Appendix A: Supplementary Information

Estrogenic activity, chemical levels and health risk assessment of municipal distribution point water from Pretoria and Cape Town, South Africa

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A.1. UPLC-MS/MS instrument and method information

The instrument and method information, MRM transitions and elution gradient for the UPLC-MS/MS analysis of the distribution point water for the selected target chemicals is summarized in Tables A.1.1, A.1.2 and A.1.3 respectively.

Table A.1.1: Instrument and method for UPLC-MS/MS analysis

Instrument	Waters Xevo TQ MS
Sample introduction	Waters Acquity UPLC (3 µL injection)
Solvent A	7.5% formic acid
Solvent B	Acetonitrile: MeOH: iPrOH 49:49:2
Source	ESCI+
MS settings:	
Capillary voltage	2.8 kV
Source	120°C
Desolvation temperature	400°C
Desolvation gas	600 L/h
Cone gas	60 L/h
Column	Waters BEH C18, 2.1 x 100 mm, @ 50°C, injection volume 3 µL

Table A.1.2: Gradient employed for elution of the selected chemicals

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.35	80	20	initial
0.04	0.35	80	20	6
1.00	0.35	65	35	5
5.00	0.35	50	50	6
6.00	0.35	20	80	6
6.10	0.35	0	100	6
10.00	0.35	0	100	6
10.01	0.35	80	20	1
11.00	0.35	80	20	6

Table A.1.3: MRM transitions for each of the selected chemicals

Compound name	Parent (m/z)	Daughter (m/z)	Cone (V)	Collision (V)
4-Nonylphenol	454.2	171	40	35
Estrone	504	171	50	35
B-estradiol	506	171	50	35
17-alpha-ethenylestradiol	530	156	50	35
17-alpha-ethenylestradiol	530	171	50	35
Bisphenol A	695.4	170	50	35

A.2. UPLC-MS/MS analysis – individual results for four sampling periods

The individual results for each of the four sampling periods are given in Table A.2.

Table A.2: Target chemical concentrations (ng/L) in distribution point water from Pretoria (PTA) and Cape Town (CPT), South Africa, using UPLC-MS/MS

Site	Month	BPA	NP	DEHA	DBP	DEHP	DINP	E_2	E_1	EE_2
PTA01	Oct 2013	0.06	<dl	2.66	200.34	55.37	44.90	<dl	<dl	<dl
	Jan 2014	1.14	<dl	3.95	220.34	92.03	43.90	<dl	<dl	<dl
	Apr 2014	0.17	<dl	2.37	175.56	118.86	26.67	<dl	<dl	<dl
	Jul 2014	0.39	<dl	1.64	260.18	46.42	<dl	<dl	<dl	<dl
PTA02	Oct 2013	0.60	<dl	3.15	258.11	52.50	25.39	<dl	<dl	<dl
	Jan 2014	<dl	<dl	2.97	302.28	332.86	187.22	<dl	<dl	<dl
	Apr 2014	<dl	<dl	2.36	372.51	81.76	<dl	0.03	2.32	<dl
	Jul 2014	0.74	<dl	2.21	155.65	<dl	<dl	<dl	<dl	<dl
PTA03	Oct 2013	4.24	<dl	2.96	291.85	64.05	10.24	<dl	3.41	<dl
	Jan 2014	5.74	<dl	3.60	402.69	152.04	56.05	<dl	<dl	<dl

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	Apr 2014	4.46	<dl	3.10	347.61	67.56	11.85	<dl	<dl	<dl
	Jul 2014	0.73	<dl	3.12	235.26	44.30	<dl	<dl	<dl	<dl
PTA04	Oct 2013	0.01	<dl	1.95	158.62	<dl	11.40	<dl	<dl	<dl
	Jan 2014	<dl	<dl	3.15	271.63	44.03	26.38	<dl	<dl	<dl
	Apr 2014	0.07	<dl	2.83	261.09	247.13	49.56	<dl	<dl	<dl
	Jul 2014	7.80	<dl	2.56	270.71	67.78	26.91	<dl	<dl	<dl
PTA05	Oct 2013	0.16	<dl	1.38	<dl	<dl	<dl	<dl	<dl	<dl
	Jan 2014	0.25	<dl	1.07	<dl	<dl	<dl	<dl	<dl	<dl
	Apr 2014	0.34	<dl	2.87	335.51	146.83	8.34	<dl	<dl	<dl
	Jul 2014	9.88	<dl	2.55	185.39	51.25	<dl	<dl	<dl	<dl
PTA06	Oct 2013	0.34	<dl	3.65	390.69	130.93	61.03	<dl	2.33	<dl
	Jan 2014	<dl	<dl	3.94	361.73	82.28	<dl	<dl	4.89	<dl
	Apr 2014	0.22	<dl	4.60	312.76	<dl	112.20	<dl	<dl	<dl
	Jul 2014	1.99	<dl	2.66	138.71	66.59	6.30	<dl	<dl	<dl
PTA07	Oct 2013	0.67	<dl	3.12	248.17	73.20	77.25	<dl	<dl	<dl
	Jan 2014	0.44	<dl	3.26	128.82	<dl	117.84	<dl	<dl	<dl
	Apr 2014	0.19	<dl	3.82	148.22	<dl	140.87	<dl	<dl	<dl

	Jul 2014	0.39	<dl	2.61	179.40	222.77	85.57	<dl	<dl	<dl
PTA08	Oct 2013	1.81	<dl	3.05	249.22	60.71	18.53	<dl	<dl	0.06
	Jan 2014	0.33	<dl	1.79	279.79	<dl	<dl	<dl	<dl	0.02
	Apr 2014	1.41	<dl	2.03	<dl	<dl	66.13	<dl	<dl	0.01
	Jul 2014	0.79	<dl	2.19	215.01	<dl	6.87	<dl	<dl	0.003
PTA09	Oct 2013	0.44	<dl	4.09	332.50	175.46	13.76	<dl	<dl	<dl
	Jan 2014	0.68	<dl	2.51	245.57	<dl	3.02	<dl	<dl	<dl
	Apr 2014	0.05	<dl	1.12	<dl	<dl	<dl	<dl	<dl	<dl
	Jul 2014	0.24	<dl	3.01	271.79	74.42	31.09	<dl	<dl	<dl
PTA10	Oct 2013	0.30	<dl	3.28	346.31	83.08	46.10	<dl	<dl	<dl
	Jan 2014	0.37	<dl	3.77	434.01	393.05	63.39	<dl	<dl	<dl
	Apr 2014	0.23	<dl	2.27	228.61	<dl	<dl	<dl	<dl	<dl
	Jul 2014	0.10	<dl	1.24	<dl	<dl	<dl	<dl	<dl	<dl
PTA	**Average**	**1.33**	**<dl**	**2.76**	**263.33**	**116.43**	**49.24**	**0.03**	**3.24**	**0.02**
	Median	**0.39**	**<dl**	**2.85**	**260.18**	**78.09**	**37.50**	**0.03**	**2.87**	**0.02**
CPT01	Oct 2013	0.12	<dl	3.25	950.08	62.36	1250.75	<dl	<dl	<dl
	Jan 2014	0.16	<dl	4.36	702.11	364.47	81.68	<dl	<dl	<dl

	Apr 2014	0.16	<dl	3.97	574.95	169.06	34.00	<dl	<dl	<dl
	Jul 2014	0.25	<dl	4.12	289.21	68.07	33.92	<dl	<dl	<dl
CPT02	Oct 2013	0.20	<dl	3.38	444.76	<dl	54.59	<dl	<dl	<dl
	Jan 2014	0.11	<dl	2.59	369.50	98.74	69.13	<dl	<dl	<dl
	Apr 2014	0.18	<dl	2.28	627.53	43.39	<dl	<dl	<dl	<dl
	Jul 2014	0.18	<dl	2.32	233.15	40.20	<dl	0.02	0.36	<dl
CPT03	Oct 2013	4.27	<dl	2.82	823.04	42.68	377.66	0.04	<dl	<dl
	Jan 2014	1.64	<dl	3.31	285.58	196.94	171.74	<dl	<dl	<dl
	Apr 2014	1.94	<dl	2.64	172.50	74.28	35.32	<dl	<dl	<dl
	Jul 2014	0.11	<dl	1.41	184.49	<dl	<dl	<dl	<dl	<dl
CPT04	Oct 2013	0.24	<dl	1.40	343.91	4119.94	<dl	<dl	<dl	<dl
	Jan 2014	1.08	<dl	4.23	656.24	4272.92	71.06	<dl	<dl	<dl
	Apr 2014	0.17	<dl	2.23	310.80	5150.76	<dl	<dl	<dl	<dl
	Jul 2014	2.62	<dl	3.51	222.90	117.14	30.44	<dl	<dl	<dl
CPT05	Oct 2013	0.36	<dl	4.18	953.24	373.60	936.99	<dl	<dl	<dl
	Jan 2014	0.26	<dl	4.06	506.56	138.00	22.59	<dl	<dl	<dl
	Apr 2014	0.28	<dl	3.59	827.83	257.73	32.86	<dl	<dl	<dl

	Jul 2014	28.83	<dl	2.00	187.03	<dl	<dl	<dl	<dl	<dl
CPT06	Oct 2013	0.38	<dl	3.14	393.55	62.50	54.28	<dl	<dl	<dl
	Jan 2014	0.10	<dl	4.13	331.26	80.87	122.54	0.05	<dl	<dl
	Apr 2014	0.12	<dl	3.02	218.33	<dl	57.17	<dl	<dl	<dl
	Jul 2014	0.43	<dl	3.44	208.46	415.01	29.02	<dl	<dl	<dl
CPT07	Oct 2013	0.22	<dl	<loq	154.35	<dl	11.32	<dl	<dl	<dl
	Jan 2014	1.34	<dl	4.61	318.93	101.34	227.12	<dl	<dl	<dl
	Apr 2014	0.19	<dl	2.62	341.33	47.15	54.77	<dl	<dl	<dl
	Jul 2014	<dl	<dl	2.56	109.48	<loq	24.85	0.04	<dl	<dl
CPT08	Oct 2013	0.41	<dl	4.53	393.76	99.53	49.68	<dl	<dl	<dl
	Jan 2014	0.50	<dl	4.97	576.97	125.49	96.18	<dl	<dl	<dl
	Apr 2014	0.17	<dl	2.97	180.47	62.05	54.84	<dl	<dl	<dl
	Jul 2014	0.56	<dl	2.60	123.63	<loq	9.66	<dl	<dl	<dl
CPT09	Oct 2013	<dl	<dl	1.76	986.10	<dl	61.61	<dl	<dl	<dl
	Jan 2014	0.04	<dl	3.83	367.73	176.19	64.55	<dl	<dl	<dl
	Apr 2014	3.47	<dl	4.70	347.41	544.54	40.78	<dl	<dl	<dl
	Jul 2014	<dl	<dl	3.29	219.67	<dl	12.06	<dl	1.14	<dl

CPT10	Oct 2013	0.15	<dl	3.44	1065.14	88.67	762.21	<dl	<dl	<dl
	Jan 2014	0.42	<dl	3.77	308.43	91.35	134.14	<dl	<dl	<dl
	Apr 2014	0.71	<dl	4.87	527.45	225.12	58.33	<dl	<dl	<dl
	Jul 2014	1.16	<dl	4.21	243.64	103.25	46.33	<dl	<dl	<dl
CPT	**Average**	**1.45**	**<dl**	**3.34**	**427.04**	**574.62**	**152.18**	**0.04**	**0.75**	**<dl**
	Median	**0.26**	**<dl**	**3.38**	**342.62**	**103.25**	**54.81**	**0.04**	**0.75**	**<dl**

<dl: Below detection limit; <loq: Below level of quantification