

**Supplementary Information: Analysis of endocrine activity in drinking water, surface water
and treated wastewater from six countries**

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Table S1: Information about the collected water samples.

Sample ID	Matrix	Country	Site Description	Sampling Date
1	WW	Germany	Urban wastewater, 875,000 PE. Applies mechanical treatment with additional phosphate precipitation, followed by biological treatment with a denitrification/nitrification unit, equipped with a trickling filter.	November 2015
2	DW	Germany	Groundwater treated by aeration for iron and manganese removal	November 2015
3	SW	Germany	River water from the Rhine River, Karlsruhe, Germany (mean discharge 1078 m ³ /s)	November 2015
4	CW	Germany	Ultrapure laboratory water	November 2015
5	WW	Australia	Urban wastewater, 450,000 PE; Secondary treated effluent, post-chlorination	November 2015
6	DW	Australia	Chlorinated drinking water, sourced from a protected surface water catchment	November 2015
7	SW	Australia	Small creek in urban area of the Gold Coast, Australia	November 2015
8	CW	Australia	Ultrapure laboratory water	November 2015
9	WW	France	Urban wastewater, secondary treated effluent, post-chlorination	November 2015
10	DW	France	Chlorinated drinking water, sourced from a protected surface water catchment	November 2015
11	SW	France	Protected surface water catchment in an urban area	November 2015
12	CW	France	Evian mineral water in glass bottle	November 2015
13	WW	South Africa	Water treatment plant filter backwash water from plant to sludge tank after settling	November 2015
14	DW	South Africa	Dam water treated by dosing with lime and flocculant, flocculation, air floatation & sand filtration simultaneously, granular activated carbon, chlorination	November 2015
15	SW	South Africa	Dam/lake in an urban nature reserve in Gauteng Province, South Africa. The waterbody is fed by small creek and a wetland, preceded upstream by an urban WWTP	November 2015
16	CW	South Africa	Ultrapure laboratory water	November 2015
17	WW	Netherlands	Urban wastewater, secondary treated effluent, no post-treatment	November 2015
18	DW	Netherlands	Non-chlorinated drinking water, sourced from open surface water	November 2015
19	SW	Netherlands	Lake in a rural area of the Netherlands	November 2015
20	CW	Netherlands	Ultrapure laboratory water	November 2015
21	WW	Spain	Treatment for nitrogen and phosphorus removal, lamellar filtration, UV and chlorination	November 2015
22	DW	Spain	Surface water (Llobregat River). Pre-oxidation with chlorine dioxide, flocculation/settling, sand filtration + UF/RO	November 2015
23	SW	Spain	Llobregat River, with important contribution of urban and industrial wastewater	November 2015
24	CW	Spain	Ultrapure laboratory water	November 2015

Sample types: CW = control water, i.e. ultrapure water; DW = drinking water; SW = surface water; WW = treated wastewater.

PE = population equivalent

Table S2: Water quality parameters of the studied water samples, along with the enrichment factor after solid phase extraction (SPE).

Sample ID	Matrix	Country	Volume (L)	pH	TOC (mg/L)	DOC (mg/L)	Conductivity ($\mu\text{S/cm}$)	Spectral absorption (m^{-1})	Free chlorine (mg/L)	Total chlorine (mg/L)	Temperature ($^{\circ}\text{C}$)	Enrichment factor after SPE
1	WW	Germany	0.2	8.0	12.0	11.0	1188	16.6	n.d.	n.d.	18.5	200
2	DW	Germany	1	7.3	0.7	0.677	694	1.0	n.d.	n.d.	15.4	4000
3	SW	Germany	4	8.0	1.6	1.4	369	3.3	n.d.	n.d.	17.0	1000
4	CW	Germany	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1000
5	WW	Australia	0.2	7.0	10.6	10.2	1664	18.5	n.d.	n.d.	16.6	200
6	DW	Australia	1	6.8	4.1	4.1	189	8.0	0.65	n.d.	22.5	4000
7	SW	Australia	4	7.4	8.5	9.8	8520	25.7	n.d.	n.d.	21.8	1000
8	CW	Australia	1	7.8	n.d.	n.d.	0	0	n.d.	n.d.	22.4	1000
9	WW	France	0.2	7.3	n.d.	7.7	938	21.0	n.d.	n.d.	20.5	200
10	DW	France	1	7.4	1.4	1.4	550	1.3	0.13	0.27	17.5	4000
11	SW	France	4	7.9	n.d.	2.2	536	10.2	0	0	15.9	1000
12	CW	France	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1000
13	WW	South Africa	0.2	9.5	6.2	n.d.	449	23.0	n.d.	n.d.	19.5	200
14	DW	South Africa	1	8.3	3.8	n.d.	426	10.1	0.98	1.15	20.1	4000
15	SW	South Africa	4	9.3	8.2	n.d.	487	22.0	n.d.	n.d.	19.0	1000
16	CW	South Africa	1	6.3	n.d.	n.d.	6.1	n.d.	n.d.	n.d.	22.9	1000
17	WW	Netherlands	0.2	6.9	5.7	5.0	280	13.0	<0.03	< 0.03	19.0	200
18	DW	Netherlands	1	8.2	1.2	1.1	600	2.4	<0.03	< 0.03	18.7	4000
19	SW	Netherlands	4	7.9	12.0	10.0	770	33.0	<0.03	< 0.03	18.9	1000
20	CW	Netherlands	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1000
21	WW	Spain	0.2	7.6	9.5	8.5	1996	20.0	n.d.	n.d.	19.5	200
22	DW	Spain	1	7.5	0.9	0.9	1022	2.4	n.d.	n.d.	15.4	4000
23	SW	Spain	4	7.9	n.d.	3.5	1452	7.6	n.d.	n.d.	17.9	1000
24	CW	Spain	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1000

Sample types: CW = control water, i.e. ultrapure water; DW = drinking water; SW = surface water; WW = treated wastewater. n.d. = not determined.

Table S3: Liquid chromatography - electrospray ionization - tandem mass spectroscopy (LC-ESI-MS/MS) target method to determine micropollutants in different water matrices. Measurements were performed in MRM (multiple reaction monitoring) mode acquiring two transitions for every micropollutant.

Instruments & injections conditions	HPLC: 1290 series, Agilent Technologies			
	Mass spectrometer : 5500 AB Sciex			
	➤ Column: Fusion RP, 250 mm × 2 mm; 4 μm			
	➤ Mobile phase: (A) 5 mM ammonium acetate in water and (B) 5 mM ammonium acetate in methanol			
	➤ Column temperature: 30 °C			
	➤ Equilibration time: 8 min			
	➤ Injection volume : 1 μL			
Chromatographic gradient for compounds measured in positive ionization mode:	Time (min)	Eluent (A) (%)	Eluent (B) (%)	Flowrate (μL/min)
	0	90	10	250
	2	90	10	250
	14	5	95	250
	19	0	100	250
	21	0	100	250
	22	90	10	250
Chromatographic gradient for compounds measured in negative ionization mode:	Time (min)	Eluent (A) (%)	Eluent (B) (%)	Flowrate (μL/min)
	0	70	30	250
	2	70	30	250
	6	5	95	250
	13	0	100	250
	19	0	100	250
	21	70	10	250
MS/MS interface parameters	Collision gas setting			7
	Curtain gas			30 psi
	Ion source gas 1			60 psi
	Ion source gas 2			90 psi
	Ion source temperature			500 °C
	Ionization voltage			-4.5 kV/5.5 kV (negative/positive Mode)

Table S4: Parameters for analysed chemicals in liquid chromatography - electrospray ionization - tandem mass spectroscopy (LC-ESI-MS/MS) target method.

Chemical	Ionization	Precursor ion	Product ion	DP	CE	CXP
17 α -Ethinylestradiol	positive	297.1	77.0	126	83	12
	positive	297.1	107.0	126	29	18
17 β -Estradiol	positive	273.1	107.1	106	29	6
	positive	273.1	135.1	106	21	10
17 β -Trenbolone	positive	271.1	165.1	100	71	12
	positive	271.1	199.0	100	29	12
2,2',4,4'-Tetrahydroxybenzophenone	negative	244.9	108.9	-55	-28	-5
	negative	244.9	134.9	-55	-18	-11
4-Nonylphenol	negative	219.1	133.0	-135	-32	-9
	negative	219.1	147.0	-135	-38	-9
4-t-Octylphenol	negative	205.1	117.0	-130	-76	-7
	negative	205.1	133.0	-130	-30	-9
5 α -Dihydrotestosterone	pos [NH4+]	308.1	255.2	51	25	12
	pos [NH4+]	308.1	291.2	51	15	4
6-Propylthiouracil	positive	171.0	112.0	86	25	8
	positive	171.0	153.8	86	23	10
Acetochlor	positive	270.1	224.1	51	13	14
	positive	270.1	148.1	51	29	24
Alachlor	positive	270.1	238.0	46	15	16
	positive	270.1	162.1	46	29	10
Amiodarone	positive	645.9	58.1	130	109	8
	positive	645.9	100.2	130	35	14
Androstenedione	positive	287.1	97.1	126	27	6
	positive	287.1	109.1	126	31	8
Atenolol	positive	267.1	74.1	61	35	0
	positive	267.1	145.0	61	35	2
Atrazine	positive	216.1	174.1	101	27	12
	positive	216.1	68.0	101	51	10
Beclomethasone	positive	409.1	91.0	96	85	14
	positive	409.1	147.0	96	37	10
Betamethasone	positive	393.1	355.2	61	17	6
	positive	393.1	373.2	61	13	4
Bisphenol A	positive	226.9	212.2	-85	-26	-9
	positive	226.9	132.8	-85	-34	-9
Carbamazepine	positive	237.0	164.9	31	55	5
	positive	237.0	193.9	31	25	5
Carbendazim	positive	192.2	160.0	81	27	26
	positive	192.2	132.0	81	45	22
Chlorpyrifos	positive	349.9	197.9	121	25	12
	positive	349.9	96.9	121	53	16
Cyproterone acetate	positive	418.1	279.2	160	33	4
	positive	418.1	357.1	160	23	10
Dexamethasone	positive	393.1	337.2	56	17	4
	positive	393.1	355.1	56	17	16

Diazepam	positive	285.2	153.9	96	39	28
	positive	285.2	192.9	96	43	12
Diazinon	positive	305.1	169.0	51	31	12
	positive	305.1	153.2	51	31	18
Diclofenac	positive	296.0	213.8	26	37	5
	positive	296.0	249.8	26	19	6
Diuron	positive	233.0	71.9	101	33	12
	positive	233.0	46.2	101	29	6
Estriol	positive	289.1	107.0	136	29	8
	positive	289.1	253.2	136	17	8
Estrone	positive	271.1	132.9	136	29	8
	positive	271.1	253.2	136	14	12
Genistein	positive	271.0	91.0	41	45	12
	positive	271.0	153.0	41	37	10
Levonorgestrel	positive	313.2	109.1	131	33	6
	positive	313.2	149.0	131	21	10
Linuron	positive	249.0	160.0	106	25	10
	positive	249.0	182.0	106	23	12
Mefenamic acid	positive	242.0	209.1	70	41	12
	positive	242.0	224.2	70	27	16
Mestranol	positive	311.1	91.0	121	67	16
	positive	311.1	121.1	121	29	6
Pentachlorophenol	negative	264.8	96.9	-110	-34	-9
	negative	264.8	205.3	-115	-16	-7
Progesterone	positive	315.1	97.0	46	27	8
	positive	315.1	109.0	46	29	8
Simazine	positive	202.1	68.1	86	47	10
	positive	202.1	104.0	86	37	18
T3 (3,3,5-Triiodothyronine)	positive	651.7	478.8	111	47	24
	positive	651.7	605.8	111	29	14
T4 (L-Thyroxine)	positive	777.6	604.7	161	53	30
	positive	777.6	633.7	161	35	12
Testosterone	positive	289.0	97.0	151	31	10
	positive	289.0	109.0	151	31	8
Tetrabromobisphenol A	negative	542.6	419.8	-180	-54	-23
	negative	540.6	417.8	-180	-54	-25
TETRAC (3,3',5,5'-Tetraiodothyroacetic acid)	negative	620.6	448.6	-130	-16	-19
	negative	620.6	576.8	-130	-6	-11
TRIAC (Triiodothyroacetic acid)	negative	746.5	447.7	-130	-32	-23
	negative	746.5	575.7	-130	-20	-25
Triclocarban	negative	314.9	126.0	-120	-36	-9
	negative	314.9	159.9	-120	-18	-1
	negative	314.9	161.9	-120	-18	-7
Triclosan	negative	286.8	35.0	-65	-36	-36
	negative	288.7	37.0	-50	-34	-34
Trifluralin	positive	336.0	236.0	31	21	16
	positive	336.0	252.0	31	23	10

CE = collision energy in eV; CXP = cell exit potential in V; DP = declustering potential in V

Table S5: Method to determine hormonal compounds using liquid chromatography - atmospheric pressure chemical ionization – high-resolution mass spectrometry (LC-APCI-HRMS).

Instruments &	UPLC: Thermo Fisher			
Injections conditions	Mass spectrometer : Orbitrap QExactive Thermo Fisher			
	➤ Column: BEH C18, 2.1 X 10 mm, 1.7 μm			
	➤ Column temperature: 30 °C			
	➤ Injection volume: 10 μL			
Chromatographic gradient for elution:	Time (min)	Water (%)	Acetonitrile (%)	Flowrate (μL/min)
	0	80	20	500
	8.5	25	75	500
	9.5	0	100	500
	11.5	0	100	500
	12	80	20	500
	14	80	20	500
Orbitrap Ionization parameters	Ionization mode	APCI - positive		
	Corona discharge	30 A		
	Sheath gas	35		
	Auxiliary gas	10		
	Source temperature	250°C		
	Capillary tube temperature	300°C		
Exact masses of target analytes	Compound	Mass (m/z)		
	Norethisterone	299.20056		
	Cortisone	361.20095		
	Cortisol	363.21660		
	Epitestosterone	289.21621		
	Androsterone	291.23186		
Calibration with internal standards	Internal standard	Mass (m/z)		
	Testosterone ¹³ C ₃	292.22627		
	Androstenedione ¹³ C ₃	290.21062		
Calibration range		Min (ng/L)	Max (ng/L)	
	Hormones	0.4	200	

Table S6: Gas chromatography - mass spectrometry (GS/MS) parameters for determination of semi-volatile compounds.

GC injections & elution conditions	Preparation: Derivatization with N-tertbutyldimethylsilyl-N-methyltrifluoroacetamide MSTBFA (MSTBFA/sample, 1 / 4, v / v)		
	Injection mode	Splitless	
	Injector temperature	250 °C	
	Injection volume	1 µL	
	Gas flow rate (helium)	1 mL/min	
	Column	Rxi -5 ms; 30 m × 0.25 mm, 0.25 µm.	
	Oven temperature	Initial: 50°C (1 min) 8°C/min till 310°C (2 min)	
Mass spec parameters:	Mass spectrometer: Thermo Fisher DSQ II		
	Source temperature: 200°C		
	Single Ion Monitoring acquisition		
	Compounds	Mass Ion	Internal standard
	Benzothiazole	108-135	PCP C13
	4- <i>t</i> -butylphenol	151-264	PCP C13
	Galaxolide	243-246-213	Tonalide D4
	Tonalide	243-246-213	Tonalide D4
	Dibutyl phthalate	149-223	DEHP D5
	Pentachlorophenol	287-323-329	PCP C13
	Triclosan	200-206-345	Triclosan C13
	DEHP	149-153-167	DEHP D5
	Bisphenol A	441-453-456	Bisphenol A C13
	2,4,6-Trichlorophenol	179	PCP C13
	Bisphenol F	253	Bisphenol A C13
Calibration range		Min (ng/L)	Max (ng/L)
	Benzothiazole	40	2000
	4- <i>t</i> -butylphenol	10	2000
	246 TCP	10	2000
	Tonalide	10	2000
	Galaxolide X10	100	20000
	Dibutyl phthalate	10	2000
	Pentachlorophenol	10	2000
	Triclosan	10	2000
	DEHP X10	200	20000
	Bisphenol F	20	2000
	Bisphenol A	10	2000

Table S7: Analysed chemicals with method quantification limit (ng/L) for the different water matrices.

Chemical	CAS No.	MW (g/mol)	Method quantification limit (ng/L)			
			Treated wastewater (WW)	Surface water (SW)	Drinking water (DW)	Ultrapure water (CW)
<i>Liquid chromatography tandem mass spectrometry (LC-MS/MS)</i>						
17 α + β -Estradiol	50-28-2/ 57-91-0	272.38	1500	300	75	300
17 α -Ethinylestradiol	57-63-6	296.40	200	40	10	40
17 β -Trenbolone	10161-33-8	270.37	50	10	2.5	10
2,2',4,4',-Tetrahydroxy- benzophenone	131-55-5	246.22	5	1	0.25	1
4-Nonylphenol	104-40-5	220.35	400	80	20	80
4- <i>t</i> -Octylphenol	140-66-9	206.32	10	2	0.5	2
5 α -Dihydrotestosterone (DHT)	521-18-6	290.44	150	30	7.5	30
6-Propylthiouracil	51-52-5	170.23	1500	300	75	300
Acetochlor	34256-82-1	269.77	10	2	0.5	2
Alachlor	15972-60-8	269.77	5	1	0.25	1
Amiodarone	19774-82-4	645.31	20	4	1	4
Androstenedione	63-05-8	286.41	15	3	0.75	3
Atenolol	56715-13-0	266.34	200	40	10	40
Atrazine	1912-24-9	215.68	2	0.4	0.1	0.4
Beclomethasone	4419-39-0	408.93	150	30	7.5	30
Betamethasone	378-44-9	392.47	2	0.4	0.1	0.4
Bisphenol A	80-05-7	228.29	5	1	0.25	1
Carbamazepine	298-46-4	236.27	5	1	0.25	1
Carbendazim	10605-21-7	191.19	5	1	0.25	1
Chlorpyrifos	2921-88-2	350.59	5	1	0.25	1
Cyproterone acetate	427-51-0	416.95	100	20	5	20
Dexamethasone	50-02-2	416.95	2	0.4	0.1	0.4
Diazepam	439-14-5	284.74	10	2	0.5	2
Diazinon	333-41-5	304.35	10	2	0.5	2
Diclofenac	15307-79-6	296.15	5	1	0.25	1
Diuron	330-54-1	233.09	5	1	0.25	1
Estriol	50-27-1	288.39	400	80	20	80
Estrone	53-16-7	270.37	600	120	30	120
Genistein	446-72-0	270.24	100	20	5	20
Levonorgestrel	17489-40-6	312.45	50	10	2.5	10
Linuron	330-55-2	249.09	5	1	0.25	1
Mefenamic acid	61-68-7	241.29	2	0.4	0.1	0.4
Mestranol	72-33-3	310.44	100	20	5	20
Pentachlorophenol	87-86-5	266.34	2	0.4	0.1	0.4
Progesterone	87-86-5	266.34	5	1	0.25	1
Simazine	122-34-9	201.66	2	0.4	0.1	0.4
T3 (3,3',5 Triiodothyronine)	6893-02-3	650.98	50	10	2.5	10
T4 (L-Thyroxine)	51-48-9	776.88	100	20	5	20
Testosterone (T)	58-22-0	288.43	5	1	0.25	1
Tetrabromobisphenol A TETRAC (3,3',5,5'- Tetraiodothyroacetic acid) TRIAC (Triiodothyroacetic acid)	79-94-7	543.88	5	1	0.25	1
	67-30-1	747.84	1500	300	75	300
	51-24-1	621.94	600	120	30	120

Chemical	CAS No.	MW (g/mol)	Method quantification limit (ng/L)			
			Treated wastewater (WW)	Surface water (SW)	Drinking water (DW)	Ultrapure water (CW)
Triclocarban	101-20-2	315.58	5	1	0.25	1
Triclosan	3380-34-5	289.54	5	1	0.25	1
Trifluralin	1582-09-8	335.29	150	30	7.5	30
<i>Liquid chromatography – atmospheric pressure chemical ionization – high resolution mass spectrometry (LC-APCI-HRMS)</i>						
Androsterone	53-41-8	290.45	6	1.2	0.3	1.2
Cortisol	50-23-7	362.47	6	1.2	0.3	1.2
Cortisone	53-06-5	360.44	6	1.2	0.3	1.2
Epitestosterone	481-30-1	288.43	6	1.2	0.3	1.2
Norethisterone	68-22-4	298.43	6	1.2	0.3	1.2
<i>Gas chromatography mass spectrometry (GC/MS)</i>						
2,4,6-Trichlorophenol (TCP)	88-06-2	197.45	100	20	5	20
4- <i>t</i> -Butylphenol	98-54-4	150.22	100	20	5	20
Benzothiazole	95-16-9	135.18	1000	200	50	200
Bisphenol A	80-05-7	228.29	400	80	20	80
Bisphenol F	620-92-8	200.24	200	40	10	40
DEHP	117-81-7	390.57	2000	400	100	400
Dibutylphthalate	84-74-2	278.34	100	20	5	20
Galaxolide	1222-05-5	258.41	1000	200	50	200
Pentachlorophenol	87-86-5	266.34	100	20	5	20
Tonalide	1506-02-1	258.41	100	20	5	20
Triclosan	3380-34-5	289.54	100	20	5	20

Section S1: Recovery by SPE.

The recovery of 44 chemicals including hormones, industrial compounds, pharmaceuticals and pesticides by StrataX SPE cartridges was evaluated using LC-ESI-MS/MS. To determine potential matrix effects, the chemical mixture was spiked into drinking water and treated wastewater. Briefly, individual chemical stock solutions were prepared in methanol, with a mixture containing all 44 chemicals prepared by diluting the individual stock solutions to concentrations of 10 and 100 µg/L in methanol. The chemical mixture was spiked into 1000 mL of drinking water and 200 mL treated wastewater, respectively, and extracted with StrataX SPE cartridges using the protocol described in Section 2.1 of the manuscript. The extracts were analysed using LC-ESI-MS/MS (details provided in Tables S3 and S4), with recovery calculated by comparing the peak areas of the spiked samples after SPE with those obtained from direct injection of the same concentration of the chemical mixture. Unspiked wastewater was also extracted and background concentrations of target analytes were subtracted when calculating recovery.

When considering recovery in drinking water, over 70% of spiked chemicals were well recovered by Strata X (75 – 125% recovery), with between 50-75% recovery for 20% of the spiked chemicals (Figure S1). Only 4 chemicals had less than 50% recovery, with poor recoveries (<10%) of hydrophilic pharmaceutical 6-propylthiouracil and positively charged pharmaceutical atenolol. Recovery was typically lower in the treated effluent, but the difference generally small for most compounds, with 55% of compounds well recovered by StrataX.

Figure S1: Recovery of chemicals spiked in drinking water and treated wastewater by StrataX SPE cartridges. The dotted line indicates 100% recovery. *genistein is a phytoestrogen, but has been grouped under hormones

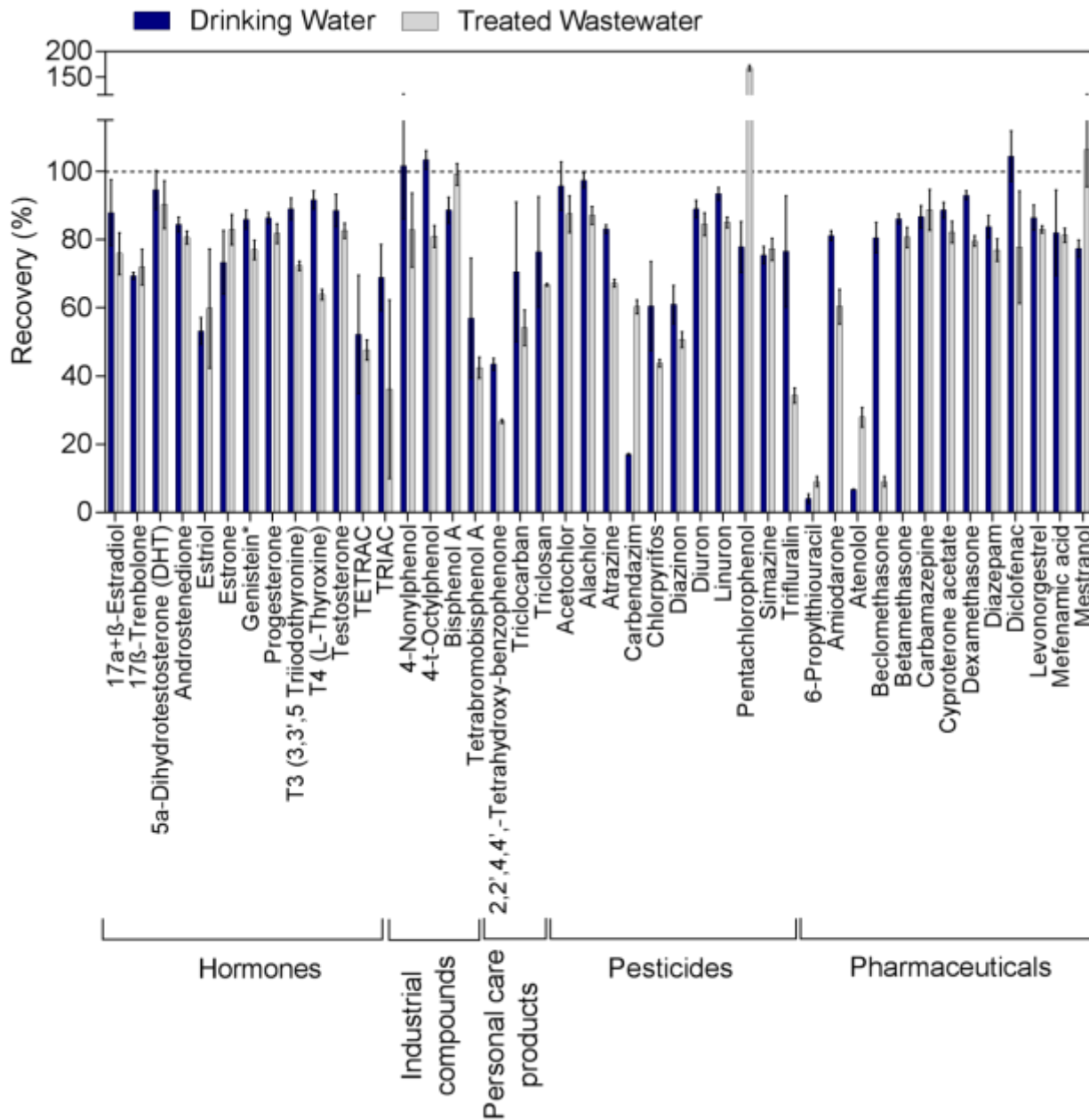
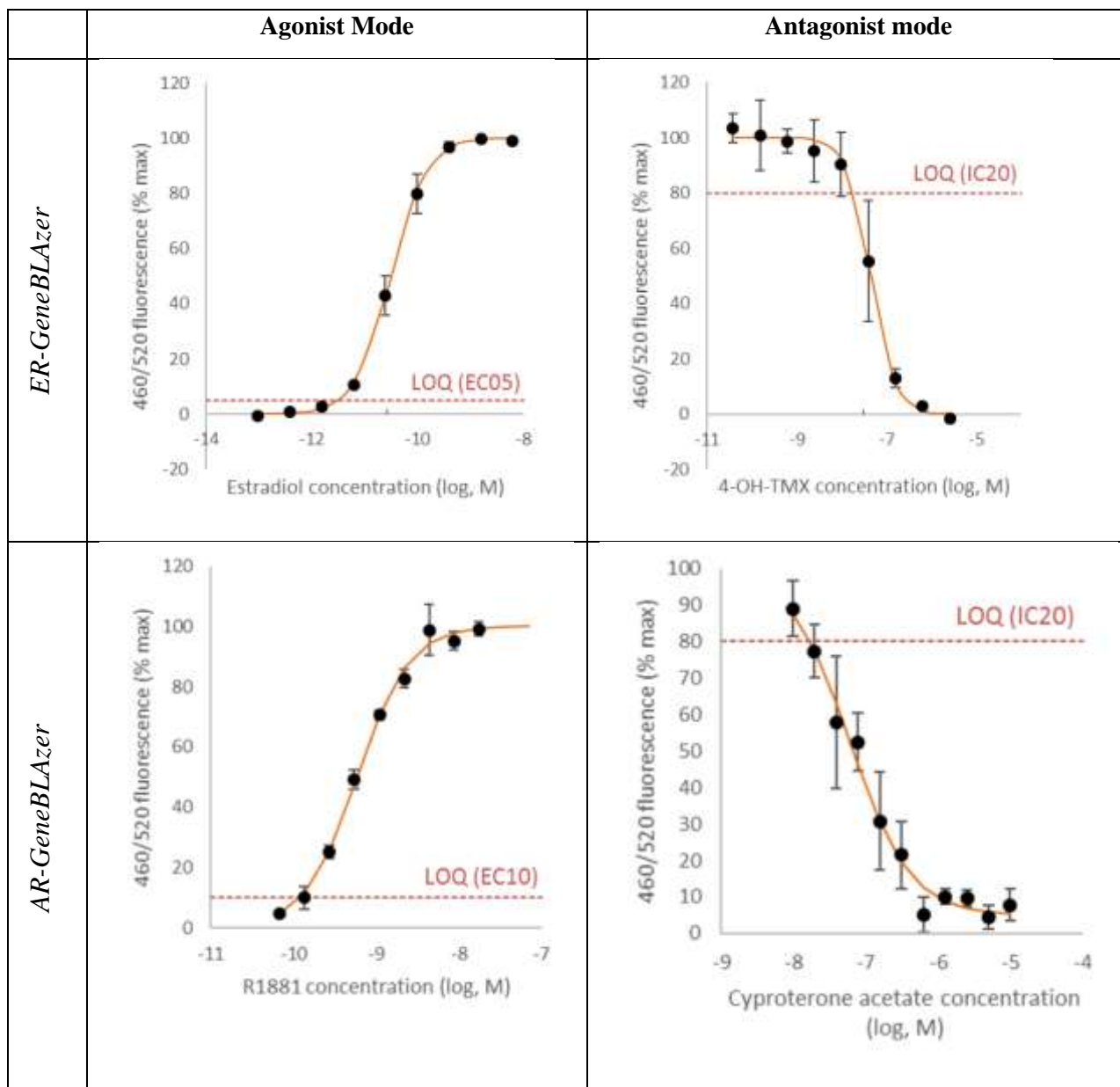


Table S8: Liquid chromatography-high resolution mass spectrometry (LC-HRMS) parameters for suspect screening.

LC-HRMS conditions	<p>Analysis was performed with an 1100 liquid chromatography system from Agilent Technologies (Santa Clara, California USA) coupled with a LTQ Orbitrap high resolution mass spectrometer (resolution of 30000 at $m/z = 400$) from Thermo Fisher Waltham MA USA.</p> <p>Samples were analysed with both positive and negative electrospray ionization (ESI+ and ESI-). For both ionization modes, the measurement was made with data-dependent acquisition in which both full scan high resolution-MS (m/z: 80-1500) and 3 low resolution-MS2 spectra were acquired (on the most three intense parent masses for MS2 analysis).</p>
Chromatographic separation parameters	<p>A generic chromatographic separation was carried out on a C_{18} column (solvent B from 5% at 0 min to 95% at 25 min). Injection volume was 20 μL. Solvents were A1: water acidified with formic acid 0.01% (v,v) and B1: methanol acidified with formic acid 0.01% (v,v) for ESI+ acquisition. Non acidified water (A2) and methanol (B2) was used for the ESI- acquisition.</p>
Data qualitative analysis	<p>To evaluate the identification confidence, a level is given to each molecule identified in a sample:</p> <p>Red level - low confidence: the identification of molecule is based only on the exact mass</p> <p>Orange Level - medium confidence: the identification is done with the exact mass and the retention time (experimental retention time is identical to the theoretical one), with fragmentation data unavailable in experimental data because of the low response of the compound in the sample</p> <p>Green level - high confidence: in addition to the exact mass and retention time, the product ions found experimentally match with the theoretical product ions (data from the literature, databases or from the injection of the commercial standard).</p>
Suspect Screening	<p>A homemade database containing more than 4000 compounds is available, with fragmentation data available for 983 compounds.</p> <p>A suspect screening was performed on these 983 compounds with fragmentation data for the 24 samples. Only compounds with a medium or high level of confidence for identification are reported in the result table (Table S9).</p>

Figure S2: Assay reference compound concentration-effect curves (n = two independent runs). GR-CALUX, PR-CALUX, GH3.TRE-Luc and RXR-CALUX run in agonist mode only. Additional information about antagonist mode: ER-GeneBLAzer run in the presence of 0.1 nM 17 β -estradiol; AR-GeneBLAzer run in the presence of 10 nM R1881, GR-GeneBLAzer run in the presence of 1 nM dexamethasone; PR-GeneBLAzer run in the presence of 1 nM levonorgestrel; HELN-RAR α -RXR run in the presence of 30 nM TTNPB; HG5LN-MR run in the presence of 10 nM aldosterone.



	Agonist Mode	Antagonist mode
<i>GR-GeneBLAzer</i>		
<i>GR-CALUX</i>		
<i>PR-GeneBLAzer</i>		

	Agonist Mode	Antagonist mode
<i>PR-CALUX</i>		
<i>GH3.TRE-Luc</i>		
<i>HELN-RARα-RXR</i>		

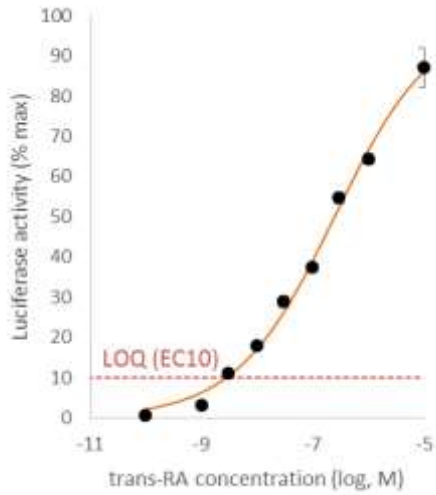
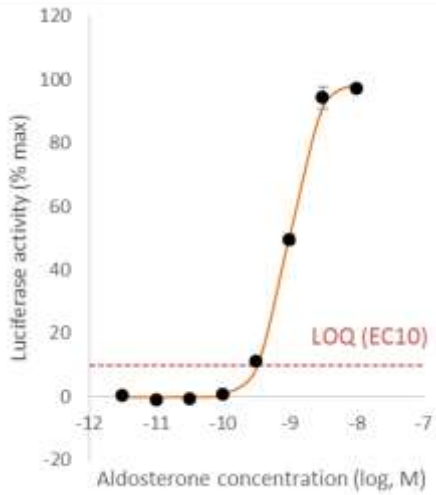
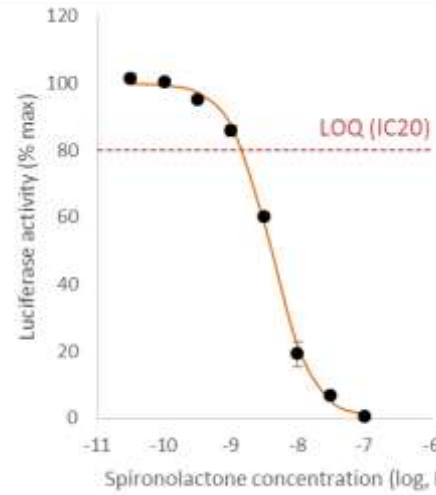
	Agonist Mode	Antagonist mode
<i>RXR-CALUX</i>	 <p>Luciferase activity (% max)</p> <p>trans-RA concentration (log, M)</p> <p>LOQ (EC10)</p>	
<i>HG5LN-MR</i>	 <p>Luciferase activity (% max)</p> <p>Aldosterone concentration (log, M)</p> <p>LOQ (EC10)</p>	 <p>Luciferase activity (% max)</p> <p>Spironolactone concentration (log, M)</p> <p>LOQ (IC20)</p>

Table S9: Qualitative identification of 149 compounds from suspect screening of 983 compounds with fragmentation data using LC-HRMS. No shading indicates that the compound was not found. Green shading indicates high confidence, with identification based on the exact mass, retention time and fragmentation data, and orange shading indicates medium confidence, with identification based on the exact mass and retention time only. See Table S8 for further information.

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
4-Acetamidoantipyrine	83-15-8	Green		Green																		Green		Green	
7H-Dodecafluoroheptanoic acid	1546-95-8					Green																			
Acebutolol	37517-30-9									Green		Orange											Green		
Acesulfame	33665-90-6	Green																							
Acide fenofibrique	42017-89-0																		Green						
Alachlor OXA	171262-17-2														Green										
Ametryn	834-12-8										Orange									Orange				Orange	
Amisulpride	71675-85-9	Green		Green						Green		Green										Green		Green	
Amitriptyline	50-48-6	Orange				Orange				Orange									Green				Orange		
Amoxapine	14028-44-5																					Green			
Aspartame	22839-47-0			Green																					
Atazanavir	198904-31-3													Orange		Orange		Green					Orange		
Atenolol acid	56392-14-4	Green		Green		Green				Green				Green	Green	Green		Green				Green		Green	
Atrazine	1912-24-9										Green			Orange	Green	Green									
Azithromycin	83905-01-5	Green								Green								Green				Orange			
Azoxystrobin	131860-33-8																			Orange					

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Bentazone	25057-89-0																								
Bicalutamide	90357-06-5																								
Bisoprolol	66722-44-9																								
Bupivacaine	38396-39-3																								
Caffeine	58-08-2																								
Candesartan	139481-59-7																								
Carbamazepine	298-46-4																								
Carbendazim	10605-21-7																								
Celiprolol	56980-93-9																								
Cetirizine	83881-51-0																								
Chlortoluron	15545-48-9																								
Ciprofloxacin	85721-33-1																								
Citalopram	59729-33-8																								
Clarithromycin	81103-11-9																								
Climbazole	38083-17-9																								
Clindamycin	18323-44-9																								
Clopidogrel carboxylic acid	14457-28-3																								
Clozapine	5786-21-0																								
Cyprodinil	121552-61-2																								
Desethylatrazine	6190-65-4																								
Desethylterbutylazine	30125-63-4																								
Desmethylcitalopram	144010-85-5																								
Desmethyltramadol	73986-53-5																								

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	147762-57-0	Green		Yellow		Yellow				Green		Yellow			Green		Yellow				Green		Green		
Desmethylvenlafaxine	149289-30-5/ 93413-62-8	Green		Yellow		Yellow				Green		Yellow				Yellow		Green				Green		Green	
Dextroprhan/Levorphanol	125-73-5/ 77-07-6	Green								Yellow												Green		Green	
Diacetolol	28197-69-5									Green		Yellow													
Diclofenac	15307-86-5	Green				Green				Green												Green			
Diltiazem	42399-41-7									Green								Green				Green		Yellow	
Diphenhydramine	58-73-1	Green																							
Disopyramide	3737-09-5									Green															
Doxepine	1668-19-5	Green																							
Ecgonine	481-37-8					Yellow				Green					Yellow								Yellow	Yellow	
EDDP	30223-73-5	Green		Yellow						Green								Green				Green		Yellow	
Enalapril	75847-73-3																							Green	
Enrofloxacin	93106-60-6																					Green			
Enterodiol	80226-00-2	Yellow				Green									Yellow								Green	Green	
Eprosartan	133040-01-4	Green																				Green		Yellow	
Erythromycine-H2O	/	Green								Yellow												Yellow			
Fexofenadine	83799-24-0	Yellow				Green		Yellow		Yellow				Yellow		Green		Yellow				Yellow			
Fipronil	120068-37-3									Green															
Flecainide	54143-55-4	Green		Green		Green				Green	Yellow	Green						Green				Green		Green	
Flonicamid	158062-67-0																		Green						
Fluconazole	86386-73-4	Yellow				Green								Yellow	Green							Yellow		Green	

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Fluoxetine	54910-89-3									Yellow								Yellow				Yellow			
Flutolanil	66332-96-5																			Green					
Fluvoxamine	54739-18-3																					Green			
Formononetin	485-72-3																			Green					
Furosemide	54-31-9																					Green			
Gabapentin	60142-96-3	Green								Green									Green						
Gemfibrozil	25812-30-0											Yellow										Green			
Gliclazide	21187-98-4																		Green						
Hydrochlorothiazide	58-93-5	Green								Green									Green			Green			
Hydroxyatrazine	2163-68-0													Green	Green	Green									
Hydroxypropafenone	86384-10-3									Green												Yellow			
Hydroxyterbutylazine	66753-07-9													Yellow	Green	Green									
Ibuprofen	15687-27-1														Green									Green	
Iomeprol	78649-41-9	Green								Yellow									Yellow						
Irbesartan	138402-11-6	Green		Green		Green				Green		Green							Green			Green		Green	
Isoproturon	34123-59-6											Green													
Lacidipine	103890-78-4																					Green			
Levetiracetam	102767-28-2					Yellow	Green	Yellow							Green	Green							Yellow		
Lidocaine	137-58-6	Green				Green				Green									Green			Green		Green	
Losartan	114798-26-4	Green								Green									Green			Green		Green	
MDMA	42542-10-9	Yellow				Yellow													Green			Yellow			
Meprobamate	57-53-4														Green	Yellow									
Metalachlor oxanilic acid	152019-73-3														Green										

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Metalaxyl	57837-19-1																								
Metazachlor ethane sulfonic acid	172960-62-2																								
Methylprednisolone	83-43-2																								
Metolachlor ESA	171118-09-5																								
Metoprolol	37350-58-6																								
Metribuzin	21087-64-9																								
Mianserine	24219-97-4																								
N,N-Diethyl-m-toluamide	134-62-3																								
Naltrexone	16590-41-3																								
Naphthalene sulphonic acid	120-18-3																								
Nebivolol	99200-09-6																								
Nefopam	13669-70-0																								
Nevirapine	129618-40-2																								
Niflumic Acid	4394-00-7																								
Nitrendipine	39562-70-4																								
Octylphenol	1806-26-4/67554-50-1																								
Ofloxacin	82419-36-1																								
Oleic acid	112-80-1																								
Oxazepam	604-75-1																								
PFBS	375-73-5																								
PFOA	335-67-1																								
Pheniramine	86-21-5																								

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Phenylbenzimidazole sulfonic acid	27503-81-7																								
Piperine	94-62-2																								
Piracetam	7491-74-9																								
Pirimicarb	23103-98-2																								
Prednisone	53-03-2																								
Primidone	125-33-7																								
Proguanil	500-92-5																								
Prometon/Secbumeton/Terbumeton	1610-18-0/26259-45-0/33693-04-8																								
Prometryn/Terbutryn	7287-19-6/886-50-0																								
Propafenone	54063-53-5																								
Propiconazole	60207-90-1																								
Propranolol	525-66-6																								
Prosulfocarb	52888-80-9																								
Quinidine	56-54-2																								
Ramipril	87333-19-5																								
Ritalinic acid	19395-41-6																								
Roxithromycine	80214-83-1																								
Sebuthylazine	7286-69-3																								
Simazine	122-34-9																								
Sitagliptin	486460-32-6																								
Sucralose	56038-13-2																								

Compound name	CAS final	Germany				Australia				France				South Africa				The Netherlands				Spain			
		Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water	Treated wastewater	Drinking water	Surface water	Ultrapure water
Sample ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Sulpiride	15676-16-1	Green														Yellow						Green		Yellow	
Telmisartan	144701-48-4	Green		Green		Green				Green				Green	Green	Green		Green				Green		Green	
Temazepam	846-50-4					Green												Green							
Terbumeton desethyl	30125-64-5									Green	Green				Yellow										
Terbutylazine	5915-41-3													Green	Green	Green									
Testosterone	58-22-0		Yellow																						
11-Nor-9-carboxy-THC	64280-14-4									Yellow				Green	Yellow	Yellow						Green			
Tiapride	51012-32-9									Green															
Tilmicosin	108050-54-0																					Green			
Tramadol	27203-92-5	Green		Yellow		Green				Green		Green				Yellow		Green				Green		Green	
Tretinoin	302-79-4							Green												Green				Yellow	
Triamterene	396-01-0	Green																Green							
Trimethoprim	738-70-5	Green								Green						Yellow		Green				Yellow		Yellow	
t-Sulfonic acid	187022-11-3													Green											
Valsartan	137862-53-4	Green				Yellow				Green												Green		Green	
Varenicline	249296-44-4	Green		Green				Green		Green		Green						Green		Green		Green		Green	
Venlafaxine	93413-69-5	Green		Green		Green				Green		Green						Green				Green		Green	
Verapamil	52-53-9									Green								Green							
Verapamil metabolite D617	34245-14-2	Green		Green		Green				Green		Yellow						Green				Yellow		Yellow	