Electronic Supplementary Information

Aryl hydrocarbon receptor potency of chlorinated parabens in the aquatic environment

Masanori Terasaki,*[a,c] Michiko Yasuda,[b,c] Masakazu Makino [b,c] and Kayoko Shimoi [b,c]

[a] Department of Environmental Sciences, Faculty of Humanities and Social Science, Iwate University, 3-18-34 Ueda, Morioka 020-8550, Japan. E-mail: terasaki@iwate-u.ac.jp; Tel/Fax: (+81)-20-621-8550.

[b] Graduate School of Integrated Pharmaceutical and Nutritional Sciences, University of Shizuoka, 52-1Yada, Suruga-ku, Shizuoka 422-8526, Japan.

[c] Institute for Environmental Sciences, University of Shizuoka, 52-1Yada, Suruga-ku, Shizuoka 422-8526, Japan.

This electronic supporting information section contains: 9 pages and 2 figures and 5 Tables

Materials and Methods

Sample Collection and Characterization. The sample characteristics are listed in Fig. 1ESI and Tables 1SI and 2ESI.

Quality Assurance and Control. Data are expressed as the mean of the values obtained from the sample carried out in duplicate runs. The calibration curves used for the chemical analysis consisted of five concentrations ranging from 0.10 mg/L to 1.6 mg/L in ethyl acetate. All standards were linear for target compounds, and their coefficient of determination ranged from 0.981 to 0.999. Quality-assurance and control data for trimethylsilyl derivatives of target compounds are listed in Tables 3 ESI. The limit of detection and limit of quantification were calculated respectively as three times and ten times the standard deviation of the replicate analyses (n = 5) of the river water spiked at the lowest calibration concentrations. During the analysis of the sample, as described above, limits of detection (LOD) ranged from 0.63−4.2 ng/L, while limit of quantifications (LOQ) ranged from 2.1−14 ng/L. The recoveries were determined by performing three separate experiments using 100 mL of river water spiked to 30 μg/L. For each compound, the recovery yield varied between 92% and 104%, and the repeatability (relative standard deviation) of the results obtained was within 11%. Average surrogate (deuterated bisphenol A) recoveries in the blanks were 90% with a relative standard deviation of 9.2%. To avoid laboratory contamination, all glassware was heated at 500°C for 3 h before use. Blank samples (surface water of the Kusanagi River; 34°59'28"N, 138°27'24"E) were routinely analyzed and assayed, and no contamination of the solvents or equipment was detected.

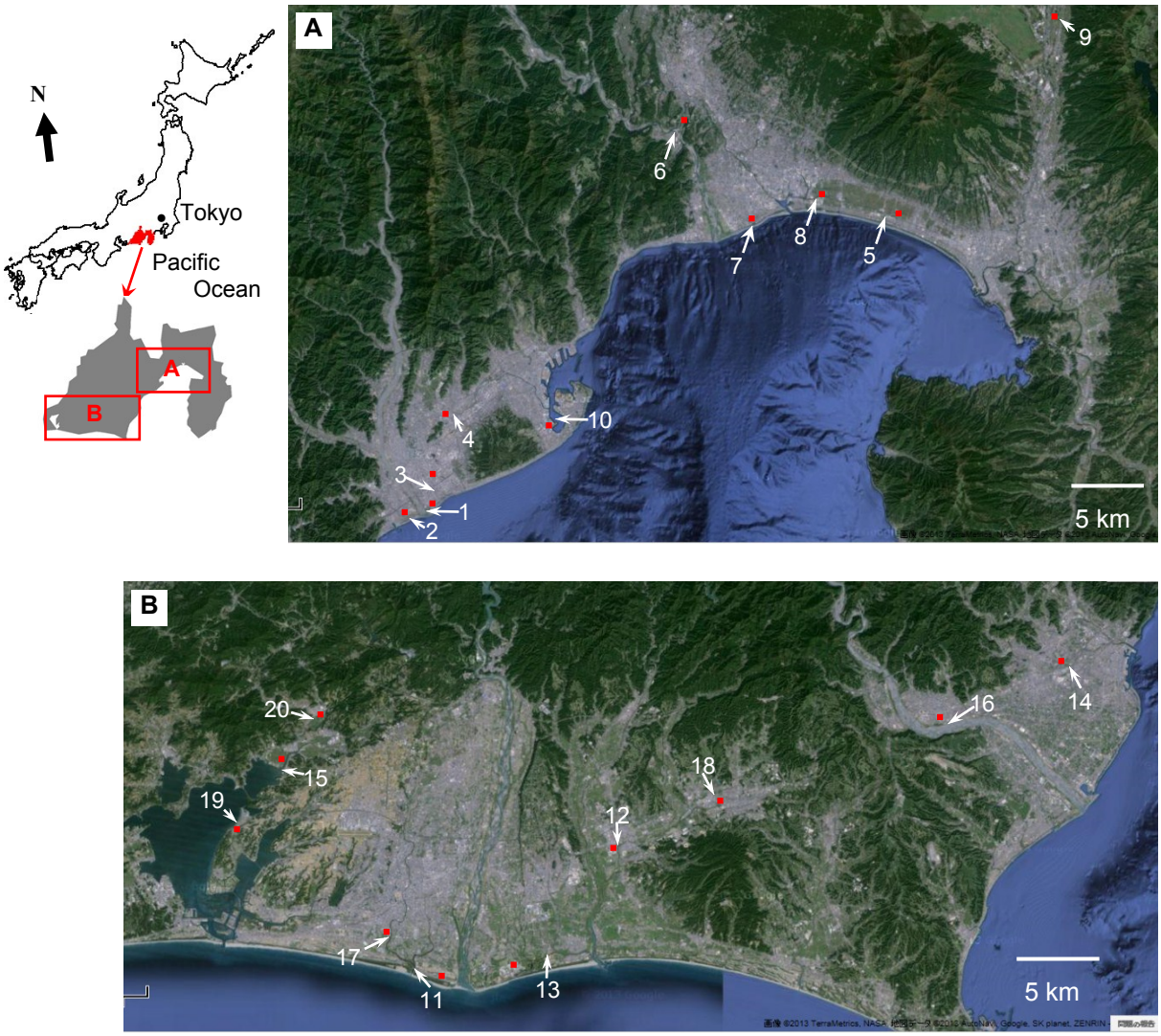

Fig. 1ESI Location of sampling sites (1–20) of water receiving effluents from sewage treatment plants (marker: ■) in Shizuoka Prefecture, Japan.

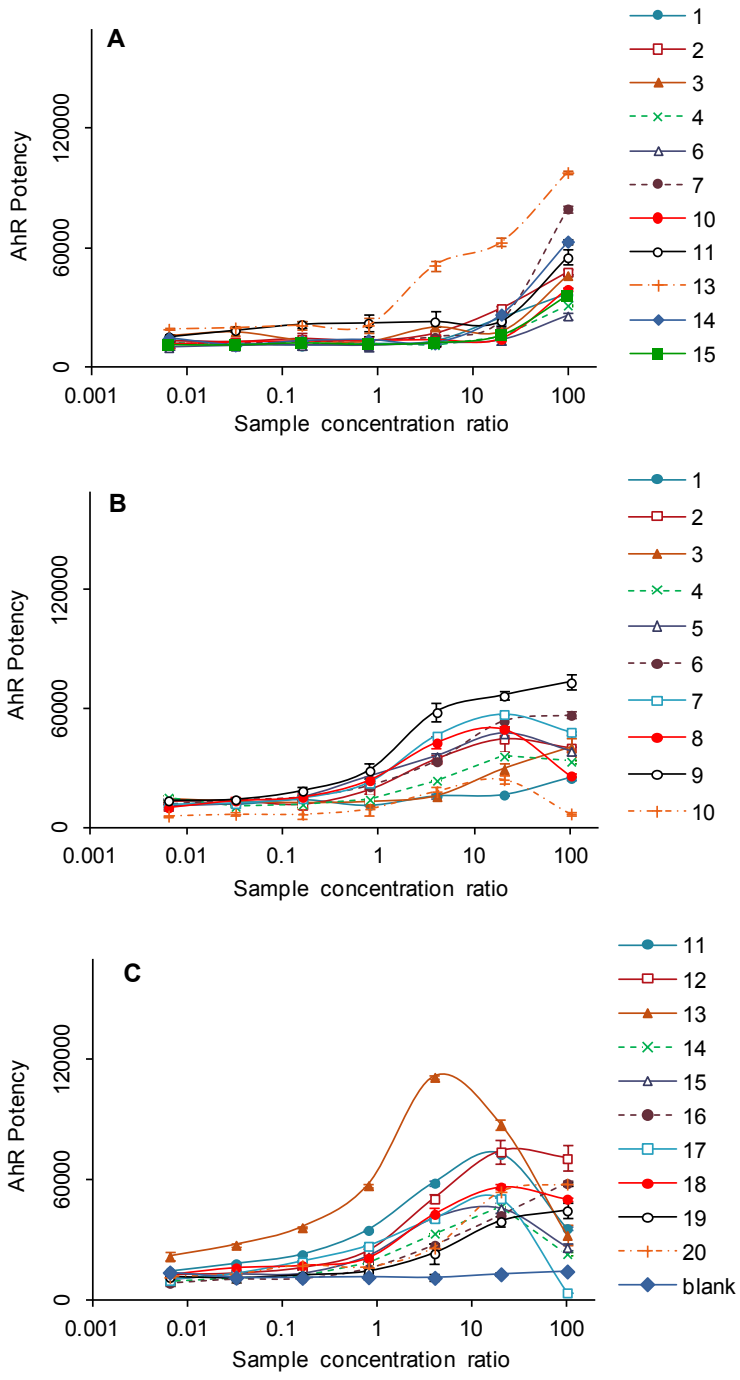

Fig. 2ESI Dose-Response curves of the SPE extracts of water samples in a yeast AhR assay; swimming pool water (A) and river water (No. 1−10) (B) and (No. 11−20) (C). Each point is represented as the mean ± standard deviation (n = 3).

Table 1ESI . Characterization of pool water samples

Site no.	date dd/mm/yy	time	temp./water temp.	pH	turbidity	free Cl	type	size
1	4-Aug-12	13:00	31/30 °C	8.0	65 mg/L	1.0 mg/L	outdoor	110 m^3
2	4-Aug-12	13:50	31/29 °C	7.4	110 mg/L	0.6 mg/L	outdoor	1,000 m^3
3	4-Aug-12	14:50	31/30 °C	7.5	156 mg/L	1.0 mg/L	indoor	590 m^3
4	4-Aug-12	15:30	31/31 °C	7.3	86 mg/L	1.0 mg/L	indoor	550 m^3
5	28-Jul-13	10:50	30/30 °C	7.4	61 mg/L	0.4 mg/L	outdoor	195 m^3
6	28-Jul-13	11:05	30/29 °C	7.4	120 mg/L	1.0 mg/L	outdoor	450 m^3
7	28-Jul-13	11:40	30/30 °C	7.0	101 mg/L	2.0 mg/L	outdoor	350 m^3
8	28-Jul-13	11:55	30/27 °C	7.0	124 mg/L	1.5 mg/L	outdoor	350 m^3
9	28-Jul-13	12:10	30/29 °C	7.0	56 mg/L	0.5 mg/L	outdoor	350 m^3
10	28-Jul-13	14:55	30/29 °C	7.7	86 mg/L	0.5 mg/L	outdoor	50 m^3
11	28-Jul-13	15:10	30/29 °C	7.0	105 mg/L	0.5 mg/L	outdoor	50 m^3
12	28-Jul-13	15:30	30/28 °C	7.0	161 mg/L	1.0 mg/L	outdoor	50 m^3
13	3-Aug-13	11:20	30/31 °C	7.5	101 mg/L	0.4 mg/L	indoor	420 m^3
14	3-Aug-13	10:40	30/29 °C	7.3	125 mg/L	0.5 mg/L	indoor	420 m^3
15	28-Jul-13	14:20	30/30 °C	7.8	153 mg/L	0.4 mg/L	outdoor	900 m^3
16	28-Jul-13	14:15	30/28 °C	7.7	143 mg/L	0.8 mg/L	outdoor	3,000 m^3
17	28-Jul-13	14:25	30/28 °C	7.8	110 mg/L	0.6 mg/L	outdoor	350 m^3

Table 2ESI. Characterization of river water samples

Site No.	coordinate data	date dd/mm/yy	time	temp./ water temp.	pH	turbidity	sewage treatment plant upstream			
							population served	capacity (m^3/day)	treatment process [a]	treatment system [b]
1	34°55'49"N 138°23'42"E	12-Dec-12	10:20	10/20 °C	6.7	166 mg/L	170,000	120,000	AS	SSS
2	34°55'49"N 138°23'17"E	12-Dec-12	10:40	10/19 °C	6.6	133 mg/L	66,000	43,000	AS	SSS
3	34°56'46"N 138°24'33"E	12-Dec-12	11:30	10/17 °C	7.0	98 mg/L	62,000	190,000	AS	CSS
4	35°00'21"N 138°24'19"E	12-Dec-12	12:10	10/17 °C	6.7	121 mg/L	77,000	54,000	AS	PCSS
5	34°08'01"N 138°46'54"E	9-May-13	14:20	21/24 °C	6.8	193 mg/L	180,000	81,000	AS	PCSS
6	35°11'34"N 138°36'24"E	9-May-13	11:30	21/23 °C	7.8	192 mg/L	68,000	36,000	AS	SSS
7	35°07'40"N 138°39'59"E	9-May-13	12:30	21/22 °C	7.4	214 mg/L	94,000	38,000	AS	SSS
8	35°08'47"N 138°43'38"E	9-May-13	13:50	21/23 °C	7.4	403 mg/L	90,000	42,000	AS	SSS
9	35°16'39"N 138°55'55"E	9-May-13	17:00	21/22 °C	6.9	197 mg/L	26,000	11,000	AS	SSS
10	34°59'32"N 138°30'07"E	9-May-13	9:30	21/21 °C	7.5	1042 mg/L	71,000	46,000	AS	SSS
11	34°39'32"N 138°45'03"E	16-May-13	13:45	25/22 °C	7.0	284 mg/L	440,000	180,000	AS	SSS
12	34°44'11"N 137°54'30"E	16-May-13	12:10	25/26 °C	7.1	151 mg/L	27,000	16,000	AS	SSS
13	34°39'58"N 137°50'16"E	16-May-13	13:20	25/24 °C	7.1	208 mg/L	120,000	66,000	AS	SSS
14	34°51'55"N 138°16'32"E	16-May-13	10:00	25/22 °C	7.1	185 mg/L	56,000	38,000	AS	SSS
15	34°47'45"N 137°38'43"E	16-May-13	17:40	25/22 °C	7.1	90 mg/L	5,500	1,200	AS	SSS
16	34°49'27"N 137°10'56"E	16-May-13	10:50	25/20 °C	7.6	63 mg/L	10,000	6,900	AS	SSS
17	34°40'50"N 137°43'52"E	16-May-13	16:10	25/23 °C	7.1	182 mg/L	170,000	110,000	AS	PCSS
18	34°46'10"N 137°59'45"E	16-May-13	11:40	25/25 °C	7.2	204 mg/L	2,000	14,000	AS	SSS
19	34°45'26"N 137°36'26"E	16-May-13	17:20	25/24 °C	6.8	465 mg/L	9,100	6,000	FCC	SSS
20	34°49'31"N 137°40'22"E	16-May-13	18:10	25/21 °C	7.6	113 mg/L	5,200	1,400	FCCS	SSS

[a] AS = activated sludge, FCC = Flocculant combination type circulation-type nitrification denitrification law, FCCS = Flocculant combination type carrier submerged-type nitrification denitrification law. [b] SSS = separated sewer system, CSS = combined sewer system, PCSS = partially combined sewer system

Table 3S. Quality-control data for trimethylsilyl derivatives of target compounds in solid phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS)

Compounds	functional group [a]			CAS No.	rt[b] (min)	recovery (%) $n = 5$	R^2 [c]	LOD[d] (ng/L)	LOQ[e] (ng/L)	MS ions m/z	
	R^1	R^2	R^3							Qnt[f]	Cnf[g]
MP	methyl	H	H	99-76-3	23.125	93 ± 3.5	0.999	0.63	2.1	209	224
EP	ethyl	H	H	120-47-8	24.850	95 ± 3.4	0.999	1.3	4.3	223	193
PP	propyl	H	H	94-13-3	27.109	96 ± 3.1	0.999	1.3	4.3	193	252
BP	isopropyl	H	H	94-26-8	29.336	97 ± 8.3	0.998	3.4	11	210	193
BnP	butyl	H	H	94-18-8	37.123	102 ± 6.1	0.990	3.7	12	193	91
HBA	isobutyl	H	H	99-96-7	26.375	100 ± 6.7	0.998	3.1	10	267	282
ClMP	benzyl	H	H	3964-57-6	26.249	92 ± 3.1	0.998	1.5	5.1	243	245
ClEP	H	H	H	16357-41-8	27.849	94 ± 7.1	0.998	2.0	6.6	257	259
ClPP	methyl	Cl	H	37470-49-8	29.941	95 ± 6.9	0.998	2.9	9.7	271	273
ClBP	ethyl	Cl	H	37470-51-2	32.033	99 ± 11	0.997	3.3	11	285	229
ClBnP	propyl	Cl	H	85303-64-6	39.503	99 ± 9.1	0.986	3.7	12	227	229
ClHBA	isopropyl	Cl	H	3964-58-7	29.173	96 ± 5.2	0.995	3.4	11	301	303
Cl_2MP	butyl	Cl	H	3337-59-5	28.995	99 ± 3.8	0.998	1.7	5.6	227	279
Cl_2EP	isobutyl	Cl	H	17302-82-8	30.447	96 ± 7.2	0.997	2.5	8.4	291	293
Cl_2PP	benzyl	Cl	H	101003-80-9	32.414	98 ± 3.5	0.997	3.0	10	305	307
Cl_2BP	H	Cl	H	909404-90-6	34.371	104 ± 4.3	0.995	3.8	13	319	321
Cl_2BnP	methyl	Cl	Cl	1116498-16-8	41.572	100 ± 7.4	0.981	4.2	14	353	355
Cl_2HBA	ethyl	Cl	Cl	3336-41-2	31.605	96 ± 6.6	0.994	3.7	12	335	337

[a] See Figure 1. [b] GC retention time. [c] Coefficient of determination for calibration curves. [d] Limits of detection. [e] Limits of quantification. [f] For quantification purpose. [g] For confirmatory purpose.

Table 4ESI. Paraben concentration [a] (ng/L) in the pool water samples and AhR potency [b] of water samples

	Site No.																
Compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
MP	658	130	141	78	3	3	LOQ[c]	LOQ	LOQ	5	LOQ	LOQ	21	4	5	3	LOQ
EP	34	32	16	74	LOQ	LOQ	LOQ	- d	LOQ	LOQ	LOQ	LOQ	201	-	LOQ	LOQ	-
PP	115	76	103	85	5	5	LOQ	LOQ	6	5	LOQ	-	LOQ	LOQ	LOQ	LOQ	LOQ
BP	18	-	-	LOQ	-	-	-	-	-	-	-	-	18	-	-	-	-
BnP	LOQ	-	LOQ	-	-	-	-	-	-	LOQ	-	-	-	-	-	-	-
HBA	72	18	16	31	LOQ	-	-	-		-	-	-	13	LOQ		-	-
ClMP	10	LOQ	6	LOQ	LOQ	-	-	-	LOQ	-	-	-	43	LOQ	LOQ	-	-
ClEP	LOQ	LOQ	-	9	-	-	-	-	-	LOQ	-	LOQ	135	-	-	-	-
ClPP	LOQ	-	-	-	-	-	-	-	-	77	-	-	-	-	-	-	-
ClBP	-	-	-	-	-	-	-	-	-	23	LOQ	LOQ	LOQ	-	-	-	-
ClBnP	-	-	-	-	-	-	-	-	-	LOQ	-	-	-	LOQ	-	-	-
ClHBA	59	LOQ	30	21	-		-	-	-		-	-	LOQ	LOQ	-	-	-
Cl_2MP	23	13	10	LOQ	-	LOQ	-	-	-	41	-	-	7	LOQ	-	LOQ	-
Cl_2EP	12	LOQ	9	LOQ	-	-	-	-	-	12	18	-	11		LOQ	-	-
Cl_2PP	19	14	18	14	LOQ	LOQ	-	-	LOQ	44	LOQ	LOQ	LOQ	LOQ	-	-	-
Cl_2BP	-	-	-	-	-	-	-	-	-	16	-	-	-	-	-	-	-
Cl_2BnP	-	-	-	-	-	-	-	-	-	LOQ	-		LOQ	15	LOQ	-	-
Cl_2HBA	36	18	23	42	LOQ	LOQ	-	-	LOQ		LOQ	LOQ	LOQ	LOQ	LOQ	-	-
Σ	1057	300	372	355	8	8			6	223	18		448	18	5	3	
AhR potency																	
REC25 (C.R.) [e]	28.94	285.58	35.60	65.93	not active	15.83	1.38	not active	not active	8.64	6.32	not active	2.07	2.08	12.19	not active	not active
SD	3.36	106.09	7.32	27.96		2.50	0.22			0.02	2.34		0.02	0.01	0.94		
As BNF (pM)	79	8	4	33		84	965			155	219		671	411	70		

[a] Means of duplication . [b] Means of triplication. [c] LOQ = Limits of quantification. Compound was detected but below the LOQ. [d] - = Not detected. [e] Concentration ratio of a sample.

Table 5ESI. Paraben concentration [a] (ng/L) in the river water samples and AhR potency [b] of water samples

	Site No.																			
Compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
MP	13	23	16	21	17	LOQ [c]	25	37	12	- d	18	-	42	20	6	8	9	25	-	6
EP	7	22	12	18	18	10	-	-	16	-	-	LOQ	-	5	17	-	18	5	8	5
PP	17	27	16	20	28	12	7	11	-	12	18	-	-	-	-	-	10	13	-	-
BP	-	LOQ	-	-	-	-	LOQ	LOQ	-	-	LOQ	-	-	-	-	-	-	-	-	-
BnP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HBA	45	106	57	97	86	64	25	29	94	32	25	12	20	17	59	69	24	21	36	29
ClMP	LOQ	LOQ	LOQ	LOQ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ClEP	-	11	LOQ	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ClPP	-	LOQ	LOQ	LOQ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ClBP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ClBnP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ClHBA	LOQ	25	LOQ	14	45	20	LOQ	LOQ	LOQ	LOQ	12	-	LOQ	LOQ	17	-	LOQ	-	LOQ	LOQ
Cl_2MP	LOQ	LOQ	LOQ	LOQ	8	-	LOQ	LOQ	6	-	LOQ	-	LOQ	LOQ	34	-	LOQ	-	-	-
Cl_2EP	LOQ	9	-	LOQ	14	-	12	9	17	LOQ	17	-	-	-	-	-	-	LOQ	-	-
Cl_2PP	LOQ	17	14	12	36	11	11	-	-	-	-	-	-	-	-	-	10	-	-	11
Cl_2BP	-	LOQ	-	LOQ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cl_2BnP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cl_2HBA	13	40	14	20	4331	417	199	184	432	207	204	93	110	71	98	147	63	54	47	33
Σ	95	281	129	211	4583	534	280	269	578	251	294	105	172	114	232	224	134	117	91	83
AhR potency																				
REC25 (C.R.) [e]	5.79	38.26	46.67	8.35	0.649	2.577	1.099	0.720	1.405	3.623	0.447	3.606	0.093	2.469	0.661	2.402	0.079	0.935	2.661	4.245
SD	2.01	9.41	4.36	1.56	0.358	1.337	0.277	0.343	1.358	0.312	0.403	0.306	0.061	1.554	0.043	0.428	0.004	0.059	0.385	0.272
As BNF (pM)	25	73	60	334	176	44	104	159	81	90	726	90	3490	132	166	46	1391	117	41	37

[a–e] See footnotes to Table 4S.