SUPPORTING INFORMATION

Determination of Parent and Hydroxy PAHs in Personal PM_{2.5} and Urine Samples Collected During Native American Fish Smoking Activities Oleksii Motorykin¹, Jill Schrlau², Yuling Jia², Barbara Harper³, Stuart Harris³, Anna Harding⁴, David Stone², Molly Kile⁴, Daniel Sudakin², Staci L. Massey Simonich^{1,2 *} ¹Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, USA

²Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon 97331, USA

³ Department of Science and Engineering, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, Oregon 97801, USA

⁴ School of Biological and Population Health Sciences, Oregon State University, Corvallis, Oregon 97331, USA

*Address correspondence to S.L.M. Simonich, 1141 Agricultural and Life Sciences Corvallis, OR 97331-7301 USA. Telephone: (541) 737-9194. Fax: (541) 737-0497. Email: staci.simonich@oregonstate.edu

SI contains:

3 Tables 12 Figures

Enzymatic hydrolysis with β -glucuronidase/arylsulfatase

The amount of β -glucuronidase/arylsulfatase needed for enzymatic hydrolysis of OH-PAHs was investigated. Three urine samples (3 mL each) were mixed with 5 mL of acetate buffer and spiked with 0 µl, 10 µl, 100 µl, and 500 µl of β -glucuronidase/arylsulfatase. The samples were extracted, analyzed, and concentrations of OH-PAHs were compared. Figure S11 shows the OH-PAH concentrations in urine after enzymatic hydrolysis with different volumes of β -glucuronidase/arylsulfatase. The results show that 10 µl of β -

glucuronidase/arylsulfatase were enough to hydrolyze the OH-PAH adducts. Larger volumes of β -glucuronidase/arylsulfatase caused the SPE cartridge to clog, resulted in greater noise in the ion chromatograms and the OH-PAH concentrations did not increase significantly (Figure S11). Although previous studies have used β -glucuronidase/arylsulfatase volumes in the range of 5µl to 25 µl¹⁻⁴. 10 µl of β -glucuronidase/arylsulfatase has been shown to be adequate for the enzymatic hydrolysis of OH-PAHs and this is what we chose to use⁵⁻⁹.

SPE column selection

Different SPE phases, including Focus, Plexa, Isolute 101, and a combination of Plexa (for OH-PAHs) and C18 (for PAHs) (in series), were tested in order to extract both PAHs and OH-PAHs from urine. Dichloromethane was used with all SPE phases as a elution solvent. Initially, Focus, Plexa, and Isolute 101 were evaluated for the extraction of OH-PAHs from urine. Later, PAHs were included in the method and the experiment was repeated in order to test Plexa vs Plexa-C18 to extract both PAH and OH-PAH. Three milliliters of UTAK urine (blank urine was analyzed before analysis and concentrations were subtracted before calculations) was spiked with 50 μ l of OH-PAHs (1 μ g/ml, to evaluate Focus vs Plexa vs Isolute 101 SPE cartridges), or PAHs (1 μ g/ml, to evaluate Plexa vs Plexa+C18 SPE cartridges), or a mixture of PAH and OH-PAH, followed by derivatization (Figure 1). The extracts were analyzed and the concentrations of PAH and OH-PAH were compared.

Figure S3 shows the OH-PAH recoveries from urine using Focus, Plexa, and Isolute 101 stationary phases and DCM as elution solvent. This experiment showed that the Plexa SPE cartridge had higher overall recoveries for the dihydroxy-PAHs, such as 2,3-, 1,5-, 1,6-, 2,7, and 2,6-dihydroxynaphthalenes, with a mean recovery of 105%. The Focus SPE cartridge had higher overall recoveries for monohydroxy-PAHs, such as hydroxynaphthalenes, hydroxyphenathrenes, some hydroxybenzo[a]pyrenes and others, with a mean recovery of 128% and were comparable to a previous study⁶. This difference in the two stationary phases may be due to differences in their polarity.

For the extraction of PAHs from urine, Plexa only and Plexa and C18 (combined in series) were tested (with DCM and EA used as elution solvents and combined) and the PAH recoveries are shown in Figure S4. The use of Plexa and C18 in combination had higher overall recoveries of PAHs, mean recovery of 71%, compared to Plexa alone, mean recovery of 56%. PAH are non-polar and the use of a non-polar octadecyl stationary phase (C18) improved PAH recoveries, especially for the more nonpolar, higher molecular weight PAH (Figure S4). *SPE elution solvent composition*

Several elution solvents were tested in order to optimize the OH-PAHs recoveries. Four aliquots of three milliliters of UTAK urine, each, were spiked with OH-PAHs (30μ l, $1 ng/\mu$ l), extracted with Focus SPE, and eluted separately with 3 mL of methanol, 3 ml of DCM + 3 ml of

EA, and the mixture of DCM and EA 1:1 (v:v, 3 ml) and 6:4 (v:v, 3 ml). The extracts were analyzed and concentrations of OH-PAHs were compared.

Figure S12 shows the OH-PAH recoveries for the Focus SPE cartridge using four different elution solvents: DCM : EA (1 : 1,v/v), DCM : EA (6 : 4, v/v), DCM followed by EA, and methanol. The elution of OH-PAH with methanol resulted in the statistically significantly lower recoveries compared to other elution solvents (DCM and EA, or the mixture of DCM and EA) and ranged from 0% to 152%. No statistically significant difference in recoveries were measured among the other elution solvents (p-value>0.05). In the end, we decided to use 3 ml of 100% DCM and 3 ml of 100% EA, in series (fractions were combined before next step), to elute the mixture of OHPAHs and PAHs from the combination of Plexa and C18 stationary phases. *Choice of derivatizing agent*

Three different derivatizing agents, BSTFA (+1%TMCS), BSA/TMCS/TMSI, and MTBSTFA, were tested to investigate which derivatized the largest number of OH-PAH. BSTFA showed poor efficiency for the dihydroxy-PAHs, especially those with hindered OHgroups. BSA worked better than MTBSTFA for hindered groups, but the latter reacts with lower temperature and takes less time to react, forms more stable adducts and shifts the molecular weight more into the higher m/z. For these reasons, it was decided to use MTBSTFA for derivatization. Table S1. The list of OH-PAH, LMW PAH, HMW PAH, and oxy-PAHs and their labeled surrogates, their abbreviations and estimated detection limits.

Class						EDL		
	#	Analyte	Abbreviation	Surrogate	Internal Standard	pg/ml in urine	pg/m ³ of air	
	1	1-Hydroxynaphthalene	1-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	14.6	12.6	
	2	2-Hydroxynaphthalene	2-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	18.0	15.4	
	3	2,3-Dihydroxynaphthalene	2,3-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	181.4	155.5	
	4	1,3-Dihydroxynaphthalene	1,3-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	141.7	121.4	
	5	1,5-Dihydroxynaphthalene	1,5-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	48.7	41.8	
	6	1,6-Dihydroxynaphthalene	1,6-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	25.1	21.5	
	7	2,7-Dihydroxynaphthalene	2,7-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	19.9	17.0	
70	8	2,6-Dihydroxynaphthalene	2,6-OH-Nap	[² H ₇]-1-OH-Nap	$[^{2}H_{10}]$ -Ace	37.5	32.1	
H	9	4-Hydroxyphenanthrene	4-OH-Phen	$[^{13}C_6]$ -4-OH-Phen	$[^{2}H_{10}]$ -Flt	6.0	5.2	
- P /	10	3-Hydroxyphenanthrene	3-OH-Phen	$[^{13}C_6]$ -4-OH-Phen	$[^{2}H_{10}]$ -Flt	30.1	25.8	
HO	11	1-Hydroxyphenanthrene	1-OH-Phen	$[^{13}C_6]$ -4-OH-Phen	$[^{2}H_{10}]$ -Flt	31.8	27.2	
•	12	2-Hydroxyphenanthrene	2-OH-Phen	$[^{13}C_6]$ -4-OH-Phen	$[^{2}H_{10}]$ -Flt	24.9	21.3	
	13	2-Hydroxyanthraquinone	2-OH-AntQn	$[^{13}C_6]$ -4-OH-Phen	$[^{2}H_{10}]$ -Flt	93.4	80.0	
	14	9-Hydroxyfluorene	9-OH-Flo	[² H ₉]-2-OH-Flo	$[^{2}H_{10}]$ -Flt	160.8	137.8	
	15	3-Hydroxyfluorene	3-OH-Flo	[² H ₉]-2-OH-Flo	$[^{2}H_{10}]$ -Flt	39.4	33.7	
	16	2-Hydroxyfluorene	2-OH-Flo	[² H ₉]-2-OH-Flo	$[^{2}H_{10}]$ -Flt	41.4	35.5	
	17	1-Hydroxy-9-fluorenone	1-OH-Flon	[² H ₉]-2-OH-Flo	$[^{2}H_{10}]$ -Flt	26.2	22.4	
	18	2-Hydroxy-9-fluorenone	2-OH-Flon	[² H ₉]-2-OH-Flo	$[^{2}H_{10}]$ -Flt	82.1	70.4	
	19	3-Hydroxyfluoranthene	3-OH-Flt	[¹³ C ₆]-3-OH-Flt	[² H ₁₂]-BkFlt	15.9	13.7	

20	1-Hydroxypyrene	1-OH-Pyr	[¹³ C ₆]1-OH-Pyr	[² H ₁₂]-BkFlt	19.4	16.6
21+ 22	2-OH-B(a)anthracen+ 3-OH-B(c)pnenanthren	2-OH-BaA+3- OH-BcPh	[¹³ C ₆]-1-OH-BaA	[² H ₁₂]-BkFlt	19.3	16.5
23	10-Hydroxybenzo(a)pyrene	10-OH-BaP	$[^{13}C_6]$ -3-OH-BcPh	[² H ₁₂]-BkFlt	44.3	38.0
24	12-Hydroxybenzo(a)pyrene	12-OH-BaP	$[^{13}C_6]$ -3-OH-BcPh	[² H ₁₂]-BkFlt	52.1	44.6
25	7-Hydroxybenzo(a)pyrene	7-OH-BaP	$[^{13}C_6]$ -3-OH-BcPh	[² H ₁₂]-BkFlt	43.7	37.4
26	9-Hydroxybenzo(a)pyrene	9-OH-BaP	$[^{13}C_6]$ -3-OH-BcPh	[² H ₁₂]-BkFlt	29.6	25.4
27	3-Hydroxybenzo(a)pyrene	3-OH-BaP	$[^{13}C_6]$ -3-OH-BcPh	[² H ₁₂]-BkFlt	36.5	31.3
28	4-Hydroxychrysene	4-OH-Chr	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	13.9	11.9
29	6-Hydroxychrysene	6-OH-Chr	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	43.9	37.7
30	3-Hydroxychrysene	3-OH-Chr	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	35.6	30.5
31	2-Hydroxychrysene	2-OH-Chr	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	20.3	17.4
32	1-Hydroxychrysene	1-OH-Chr	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	9.5	8.1
33	2,6-Hydroxyanthraquinone	2.6-OH-AntQn	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	6.8	5.8
34	11-Hydroxybenzo(b)fluoranthene	11-OH-BbFlt	[¹³ C ₆]-3-OH-Chr	[² H ₁₂]-BkFlt	38.6	33.1
35	Naphthalene	Nap	$[^{2}H_{10}]$ -Flo	$[^{2}H_{10}]$ -Ace	9.2	7.9
36	Acenaphtylene	Асу	$[^{2}H_{10}]$ -Flo	$[^{2}H_{10}]$ -Ace	31.6	27.1
37	Acenaphthene	Ace	$[^{2}H_{10}]$ -Flo	$[^{2}H_{10}]$ -Ace	18.5	15.8
38	Fluorene	Flo	$[^{2}H_{10}]$ -Flo	$[^{2}H_{10}]$ -Ace	23.1	19.8
39	Phenanthrene	Phen	[² H ₁₀]-Phen	$[^{2}H_{10}]$ -Ace	3.0	2.5
40	Anthracene	Ant	[² H ₁₀]-Phen	$[^{2}H_{10}]$ -Ace	19.6	16.8
41	Fluoranthene	Flt	$[^{2}H_{10}]$ -Pyr	$[^{2}H_{10}]$ -Flt	15.2	13.0
42	Pyrene	Pyr	$[^{2}H_{10}]$ -Pyr	$[^{2}H_{10}]$ -Flt	22.6	19.3
43	Retene	Ret	$[^{2}H_{10}]$ -Pyr	$[^{2}H_{10}]$ -Flt	60.5	51.8
44	Benzo(a)anthracene	BaA	[² H ₁₂]-TriPh	$[^{2}H_{10}]$ -Flt	27.9	23.9

		~	~	-2	-2	l	
	45	Chrysene	Chr	[² H ₁₂]-TriPh	[⁻ H ₁₀]-Flt	19.7	16.9
	46	Triphenylene	TriPh	[² H ₁₂]-TriPh	$[^{2}H_{10}]$ -Flt	19.7	16.9
	47	Benzo(b)fluoranthene	BbFlt	$[^{2}H_{12}]$ -BaP	[² H ₁₂]-BkFlt	52.1	44.6
	48	Benzo(k)fluoranthene	BkFlt	$[^{2}H_{12}]$ -BaP	[² H ₁₂]-BkFlt	56.9	48.8
	49	Benzo(e)pyrene	BeP	$[^{2}H_{12}]$ -BaP	[² H ₁₂]-BkFlt	72.5	62.2
	50	Benzo(a)pyrene	BaP	$[^{2}H_{12}]$ -BaP	[² H ₁₂]-BkFlt	89.7	76.9
	51	Indeno(1,2,3-cd)pyrene	I(1,2,3-cd)Pyr	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt	36.1	30.9
	52	Dibenz(a,h)anthracene	BahA	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt	43.7	37.5
	53	Benzo(ghi)perylene	BghiPer	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt	29.7	25.5
	54	Picene	Pic	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		142.9
	55	Naphtho[1,2-b]fluoranthene	N12bF	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		142.9
	56	Naphtho[2,3-j]fluoranthene	N23jF	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		285.7
	57	Naphtho[2,3-b]fluoranthene	N23bF	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		285.7
AHs	58+ 59	Dibenzo[a,e]fluoranthene+ Dibenzo[b,k]fluoranthene	DBaeF+DBbkF	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		142.9
/ P .	60	Dibenzo[j,l]fluoranthene	DBjlF	[² H ₁₂]- BghiPer	$[^{2}H_{12}]$ -BkFlt		142.9
ММ	61	Dibenzo[a,l]pyrene	DBalP	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		142.9
H	62	Naphtho[2,3-e]pyrene	N23eP	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		142.9
	63	Dibenzo[a,e]pyrene	DBaeP	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		285.7
	64	Coronene	Cor	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		142.9
	65	Dibenzo[e,l]pyrene	DBelP	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		285.7
	66	Benzo[b]perylene	BbPer	[² H ₁₂]- BghiPer	[² H ₁₂]-BkFlt		285.7
' s	67	9-fluorenone		[² H ₈]- 1,4-NQn	$[^{2}H_{8}]$ - 9-Flon		0.33
)xy AH	68	9,10-anthraquinone		[² H ₈]- 9,10-AntQn	$[^{2}H_{8}]$ - 9-Flon		0.42
	69	2-methyl-9.10-anthraquinone		$[^{2}H_{8}]$ - 9,10-AntQn	$[^{2}H_{8}]$ - 9-Flon		1.4

70	benzanthrone	$[^{2}H_{8}]$ - 9,10-AntQn	$[^{2}H_{8}]$ - 9-Flon	0.56
71	benz[a]anthracene-7,12-dione	$[^{2}H_{8}]$ - 9,10-AntQn	$[^{2}H_{8}]$ - 9-Flon	0.15

Window	Retention times, min	#	OH-PAH or PAH	MW	m/z 1	m/z 2	m/z 3	m/z 4
1	9.8-11.0	1	Naphthalene	128	128	127		
		2	Acenaphthylene	152	152	151	76	
		3	Acenaphthene-d10-IS	164	164	162		
3	16.0-24.0	4	Acenaphthene	154	154	153	152	
		5	Fluorene-d10	176	176	174		
		6	Fluorene	166	166	165	163	
		7	1-Hydroxynaphthalene	144	258	201	185	
		8	1-Hydroxy[2H7]naphthalene	151	265	208	192	
		9	2-Hydroxynaphthalene	144	258	201	185	
4	24.0-26.50	10	2-Hydroxy[2H7]naphthalene	151	265	208	192	
		10	Phenanthrene-d10	188	188	189		
		11	Phenanthrene	178	178	176	179	
		12	Anthracene	178	178	176	179	
	31.0-35.5	13	9-Hydroxyfluorene	182	296	239		165
		14	Fluoranthene-d10-IS	212	212	213		
6		15	Fluoranthene	202	202	200	203	
		16	Pyrene-d10	212	212	213		
		17	Pyrene	202	202	203	200	
		18	3-Hydroxyfluorene	182	296	239		165
7		19	Retene	234	219	234	204	
(35.5-37.5	20	2-hydroxy[2H9]fluorene	191	305	248		174
		21	2-hydroxyfluorene	182	296	239		165
		22	2,3-Dihydroxynaphthalene	160	388	331		273
		23	1,3-Dihydroxynaphthalene	160	388	331		273
8	37.5-39.7	24	4-Hydroxyphenanthrene	194	308	251	235	
		25	4-hydroxy[13C6]phenanthrene	200	255	239	312	
		26	1-hydroxy-9-fluorenone	196		253		223
		27	1,5-Dihydroxynaphthalene	160	388	331		273
9	39.7-41.5	28	1,6-Dihydroxynaphthalene	160	388	331		275
		29	2-hydroxy-9-fluorenone	196	310	253		

Table S2. Selected Ion Monitoring (SIM) windows and m/z ions monitored. The surrogates for OH-PAHs and PAHs are in bold and IS indicates internal standards

		30	3-Hydroxyphenanthrene	194	308	251	235	
		31	2,7-Dihydroxynaphthalene	160	388	331		275
		32	2,6-Dihydroxynaphthalene	160	388	331		275
		33	1-Hydroxyphenanthrene	194	308	251	235	
		34	2-Hydroxyphenanthrene	194	308	251	235	
		35	Triphenylene-d12	240	240	241		
10	41.5-44.5	36	Benzo(a)anthracene	228	228	226	229	
		37	Chrysene+triphenylene	228	228	226	229	
		38	2-Hydroxyanthraquinone	224	338	281		253
		39	3-hydroxy[13C6]flouranthene	224	338	281	265	221
11	44.5-48.5	40	3-Hydroxyfluoranthene	218	332	275	259	189
		41	1-hydroxy[13C6]pyrene	224	338	281	265	250
		42	1-Hydroxypyrene	218	332	275	259	
		43	Benzo(b)fluoranthene	252	252	250	253	
	48.5-52.5	44	Benzo(k)fluoranthene-d12-IS	264	264	265		
12		45	Benzo(k)fluoranthene	252	252	250	253	
12		46	Benz(e)pyrene	252	252	250	253	
		47	Benzo(a)pyrene-d12	264	264	265	263	
		48	Benzo(a)pyrene	252	252	250	253	
		49	1-hydroxy[13C6]benzo(a)anthracene	250	364	307	291	276
		50	4-Hydroxychrysene	244	358	301	285	270
		51	6-Hydroxychrysene	244	358	301	285	270
		52	2-hydroxybenzo(a)anthracene	244	358	301	285	
13	52 5-58 00	53	3-Hydroxybenzo(c)phenanthrene	244	358	301	285	
15	52.5-50.00	54	3-Hydroxy[13C6]benzo(c)phenanthrene	250	364	307	291	
		55	3-Hydroxychrysene	244	358	301	285	
		56	3-hydroxy[13C6]chrysene	250	364	307	291	
		57	1-Hydroxychrysene	244	358	301	285	
		58	2-Hydroxychrysene	244	358	301	285	
		59	Indeno(1,2,3-cd)pyrene	276	276	274	277	
1/	58 0-61 5	60	Dibenz(a,h)anthracene	278	278	276	279	
14	30.0-01.3	61	Benzo(ghi)perylene-d12	288	288	289		
		62	Benzo(ghi)perylene	276	276	274	277	
15	61 5-67 2	63	2,6-Dihydroxyanthraquinone	240	468	411	395	
15	01.5-67.2	64	10-hydroxybenzo(a)pyrene	268	382	325	309	

65	12-hydroxybenzo(a)pyrene	268	382	325	309	
66	11-hydroxybenzo(b)fluoranthrene	268	382	325	309	
67	7-Hydroxybenzo(a)pyrene	268	382	325	309	
68	9-Hydroxybenzo(a)pyrene	268	382	325	309	
69	3-Hydroxybenzo(a)pyrene	268	382	325	309	

Figure S1. Experimental timeline for the collection of urine and personal $PM_{2.5}$ samples. SS – smoke shed; TP – tipi; U – urine sample; A – air sample; the number at the end of sample name represents the length of exposure, in hours; red arrow shows the duration of fish smoking activity;

			5/15/2011	5/16/2011
	Smoke	Urine	Apple-SS-U-0> App	le-SS-U-5.5
	shed	Air	Time zero 🗲	Apple-SS-A-14.2
Apple	Smoking	activity	←	→
	Tini	Urine	Apple-TP-U-0> Appl	e-TP-U-4.3> Apple-TP-U-12.1> Apple-TP-U-21.8
	прі	Air	Time zero 🗲	Apple-TP-A-12.8
	Smoking	activity	←	→
			5/17/2011	5/18/2011
	Smoke	Urine		Alder-SS-U-10.0 — Alder-SS-U-21.8 — Alder-SS-U-33.3
	shed	Air	Time zero 🗲	> Alder-SS-A-34.8
Alder	Smoking	activity	←	→
	Tini	Urine		Alder-TP-U-10.0
		Air	Time zero 🗲	> Alder-TP-A-34.8
	Smoking activity		<	→

Figure S2. Tipi (upper left) and smoke shed (upper right), and fish positioning inside (lower left and lower right, respectively)









Figure S4. Mean PAH recoveries for the entire analytical method using spiked UTAK urine and different SPE phases (three independent extractions, error bars represent standard deviation).

Figure S5. Mean PLE recoveries for the extraction of PAH and OH-PAH from PM_{2.5} (three independent extractions, error bars represent standard deviation). * - statistically different (p-value<0.05)





Figure S6. Stability of spiked OH-PAHs in frozen UTAK urine (one extraction, storage temperature -20°C).

Figure S7. Mean stability of the products of OH-PAH and MTBSTFA derivatization in the extract (frozen at -20°C) (three independent extractions, error bars represent standard deviation). * - statistically (p-value<0.05) different from Day 1.



Figure S8. Mean intra-day variability of the Parent and OH-PAH method for urine (three independent extractions, error bars represent standard deviation). The solutions of three concentrations (10, 100, and 1000 pg/ul) were prepared and analyzed three times during 24 hours.



Figure S9. Mean inter-day variability of the OH-PAH method for urine (three independent extractions, error bars represent standard deviation). The solutions of three concentrations (10, 100, and 1000 pg/ul) were prepared and analyzed three times over three consecutive days.



SRM 3672. Organic	Our Result	s, ng/L	NIST Resul		
contaminants in smoker's urine	Concentration	Standard Deviation	Concentration	Standard Deviation	% diff
1-Hydroxynaphthalene*	25630	949	34442	4382	-26%
2-Hydroxynaphthalene	11949	1127	8733	163	37%
1,5-Dihydroxynaphthalene	668	367	NM	NM	NA
1,6-Dihydroxynaphthalene	302	15	NM	NM	NA
9-Hydroxyphenanthrene	NM	NM	977	62	NA
4-Hydroxyphenanthrene*	82	68	49	5	67%
3-Hydroxyphenanthrene	195	59	125	7	56%
2-Hydroxyphenanthrene	252	40	84	1	76%
1-Hydroxyphenanthrene	148	24	136	14	86%
9-Hydroxyfluorene	2384	1102	337	78	607%
3-Hydroxyfluorene	530	61	428	18	24%
2-Hydroxyfluorene*	892	67	870	15	3%
1-Hydroxypyrene*	211	36	173	10	22%
Phenanthrene	987	128			NA
Fluoranthene	76	33	NM	NM	NA
Pyrene*	111	25	NM	NM	NA
Retene	444	67			NA

Table S3. Analysis of NIST SRM samples. (NM – not measured; NA – not available; * - Surrogate was available for this compound)

SRM 3673. Organic	Our Result	s, ng/L	NIST Resul		
contaminants in non- smoker's urine	Concentration	Standard Deviation	Concentration	Standard Deviation	% diff
1-Hydroxynaphthalene*	150943	10925	210933	33627	-28%
2-Hydroxynaphthalene	2151	305	1345	31	60%
1,5-Dihydroxynaphthalene	1727	231			NA
9-Hydroxyphenanthrene	NM		12	1	NA
4-Hydroxyphenanthrene*	74	62	10	1	611%
3-Hydroxyphenanthrene	74	45	28	1	169%
2-Hydroxyphenanthrene	68	18	25	4	177%
1-Hydroxyphenanthrene	112	7	49	8	129%
9-Hydroxyfluorene	965	865	110	26	776%
3-Hydroxyfluorene	142	43	39	4	264%
2-Hydroxyfluorene*	163	38	107	7	52%
1-Hydroxypyrene*	44	24	30	2	43%
Phenanthrene	851	21			NA
Fluoranthene	99	39	NM	NM	NA
Pyrene*	120	24	NM	NM	NA
Retene	339	64			NA



Figure S10. PAH profile from Personal Air Samples

Figure S11. Mean concentrations of selected OH-PAHs in urine extracts after addition of different volumes of β -glucuronidase/arylsulfatase (three independent extractions, points represent average values, whiskers – standard deviation).



Figure S12. OH-PAHs recoveries from standard solution using Focus SPE with different eluent compositions (one extraction).



References

- (1) Klotz, K., Schindler, B. K., and Angerer, J. (2011) 1,2-Dihydroxynaphthalene as biomarker for a naphthalene exposure in humans. *Int. J. Hyg. Environ. Health.*, 214, 110-114.
- (2) Smith, C. J., Huang, W. L., Walcott, C. J., Turner, W., Grainger, J., and Patterson, D. G. (2002) Quantification of monohydroxy-PAH metabolites in urine by solid-phase extraction with isotope dilution-GC-MS. *Anal. Bioanal. Chem.*, *372*, 216-220.
- (3) Xu, X., Zhang, J. F., Zhang, L., Liu, W. L., and Weisel, C. P. (2004) Selective detection of monohydroxy metabolites of polycyclic aromatic hydrocarbons in urine using liquid chromatography/triple quadrupole tandem mass spectrometry. *Rapid Commun. Mass Spectrom.*, *18*, 2299-2308.
- Hagedorn, H. W., Scherer, G., Engl, J., Riedel, K., Cheung, F., Errington, G., Shepperd, J., and McEwan, M. (2009) Urinary Excretion of Phenolic Polycyclic Aromatic Hydrocarbons (OH-PAH) in Nonsmokers and in Smokers of Cigarettes with Different ISO Tar Yields. J. Anal. Toxicol., 33, 301-309.
- (5) Fan, R. F., Wang, D. L., Ramage, R., and She, J. W. (2012) Fast and Simultaneous Determination of Urinary 8-Hydroxy-2 '-deoxyguanosine and Ten Monohydroxylated Polycyclic Aromatic Hydrocarbons by Liquid Chromatography/Tandem Mass Spectrometry. *Chem. Res. Toxicol.*, 25, 491-499.
- (6) Li, Z., Sandau, C. D., Romanoff, L. C., Caudill, S. P., Sjodin, A., Needham, L. L., and Patterson, D. G. (2008) Concentration and profile of 22 urinary polycyclic aromatic hydrocarbon metabolites in the US population. *Environ. Res.*, *107*, 320-331.
- Onyemauwa, F., Rappaport, S. M., Sobus, J. R., Gajdosova, D., Wu, R. A., and Waidyanatha, S. (2009) Using liquid chromatography-tandem mass spectrometry to quantify mono hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. J. *Chromatogr. B*, 877, 1117-1125.
- (8) Romanoff, L. C., Li, Z., Young, K. J., Blakely, N. C., Patterson, D. G., and Sandau, C. D. (2006) Automated solid-phase extraction method for measuring urinary polycyclic aromatic hydrocarbon metabolites in human biomonitoring using isotope-dilution gas chromatography high-resolution mass spectrometry. *J. Chromatogr. B*, 835, 47-54.
- (9) Schummer, C., Delhomme, O., Appenzeller, B. M. R., Wennig, R., and Millet, M. (2009) Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta*, 77, 1473-1482.