Supporting Information for

Bioaccessibility of PAHs in Fuel Soot Assessed by an *in vitro* Digestive Model with Absorptive Sink: Effect of Food Ingestion

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There are 9 pages, including 10 tables and 5 figures.

Contents:

This supporting information contains 9 pages, including:

SI-1. Parameters of the digestive fluid in default and fed conditions

The default conditions are summarized as follows. Fuel soot (50.0 mg) was first incubated with 5.0 mL salivary fluid (2.8 g/L α-amylase in 0.02 M phosphate buffer at pH 6.5) for 5 min in a centrifuge tube. Then 7.5 mL gastric fluid (10.0 g/L pepsin in 0.2 M KCl adjusted to pH 1.0 with HCl) was added and incubation was continued for 2 h. Lastly, 20.0 mL small intestinal fluid (20.0 g/L pancreatin, 3.0 g/L lipase, and 14.0 g/L porcine bile extract in 0.2 M phosphate buffer at pH 7.8) and 2.0 g silicone sheet were added and incubation was continued for another 4 h. The pH measured after the gastric stage (gastric pH) was 1.21 and after the small intestinal stage (small intestinal pH) was 6.82. The default bile acid concentration in the digestive fluid was 8.62 g/L. The unadjusted ionic strength of the digestive fluid was calculated to be 0.37 M, not including soluble substances from the soot. The soot contains a large amount of soluble sulfate; assuming complete dissolution of these sulfate acids/salts under the default digestion conditions, the contribution to ionic strength would be somewhere between 0.0197 M and 0.0262 M, depending on whether the counterion(s) are univalent or divalent.

The default conditions were varied to mimic the change in physiological conditions expected with food ingestion, including bile acids concentration (2.0–10 g/L), gastric pH (1.21–4.30, **Table S1**), small intestinal pH (5.00–7.35, **Table S2**), ionic strength (0.056–0.933 M, **Table S3**), and the presence of food nutrients. Different gastric and small intestinal pH, and varied ionic strength were obtained by changing the buffer solutions making the digestive fluid. For example, to obtain a lower small intestinal pH of 5.0, 5.5 mL of 0.2 M phosphate buffer at pH 7.8 was used to make the small intestinal fluid instead of 20.0 mL, while the remaining 16.0 mL was replaced by ultrapure water. KCl was added to the digestive fluid to get a higher ionic strength. NaOH was spiked to the digestive fluid at the small intestinal stage to obtain a final pH of 7.35. When non-lipid milk was tested, 4.0 mL were combined with 1.0 mL of phosphate buffer (0.02 M, pH 6.5) and 2.8 g/L α-amylase to comprise the salivary fluid. When soybean oil was tested, 0.17–0.68 mL was added to supplement the 5 mL of default salivary fluid.

The 24-h partition coefficients of individual PAHs between the silicone sheet and the digestive fluid at 37 °C were determined with different bile acid concentration, small intestinal pH (**Table S2**), and ionic strength (**Table S3**). Mixed PAH standards in *n*-hexane were deposited into a 35 mL amber tube and the solvent allowed to evaporate in a fume hood. Next, 20 mL of specific digestive fluid and 1.0 g of silicone sheet were added. The tube was shaken for 24 h and the concentrations of individual PAHs in the silicone sheet and digestive fluid were subsequently measured to determine $K_{s,24\,h}$.

Table S1.

Parameters of the artificial digestive fluids with different gastric pH. Mean and standard deviation of the pH values are shown.

0.2 M phosphate buffer at pH 6.5, mL	0.2 M KCl solution at pH 1.0, mL	0.2 M phosphate buffer at pH 7.8, mL	Gastric pH	Small intestinal pH	Ionic strength, M
5.0*	7.5	8	1.21 (0.00)	6.58 (0.01)	0.191
5.0	7.5	20.0	1.61 (0.02)	6.59 (0.01)	0.347
5.0	3.5	20.0	2.34 (0.00)	6.87 (0.01)	0.338
5.0	1.5	20.0	3.37 (0.02)	6.98 (0.02)	0.333
5.0	1.0	20.0	4.30 (0.01)	7.02 (0.02)	0.333

* 0.02 M phosphate buffer at pH 6.5 was used.

Table S2.

Parameters of the artificial digestive fluids with different small intestinal pH. Mean and standard deviation of the pH values are shown.

0.02 M phosphate buffer at pH 6.5, mL	0.2 M KCl solution at pH 1.0, mL	0.2 M phosphate buffer at pH 7.8, mL	Gastric pH	Small intestinal pH	Ionic strength, M
5.0	7.5	5.5	1.21 (0.00)	5.00 (0.08)	0.152
5.0	7.5	6.2	1.21 (0.00)	5.62 (0.01)	0.161
5.0	7.5	7.5	1.21 (0.00)	6.16 (0.01)	0.179
5.0	7.5	12.0	1.21 (0.00)	6.58 (0.01)	0.243
5.0	7.5	20.0*	1.21 (0.00)	7.35 (0.12)	0.433

* 0.6 mL of 2 M NaOH was also added.

Table S3.

Parameters of the artificial digestive fluids with different ionic strength. Mean and standard deviation of the pH values are shown.

0.02 M phosphate buffer at pH 6.5, mL	0.2 M KCl solution at pH 1.0, mL	0.2 M phosphate buffer at pH 7.8, mL	1 M KCl, mL	pH	Ionic strength, M
5.0	0.4	1.0	0.0	6.41 (0.01)	0.056
5.0	1.5	3.0	0.0	6.51 (0.01)	0.089
5.0	5.0	10.0	0.0	6.60 (0.01)	0.202
5.0	7.5	20.0	0.0	6.59 (0.01)	0.347
5.0	5.0	10.0	10.0	6.48 (0.00)	0.661
5.0	0.4	1.0	19.0	6.37 (0.00)	0.933

SI-2. Bile acids concentration

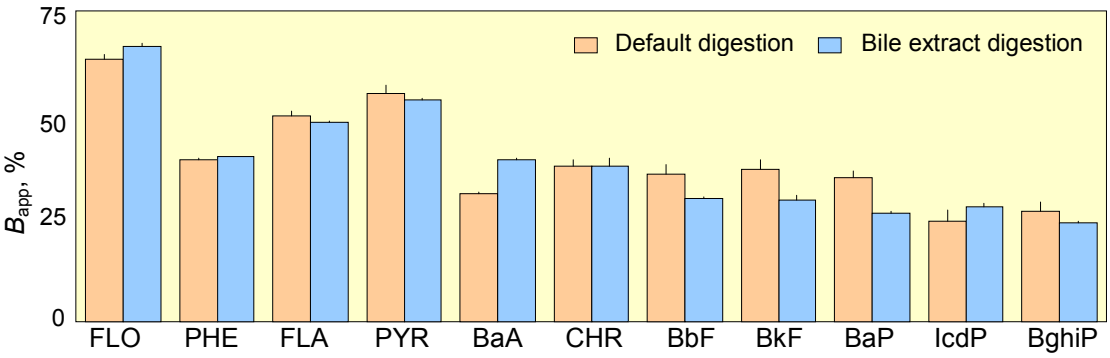

Figure S1. Comparison of the apparent bioaccessibility (B_{app}) of individual PAHs in the fuel soot after digestion with buffered bile acids alone with that after a default digestion. Mean and standard deviation of triplicates are shown.

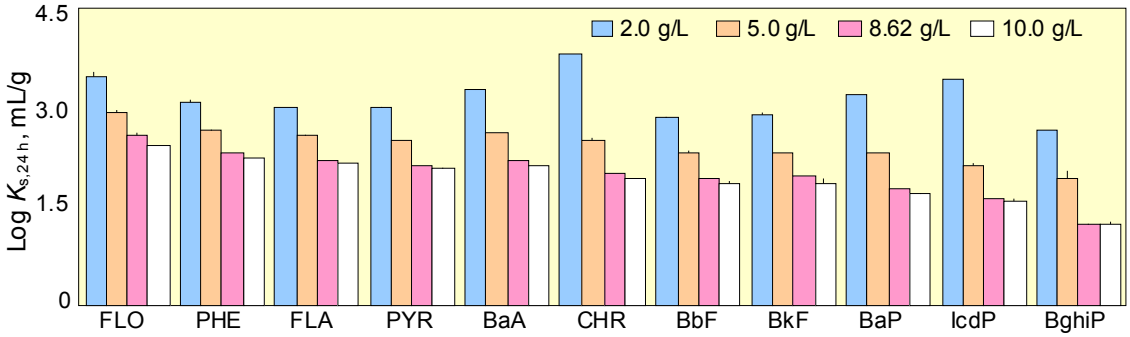

Figure S2. The 24-h partition coefficient (log $K_{s,24\,h}$) of individual PAHs between silicone sheet and buffered bile acids fluids at varied concentrations. The average pH and ionic strength of the digestive fluids are pH 6.95 and 0.368 M, respectively. Mean and the range of duplicates are shown.

Table S4.

The values of the parameters after linear regression of the 24-h sheet-bile acids fluids partition coefficient (log $K_{s,24\,h}$) of individual PAHs against the concentration of the bile acids (log C_{bile}, C_{bile} = 2.0–10 g/L) and the coefficient of determination (R^2) derived based on the least square regression.

Regression parameter	FLO	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Slope	-1.47	-1.23	-1.23	-1.36	-1.68	-2.72	-1.40	-1.48	-2.18	-2.69	-2.12
Intercept	3.92	3.46	3.37	3.42	3.76	4.55	3.25	3.33	3.82	4.15	3.30
R^2	0.9987	0.9942	0.9926	0.9965	0.9991	0.9848	0.9983	0.9981	0.9967	0.9783	0.9846

Table S5. The estimated labile concentrations (c_{LAB}) and the digestion residue-digestive fluid partition coefficients ($K_{r,lab}$) for individual PAHs under various digestion conditions. The values are derived from nonlinear regression of equation 3.

Regression parameters	FLO	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Bile extract 2.0 g/L, small intestinal pH 6.48 – 6.90											
c_{LAB} (μg/g)	--	4.93	7.53	11.3	1.04	0.868	1.97	1.15	1.40	1.94	5.12
$K_{r,lab}$, mL/g	--	1385	1699	1603	1621	1475	2086	2131	2128	1381	2395
Log $K_{r,lab}$, log (mL/g)	--	3.14	3.23	3.20	3.21	3.17	3.32	3.33	3.33	3.14	3.38
R^2	--	1.000	1.000	1.000	0.982	1.000	0.999	0.997	0.994	0.995	0.996
Bile extract 5.0 g/L, small intestinal pH 6.48 – 6.89											
c_{LAB} (μg/g)	0.327	4.83	8.16	13.2	0.765	1.06	1.79	2.32	1.25	1.92	8.20
$K_{r,lab}$, mL/g	377	532	831	803	972	1302	1673	1861	1865	1360	2073
Log $K_{r,lab}$, log (mL/g)	2.58	2.73	2.92	2.90	2.99	3.11	3.22	3.27	3.27	3.13	3.32
R^2	0.997	1.000	1.000	1.000	0.995	0.998	1.000	0.991	0.998	0.992	0.995
Default condition, bile extract 8.62 g/L, small intestinal pH 6.52 – 7.01											
c_{LAB} (μg/g)	0.264	4.77	8.69	14.1	0.426	0.816	1.54	1.41	1.28	2.23	6.38
$K_{r,lab}$, mL/g	0	112	338	314	705	440	698	554	405	1380	961
Log $K_{r,lab}$, log (mL/g)	--	2.05	2.53	2.50	2.85	2.64	2.84	2.74	2.61	3.14	2.98
R^2	0.997	1.000	1.000	1.000	0.998	0.999	0.999	0.999	1.000	0.999	0.999
Bile extract 8.62 g/L, small intestinal pH 5.81											
c_{LAB} (μg/g)	--	3.34	9.96	12.4	0.627	0.748	1.47	0.425	0.790	1.42	5.48
$K_{r,lab}$, mL/g	--	0	805	536	1630	1903	988	945	2000	2001	1771
Log $K_{r,lab}$, log (mL/g)	--	--	2.91	2.73	3.21	3.28	2.99	2.98	3.30	3.30	3.25
R^2	--	1.000	0.998	0.999	0.992	0.964	0.970	0.916	0.922	0.950	0.948
Bile extract 8.62 g/L, small intestinal pH 5.00											
c_{LAB} (μg/g)	0.0130	1.37	2.94	4.82	0.613	0.431	1.04	0.955	0.322	0.865	2.60
$K_{r,lab}$, mL/g	0	970	1789	1559	4344	2095	2609	1884	441	1867	1065
Log $K_{r,lab}$, log (mL/g)	--	2.99	3.25	3.19	3.64	3.32	3.42	3.28	2.64	3.27	3.03
R^2	0.788	0.998	0.997	0.996	0.960	0.983	0.980	0.910	0.994	0.850	0.737
Bile extract 8.62 g/L, small intestinal pH 6.46-6.82, lipid concentration 11.5 g/L											
c_{LAB} (μg/g)	--	15.6	21.3	36.1	1.07	1.43	5.28	4.67	1.58	4.73	15.2
$K_{r,lab}$, mL/g	--	0.00	0.00	23.4	12.4	72.0	22.9	47.6	100	24.6	53.5
Log $K_{r,lab}$, log (mL/g)	--	--	--	1.37	1.09	1.86	1.36	1.68	2.00	1.39	1.73
R^2	--	0.972	0.989	0.998	1.000	1.000	0.996	1.000	0.986	0.960	0.944

SI-3. Gastric pH

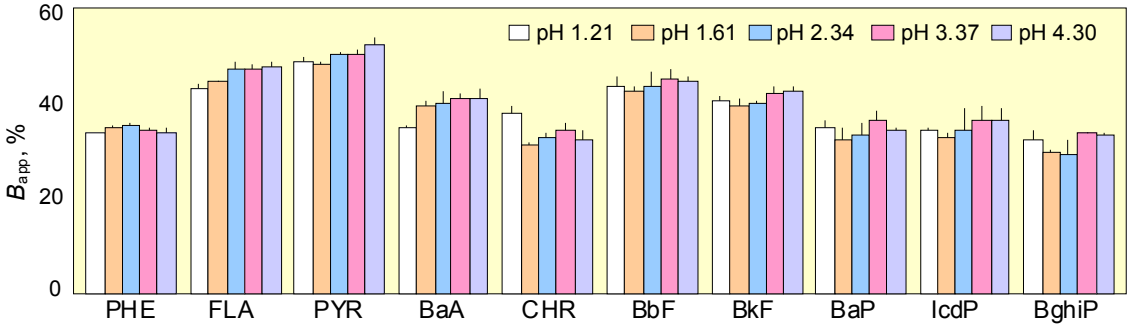

Figure S3. The influence of gastric pH on the apparent bioaccessibility (B_{app}) of individual PAHs in the fuel soot. Mean and standard deviation of triplicates are shown.

SI-4. Small intestinal pH and ionic strength

Table S6.

The values of the parameters after linear regression of the apparent bioaccessibility (B_{app}) of individual PAHs in the fuel soot with small intestinal pH (5.00–7.35) and the coefficient of determination (R^2) derived based on the least square regression.

Regression parameter	FLO	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Slope	17.5	13.2	17.1	18.6	12.3	14.9	15.6	12.3	12.7	11.0	8.05
Intercept	-61.4	-53.6	-69.3	-74.9	-46.3	-61.9	-59.0	-41.9	-49.9	-40.1	-24.7
R^2	0.9788	0.9875	0.9962	0.9895	0.9979	0.9923	0.9627	0.8722	0.9370	0.9010	0.7956

Table S7.

The values of the parameters after linear regression of the determined 4-h sheet-fluid partition coefficients ($K_{s,4\,h}$) after simulated digestion with small intestinal pH (5.62–7.35) and the coefficient of determination (R^2) derived based on the least square regression.

Regression parameter	FLO	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Slope	58.0	38.0	29.4	23.4	38.5	25.3	19.2	9.1	10.7	11.9	8.94
Intercept	-193	-92.1	-69.8	-52.9	-80.0	-69.6	-70.1	-22.7	-45.7	-42.7	-37.9
R^2	0.9920	0.9992	0.9968	0.9581	0.8547	0.9603	0.9864	0.9774	0.9675	0.9837	0.9850

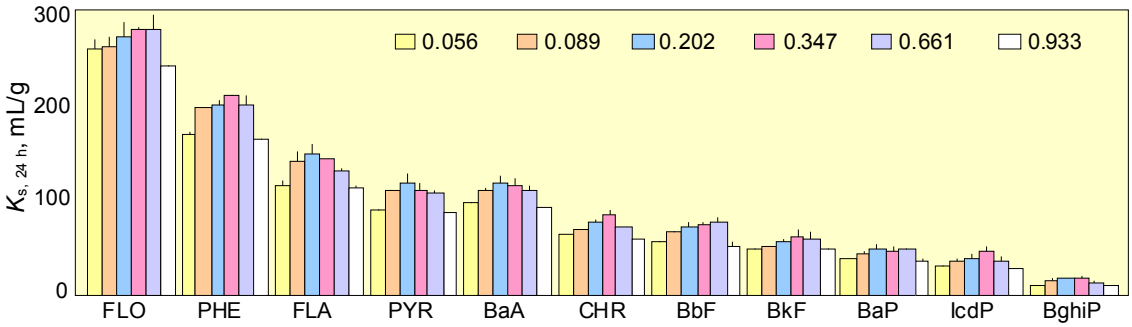

Figure S4. The 24-h partition coefficients of individual PAHs between silicone sheet and digestive fluid ($K_{s,24\ h}$) at various ionic strengths in the digestive fluid. Mean and the range of duplicates are shown.

Table S8.

The values of the parameters after linear regression of the 24-h sheet-fluid partition coefficients of individual PAHs ($K_{s,24\ h}$) with pH of the digestive fluid (5.76–7.29) at relatively constant ionic strength (0.324–0.359 M) and the coefficient of determination (R^2) derived based on the least square regression.

Regression parameter	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Slope	66.5	69.6	60.2	45.9	36.9	31.2	27.7	26.0	22.8	12.6
Intercept	-221	-317	-285	-186	-160	-133	-131	-121	-116	-65.4
R^2	0.9710	0.9744	0.9576	0.9909	0.9955	0.9506	0.9468	0.9802	0.9522	0.9651

SI-5. Lipid addition

Table S9.

Spike recoveries of individual PAHs for digestive fluid with a lipid concentration of 19.5 g/L by liquid-to-liquid extraction. Means and standard deviations of triplicates are shown.

Spike recovery, %	FLO	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Average	95.5	81.5	96.2	96.3	86.4	90.7	88.1	87.2	37.2	83.9	89.8
Standard deviation	2.13	5.99	8.59	6.98	1.04	3.72	5.21	6.22	1.30	3.85	18.6

Table S10.

The values of the parameters after linear regression of the 4-h sheet-fluid partition coefficients of individual PAHs (log $K_{s,4\ h}$) with lipid concentration (log C_{lipid}) of the digestive fluid (4.88–19.5 g/L) and the coefficient of determination (R^2) derived based on the least square regression.

Regression parameter	FLO	PHE	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	BghiP
Slope	-2.42	-1.10	-0.92	-1.74	-1.10	-1.13	-1.37	-1.35	-1.03	-1.24	-1.28
Intercept	3.26	2.03	1.67	2.55	1.88	1.90	1.96	2.05	1.77	1.68	1.73
R^2	0.7926	0.9854	0.9482	0.9963	0.9788	0.9997	0.9911	0.9515	0.9703	0.9996	0.9999

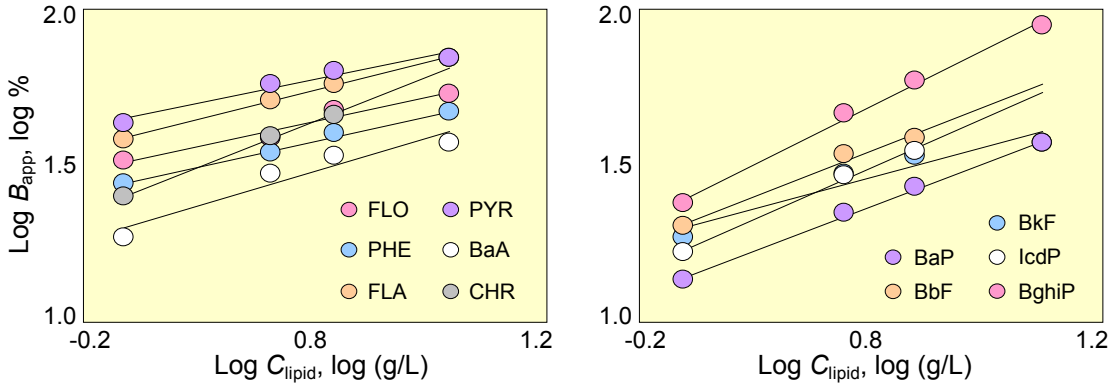

Figure S5. The linear relationship between apparent bioaccessibility (log B_{app}) and lipid concentration in the digestive fluid (log C_{lipid}, C_{lipid} = 0.98–34.5 g/L) for individual PAHs in the fuel soot. All the digestion was conducted in the absence of silicone sheet.