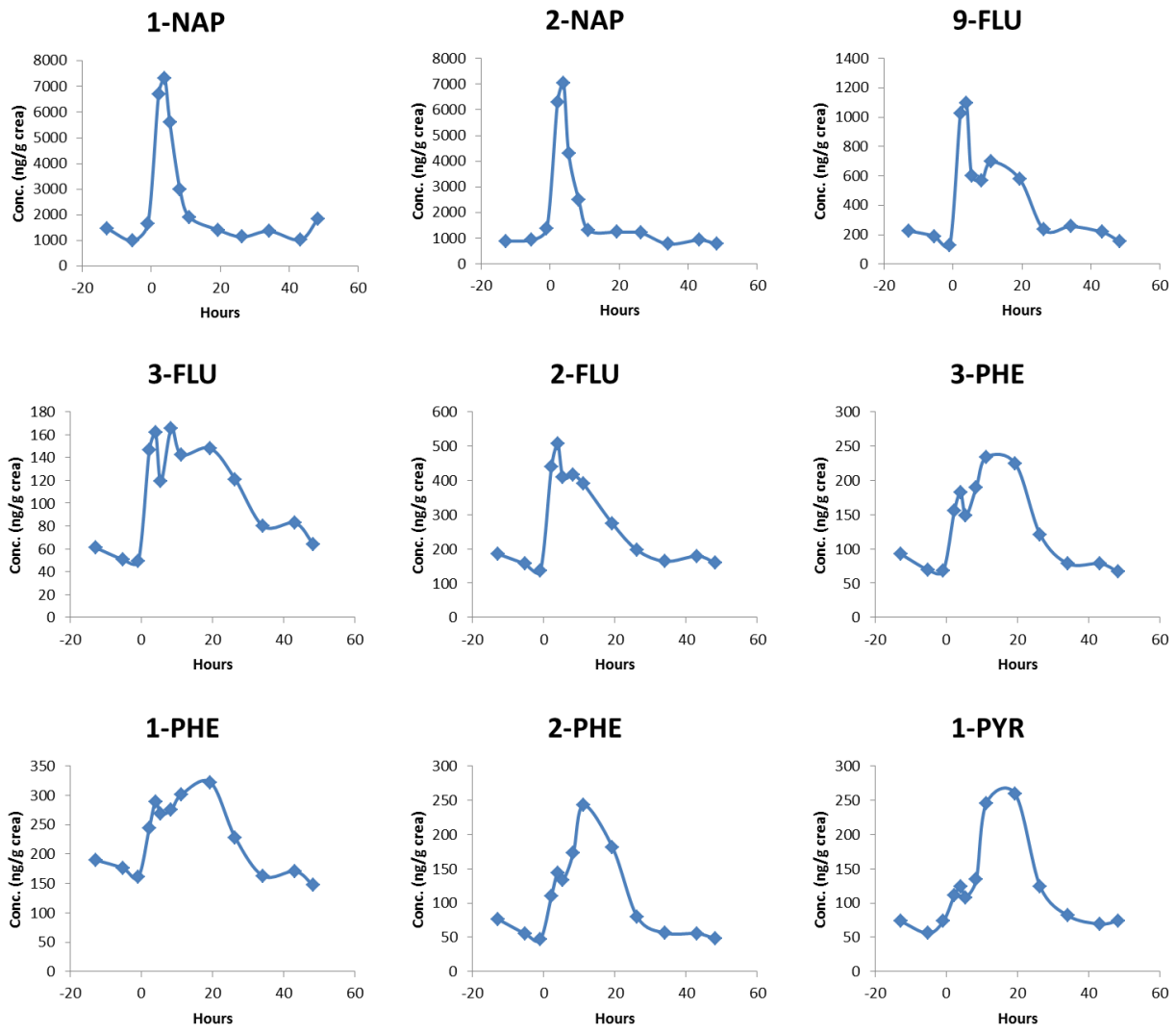

Figure S1. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant A who was exposed to wood smoke for 2 hours (0-2 h).

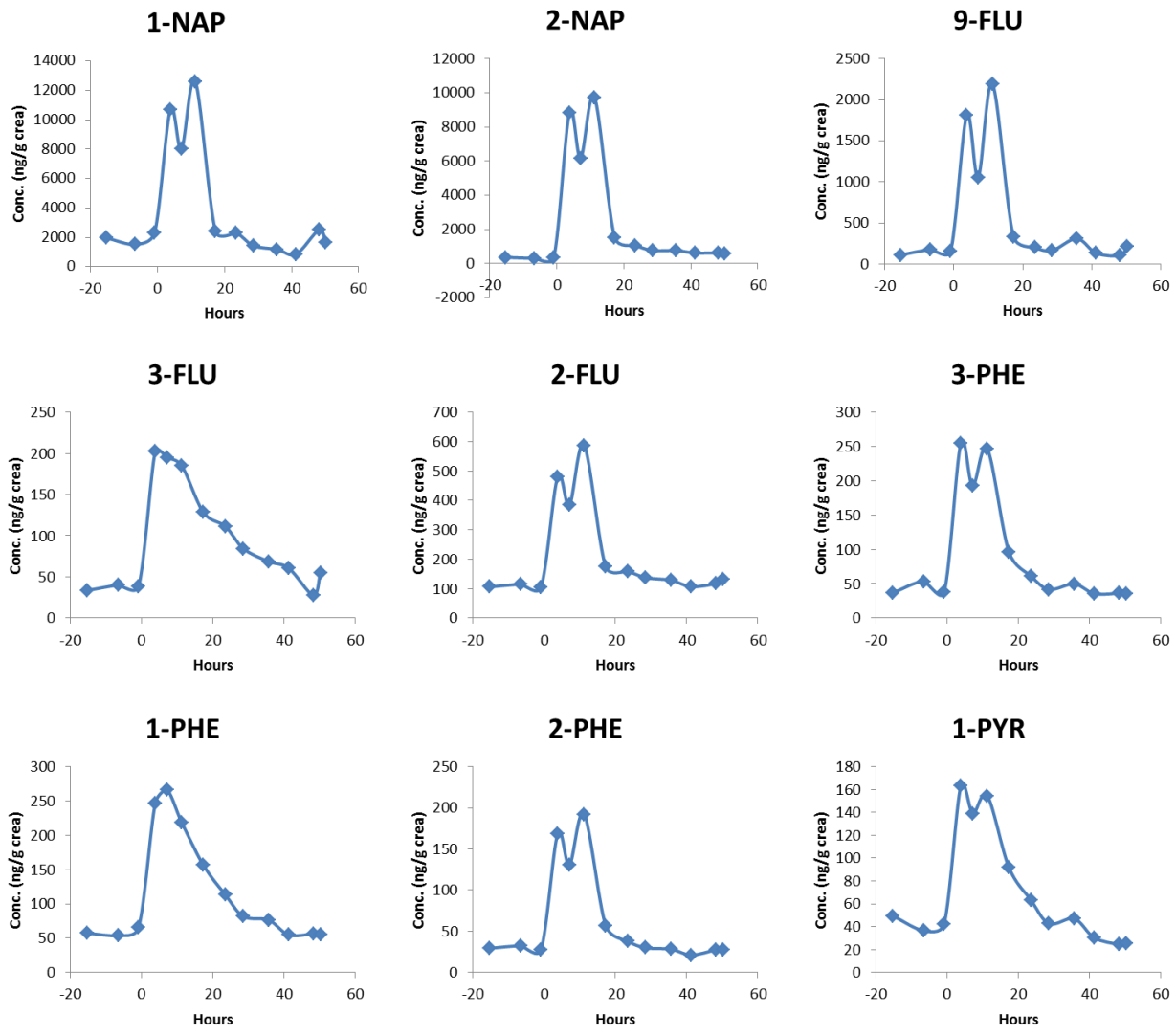

Figure S2. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant B who was exposed to wood smoke for 2 hours (0-2 h).

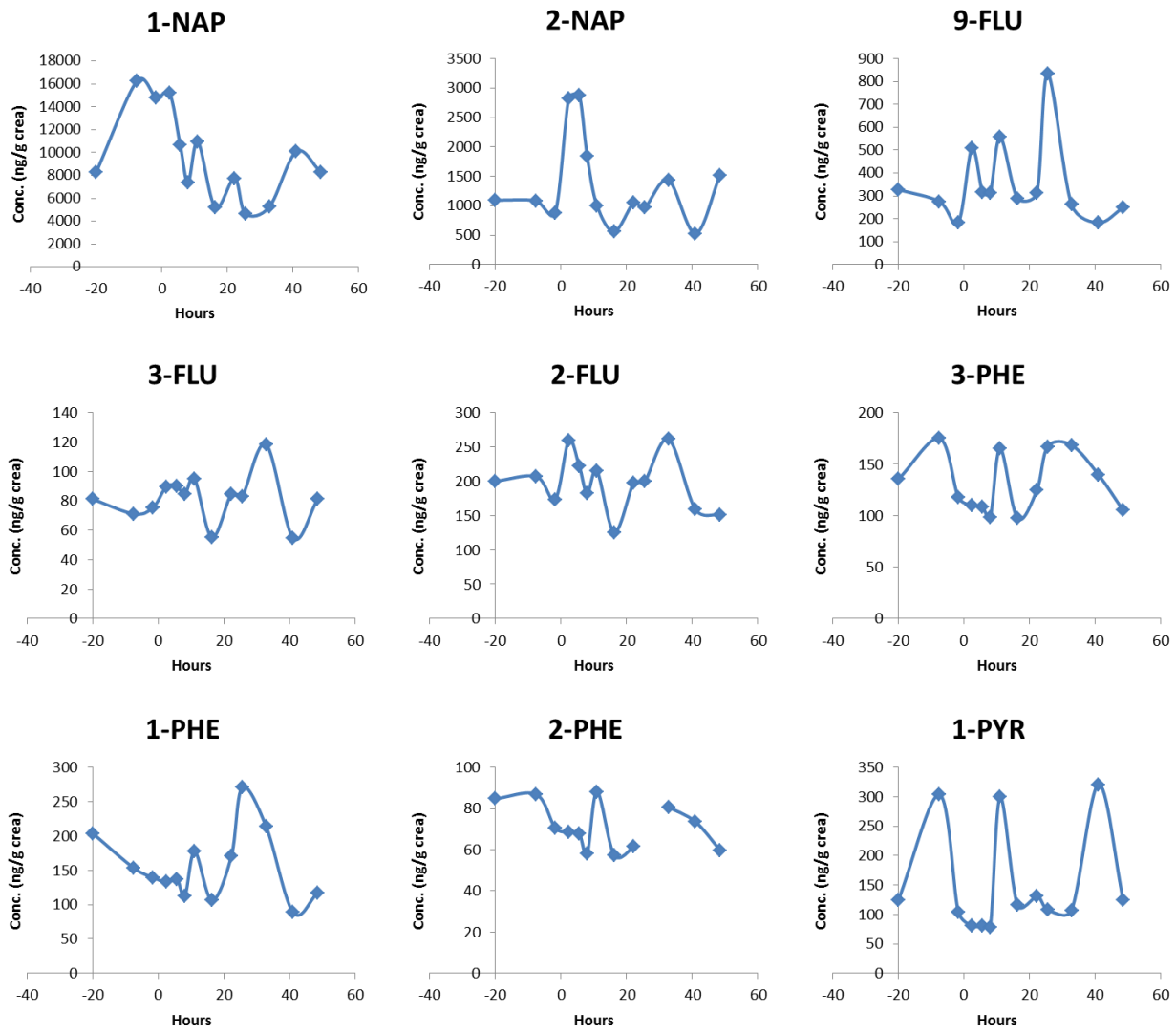

Figure S3. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant C who was exposed to wood smoke for 2 hours (0-2 h).

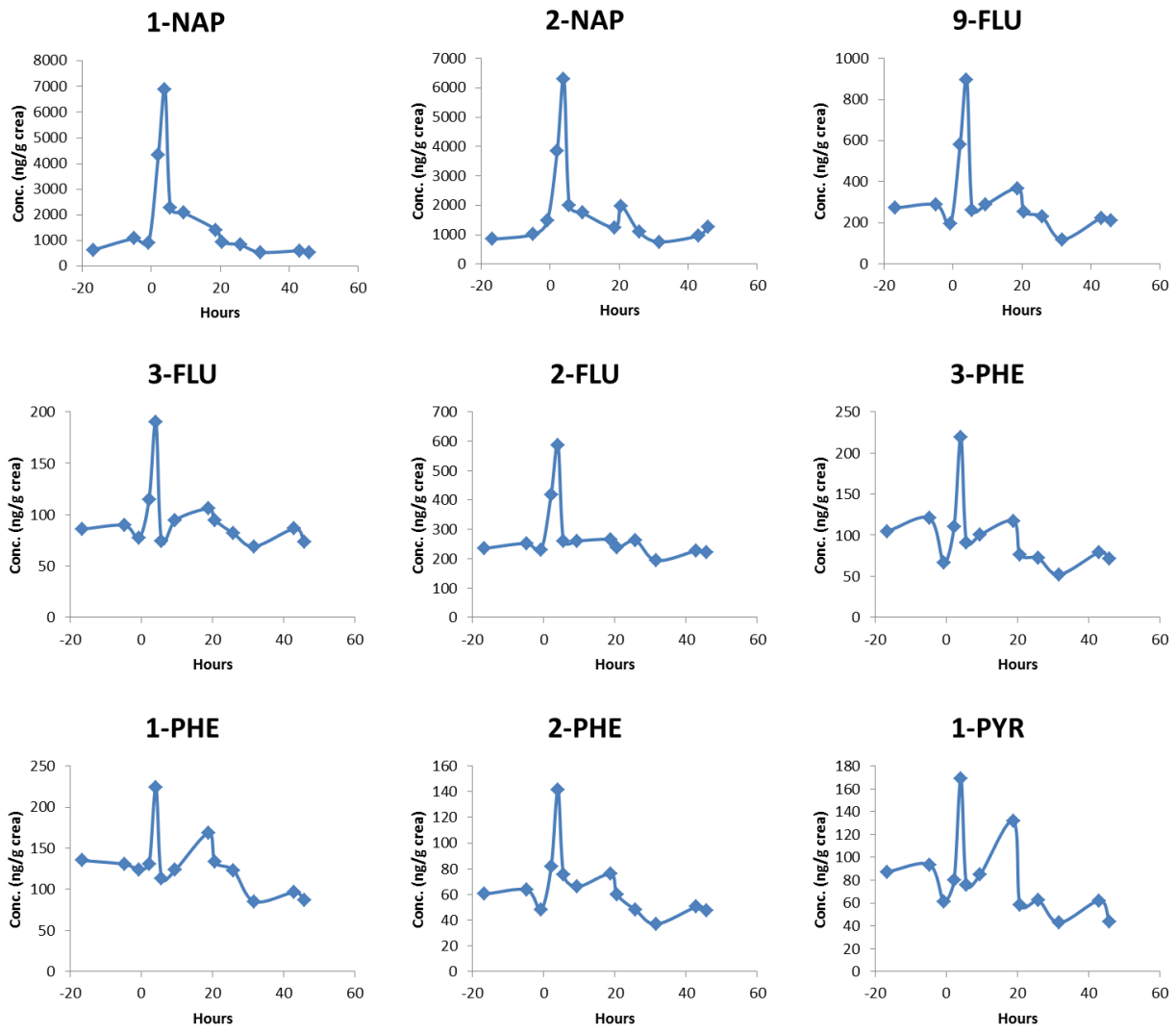

Figure S4. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant D who was exposed to wood smoke for 2 hours (0-2 h).

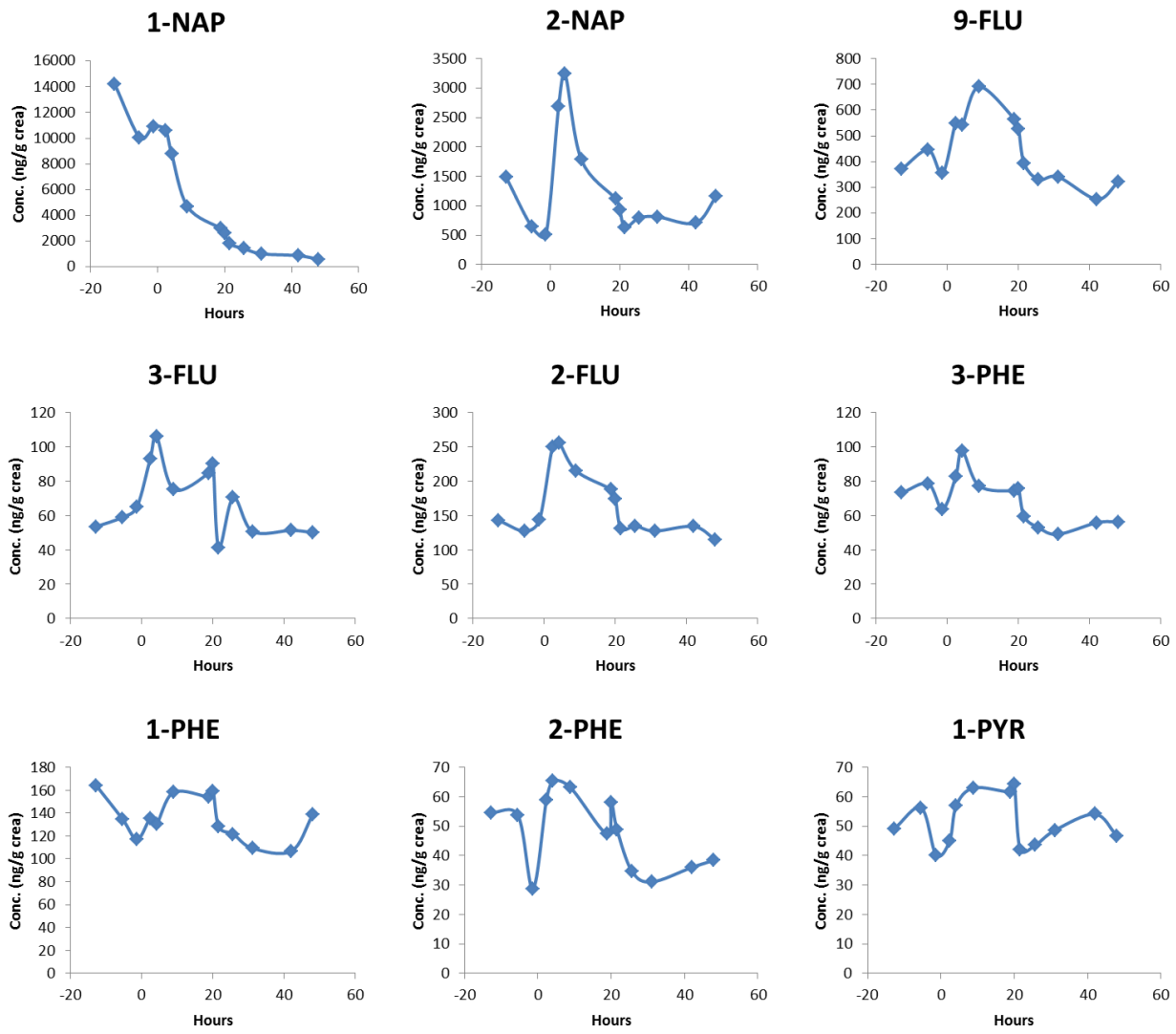

Figure S5. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant E who was exposed to wood smoke for 2 hours (0-2 h).

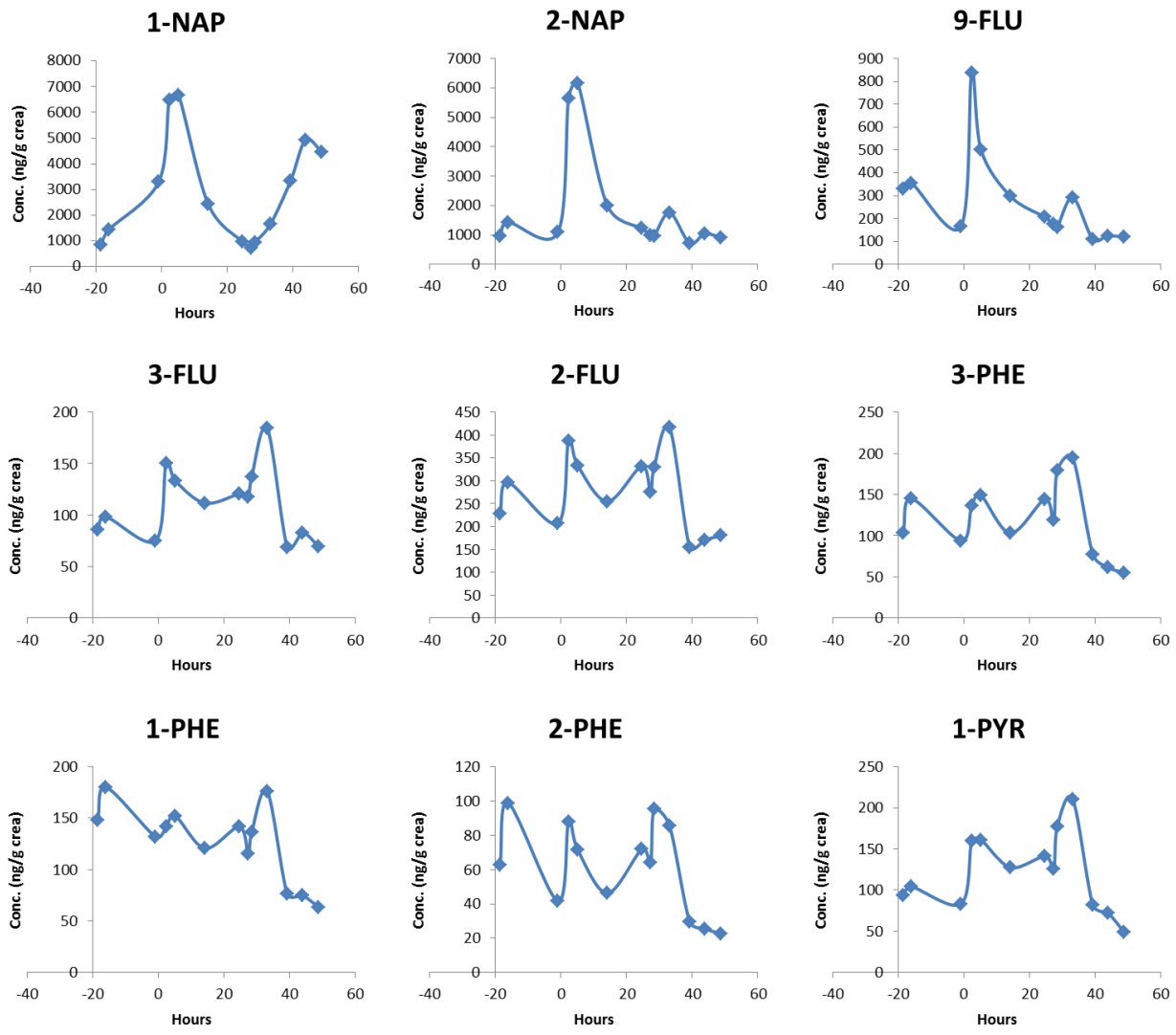

Figure S6. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant F who was exposed to wood smoke for 2 hours (0-2 h).

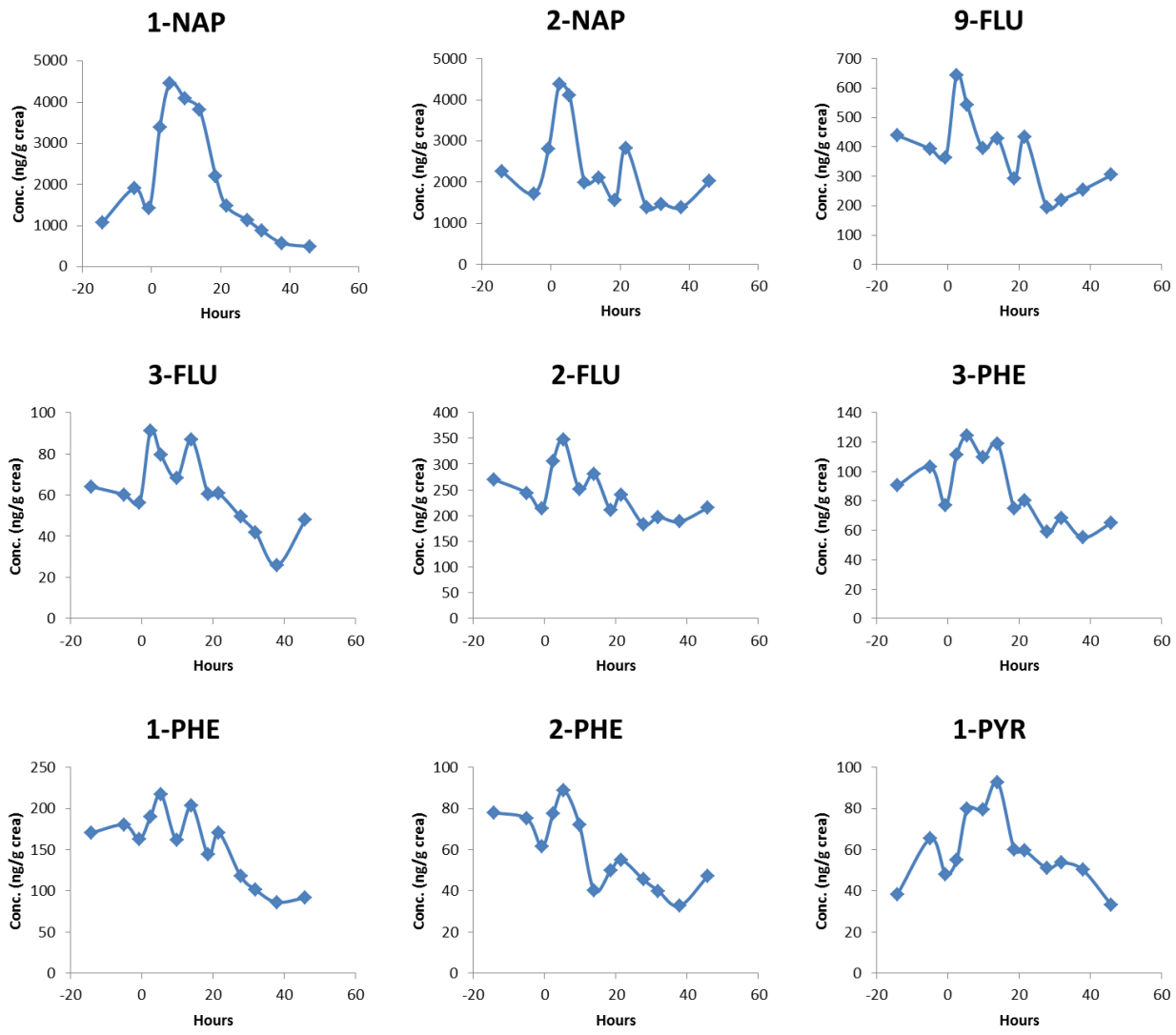

Figure S7. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant H who was exposed to wood smoke for 2 hours (0-2 h).

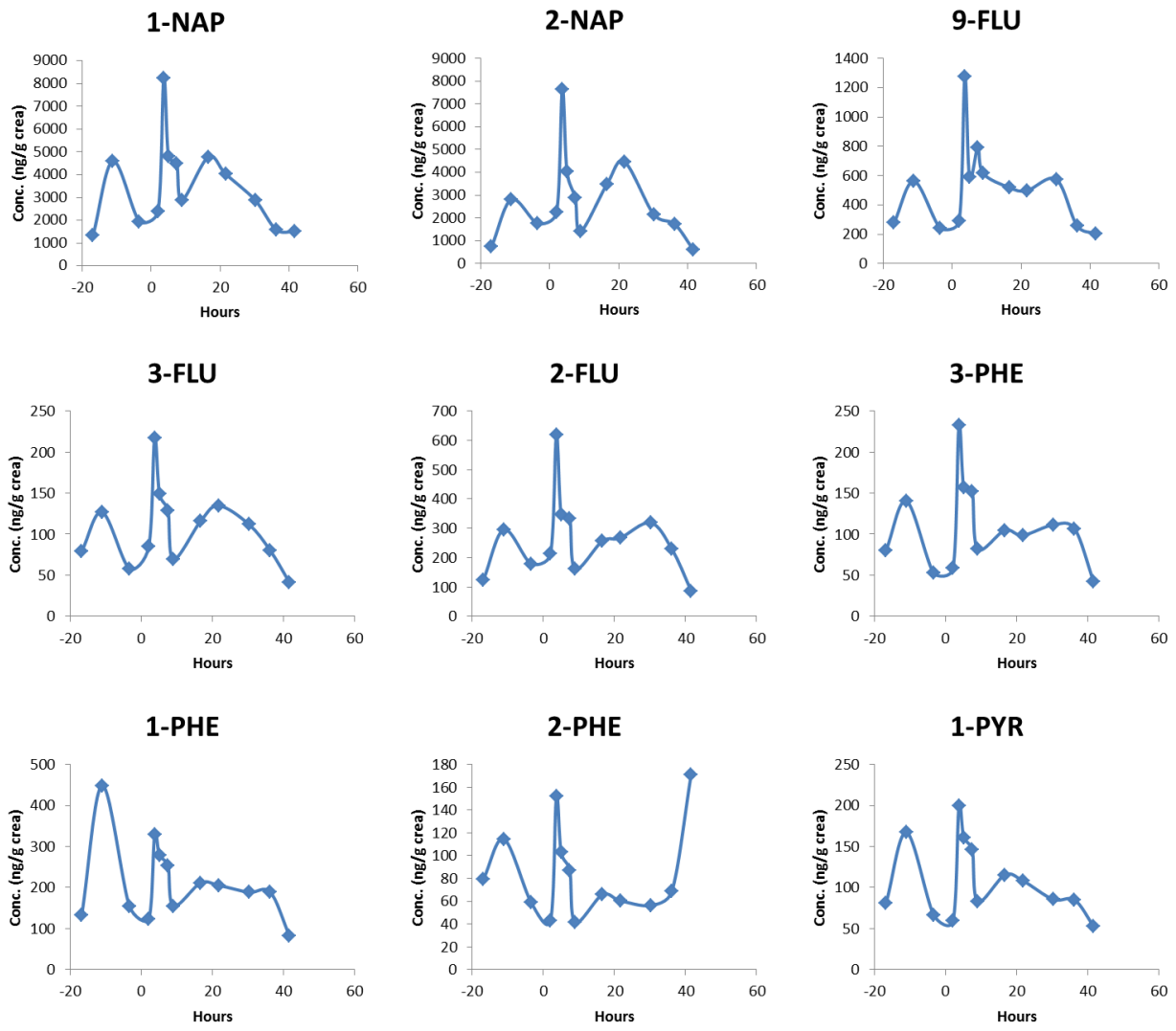

Figure S8. Creatinine-adjusted concentration of 9 urinary PAH metabolites from participant I who was exposed to wood smoke for 2 hours (0-2 h).

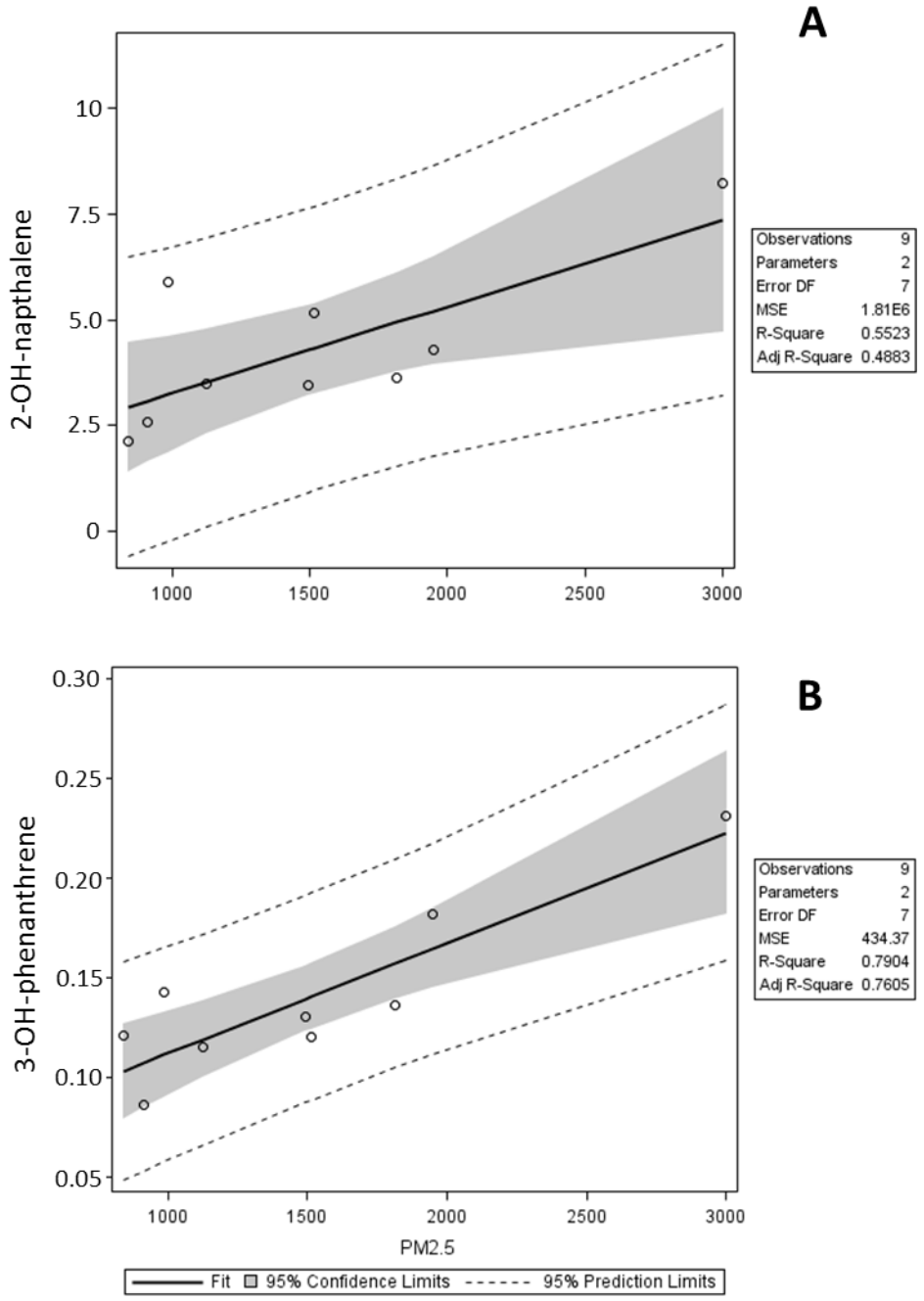

Figure S9. Linear regression plots of urinary 2-naphthol (A), 3-hydroxyphenanthrene (B) against $PM_{2.5}$ (μg/m^3) in personal air samples collected by nine participants. Average concentration (μg/g creatinine) during 0-12 h post exposure was used in this analysis.

Urinary OH-PAH Stability Test

Objective

We had observed that urinary OH-PAHs (free or conjugated) are stable for several years when stored frozen and several weeks when refrigerated (data not shown). In this experiment, we tested the stability of conjugated (naturally present) and unconjugated "free" (manually fortified) OH-PAHs in urine under various storage conditions (~20ºC and 37ºC, under light or kept dark) for up to two days.

Experimental

We chose three urine samples for this experiment, i.e., two anonymously collected urine specimens and a urine pool fortified with a mixture of free OH-PAH standards. Each urine sample was prepared and analyzed in triplicates for each part of the experiment.

1. On the first day of the experiment, we homogenized each urine sample and aliquot it into 15 tubes (2mL in each tube).

2. We placed one triplicate set of each urine at room temperature (~20ºC and under light) and another triplicate set of each urine in an incubation oven (set at 37ºC, temperature verified daily by a thermometer). We placed the remaining tubes in the fridge.

3. On the second day, we placed a second set of urine at room temperature and in the 37ºC oven, while keeping the last triplicate set in the refrigerator.

4. On the third day, we prepared all samples (15 tubes for each urine sample) and analyzed for OH-PAHs.

Results

Results from each triplicate set are presented as mean with standard deviations (error bars in the graphs). The OH-PAH conjugates naturally present in both urine specimens were stable during the testing period under various conditions. Therefore, results from one urine specimen are presented (Figure S10, top graphs). Several free OH-PAHs spiked into the urine pool, on the other hand, degraded during the two-day testing period (Figure S10, bottom graphs).

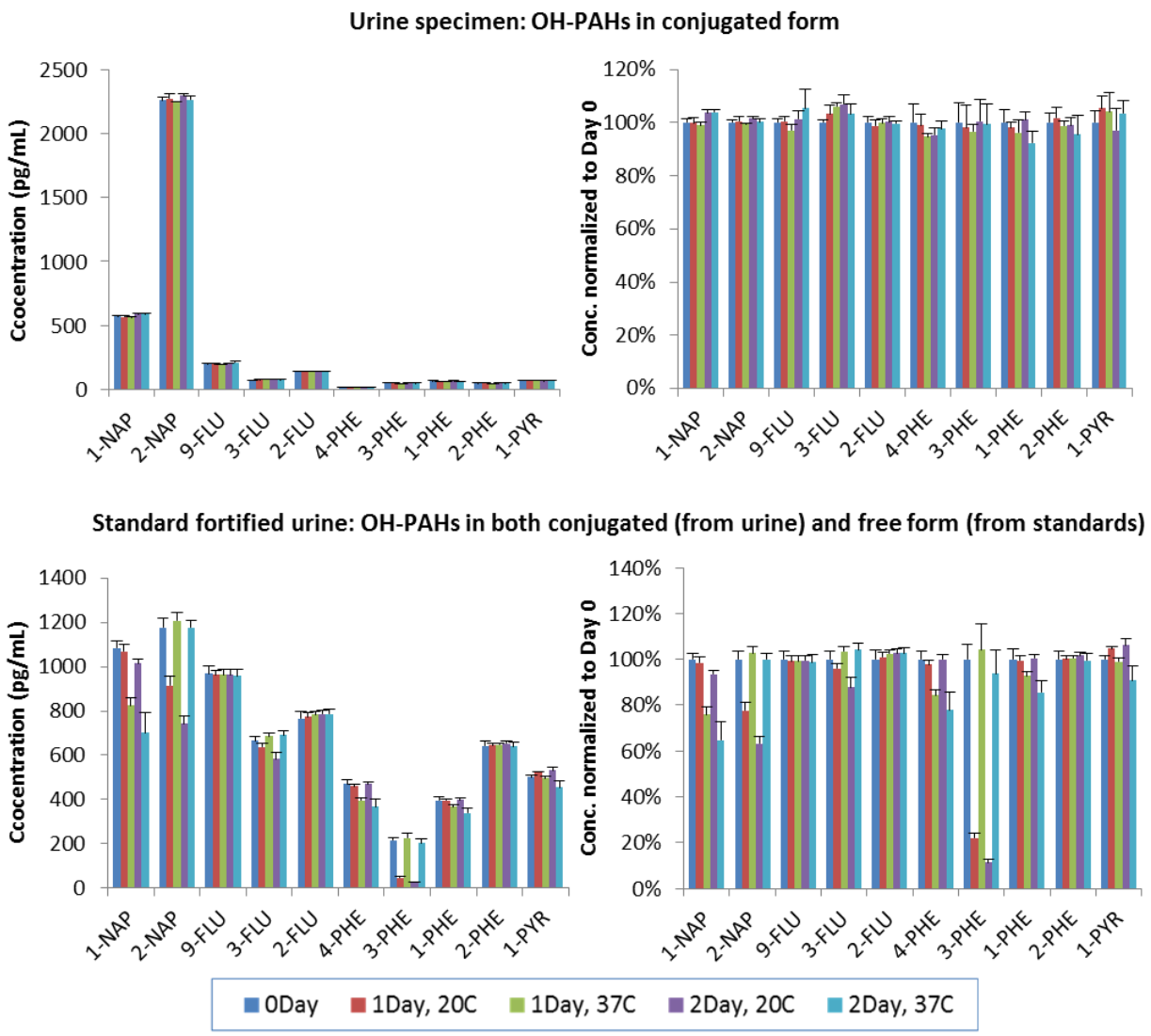

Figure S10. Stability of urinary OH-PAHs in a urine specimen (top) and a urine pool fortified with OH-PAH standards (bottom), stored at 20ºC (under light) or 37ºC (in an oven set at 37ºC) for up to two days.