

Supporting Information

Levels of Polycyclic Aromatic Hydrocarbons in Maternal Serum and Risk of Neural Tube Defects in Offspring

Bin Wang ^{†, #}, Lei Jin ^{†, #}, Aiguo Ren ^{†, *}, Yue Yuan [†], Jufen Liu [†], Zhiwen Li [†], Le Zhang [†], Deqing Yi [†], Lin-lin Wang [†], Yali Zhang [†], Xilong Wang [§], Shu Tao [§], Richard H. Finnell ^{†, ‡}

[†] Institute of Reproductive and Child Health / Ministry of Health Key Laboratory of Reproductive Health, School of Public Health, Peking University, Beijing 100191, P. R. China

[§] Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing 100871, P. R. China

[‡] Dell Pediatric Research Institute, Department of Nutritional Sciences, University of Texas at Austin, Austin, TX 78723, USA

[#] These authors contributed equally to this work.

^{*} Corresponding author. Aiguo Ren, Institute of Reproductive and Child Health, Peking University Health Center, Beijing 100191, China. Phone: +86-10-82801140, Fax: +86-10-82801141, E-mail: renag@pku.edu.cn.

Number of tables: 4

Number of figure: 1

32 **Solvents and Reagents.**

33 Acetonitrile and *n*-hexane of Ultra Resi-Analyzed[®] were purchased from Merck,
34 Germany, and acetone and dichloromethane of Suprasolv[®] from J.T. Baker[®], USA.
35 Silica gel (100-200 mesh), neutral aluminum oxide (200-300 mesh), and granular
36 anhydrous sodium sulfate were all obtained from Beijing Chemical Reagent Co.,
37 China. Anhydrous sodium sulfate was baked at 600°C for 6 h and silica gel and
38 neutral aluminum oxide were heated at 450°C for 4 h to remove impurities. Silica gel
39 was further reactivated and kept in a sealed desiccator and heated at 130°C for at least
40 16 h before use. Granular anhydrous sodium sulfate was stored in a sealed glass bottle
41 after cleaning. All glassware was cleaned with an ultrasonic cleaner (KQ-500B,
42 Kunshan, China) for 30 min with liquid detergent, rinsed with distilled water, and
43 finally baked at 400°C for 6 h.

44 **Extraction and Cleanup for PAH Analysis**

45 PAH extraction was modified by using a microwave extraction system. Briefly,
46 0.6-1.5 ml serum spiked with two recovery surrogates (2-fluorobiphenyl and
47 *p*-terphenyl D₁₄, J&K Chemical, USA) was extracted with 20 ml acetonitrile using
48 microwave extraction system (CEM Mars Xpress, USA, 1200 W). The extraction
49 temperature program was increased from room temperature to 110°C over the course
50 of 10 min, and held at this temperature for another 10 min. The precipitated lipid was
51 filtered using a 37 mm (diameter) glass fiber filter (0.22 μm). The filtrate was
52 transferred to a 250-ml separatory funnel and then extracted with 30 ml *n*-hexane with
53 a 4% sodium sulfate solution, twice. The extraction (about 60 ml *n*-hexane) was
54 concentrated to about 1 ml in a rotary evaporator and transferred to a silica/alumina
55 gel cleanup column (30 cm×10 mm i.d.) containing: 12 cm alumina, 12 cm silica gel,
56 and 1 cm anhydrous sodium sulfate from bottom to top; conditioned with 10 ml
57 *n*-hexane). The column was eluted with 50 ml *n*-hexane/dichloromethane (1:1, v/v),
58 and the eluate was concentrated to about 1 ml and spiked with five mixed internal
59 standards (naphthalene-d₈, acenaphthene-d₁₀, anthracene-d₁₀, chrysene-d₁₂, and
60 perylene-d₁₂, J&K Chemical, USA). The extract was further concentrated to about
61 100 μL in a 250 μL glass intubation tube prior to quantitative analysis. All the

62 extraction and cleanup processes were operated in the fume hood and all the sample
63 containers were sealed when they were waiting for the next treatment to avoid the
64 PAH contamination from ambient environment.
65

66 Table S1. Median concentrations (ng/g lipid) of polycyclic aromatic hydrocarbons (PAHs) in maternal serum by population characteristics in
 67 Shanxi Province, China, 2010-2013

Population characteristic	Number of subjects ^a	ANY	ACE	FLE	PHE	ANT	FLU	RET	PYR	BAA	CHR	BBF	BKF	BAP	ΣL-PAHs	ΣH-PAHs	ΣPAHs
Maternal age(y)																	
< 25	114	168	188	592	937	78.9	181	344	293	83.9	109	77.6	23.2	34.0	2851	791	3421
25 - 29	64	136	190	644	1027	104	234	343	296	106	104	50.6	12.8	31.6	2713	856	3666
≥ 30	55	101	211	527	1328	121	330	412	251	103	86.9	67.9	17.4	61.5	3533	808	4341
<i>p</i> ^b		0.568	0.877	0.806	0.186	0.026	0.225	0.797	0.837	0.515	0.828	0.774	0.557	0.128	0.536	0.665	0.618
Maternal prepregnancy BMI (kg/m²)																	
< 18.5	24	210	208	634	969	101	214	482	333	92	134	39.7	11.1	33.5	4104	994	5143
18.5-24.9	142	132	158	514	1019	84.0	191	378	262	70.3	85.9	71.1	19.8	35.3	2644	741	3397
≥ 25	61	174	266	765	1143	117	218	388	332	120	185	86.5	23.4	50.7	3621	958	4625
<i>p</i> ^b		0.717	0.022	0.062	0.310	0.050	0.466	0.898	0.433	0.084	0.031	0.457	0.575	0.663	0.209	0.026	0.120
Maternal education																	
Primary or lower	11	19.9	165	606	1542	141	303	581	272	101	84.9	55.4	13.7	100	3592	749	4341
Junior high	152	163	191	592	1024	96.8	229	426	321	103	118	67.0	17.0	44.2	3018	836	3853
High school or above	74	99.3	200	521	1071	89.4	124	288	187	74.4	84.6	78.9	26.0	22.0	2555	728	2967
<i>p</i> ^b		0.130	0.777	0.304	0.376	0.356	0.181	0.138	0.228	0.318	0.657	0.622	0.297	0.251	0.236	0.260	0.249
Maternal occupation																	
Farmer	177	144	201	606	1011	96.5	224	427	292	95.2	113	61.0	19.4	54.6	2915	836	3679
Non-farmer	60	137	171	476	1202	91.8	139	326	273	94.7	97.3	87.0	25.1	20.3	2644	656	3469
<i>p</i> ^c		0.808	0.514	0.112	0.743	0.649	0.127	0.076	0.403	0.626	0.949	0.152	0.242	0.002	0.305	0.072	0.225
Previous birth defects history																	
No	222	194	303	1050	1198	130	308	688	375	115	200	77.3	109	50.7	3844	886	4625
Yes	7	149	194	603	1071	93.9	209	399	299	95.6	107	70.9	19.8	38.0	2954	812	3810
<i>p</i> ^c		0.621	0.921	0.995	0.531	0.141	0.609	0.713	0.706	0.954	0.767	0.476	0.292	0.488	0.768	0.281	0.664

68

69

70 Table S1(Continued)

Population characteristic	Number of subjects ^a	ANY	ACE	FLE	PHE	ANT	FLU	RET	PYR	BAA	CHR	BBF	BKF	BAP	ΣL-PAHs	ΣH-PAHs	ΣPAHs
Gravidity																	
1 time	95	170	180	552	1027	89.3	194	349	319	86.9	106	83.8	24.7	28.5	2717	820	3790
2 times	114	131	201	600	1220	104	212	399	274	103	118	60.9	16.4	44.2	3018	817	3778
<i>p</i> ^c		0.995	0.552	0.209	0.200	0.107	0.313	0.750	0.615	0.335	0.425	0.473	0.314	0.094	0.227	0.350	0.276
Parity																	
1 time	196	140	191	567	1051	91.3	196	378	289	92.3	107	73.6	20.2	35.0	2789	816	3734
2 times	13	207	367	1050	1285	141	308	566	375	120	178	66.5	16.5	43.2	3844	877	4625
<i>p</i> ^c		0.390	0.278	0.384	0.394	0.024	0.476	0.600	0.433	0.300	0.600	0.678	0.760	0.881	0.476	0.366	0.444
Periconceptional folate supplementation																	
No	112	157	195	629	1135	99.0	218	412	319	86.9	95.7	71.3	20.2	47.2	3107	802	4085
Yes	119	146	191	532	1071	94.3	199	346	286	101	125	73.0	17.4	28.9	2851	823	3734
<i>p</i> ^c		0.81	0.88	0.66	0.94	0.79	0.92	0.74	0.80	0.91	0.81	0.94	0.86	0.04	0.95	0.50	0.87
Fever or flu during early pregnancy																	
No	140	149	276	729	1254	119	259	497	301	117	143	92.5	32.1	52.7	3815	956	5223
Yes	90	137	155	484	947	87.2	186	306	271	70.5	81.9	50.6	14.8	34.6	2477	725	3052
<i>p</i> ^c		0.658	0.017	0.171	0.236	0.025	0.035	0.018	0.260	0.025	0.028	0.037	0.060	0.331	0.039	0.011	0.028
Maternal smoking or passive smoking																	
No	121	127	196	591	1234	111	241	411	282	101	86.9	69.2	18.4	63.0	3318	845	4109
Yes	104	141	186	496	905	77.5	168	343	275	78.4	106	71.3	20.4	28.9	2550	769	3468
<i>p</i> ^c		0.392	0.867	0.512	0.119	0.005	0.102	0.716	0.752	0.970	0.432	0.204	0.681	0.007	0.257	0.436	0.328
Alcohol drinking																	
No	214	132	191	716	1121	103	212	452	309	92.5	99.1	59.8	17.4	56.3	2954	824	3729
Yes	24	143	194	568	1037	93.4	199	365	283	92.3	106	71.9	20.1	35.0	2789	787	3656
<i>p</i> ^c		0.77	0.72	0.87	0.73	0.64	0.62	0.91	0.88	0.67	0.77	0.24	0.63	0.21	0.70	0.86	0.67

71 The abbreviations of individual PAHs are acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLU), retene (RET), pyrene
72 (PYR), benz[a]anthracene (BAA), chrysene (CHR), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF), benzo[a]pyrene (BAP). ΣL-PAHs is the sum of ANY, ACE, FLE, PHE, ANT,
73 FLU and RET; ΣH-PAHs of PYR, BAA, CHR, BBF, BKF and BAP; ΣPAHs of ΣL-PAHs and ΣH-PAHs.

74 ^a Total number may not be equal to the number of cases or controls due to missing or unknown data.

75 ^b Calculated by Kruskal-Wallis H Test.

76 ^c Calculated by Mann-Whitney *U* test.

77 **Table S2.** Risks of neural tube defects (NTDs) in association with levels of polycyclic aromatic hydrocarbons (PAHs) of high-molecular weight
 78 (H-PAHs) and low-molecular weight (L-PAHs) above the median concentration in maternal serum of controls in Shanxi Province, China,
 79 2010-2013

PAHs ^a	Median (IQR) ^b	Total NTDs		Anencephaly		Spina bifida	
		Crude OR ^c (95% CI)	Adjusted OR ^{c, d} (95% CI)	Crude OR ^c (95% CI)	Adjusted OR ^{c, d} (95% CI)	Crude OR ^c (95% CI)	Adjusted OR ^{c, d} (95% CI)
ΣL-PAHs	2851 (1307-6683)	1.22 (0.62-2.40)	1.20 (0.49 -2.92)	0.88 (0.35-2.23)	1.09 (0.27 -4.43)	0.91 (0.41-2.01)	0.94 (0.35-2.55)
ΣH-PAHs	805 (420-1514)	4.14 (2.04-8.42) ^{***}	5.03 (1.93-13.1) ^{**}	3.00 (1.13-8.02) [*]	4.25 (0.94-19.3)	4.95 (2.06-11.9) ^{***}	4.47 (1.49-13.44) ^{**}

80 OR, odds ratio; IQR, interquartile range; ^{*} $p < 0.05$ and ^{***} $p < 0.001$.

81 ^a ΣL-PAHs is the sum of acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, retene; ΣH-PAHs of pyrene, benz[a]anthracene, chrysene,
 82 benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene.

83 ^b Statistical results of all subjects.

84 ^c Calculated by taking both ΣH-PAHs and ΣL-PAHs together into binary logistic regression model.

85 ^d Adjust for maternal general characteristics and exposure, including BMI, parity, fever of flu during early pregnancy, and active or passive smoking and drinking.

86

87 Table S3. Risks of fetal total neural tube defects (NTDs) in four quartile ranges of controls in Shanxi Province, China, 2010-2013

PAHs ^a	Concentration group (ng/g lipid)	Total NTDs		Anencephaly		Spinal bifida	
		Unadjusted OR ^b (95% CI)	Adjusted OR ^{b,c} (95% CI)	Unadjusted OR ^b (95% CI)	Adjusted OR ^{b,c} (95% CI)	Unadjusted OR ^b (95% CI)	Adjusted OR ^{b,c} (95% CI) ^{b,c}
ΣL-PAHs	< 1007	1.00	1.00	1.00	1.00	1.00	1.00
	1007 - 2068	1.37 (0.58 - 3.26)	1.795 (0.550- 5.86)	0.48 (0.15 - 1.59)	0.50 (0.07 - 3.41)	1.79 (0.62 - 5.15)	2.91 (0.74 - 11.53)
	2068 - 3818	1.29 (0.55 - 3.03)	1.710 (0.531- 5.51)	0.37 (0.11 - 1.33)	0.88 (0.17 - 4.54)	1.47 (0.50 - 4.31)	2.41 (0.57 - 10.15)
	≥ 3818	4.95 (2.30 - 10.6)***	5.309 (1.929- 14.61)**	2.06 (0.85 - 5.00)	3.07 (0.86 - 11.01)	4.24 (1.64 - 11.0)**	4.50 (1.32 - 15.34)*
ΣH-PAHs	< 300.2	1.00	1.00	1.00	1.00	1.00	1.00
	300.2 - 556.8	1.57 (0.57 - 4.33)	1.147 (0.293 - 4.49)	1.63 (0.43 - 6.19)	0.87 (0.12 - 6.07)	1.12 (0.31 - 4.08)	1.56 (0.32 - 7.57)
	556.8 - 944.3	3.27 (1.28 - 8.32)*	3.481 (1.068 - 11.34)*	1.91 (0.53 - 6.87)	1.65 (0.30 - 9.15)	2.72 (0.88 - 8.35)	3.06 (0.75 - 12.47)
	≥ 944.3	8.92 (3.66 - 21.8)***	8.272 (2.727- 25.09)***	5.60 (1.73 - 18.2)**	7.39 (1.55 - 35.17)*	7.47 (2.58 - 21.6)***	8.10 (2.14 - 30.64)**
ΣPAHs	< 1406	1.00	1.00	1.00	1.00	1.00	1.00
	1406 - 2668	1.27 (0.53 - 3.05)	1.804 (0.549- 5.93)	0.39 (0.11 - 1.40)	0.59 (0.09 - 4.05)	1.62 (0.56 - 4.64)	2.44 (0.63 - 9.52)
	2068 - 5065	1.59 (0.67 - 3.78)	2.579 (0.756 - 8.80)	0.64 (0.20 - 2.05)	1.88 (0.34 - 10.47)	1.79 (0.62 - 5.17)	3.74 (0.86 - 16.32)
	≥ 5065	4.99 (2.29 - 10.9)***	5.485 (1.944- 15.48)**	2.14 (0.86 - 5.34)	3.68 (0.93 - 14.52)	3.75 (1.44 - 9.75)**	3.99 (1.17 - 13.66)*

88 OR, odds ratio

89 ^a ΣL-PAHs is the summery of acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and retene; ΣH-PAHs of pyrene, benz[a]anthracene,
 90 chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, and benzo[a]pyrene; ΣT-PAHs of ΣL-PAHs and ΣH-PAHs.

91 ^b Calculated by using binary logistic regression

92 ^c Adjusted by maternal general characteristics, including BMI, parity, fever of flu during early pregnancy, and active or passive smoking and drinking

93

94

95

96

97 Table S4. Median concentrations (ng/g lipid) of polycyclic aromatic hydrocarbons (PAHs) in the maternal serum in subgroups of life styles and
 98 energy usage characteristics in Shanxi Province, China, 2010-2013

Indoor life style or energy usage characteristic	Number of subjects ^a	ANY	ACE	FLE	PHE	ANT	FLU	RET	PYR	BAA	CHR	BBF	BKF	BAP	ΣL-PAHs	ΣH-PAHs	ΣPAHs
Separate kitchen from living room/bedroom																	
Yes	189	155	205	606	1099	93.6	200	395	301	102	128	73.5	20.7	35.5	3117	820	4085
No	49	95.0	153	552	906	108	176	288	236	70.0	70.9	54.8	13.8	38.9	2217	702	2790
<i>p</i> ^b		0.386	0.153	0.967	0.66	0.945	0.729	0.246	0.474	0.152	0.066	0.118	0.304	0.336	0.401	0.124	0.297
Primary fuel used for cooking																	
Soft coal	39	170	216	926	1420	102	263	473	413	110	98.2	72.4	16.3	43.3	3818	886	5299
Hard coal	86	103	223	592	914	93.9	194	358	237	83.9	102	55.1	13.1	34.2	2789	773	3598
Liquefied gas	97	141	163	477	1080	94	199	349	301	88	108	71.3	20.1	36	2662	735	3392
<i>p</i> ^c		0.355	0.462	0.159	0.355	0.751	0.373	0.486	0.297	0.705	0.455	0.654	0.878	0.222	0.574	0.513	0.556
Conception during the heating season																	
Yes	134	131	148	480	968	89	187	312	282	70.3	87.2	67.7	17.4	40.8	2555	811.67	3307
No	100	201	233	772	1306	106	263	426	331	110	138	74.2	24.1	35.3	3665	831	4440
<i>p</i> ^b		0.151	0.028	0.077	0.292	0.353	0.147	0.332	0.215	0.189	0.253	0.693	0.918	0.583	0.294	0.348	0.356
Using a stove for heating ^d																	
Yes	77	97.9	133	483	847	77.5	137	273	267	62.7	79.9	50.8	13.7	50.7	2318	790	2790
No	58	137	151	444	1127	99.3	204	368	276	86.5	103	71.1	20.5	34.4	2611	808	3629
<i>p</i> ^b		0.164	0.496	0.779	0.099	0.709	0.225	0.533	0.578	0.262	0.179	0.430	0.629	0.425	0.411	0.919	0.408
Stove in the living room/ bedroom ^d																	
Yes	47	79.3	133	468	709	79.1	119	273	216	70.6	71.8	42.2	6.42	44.7	2169	766	2661
No	31	126	97.5	485	967	65.2	188	331	284	55.2	84.9	72.4	17.4	60.0	2474	826	2867
<i>p</i> ^b		0.849	0.858	0.798	0.787	0.649	0.833	0.975	0.866	0.247	0.597	0.863	0.954	0.716	0.744	0.842	0.683
Primary fuel used for heating ^d																	
Soft coal	38	90.5	125	445	638	72.6	110	260	186	71.5	68.4	56.7	13.4	30.6	1862	724	2717
Hard coal	38	61.9	147	525	902	82.5	136	371	282	58.7	77.0	48.6	13.1	77.8	2551	824	3468
<i>p</i> ^b		0.797	0.410	0.803	0.540	0.516	0.954	0.431	0.942	0.665	0.601	0.741	0.983	0.472	0.245	0.519	0.299

100 Table S4 (Continued)

Indoor life style or energy usage characteristic	Number of subjects ^a	ANY	ACE	FLE	PHE	ANT	FLU	RET	PYR	BAA	CHR	BBF	BKF	BAP	ΣL-PAHs	ΣH-PAHs	ΣPAHs
Frequency of ventilation in the living room/bedroom ^d																	
Everyday	58	143	169	476	812	72.1	143	295	276	69.5	82.4	49.8	13.4	39.4	2471	757	2781
1 - 6 times per week	15	38.0	30.1	419	906	77.5	114	149	179	58	65.4	22.2	5.8	132	2087	811	2817
<i>p</i> ^b		0.052	0.005	0.265	0.795	0.816	0.778	0.122	0.185	0.255	0.411	0.313	0.469	0.593	0.409	0.712	0.517
Frequency of combustion exhausted gas in living room ^d																	
Everyday	18	72.1	223	381	561	91.0	105	263	181	64.5	75.9	0.0	0.0	137	1862	641	2514
1 - 6 times per week	9	0	0	483	906	79.1	303	261	248	72.4	65.4	41.7	0.5	43.3	1719	790	2313
≤ 1 time per week	45	151	118	601	907	71.1	135	273	323	62.7	63.7	72.4	19.4	25.6	2634	820	3321
<i>p</i> ^c		0.163	0.052	0.811	0.810	0.970	0.336	0.745	0.710	0.555	0.883	0.171	0.194	0.587	0.717	0.805	0.580

101 The abbreviations of individual PAHs are acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLU), retene (RET), pyrene
 102 (PYR), benz[a]anthracene (BAA), chrysene (CHR), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF), benzo[a]pyrene (BAP). ΣL-PAHs is the sum of ANY, ACE, FLE, PHE, ANT,
 103 FLU and RET; ΣH-PAHs of PYR, BAA, CHR, BBF, BKF and BAP; ΣPAHs of ΣL-PAHs and ΣH-PAHs.

104 ^a Total number may not be equal to the number of cases or controls due to missing or unknown data.

105 ^b Calculated by Mann-Whiney *U* test

106 ^c Calculated by Kruskal-Wallis H Test

107 ^d For women whose pregnancies occurred during the winter heating season.

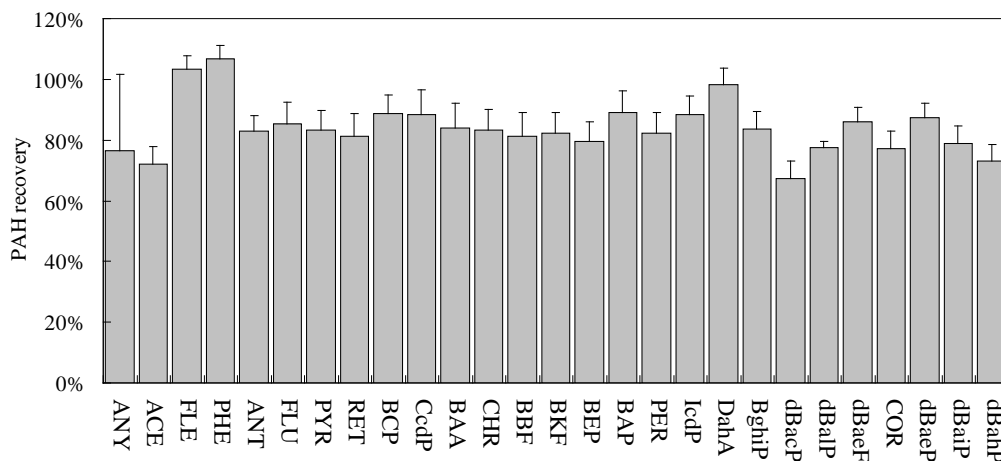


Figure S1. The method recoveries of the twenty-seven spiked PAHs with serum. The data were shown as mean \pm standard error. The abbreviation of PAHs are acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLU), retene (RET), pyrene (PYR), benzo(c)phenanthrene (BCP), cyclopenta(c,d)pyrene (CcdP), benzo(a)anthracene (BAA), chrysene (CHR), benzo(b)fluoranthene (BBF), benzo(k)fluoranthene (BKF), benzo(e)pyrene (BEP), benzo(a)pyrene (BAP), Perylene (PER), indeno(1,2,3-cd)pyrene (IcdP), dibenz(a,h)anthracene (DahA), benzo(g,h,i]perylene (BghiP), di-benzo(a,c)pyrene (dBacP), di-benzo(a,l)pyrene (dBaIP), dibenzo(a,e)fluoranthene (dBaeF), coronene (COR), di-benzo(a,e)pyrene (dBaeP), di-benzo(a,i)pyrene (dBaiP), and di-benzo(a,h)pyrene (dBahP).