National & Kapodistrian
University of Athens

Department of Chemistry
Laboratory of Analytical Chemistry

TREMEPOL

Transformation Products of Emerging Pollutants in the Aquatic Environment

Work Package 5 – Results dissemination

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Summary

The chemical pollutants that are regulated under international and EU legislation represent a very small fraction of the universe of chemicals that occur in the environment as a result of human activities. So far, most regulating and implementation bodies, responsible for water and wastewater treatment, are working on the assumption that the so-called priority pollutants are responsible for the most significant share of environmental, human health and economic risk, even though they are representing a minor fraction of the universe of both known and yet-to be identified chemicals. Thus, the study of the fate of the emerging pollutants and their transformation products in wastewater-treatment plants (WWTP) is of paramount importance.

As the power of analytical chemistry increases, the types of chemicals that can be detected increase, and the limits of concentration at which they can be measured are continually lowered. With respect to obtaining a holistic view of risk, target-based environmental monitoring should necessarily be accompanied by non-targeted analysis using high resolution hybrid mass spectrometers.

The main objective of this research is the contribution to the current knowledge on the actual burden of micropollutants on the aquatic environment and comprehensive conclusions will be reached for the possible measures to be taken for its future protection.

The investigation carried out has a significant practical interest and application since the results can be directly used by authorities and operators of WWTPs.

Introduction

Contamination of water bodies has been the spotlight of scientific community concerning the preservation and sustainability of the environment. Due to the advances of analytical chemistry, a great number of regulated and non-regulated compounds are detected in wastewater samples. Emerging contaminants is the term for compounds that are newly released in the environment or have recently been discovered by water quality controls and have yet to be studied. The EPs encompass a diverse group of compounds, for which nowadays many information are available. However, only a small proportion of the chemical compounds have been sufficiently monitored in the water bodies (Loos et al., 2013).

The determination of organic contaminants in environmental samples constitutes a great challenge, since many matrix components may interfere the analysis and mostly due to the increased number of compounds with various physico-chemical properties. The most common choice for the determination of EPs are multi-residue methods, including however only a few hundreds of compounds. This automatically reveals a gap in environmental analysis concerning methods and techniques that can analyze simultaneously a great number of emerging pollutants. Multi-residue, wide-scope screening methods offer this possibility, for the determination of more analytes. The development of high resolving power mass analyzers (HRMS) has contributed a lot towards the wide-scope screening of analytes (Hernandez et al., 2012) and more complete information on undesirable compounds present in the sample is feasible.

HRMS full scan acquisition technique offers the possibility of retrieving all the information concerning the analytes in post-acquisition approaches. Furthermore, HRMS is continually evolving, giving the possibility for quantitative analysis with constantly increasing sensitivity. Limited linear range, traditionally attributed to QTOF systems because of saturation effects, has been overcome by modern instruments (Aguera et al., 2013).

The main approaches for post-acquisition data evaluation are target, suspect and non-target screening (Krauss et al., 2010). The

main difference between the first approach and the latter ones is the presence of reference standard available.

The aims of this project have 2 main directives. The first study will try to identify the organic loading that end up in water bodies, by 3 main workflows in order to cover the majority of compounds present in the samples. In the second part, we try to extend our knowledge not only to emerging pollutants, but also to their transformation products and metabolites, that are formed during the wastewater treatment and may be of risk to human or the environment.

The first study is a comprehensive quantitative target screening of emerging pollutants in environmental samples, which involves a generic sample preparation, a UPLC-QTOF-MS/MS method and post-acquisition evaluation of the data. An in-house database was built with information of retention time, MS and MS/MS ions for 2327 compounds, including pesticides, pharmaceuticals, drugs of abuse, industrial chemicals, doping compounds, as well as some metabolites and transformation products. Optimization was performed in order to minimize false negative results and a validation protocol is proposed in order to evaluate the performance criteria of the HRMS method. The method was applied in an influent and an effluent wastewater from a wastewater treatment plant of Greece. Additional emerging pollutants were detected by suspect screening, where specific validated workflow was applied for the identification of human metabolites of well consumed drugs. Non-target screening was the last step to identify compounds that are abundant in the samples and yet no previous knowledge existed.

In the second study, batch experiments were conducted in the laboratory to simulate the processes during the wastewater treatment plant. Biotransformation and chlorination of analytes that are removed during the treatment was performed in order to find out whether more toxic transformation products are formed.

Samples

Influent and effluent wastewater samples, as well as sludge samples were collected from the WWTP of Athens (Greece) during two sampling campaigns conducted on 2014 and 2015.

The WWTP of Athens is designed with primary sedimentation, activated sludge process with biological nitrogen and phosphorus removal and secondary sedimentation. The average sewage flow for the periods of study was 720,000 m^3 day^{-1} for a typical dry day. For the load calculations the specific day flow has been used. As for the travel distances of wastewater in the sewer, the closest connected household is 0.5 km and the most remote is 30 km away. The residential population connected to the WWTP based on official census excluding the commuters is 3,700,000 and the number of people estimated based on number of house connections is 4,562,500. This WWTP has a design capacity to serve a population equivalent of 5,200,000, being by far the largest of Greece and one of the largest in the world. For the drug use estimations, we used the census value for the residential population.

In each campaign influent and effluent wastewater samples (24-hour composite flow proportional samples) were collected during 7 consecutive days from 11/3 to 18/3 in 2014 and from 4/3 to 11/3 in 2015. Moreover, the respective sludge for each day was also collected in plastic bags.

This allows investigation of drug use trends throughout the week and the partitioning of the analytes on particulate matter. All wastewater samples were collected in pre-cleaned high-density polyethylene (HDPE) bottles. Untreated and treated wastewater samples were immediately filtered with glass fiber filters (pore size 0.7 μm) after arrival at the laboratory. Samples were stored in the dark at 4 °C until analysis.

1st Study- Target, Suspect & Non-target Screening

Analysis and Evaluation of the data

Optimization was performed in order to minimize false negative results and a validation protocol is proposed in order to evaluate the performance criteria of the HRMS method. Analytes were extracted from wastewater sample by mixed-bed SPE protocol (Bletsou et al., 2015) and from sludge sample with solid-liquid extraction (Gago-Ferrero et al., 2015a). The samples were analyzed and evaluated with sophisticated software. Identification points were attributed to each analyte and quantitation was also carried out.

Results in wastewater

Target Analysis

Reversed-Phase (RP) liquid chromatography

The optimized and validated method was applied to influent and effluent wastewater samples from the wastewater treatment plant of Athens. For the identification of the compound, studying the MS spectrum, the retention time, mass accuracy criteria should be met, gaining thus 2 IPs. When studying the MS/MS spectrum, if fragments are also available, additional 2.5 IPs are earned. For screening of the compounds, at least 2 (≥ 2) IPs are required, and for identification at least 4 (≥ 4) IPs. In Table 1, all the detected analytes in influent and effluent are presenting, accompanied by the identification points earned.

In total, 371 compounds were detected in the samples; 338 in influent wastewater and 301 in effluent wastewater. In influent wastewater, 219 compounds were identified, earning at least 2 IPs, and the rest 188 were identified with additional MS/MS information. 61 pesticides were detected, 205 drugs, including pharmaceuticals, illicit and drugs of abuse, 4 sweeteners, 10 perfluorinated compounds (PFCs), 8 aminoacids, 47 transformation products and other chemicals. In effluent wastewater, 192 compounds were identified and the 109 were identified. 51 pesticides were present, 191 drugs, 4 sweeteners, 11 PFCs, 4 aminoacids, 49 transformation products and other chemicals. It is worth mentioning that more TPs are detected in effluent wastewater, because they are formed through the wastewater procedure in the plant.

This is the first study reported the presence of 371 organic micropollutants in wastewaters, belonging in various classes. 66 pesticides, belonging to different classes were detected in total. 29 stimulants, most of them being amphetamine derivatives and 9 sympathomimetics (ephedrine derivatives) are reported. Moreover 9 anesthetics, closely related to lidocaine, which is well reported in the literature, are present in the samples. Various other drugs are identified, categorized as drugs against high blood pressure, cardiovascular diseases, diuretics, anti-diabetics, antiviral, anti-histamin, etc. This categorization, as presented in Table 1 can provide a valuable and holistic information for the consumption of drugs in the area of Athens.

Table 1. Quantitative results of wastewater samples

	Compound Name	CAS number	influent wastewater		effluent wastewater	
			IPs	C (μg/L)	IPs	C (μg/L)
	Pesticides					
1	Acetochlor	34256-82-1	2.5	0.0003	2.5	0.0002
2	Dimethachlor	50563-36-5	2	0.07	n.d.	
3	Dimethachlor-ESA	-	2.5	2.0	2.5	1.1
4	Dimethachlor-OXA	1086384-49-7	2.5	0.54	2	0.09
5	Metolachlor	51218-45-2	5	0.002	5	0.007
6	Metolachlor-ESA	171118-09-5	2.5	0.43	2.5	0.05
7	Diuron	330-54-1	≥5	0.011	≥5	0.02
8	Fenuron	101-42-8	n.d.		2	0.088
9	Difenoxuron	14214-32-5	2.5	0.02	2.5	0.19
10	Diflubenzuron	35367-38-5	2.5	0.005	n.d.	
11	Fluometuron	2164-17-2	≥5	0.58	≥5	13.56
12	Metobromuron	3060-89-7	2	0.004	n.d.	
13	Dimethoate	60-51-5	2	0.03	2	0.04
14	Fludioxonil	131341-86-1	2.5	0.005	2	0.004
15	Cyprodinil	121552-61-2	n.d.		2	0.002
16	Flutolanil	66332-96-5	2	0.01	2	0.01
17	Fipronil	120068-37-3	≥5	0.02	≥5	0.01
18	Fipronil sulfone	120068-36-2	2.5	0.0012	2.5	0.002
19	Imidacloprid	138261-41-3	2	0.02	5	0.12
20	Terbutryn	886-50-0	2	0.003	2.5	0.0006
21	Prometryn (Caparol)	7287-19-6	2	0.04	2	0.04
22	Thiodicarb	59669-26-0	2	0.009	2.5	0.04
23	Propamocarb	24579-73-5	2.5	0.010	2.5	0.004
24	Dioxacarb	6988-21-2	2	0.004	n.d.	
25	Isoprocarb	2631-40-5	2.5	0.003	n.d.	

26	Iprovalicarb	140923-17-7	n.d.		2	0.02
27	Methiocarb (Mercaptodimethur)	2032-65-7	2	0.01	n.d.	
28	Propham	122-42-9	2.5	0.05	2.5	0.08
29	Temephos	3383-96-8	2.5	0.45	n.d.	
30	Pirimiphos-methyl	29232-93-7	2	0.01	4.5	0.02
31	Monocrotophos	6923-22-4	2	0.01	n.d.	
32	Carbendazim	10605-21-7	2	0.01	2	0.02
33	Carbofuran-3-hydroxy	16655-82-6	5	0.02	2.5	0.007
34	Chlormequat	7003-89-6	2.5	0.02	4.5	0.007
35	Napropamide	15299-99-7	2	0.007	2	0.02
36	Climbazole	38083-17-9	5	0.15	≥5	0.19
37	Difenoconazole	119446-68-3	2	0.01	n.d.	
38	Penconazole	66246-88-6	2	0.03	≥5	0.09
39	Cyproconazole	94361-06-5	n.d.		2	0.02
40	Fluconazole	86386-73-4	≥5	0.09	≥5	0.75
41	Thiabendazole	148-79-8	2	0.010	2.5	0.01
42	Atrazine	1912-24-9	2	0.03	n.d.	
43	Atrazine-desisopropyl	1007-28-9	2.5	0.16	2.5	0.39
44	Simazine	122-34-9	2	0.05	2.5	0.11
45	Azoxystrobin	131860-33-8	≥5	0.60	≥5	1.9
46	Azoxystrobin acid	1185255-09-7	5	0.04	≥5	0.09
47	Dalapon	75-99-0	2.5	0.01	n.d.	
48	Dazomet	533-74-4	2.5	0.05	2.5	0.11
49	Dikegulac	18467-77-1	2	0.0003	n.d.	
50	Famoxadone	131807-57-3	2	0.0006	n.d.	
51	Imazapyr	81334-34-1	2	0.01	2.5	0.01
52	Methoxyfenozide	161050-58-4	4.5	0.21	≥5	0.94
53	N-2,4-Dimethylphenylformamide (DMF. Metabolite Amitraz)	60397-77-5	2.5	0.008	2	0.0004
54	Naptalam (N-1-Naphthylphthalamicacid)	132-66-1	n.d.		2.5	0.06
55	Thiamethoxam	153719-23-4	2.5	0.0006	5	0.01
56	Cycloheximide	66-81-9	2.5	0.11	n.d.	
57	Carboxin	5234-68-4	2.5	0.0009	n.d.	
58	Oxycarboxin	5259-88-1	2.5	0.01	2	0.011
59	Picaridin (Icaridin)	119515-38-7	2	0.03	2	0.07
60	DEET (Diethyltoluamide)	134-62-3	5	0.07	5	0.02
61	Metalaxyl	57837-19-1	≥5	0.003	≥5	0.08
62	Amitrole	61-82-5	2.5	1.17	2	0.04
63	Dinoterb	1420-07-1	5	0.03	2.5	0.01
64	Fluazifop	69335-91-7	2.5	0.02	2.5	0.05
65	Propoxur	114-26-1	≥5	0.003	≥5	0.003
66	Piperonyl butoxide	51-03-6	≥5	0.11	2	0.003
	Opiates, opioids					
67	Morphine	57-27-2	≥5	0.64	2	0.0012

68	Normorphine	466-97-7	2.5	0.02	n.d.	
69	Methadone (METH)	76-99-33	2	0.08	2.5	0.04
70	Codeine (COD)	76-57-3	2	0.04	5	0.44
71	Norcodeine	467-15-2	n.d.		2.5	0.08
72	EDDP	30223-73-5	2	0.12	2	0.10
73	Hydrocodone	125-29-1	n.d.		2.5	0.02
	Stimulants- Amphetamins					
74	Cocaine (COC)	50-36-2	5	0.11	n.d.	
75	Benzoylecgonine (BECG)	519-09-5	≥5	0.30	2	0.05
76	Ecgonine methyl ester (EME)	7143-09-01	2	0.11	n.d.	
77	Amphetamine	300-62-9	≥5	0.27	n.d.	
78	Methamphetamine (MA)	537-46-2	2.5	0.07	n.d.	
79	Dimethylamphetamine	1009-69-4	2.5	0.12	2.5	0.95
80	Ethylamphetamine	457-87-4	2	0.18	2.5	1.3
81	3,4-methylenedioxy-amphetamine (MDA)	4764-17-4	≥5	2.1	2	0.22
82	3,4-methylenedioxy-N-methylamphetamine (MDMA)	42542-10-9	2	0.16	2	0.09
83	PMMA (para-Methoxy-N-methylamphetamine)	3398-68-3	5	6.1	5	19
84	Metaraminol (3,β-dihydroxyamphetamine)	337376-15-5	2.5	1.2	n.d.	
85	Pholedrine (p-hydroxy-methylamphetamine)	6114-26-7	n.d.		2.5	1.1
86	4-methyl-2-hexanamine	105-41-9	2	0.59	2	0.11
87	Mephentermine	100-92-5	2	0.74	2.5	0.97
88	Phenelzine	51-71-8	2	3.6	2.5	0.75
89	Pyrovalerone	3563-49-3	n.d.		2.5	0.31
90	Phendimetrazine	17140-98-6	2	0.46	2	0.26
91	Midodrine	133163-28-7	2	0.20	2.5	1.08
92	Heptaminol	372-66-7	2.5	0.49	2.5	0.64
93	Cathine/ norpseudoephedrine	492-39-7	2.5	0.12	2.5	0.05
94	Nikethamide	59-26-7	n.d.		2.5	0.72
95	Pemoline	2152-34-3	2	0.05	n.d.	
96	Aminorex	2207-50-3	2	0.43	2	2.23
97	Dimefline	1165-48-6	n.d.		2.5	0.46
98	Ethamivan	304-84-7	n.d.		≥5	0.48
99	TMA (trimethoxyamphtamine)	1082-23-1	2	0.26	2	0.26
100	3,4-DMA (dimethoxyamphtamine)	120-26-3	2	0.22	2	0.17
101	4-Methyl-pyrrolidino-propiophenone (MPPP)	28117-80-8	2	0.01	2	0.01
102	2 C-D (2,5-dimethoxy-4-methylphenethylamine)	24333-19-5	2	3.41	2.5	1.41
	Sympathomimetics					
103	Ephedrine	299-42-3	≥5	0.34	≥5	0.03

104	Norephedrine	492-41-1	2.5	0.82	≥5	0.22
105	Etafedrine	48141-64-6	2.5	0.02	2.5	0.12
106	Metanephrine	5001-33-2	2.5	0.005	n.d.	
107	Phenylephrine	1416-03-1	n.d.		2	0.01
108	Apophedrin (Phenylethanolamine)	7568-93-6	5	0.07	5	0.03
109	Isoetharine	7279-75-6	2.5	0.01	2.5	0.03
110	Methoxamine	337376-15-5	2	0.00	2.5	0.02
111	Nylidrin	447-41-6	2.5	0.00	2	0.01
	Hallucinogenic (cannabinoids)					
112	Δ9-Tetrahydrocannabinol (THC)	1972-08-3	n.d.		2	0.01
	Benzodiazepines tranquilizers					
113	Alprazolam	92623-85-3	2	0.03	≥5	0.04
114	Clobazam	22316-47-8	2	0.009	2	0.001
115	Diazepam	439-14-5	2.5	0.04	2.5	0.04
116	Nordiazepam	1088-11-5	2.5	0.008	2.5	0.009
117	7-amino-flunitrazepam	34084-50-9	2	0.03	2	0.04
118	Lorazepam	846-49-1	n.d.		2.5	0.01
119	Midazolam	59467-70-8	n.d.		2	0.02
120	temazepam	846-50-4	≥5	0.03	5	0.03
121	Oxazepam	604-75-1	2.5	0.01	2.5	0.02
	Barbiturates					
122	Phenobarbital	50-06-6	2	0.01	2.5	0.02
123	Primidone	125-33-7	n.d.		2.5	0.10
124	Bemegride	64-65-3	2.5	0.68	2	0.05
	Antipsychotics					
125	Clozapine	5786-21-0	2	0.15	2	0.08
126	Quetiapine	111974-69-7	≥5	0.02	2	0.01
127	Amisulpride	71675-85-9	≥5	0.07	≥5	0.07
128	Amisulpride-N-Oxide	71675-85-9	2	0.004	5	0.01
129	Sulpiride	15676-16-1	≥5	0.04	≥5	0.08
130	Haloperidol	52-86-8	2.5	0.0001	≥5	0.0005
131	Risperidone	106266-06-2	n.d.		2	0.003
132	Paliperidone (9-OH-Risperidone)	147687-18-1	n.d.		2	0.01
133	Buspirone	36505-84-7	≥5	0.02	n.d.	
134	Levomepromazine sulfoxide	7052-08-6	2	0.09	2.5	0.09
	Antiepileptic					
135	Carbamazepine	298-46-4	5	0.61	≥5	1.7
136	Carbamazepine-10,11-epoxid	36507-30-9	5	0.19	4.5	0.25
137	10-Hydroxycarbamazepine	29331-92-8	5	0.42	5	1.00
138	Oxcarbazepine	28721-07-5	5	0.04	5	0.05
139	Topiramate	97240-79-4	5	0.40	≥5	0.61
140	Lamotrigine	84057-84-1	≥5	0.06	2.5	1.6
141	Levetiracetam	102767-28-2	≥5	0.59	2.5	0.12

142	Valproic acid	99-66-1	2.5	25	2.5	0.18
143	Phenytoin	57-41-0	n.d.		2	0.03
	Antidepressants					
144	Amitriptyline	50-48-6	≥5	0.23	5	0.11
145	Nortriptyline	894-71-3	2	0.004	2	0.01
146	Doxepine	1668-19-5	2	0.05	2	0.04
147	Mirtazapine	61337-67-5	≥5	0.33	≥5	0.49
148	8-OH-Mirtazapine	-	2.5	0.09	n.d.	
149	Desmethyl mirtazapine	61337-68-6	2	0.03	2	0.04
150	Maprotiline	10262-69-8	2	0.01	n.d.	
	SSRIs (serotonin replacing inhibitors)					
151	Citalopram	59729-33-8	≥5	1.0	≥5	1.0
152	Norcitalopram	144025-14-9	≥5	0.23	≥5	0.32
153	Sertraline	79617-96-2	2	0.10	2.5	0.02
154	Fluoxetine	54910-89-3	2.5	0.10	2.5	0.07
	SNRIs (serotonin-norepinephrine reuptake inhibitors)					
155	Duloxetine	116539-59-4	n.d.		2.5	0.005
156	Venlafaxine	93413-69-5	≥5	0.92	≥5	2.0
157	Venlafaxine-N-oxide	1094598-37-4	2	0.01	2.5	0.06
158	N,O- bisdesmethylvenlafaxine	135308-74-6	5	0.12	5	0.16
159	N-Desmethylvenlafaxine	149289-30-5	4.5	1.9	5	6.5
160	O-desmethylvenlafaxine	93413-62-8	≥5	0.89	≥5	1.1
	Anesthetics					
161	Benzocaine	94-09-7	2	0.01	n.d.	
162	Bupivacaine	38396-39-3	2	0.004	n.d.	
163	Lidocaine	137-58-6	2.5	0.17	2.5	0.69
164	Mepivacaine	96-88-8	2.5		n.d.	
165	Prilocaine	721-50-6	2	0.005	2.5	0.006
166	Procaine	59-46-1	2.5	0.002	2.5	0.006
167	Para-fluorofentanyl	90736-23-5	2	0.002	2.5	0.004
168	Norfentanyl	1609-66-1	n.d.		2.5	0.001
	Antiviral drugs					
169	Amantadine	768-94-5	5	0.06	5	0.09
170	Atazanavir	198904-31-3	2.5	0.02	2.5	0.05
171	Darunavir	206361-99-1	5	0.15	5	0.10
172	Ritonavir	155213-67-5	≥5	0.014	2.5	0.009
173	Emtricitabine	143491-57-0	5	0.33	5	0.15
174	Tenofovir	147127-20-6	2.5	0.30	n.d.	
	Hypertension- diuretic drug					
175	Aliskiren	173334-57-1	≥5	0.27	≥5	0.25
176	Valsartan	137862-53-4	≥5	0.66	5	0.92
177	Candesartan	139481-59-7	2	0.29	2	0.42
178	Telmisartan	144701-48-4	2.5	0.22	5	0.18
179	Verapamil	52-53-9	2.5	0.02	2	0.02
180	D617 (met. of verapamil)	34245-14-2	5	0.07	5	0.10
181	Eprosartan	133040-01-4	5	0.84	2.5	0.22

182	Irbesartan	138402-11-6	2	0.40	2	0.40
183	Diltiazem	42399-41-7	5	0.10	5	0.07
184	Nordiltiazem	-	≥5	0.03	2.5	0.02
185	Deacetyldiltiazem	42399-40-6	5	0.13	5	0.28
186	Phenoxybenzamine	59-96-1	2.5	0.41	2.5	0.45
187	Furosemide	54-31-9	≥5	0.03	2.5	0.03
188	Hydrochlorothiazide	58-93-5	≥5	0.28	≥5	0.32
189	Bendroflumethiazide	73-48-3	2.5	0.01	n.d.	
190	Acetazolamide	59-66-5	5	0.03	5	0.004
191	Amiloride	2016-88-8	2	0.05	2.5	0.03
192	Chlorthalidone	77-36-1	n.d.		2	0.008
	Antidiabetic drugs					
193	Sitagliptin	486460-32-6	≥5	0.48	n.d.	
194	Vildagliptin	274901-16-5	5	0.29	5	0.51
195	Pioglitazone	111025-46-8	2	0.004	2	0.004
196	Lacosamide	175481-36-4	4.5	0.02	5	0.04
197	Nateglinide	105816-04-4	2	0.005	n.d.	
198	Metformin	657-24-9	≥5	93	≥5	35
199	Guanylurea	926-72-7	2	0.74	≥5	5.0
	Antihistamine					
200	Hydroxyzine	68-88-2	2.5	0.004	n.d.	
201	Cetirizine	83881-52-1	≥5	0.14	5	0.18
202	Chlorpheniramine	132-22-9	2.5	0.01	2.5	0.008
203	Crotamiton	483-63-6	2.5	0.01	5	0.01
204	Diphenhydramine	58-73-1	≥5	0.04	≥5	0.04
205	Orphenadrine	83-98-7	≥5	0.05	≥5	0.04
206	Norорphenadrine (Tofenacin, Elamol)	15301-93-6	2	0.007	2.5	0.01
	Antiulcer					
207	Cimetidine	51481-61-9	2	0.07	2.5	0.50
208	Ranitidine	66357-35-5	≥5	2.6	≥5	1.0
209	Ranitidine-S-oxide	73851-70-4	2	0.17	2.5	0.11
	Cardiovascular diseases-intravascular					
210	Rosuvastatin	287714-41-4	2.5	0.17	2	0.13
211	Atorvastatin	134523-00-5	≥5	1.5	n.d.	
212	Gemfibrozil	25812-30-0	≥5	0.24	2	0.05
213	Fenofibric acid	49562-28-9	≥5	0.61	2	0.30
214	Propafenone	54063-53-5	≥5	0.56	≥5	0.53
215	Iopromide	73334-07-3	≥5	1.6	≥5	0.94
216	Clopidogrel Carboxylic acid	144457-28-3	≥5	0.60	≥5	0.56
	CNS stimulants					
217	Caffeine	58-08-2	≥5	9.6	5	3.0
218	Paraxanthin (1,7-dimethylxanthine)	611-59-6	≥5	5.9	≥5	0.92
219	Theophylline (1,3-dimethylxanthine)	58-55-9	≥5	2.0	n.d.	
220	Pentoxyfylline	*6493-05-06	5	0.64	n.d.	

221	Nicotine	54-11-5	≥5	13.0	≥5	0.93
222	Cotinine	486-56-6	5	9.1	5	0.54
223	Hydroxycotinine	34834-67-8	≥5	11.8	2.5	0.07
	Analgesics-NSAIDs					
224	O-N-bisdesmethyltramadol	-	2.5	0.02	2.5	0.02
225	O-desmethyltramadol	73986-53-5	2.5	0.03	5	0.01
226	N-desmethyltramadol	75377-45-6	2.5	0.01	2.5	0.01
227	Tramadol-N-oxide	147441-56-3	2	0.10	2.5	0.12
228	Salicylic acid	69-72-7	5	5.4	≥5	0.14
229	Paracetamol	103-90-2	≥5	4.8	2.5	0.14
230	4-Acetamidoantipyrine	83-15-8	5	0.07	5	0.09
231	4-Formylaminoantipyrine	1672-58-8	2.5	0.02	≥5	0.03
232	Isopyrin (4-Isopropyl-aminoantipyrine)	3615-24-5	n.d.		2.5	0.02
233	Meptazinol	54340-58-8	n.d.		2.5	0.003
234	Pethidine	57-42-1	2	0.001	2.5	0.003
235	Salicylamide	65-45-2	2	0.01	2.5	0.11
236	Diclofenac	15307-86-5	2.5	0.08	n.d.	
237	Fenbufen	36330-85-5	≥5	1.7	5	0.39
238	Fenoprofen	29679-58-1	2.5	5.1	2.5	2.0
239	Flufenamic acid	530-78-9	2.5	0.02	2.5	0.03
240	Flurbiprofen	51543-39-6	2	0.48	n.d.	
241	Ibuprofen	15687-27-1	≥5	1.1	n.d.	
242	Indoprofen	31842-01-0	2	0.22	n.d.	
243	Ketoprofen	22071-15-4	2.5	0.11	n.d.	
244	Meclofenamic Acid	644-62-2	2.5	0.02	n.d.	
245	Mefenamic acid	61-68-7	5	0.51	5	0.05
246	Naproxen	22204-53-1	5	0.93	2	0.05
247	Niflumic acid	4394-00-7	≥5	0.14	≥5	0.27
248	Nimesulide	51803-78-2	n.d.		2.5	0.098
249	Sulindac	38194-50-2	2	0.002	n.d.	
250	Oxaprozin	21256-18-8	2.5	0.44	n.d.	
251	Antipyrine /Phenazone	60-80-0	n.d.		2	0.05
	beta-blockers					
252	Albuterol	18559-94-9	2	0.005	2.5	0.02
253	Atenolol	29122-68-7	≥5	1.65	≥5	1.07
254	Atenolol acid (Metoprolol acid)	63659-18-7	5	0.47	≥5	0.12
255	Betaxolol	63659-18-7	2	0.008	n.d.	
256	Bisoprolol	66722-44-9	≥5	0.03	≥5	0.07
257	Carteolol	51781-06-7	2.5	0.002	n.d.	
258	Celiprolol	56980-93-9	≥5	0.42	≥5	0.33
259	Metoprolol	37350-58-6	5	0.81	≥5	1.3
260	Pindolol	13523-86-9	2	0.001	n.d.	
261	Propranolol	525-66-6	5	0.13	2.5	0.21
262	Salbutamol	18559-94-9	2.5	1.2	2.5	0.72
263	Sotalol	3930-20-9	5	0.43	5	0.55

264	Esmolol	103598-03-4	2.5	0.002	2.5	0.01
	Antibiotics					
265	Azithromycin	83905-01-5	2.5	0.03	≥5	0.06
266	Roxithromycin	80214-83-1	5	0.02	2	0.03
267	Clarithromycin	81103-11-9	≥5	2.7	≥5	2.4
268	N-desmethyl Clarithromycin	101666-68-6	≥5	0.72	5	0.93
269	Sulfadiazine	68-35-9	5	0.04	5	0.02
270	N4-Acetylsulfadiazine	127-74-2	2.5	0.07	2.5	0.46
271	Sulfadimidine	57-68-1	2	0.0008	5	0.005
272	N4-Acetylsulfamethazine (N4-Acetylsulfadimidine)	100-90-3	2	0.03	2.5	0.08
273	Sulfamethoxazole	723-46-6	5	0.09	≥5	0.36
274	N4-Acetylsulfamethoxazole	21312-10-7	2.5	0.03	2	0.02
275	Sulfapyridine	144-83-2	≥5	0.03	≥5	0.06
276	Trimethoprim	738-70-5	5	0.06	≥5	0.41
277	Linezolid	165800-03-3	2	0.03	2.5	0.06
278	Metronidazole	443-48-1	2.5	0.17	5	0.13
279	Ternidazol	1077-93-6	2	0.03	n.d.	
280	Nigericin	28380-24-7	2	0.41	2.5	0.84
281	Levamisole	14769-73-4	2	0.06	≥5	0.10
	Antibacterial - veterinary drugs					
282	Enrofloxacin	93106-60-6	2.5	0.02	n.d.	
283	Marbofloxacin	115550-35-1	2	0.05	n.d.	
284	Triclocarban	101-20-2	2	0.11	2.5	0.35
285	Triclosan	3380-34-5	2	0.08	2.5	0.08
286	Decoquinate	18507-89-6	n.d.		2	0.099
	Anticonvulsant					
287	Pregabalin	148553-50-8	5	0.68	5	0.45
288	Gabapentin	60142-96-3	≥5	0.79	≥5	0.24
289	Warfarin	81-81-2	2.5	1.9	n.d.	
	Chemotherapeutic-anti-cancer drugs					
290	Cytarabin	147-94-4	2	0.90	n.d.	
291	Ifosfamide	3778-73-2	2.5	0.10	2.5	0.28
292	Cyclophosphamide	50-18-0	2.5	0.009	2.5	0.03
	Other drugs					
293	Memantine	19982-08-2	2.5	0.04	2.5	0.06
294	Acamprosate	77337-76-9	2	0.52	2	0.84
295	Fluocinolone acetonide	67-73-2	n.d.		2.5	0.01
296	Benserazide	14919-77-8	2	0.48	2	1.47
297	Benzamidine	618-39-3	2.5	0.70	2	0.65
298	dextromethorphan	125-71-3	n.d.		2	0.0017
299	Vigabatrin	60643-86-9	2	0.18	n.d.	
300	Guaifenesin	93-14-1	2.5	0.56	n.d.	
301	Piracetam	7491-74-9	2.5	0.03	2.5	0.33
	Steroids					
302	17β-Estardiol (E2)	50-28-2	2.5	0.59	2.5	0.60
303	Prednisolone	50-24-8	2.5	2.2	n.d.	

304	Drostanolone metabolite	-	2.5	0.02	2.5	0.03
305	Mesterolone metabolite	-	2.5	0.07	2.5	0.13
306	Progesterone	57-83-0	2.5	1.71	n.d.	
307	19-Norandrosterone	1225-01-0	n.d.		2.5	1.04
308	allo-THF (Allotetrahydrocortisol)	302-91-0	2.5	1.47	n.d.	
309	THE (Tetrahydrocortisone)	200-161-9	≥5	0.98	n.d.	
310	THF (Tetrahydrocortisol)	53-02-1	2.5	2.8	n.d.	
	PFCs					
311	PFBuS	375-73-5	2.5	0.007	5	0.006
312	PFDeA	335-76-2	5	0.05	2	0.04
313	PFHpA	375-85-9	2	0.006	2	0.006
314	PFHps	335-77-3	2.5	0.0007	2	0.0005
315	PFHxA	307-24-4	2	0.002	5	0.004
316	PFHxS	355-46-4	2.5	0.005	2	0.004
317	PFNA	375-95-1	2	0.010	2	0.01
318	PFOA	2395-00-8	≥5	0.008	5	0.006
319	PFOS	1763-23-1	2.5	0.03	2.5	0.004
320	PFPeA	2706-90-3	2	0.002	4.5	0.002
321	PFUnA	2058-94-8	n.d.		2	0.0003
	sweeteners					
322	Acesulfame	33665-90-6	≥5	1.9	≥5	0.64
323	Cyclamate	139-05-9	5	24	2.5	1.0
324	Saccharine	81-07-2	2.5	3.1	2.5	0.011
325	Sucralose	56038-13-2	2.5	0.60	2.5	0.98
	Industrial Chemicals					
326	Benzotriazole (BTR)	95-14-7	2	0.04	5	0.49
327	1-Hydroxy-Benzotriazole	2592-95-2	2	0.17	2.5	0.16
328	4-Hydroxy-Benzotriazole	26725-51-9	2	0.27	2.5	0.64
329	4-Me-Benzotriazole/ 5-Me Benzotriazole	29385-43-1	2	0.0009	≥5	1.2
330	Benzothiazole (BTH)	95-16-9	2.5	0.05	2.5	0.07
331	2-Amino-Benzothiazole	136-95-8	≥5	0.07	≥5	0.09
332	2-Me-S-Benzothiazole	615-22-5	≥5	0.06	≥5	0.03
333	2-OH-Benzothiazole	934-34-9	2	0.15	2.5	0.21
334	4-tert-octylphenol (4-t-OP)	27193-28-8	≥5	1.2	≥5	0.51
335	4-nonylphenol (4-NP)	104-40-5	≥5	0.07	2	0.03
336	4-Nonylphenol-mono-ethoxylate	104-35-8	2	0.01	2	0.004
337	Melamine	108-78-1	2	0.65	2	0.66
338	Bisphenol A	80-05-7	5	0.03	n.d.	
339	Benzenesulfonamide	98-10-2	2.5	0.12	2	0.07
340	o-toluenesulfonamide	88-19-7	2	0.13	2	0.09
341	Galaxolidone	-	2.5	0.59	2.5	0.60
342	Diethyl phthalate	84-66-2	5	2.0	5	1.8
343	Dimethyl phthalate	131-11-3	2.5	0.02	2.5	0.02
344	Di-n-butyl phthalate	84-74-2	5	0.33	5	1.1

345	Benzyl butyl phthalate	85-68-7	≥5	0.05	≥5	0.08
346	Triethylphosphate	78-40-0	2.5	0.05	5	0.05
347	Triphenyl phosphate (TPP)	115-86-6	5	0.05	5	0.12
348	Benzophenon 3 (2-Hydroxy-4-methoxybenzophenon)	131-57-7	2.5	1.5	n.d.	0.20
349	Prolinamide	51-06-9	2	4.1	2	1.5
350	Benzoic acid	65-85-0	2.5	49	2.5	29
351	2-Aminobenzimidazole	934-32-7	n.d.		2.5	0.13
352	Tributylamine	102-82-9	2.5	0.008	2.5	0.01
353	Benzyl-dimethyl-dodecylammonium	139-07-1	2	0.07	n.d.	
354	Didecyldimethylammonium (DADMAC (C10:C10))	2390-68-3	2	0.02	2	0.01
355	2-aminoheptane	123-82-0	5	0.64	2	0.16
356	4-Piperidin carboxamide	39546-32-2	2	1.0	2	0.95
357	Ethyl sulfate	540-82-9	2.5	3.6	2	1.3
	Aminoacids - Naturally occurring compounds					
358	Alanine (Ala)	56-41-7	2.5	11	n.d.	
359	g-aminobutiric acid (GABA)	56-12-2	2.5	7.2	2.5	13
360	Glutamic acid (Glu)	56-86-0	2	13	2	4.5
361	Methionine (Met)	63-68-3	2.5	2.3	2	0.06
362	Proline (Pro)	147-85-3	2	7.8	n.d.	
363	Serine (Ser)	56-45-1	2	2.8	n.d.	
364	Valine (Val)	72-18-4	≥5	59	2	48
365	Leucine (Leu)	328-39-2	2.5	12	n.d.	
366	Adenosine	58-61-7	≥5	0.61	2.5	0.58
367	Resveratrol	501-36-0	2.5	0.11	2.5	0.12
368	1,4-butanediol (1,4 BD)	110-63-4	2	0.0006	n.d.	
369	2-Phenylphenol	90-43-7	2.5	0.09	n.d.	
370	Dimethylaniline	95-68-1	2.5	0.02	n.d.	
371	2-Phenethylamine	64-04-0	2.5	0.09	2	0.12

Quantification of the analytes was performed, as well. As shown in Table 1, the concentrations of the analytes range in influent from 93 µg/L (metformin) to 0.14 ng/L (haloperidol) and in effluent from 48 µg/L (valine) to 0.22 ng/L (acetochlor). In Figure 1, the distribution of the concentrations of the analytes from the sub-ng level until some mg is presented. Apart from metformin, also valproic acid and caffeine are the most abundant drugs in influent wastewater. In effluent, metformin together with its metabolite gualynurea and N-desmethylvenlafaxine present the higher concentrations. For pesticides, most abundant compounds were fluometuron, azoxystrobin and a metabolite of dimethachlor, both in

influent and effluent wastewaters. Sweeteners are also present in high concentration (0.6 μg/L sucralose to 24 μg/L cyclamate), but they are removed more than 60 % during the treatment. Benzoic acid is the most abundant from the rest of the chemicals, at concentrations 49 μg/L and 29 μg/L in influent and effluent wastewater, respectively. It is mainly used mostly in the production of other chemicals. An interesting chemical that is present in high concentrations is ethyl sulfate, which is a minor metabolite of human after alcohol consumption (Rodríguez-Álvarez et al., 2014). Aminoacids are not emerging pollutants, but they are present in wastewater in very high concentrations. In influent, the concentrations are above 2 μg/L for the aminoacids detected and while significant removal is observed, in effluent, valine and g-aminobutiric acid (GABA) are at concentrations over 10 μg/L.

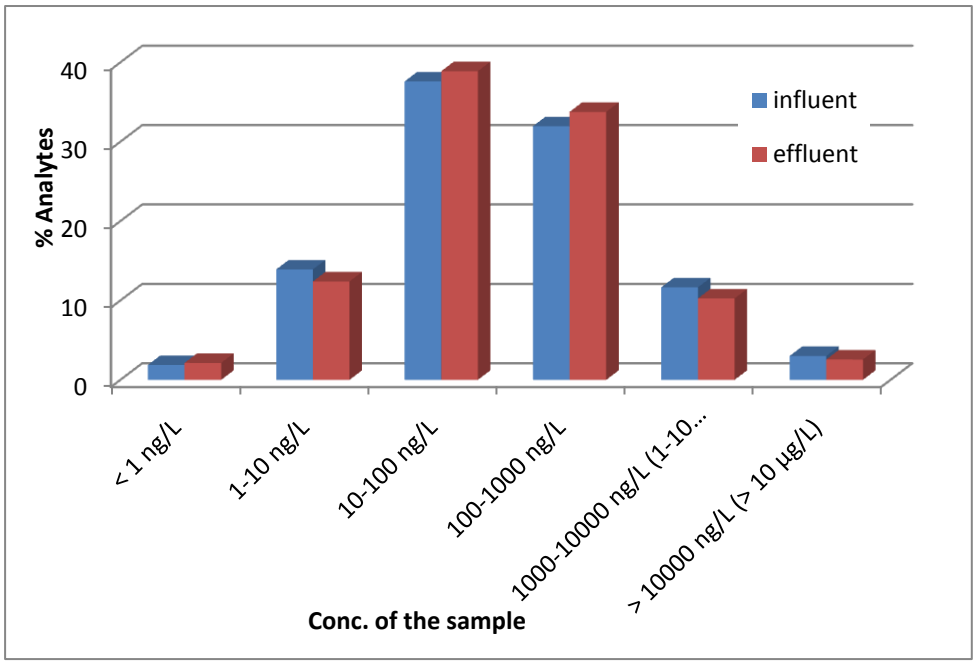


Figure 1. Distribution of concentration of analytes detected in samples

Hydrophilic interaction liquid chromatography (HILIC)

Hydrophilic interaction liquid chromatography (HILIC) has been a valuable complementary, orthogonal approach to reverse phase (RP) chromatography. Hyphenated to high resolution mass spectrometry it can provide additional information and selectivity for the target screening of polar micropollutants in the environment.

Additional to the RP-QToF-MS/MS method, a HILIC-QToF-MS/MS method was developed, optimized and validated for the

determination of 902 polar and semi-polar micropollutants in wastewater samples. In Table 2, the additional compounds that are detected only in HILIC method are presented.

Table 2. Additional compounds detected in wastewater samples after the application of HILIC method

1	1-(3-Trifluoromethylphenyl)-piperazine
2	2-Methyl-4-amino-6-methoxy-s-triazine
3	2-Naphthalene sulfonic acid
4	3 4-methylenedioxy-N-ethylamphetamine (MDEA)
5	3 phenoxy benzoic acid
6	3,5,6 trichloro-2-pyridinol
7	4-nonylphenoxy acetic acid
8	4-Trifluoromethylphenol
9	5,6-di-Me-Benzotriazole
10	6-methyl-2-thiouracil
11	Amineptine
12	Amlodipine
13	Ancymidol
14	Aspartame
15	Atomoxetin
16	Atrazine-desethyl
17	Bamethane
18	Benzylamphetamine
19	Benzylpiperazine (BZP)
20	Bufexamac
21	Capsaicine
22	Carbamazepine-10-11-dihydro-10-11-dihydroxy
23	Cefalexin
24	Cefazolin
25	Chlrothalonil-4-hydroxy
26	Cimaterol
27	Clindamycin
28	Clopidol
29	Cloranolol
30	Clorprenaline
31	Crotethamide
32	Cyclobenzaprine
33	Cyromazine
34	Hydrocodone
35	Deprenyl-N-oxide
36	Desipramine

37	Dexamethasone
38	Diflunisal
39	Dimethyldioctadecyl ammonium
40	Dimoxystrobin
41	Dinotefuran
42	Diprophylline
43	Doxylamine
44	Dropropizine
45	Eplerenone
46	Etilefrine
47	Etodolac
48	Exemestan
49	Fenproporex
50	Fenspiride
51	Fluorometholone
52	Fluoxymesterone
53	Hydromorphone
54	Hyoscine
55	Irgarol
56	Isproturon- didemethyl
57	Ketamine
58	Levallorphan
59	Lidocaine-N-oxide
60	LMG
61	Lovastatin
62	Mabuterol
63	Mazindol
64	Meclofenoxate
65	Mefenorex
66	Mephedrone
67	Mephenesine
68	Metamitron desamino
69	Methotrexate
70	Methoxyphenamine
71	Metipranolol
72	Nadolol
73	Naphazoline
74	Nefopam
75	N-ethyl-4-methoxybenzamide
76	N-bisdesmethyl tramadol
77	N-N-Didesmethylvenlafaxine
78	Tramadol
79	Norfenefrine
80	Octopamine

81	Norbuprenorphine
82	Norfenfluramine
83	Norketamine
84	Norlidocaine
85	Nornicotine
86	Oseltamivir carboxylate
87	Procainamide
88	Oxilofrine
89	Oxprenolol
90	Oxycodone (OC)
91	Oxymetazoline
92	Pencyclovir
93	Pentylenetetrazol
94	Phenylalanine (Phe)
95	Phenylbutazone
96	Prolinamide
97	Prolintane
98	Propachlor-OXA
99	Propazine-2-hydroxy
100	Propionylpromazine
101	Propoxycaine
102	Propylhexedrine
103	Pyrilamine
104	Pyrimidinol
105	Ritalinic acid
106	Rivastigmin
107	Stanozolol
108	Sudan II
109	Sulfamoxole
110	Sulfanilamide
111	Terbutaline
112	Tiamulin
113	Tolnaftat
114	Torasemide
115	Tralkoxydim
116	Triamterene
117	Triethylamine
118	Trimeprazine
119	Trimethylamine
120	Tripropylamine
121	Tryptamine
122	Tyramine
123	Tyrosine (Tyr)
124	Ursodeoxycholic acid

125	Vedaprofen
126	Zidovudine
127	Zonisamide
128	Zopiclone

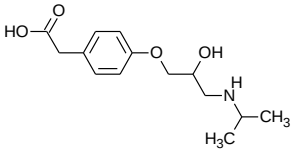
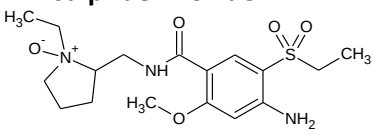
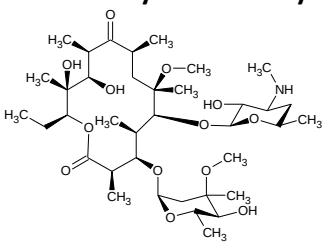
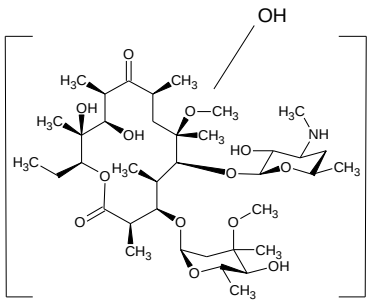
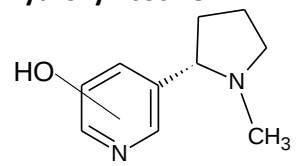
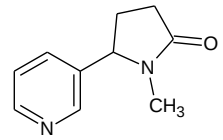
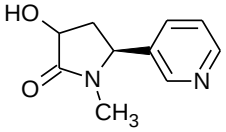
Communication of the achieved levels of confidence for Suspect & Non-target Screening

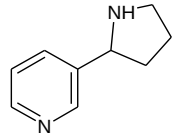
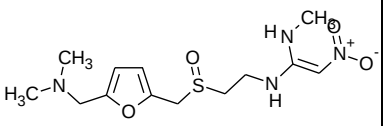
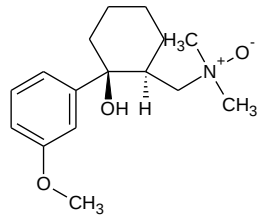
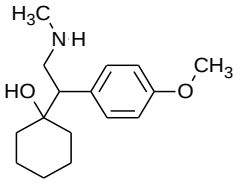
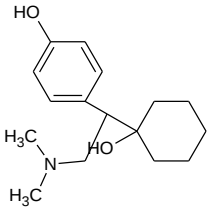
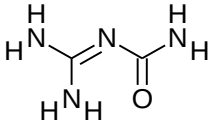
The system presented by Schymanski et al. (2014) to communicate the level of confidence achieved in the identification of the detected compounds was used. Level 1 corresponds to confirmed structures where a reference standard is available, level 2 to probable structures, level 3 for tentative candidate(s), Level 4 to unequivocal molecular formulas and level 5 to exact mass(es) of interest.

Suspect Screening

Suspect screening of the influent wastewater samples using the "metabolite suspects" yielded 1660 hits in positive ESI mode (PI) and 864 in negative mode (NI) applying only accuracy threshold (2 mDa). After the application of filtering steps this number decreased to 79 hits (PI, corresponding to 37 compounds) and 71 hits (NI, corresponding to 21 substances). The 37 and 21 substances (PI and NI, respectively) remaining were then investigated closer according to steps 6 and 7 above (presence of characteristic adducts and spectral interpretation). After the evaluation of all steps, 13 suspect compounds were tentatively identified that fulfilled all criteria, all of them in PI. None of the evaluated substances was tentatively identified in NI, most probably due to the lower sensitivity of this operational mode. The identified suspects are given in Table 3. In cases of Level 1 Identification Confidence, which means that reference standard was purchased for confirmation, the analyte was also included in the target database, as well.

Table 3. Details on the 13 suspect metabolites.

Name, Structure and Formula	Parent	Exp. t_R (Pred. t_R)[a]	Additional Evidence[b]	Level
Atenolol acid  $C_{14}H_{21}NO_4$	*Atenolol*	5.1 (5.5)	-Similarity 0.92 with MassBank record EA069710[c]. -Confirmation with standard	1
Amisulpride-N-oxide  $C_{17}H_{27}N_3O_5S_1$	*Amisulpride*	5.6 (6.5)	- Confirmation with standard	1
N-desmethyl clarithromycin  $C_{37}H_{67}NO_{13}$	*Clarithromycin*	10.1 (9.6)	-Intra-day trend consistent with the parent compound[d] -Intra-week trend consistent with the parent compound[e] -Confirmation with standard	1
Hydroxyclarithromycin  $C_{38}H_{69}NO_{14}$	*Clarithromycin*	8.5 (9.2)	-Intra-day trend consistent with the parent compound[d] -Intra-week trend consistent with the parent compound[e]	3
Hydroxynicotine  $C_{10}H_{14}N_2O_2$	*Nicotine*	3.1 (2.3)	-	3
Cotinine  $C_{10}H_{12}N_2O$	*Nicotine*	4.9 (5.1)	-Similarity 0.99 with MassBank record WA000998[c] -Confirmation with standard	1
Hydroxycotinine 	*Nicotine*	3.9 (4.0)	- HILIC/RP elution supports properties[f] -Confirmation with standard	1

$C_{10}H_{12}N_2O_2$				
Nornicotine  $C_9H_{12}N_2$	*Nicotine*	2.5 (2.6)	-Similarity 0.77 with NIST record 1185301[b] -Feasible t_R[c]	2b
Ranitidine-S-oxide  $C_{13}H_{22}N_4O_4S_1$	*Ranitidine*	2.0 (2.9)	-Intra-week trend consistent with the parent compound[e] -Confirmed with reference standard	1
Tramadol-N-oxide  $C_{16}H_{25}NO_3$	*Tramadol*	6.0 (8.0)	-Intra-week trend consistent with the parent compound[e]	2b
N-desmethyl venlafaxine  $C_{16}H_{25}NO_2$	*Venlafaxine*	7.4 (6.3)	-Similarity 0.96 with MassBank record EA103410[c] -Intra-day trend consistent with the parent compound[d] -Intra-week trend consistent with the parent compound[e] -Confirmation with standard	2a
O-desmethyl venlafaxine  $C_{16}H_{25}NO_2$	*Venlafaxine*	6.0 (6.4)	-Similarity 0.98 with MassBank record EA105304[c] -Intra-day trend consistent with the parent compound[d] -Intra-week trend consistent with the parent compound[e] -Confirmation with standard	2a
Guanylurea  $C_2H_6N_4O$	*Metformin*	1.3 (1.2)	- HILIC/RP elution supports properties[f] Confirmation with standard	1

Mass spectra were available for several of these compounds, allowing the assignment of a confidence level of 2a initially where the measured spectra matched the database spectra.

Where mass spectra were not available in libraries, other evidence was pursued to increase the confidence of the suspect

identification. The spectrum of the tentatively identified metabolite was compared with that of the parent compound.

The complementary nature of HILIC and RP elution as well as the presence in influent and effluent samples was also exploited in the identification of suspect analytes.

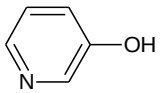
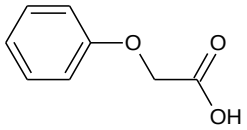
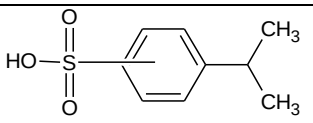
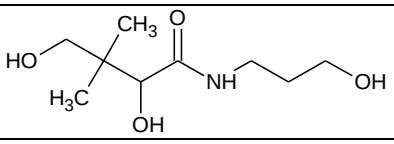
The intra-day concentration profile of parent and metabolites can also be used to provide additional evidence for the identification. Similar conclusions were reached from the comparison of the intra-week concentration profiles among parent compounds and related metabolites.

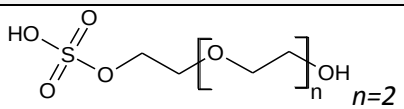
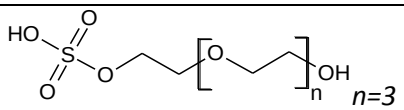
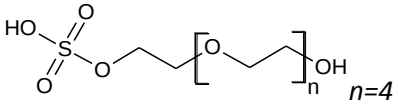
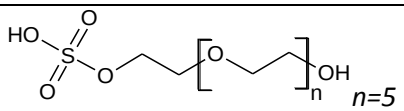
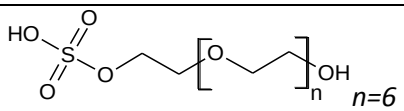
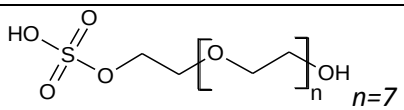
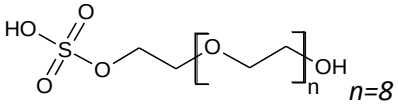
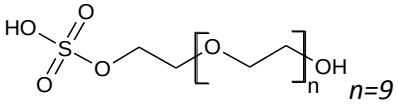
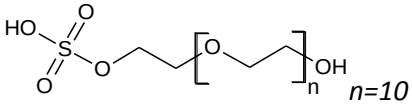
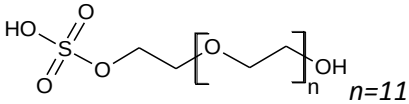
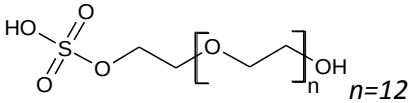
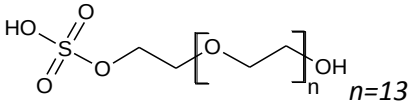
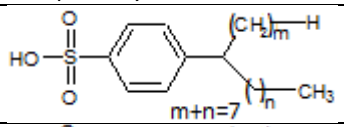
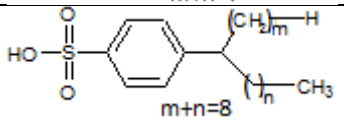
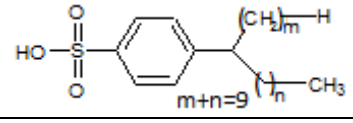
Non-target Screening

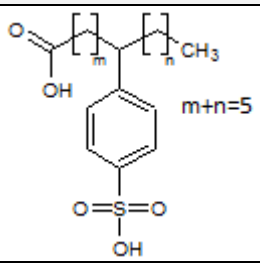
The non-target screening approach was applied to masses selected from among the most intense masses detected in the influent wastewaters from the WWTP of Athens. The workflow applied for the identification of non-target compounds is presented by Gago-Ferero et al. (2015b).

Table 4 and 5 summarizes the results obtained for 25 of the highest intensity masses detected in negative and positive ionization mode, respectively.

Table 4. Details on non-target compounds in ESI(-) mode.

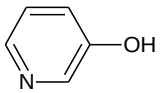
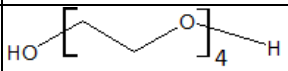
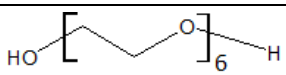
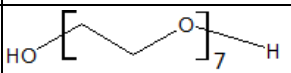
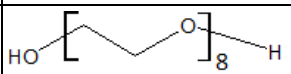
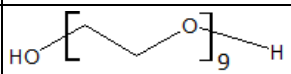
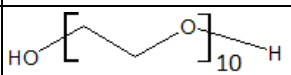
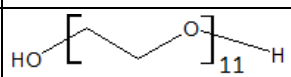
m/z	Unequivocal molecular formula	t_R	Pred. t_R	Level	Adduct	Tentative Candidate
Individual substances Levels 1-3						
94.02970	**C5H5NO (3-hydroxy pyridine)**	3.3	4.2	1	$[M-H]^-$	
151.0404	**C8H8O3 (Phenoxy acetic acid)**	3.6	5.5	2ª	$[M-H]^-$	
199.0439	**C9H12O3S (4-Isopropyl benzene sulfonic acid)**	5.3	5.1	3	$[M-H]^-$	
204.1244	**C9H19NO4 (Panthenol)**	3.3	4.1	1	$[M-H]^-$	
Glycol ether sulfates (GES)						

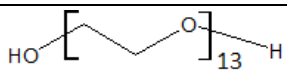
m/z	Unequivocal molecular formula	t_R	Pred. t_R	Level	Adduct	Tentative Candidate
229.0376	**C6H14O7S (2GES)**	1.8	3.6	2b	$[M-H]^-$	 *n=2*
273.0650	**C8H18O8S (3GES)**	2.4	3.8	2b	$[M-H]^-$	 *n=3*
317.0900	**C10H22O9S (4GES)**	2.9	4.4	2b	$[M-H]^-$	 *n=4*
361.1162	**C12H26O10S (5GES)**	3.2	5.2	2b	$[M-H]^-$	 *n=5*
405.1422	**C14H30O11S (6GES)**	3.5	6.1*	2b	$[M-H]^-$	 *n=6*
449.1689	**C16H34O12S (7GES)**	3.7	6.7*	2b	$[M-H]^-$	 *n=7*
493.1955	**C18H38O13S (8GES)**	3.9	7.2*	2b	$[M-H]^-$	 *n=8*
537.2217	**C20H42O14 (9GES)**	4.1	7.6*	2b	$[M-H]^-$	 *n=9*
581.2470	**C22H46O15S (10GES)**	4.3	7.9*	2b	$[M-H]^-$	 *n=10*
625.2729	**C24H50O16S (11GES)**	4.4	8.1*	2b	$[M-H]^-$	 *n=11*
669.2990	**C26H54O17S (12GES)**	4.5	8.2*	2b	$[M-H]^-$	 *n=12*
713.3259	**C28H58O18S (13GES)**	4.6	8.2*	2b	$[M-H]^-$	 *n=13*
Linear Alkylbenzyl Sulfonates (LAS) and SulfoPhenyl Alkyl Carboxylic acids (SPACs)						
297.1530	**C16H26O3S (C10-LAS)**	11.5	11.7	2a[§]	$[M-H]^-$	 m+n=7
311.1684	**C17H28O3S (C11-LAS)**	12	12.1	2a[§]	$[M-H]^-$	 m+n=8
325.1842	**C18H30O3S (C12-LAS)**	12.5	12.3	1	$[M-H]^-$	 m+n=9

m/z	Unequivocal molecular formula	t_R	Pred. t_R	Level	Adduct	Tentative Candidate
299.0965	**C14H20O5S (SPA-8C)**	4.8	7.2	3	$[M-H]^-$	
Remaining substances (Levels 3-5)						
121.0291	**C7H6O2**	2.8		4	$[M-H]^-$	57 hits chemspider
121.0290	**C7H6O2**	4.5		4	$[M-H]^-$	57 hits Chemspider
186.1139	**C9H17NO3**	3.3		4	$[M-H]^-$	789 hits Chemspider
405.1436	**C14H29O11S**	3.5		3	$[M-H]^-$	Branched isomer of hexaglycol ether sulfate

Pred. t_R: predicted retention time; *Out of the applicability domain of the t_R prediction model; [§]mix of isomers (spectra present in MassBank as a mix of isomers).

Table 5. Details on non-target compounds in ESI(+) mode.

m/z	Unequivocal molecular formula	t_R	Pred. t_R	Level	Adduct	Tentative Candidate
Confirmed non-targets						
96.0452	C_5H_5NO	2.27	2.9	1	$[M+H]^+$	
195.1233	$C_8H_{18}O_5$ (4-PEG)	4.19	6.2	1	$[M+H]^+$	
Polyethylene glycol suspects						
239.1489	$C_{10}H_{22}O_6$ (5-PEG)	4.8	6.8	3	$[M+H]^+$	
300.2016	$C_{12}H_{26}O_7$ (6-PEG)	5.16	7.3	3	$[M+NH_4]^+$	
344.2278	$C_{14}H_{30}O_8$ (7-PEG)	5.4	7.6	3	$[M+NH_4]^+$	
388.2541	$C_{16}H_{34}O_9$ (8-PEG)	5.6	7.8	3	$[M+NH_4]^+$	
432.2803	$C_{18}H_{38}O_{10}$ (9-PEG)	5.8	8.0	3	$[M+NH_4]^+$	
476.3065	$C_{20}H_{42}O_{11}$ (10-PEG)	5.9	8.2	3	$[M+NH_4]^+$	
520.3327	$C_{22}H_{46}O_{12}$ (11-PEG)	6.13	8.3	3	$[M+NH_4]^+$	

m/z	Unequivocal molecular formula	t_R	Pred. t_R	Level	Adduct	Tentative Candidate
564.3589	$C_{24}H_{50}O_{13}$ (12-PEG)	6.3	8.4	3	$[M+NH_4]^+$	
608.3851	$C_{26}H_{54}O_{14}$ (13-PEG)	6.44	8.5	3	$[M+NH_4]^+$	
652.4113	$C_{28}H_{58}O_{15}$ (14-PEG)	6.6	8.6	3	$[M+NH_4]^+$	
696.4376	$C_{30}H_{62}O_{16}$ (15-PEG)	6.7	8.7	3	$[M+NH_4]^+$	
740.4638	$C_{32}H_{66}O_{17}$ (16-PEG)	6.9	8.8	3	$[M+NH_4]^+$	
784.49	$C_{34}H_{70}O_{18}$ (17-PEG)	7	8.9	3	$[M+NH_4]^+$	
Remaining non-targets (Level 4 and Level 5)						
145.0977	$C_6H_{12}N_2O_2$	1.91		4	$[M+H]^+$	*523 hits Chemspider*
149.1176	$C_7H_{16}O_3$	5.73		4	$[M+H]^+$	*103 hits Chemspider*
191.1647	$C_{10}H_{22}O_3$	9.4		4	$[M+H]^+$	*106 hits Chemspider*
135.1018	$C_6H_{14}O_3$	4.68		4	$[M+H]^+$	*106 hits Chemspider*
164.1282	$C_7H_{17}NO_3$	1.28		5	*	
232.1913	$C_{12}H_{25}NO_3$	9.1		5	*	
316.1955	4 possibilities	12.69		5		
358.2078	3 possibilities	5.09		5		
374.2390	7 possibilities	5.24		5		
424.1857	12 possibilities	4.98		5		
468.2108	6 possibilities	5.2		5		

Pred. t_R: predicted retention time.*The identity of the adduct could not be confirmed.

Retrospective suspect screening of surfactants

As a high number of tentatively identified surfactant substances of different types were among the most intense peaks, the presence of surfactants, originally found as non-targets, was studied in detail through retrospective suspect screening using the surfactants list described above.

In total, 82 substances out of 398 suspects were tentatively identified, as shown in Table 6. 38 out of these 82 compounds were tentatively identified with MS/MS evidence and 44 without MS/MS evidence, but with additional information (plausible t_R and chromatographic peak shape among the homologue series), supporting their presence.

Ten compounds from the SPCs class of surfactants were identified. Other groups of surfactants were identified similarly, including DATS (7 substances tentatively identified), LAS (4 substances tentatively identified), AS (4 substances tentatively identified), AEOs (13 substances tentatively identified) or DEAs (3 substances tentatively identified).

Table 6. Suspect surfactants results

Color code:

GREEN: *Tentatively identified*. t_R is plausible among the analogue series (also in HILIC) + MS/MS match with *MassBank*, MS/MS is consistent with one of the analogues present in *MassBank* or at least is plausible according to the structure.
ORANGE: *Tentatively identified*. t_R is plausible among the analogue series (also in HILIC) but there was not possible to obtain MS/MS information because of the intensity of the peaks. We consider them tentatively identified also based on chromatographic criteria and the information present in other studies.
RED: No evidences. No MS/MS information.

Suspect surfactants results - Negative ESI mode:

Name	Neutral formula	Monoisotopic mass	RP t_R (min)	HILIC t_R (min)	Plausible t_R among the analogue series	MS/MS data available	MS/MS plausibility	Tentatively identified
C5-DATS	C11H14O3S1	225.0591	7.4	1.21	*Yes*	*Yes*	*Yes*	YES
C8-DATS	C14H20O3S1	267.1060	10.5	1.75	*Yes*	*No*	*n.e.*	YES
C9-DATS	C15H22O3S1	281.1217	11	1.73	*Yes*	*Yes*	*Yes*	YES
C10-DATS	C16H24O3S1	295.1373	11.8	1.71	*Yes*	*Yes*	*Yes**	YES
C11-DATS	C17H26O3S1	309.1530	12.3	1.70	*Yes*	*Yes*	*Yes*	YES
C12-DATS	C18H28O3S1	323.1686	12.9	1.67	*Yes*	*Yes*	*Yes*	YES
C13-DATS	C19H30O3S1	337.1843	13.4	*n.d.*	*Yes*	*No*	*n.e.*	YES
C15-DATS	C21H34O3S1	365.2156	6	*n.d.*	*No*	*No*	*No*	NO
C10-LAS	C16H26O3S1	297.1530	12.5	1.65	*Yes*	*Yes*	*YES*	YES
C11-LAS	C17H28O3S1	311.1686	13.0	1.55	*Yes*	*Yes*	*YES*	YES
C12-LAS	C18H30O3S1	325.1843	13.6	1.42	*Yes*	*Yes*	*YES*	YES
C13-LAS	C19H32O3S1	339.1999	14.1	1.37	*Yes*	*Yes*	*YES*	YES
C4-SPC	C10H12O5S1	243.0333	1.8	7.02	*Yes*	*Yes*	*Yes*	YES
C5-SPC	C11H14O5S1	257.0489	2.3	6.58	*Yes*	*Yes*	*Yes*	YES
C6-SPC	C12H16O5S1	271.0646	4.7	6.24	*Yes*	*Yes*	*Yes*	YES
C7-SPC	C13H18O5S1	285.0802	5.3	5.24	*Yes*	*Yes*	*Yes**	YES

Name	Neutral formula	Monoisotopic mass	RP t_R (min)	HILIC t_R (min)	Plausible t_R among the analogue series	MS/MS data available	MS/MS plausibility	Tentatively identified
C8-SPC	C14H20O5S1	299.0959	6.0	4.33	*Yes*	*Yes*	*Yes**	YES
C9-SPC	C15H22O5S1	313.1115	6.7	4.07	*Yes*	*Yes*	*Yes*	YES
C10-SPC	C16H24O5S1	327.1272	7.6	3.38	*Yes*	*Yes*	*Yes**	YES
C11-SPC	C17H26O5S1	341.1428	8.6	3.32	*Yes*	*Yes*	*Yes*	YES
C12-SPC	C18H28O5S1	355.1585	9.3	*n.d.*	*Yes*	*Yes*	*Yes*	YES
C13-SPC	C19H30O5S1	369.1741	8.5	*n.d.*	*No*	*No*	*n.e.*	NO
C13-SPC	C19H30O5S1	369.1741	10.1	*n.d.*	*Yes*	*Yes*	*Yes*	YES
C15-SPC	C21H34O5S1	397.2054	10.2	*n.d.*	*No*	*No*	*n.e.*	NO
C12-AS	C12H26O4S1	265.1479	11.2	1.24	*Yes*	*Yes*	*Yes**	YES
C13-AS	C13H28O4S1	279.1636	12.8	1.21	*Yes*	*Yes*	*Yes*	YES
C14-AS	C14H30O4S1	293.1792	13.6	1.21	*Yes*	*Yes*	*Yes*	YES
C15-AS	C15H32O4S1	307.1949	11.8	1.20	*No*	*No*	*n.e.*	NO
C16-AS	C16H34O4S1	321.2105	14.5	1.20	*Yes*	*Yes*	*Yes*	YES
C12-AE1S	C14H30O5S1	309.1741	10.7	*n.d.*	*No*	*Yes*	*No*	NO
C12-AE1S	C14H30O5S1	309.1741	11.6	1.21	*Yes*	*No*	*n.e.*	YES
C12-AE2S	C16H34O6S1	353.2003	12.0	1.25	*Yes*	*No*	*n.e.*	YES
C12-AE3S	C18H38O7S1	397.2265	12.9	1.29	*Yes*	*No*	*n.e.*	YES
C12-AE4S	C20H42O8S1	441.2528	13.4	1.35	*Yes*	*No*	*n.e.*	YES
C12-AE5S	C22H46O9S1	485.2790	13.5	1.39	*Yes*	*No*	*n.e.*	YES
C12-AE6S	C24H50O10S1	529.3052	13.6	1.50	*Yes*	*No*	*n.e.*	YES
C12-AE7S	C26H54O11S1	573.3314	13.4	*n.d.*	*Yes*	*No*	*n.e.*	YES
C13-AE1S	C15H32O5S1	323.1898	13.2	1.20	*Yes*	*No*	*n.e.*	YES
C13-AE2S	C17H36O6S1	367.2160	13.5	1.24	*Yes*	*YES*	*Yes*	YES
C13-AE3S	C19H40O7S1	411.2422	13.6	1.29	*Yes*	*No*	*n.e.*	YES
C13-AE5S	C23H48O9S1	499.2946	13.8	1.38	*Yes*	*No*	*n.e.*	YES
C13-AE6S	C25H52O10S1	543.3208	13.8	*n.d.*	*Yes*	*No*	*n.e.*	YES

Name	Neutral formula	Monoisotopic mass	RP t_R (min)	HILIC t_R (min)	Plausible t_R among the analogue series	MS/MS data available	MS/MS plausibility	Tentatively identified
C13-AE7S	C27H56O11S1	587.3471	13.9	*n.d.*	*Yes*	*No*	*n.e.*	YES
C13-AE8S	C29H60O12S1	631.3733	13.9	*n.d.*	*Yes*	*No*	*n.e.*	YES
C14-AE1S	C16H34O5S1	337.2054	14.0	1.20	*Yes*	*No*	*n.e.*	YES
C14-AE2S	C18H38O6S1	381.2316	14.2	1.22	*Yes*	*No*	*n.e.*	YES
C14-AE3S	C20H42O7S1	425.2578	14.3	1.25	*Yes*	*No*	*n.e.*	YES
C14-AE4S	C22H46O8S1	469.2841	14.3	*n.d.*	*Yes*	*No*	*n.e.*	YES
C14-AE5S	C24H50O9S1	513.3103	14.5	*n.d.*	*Yes*	*No*	*n.e.*	YES
C16-AE1S	C18H38O5S1	365.2367	14.9	*n.d.*	*Yes*	*No*	*n.e.*	YES
C16-AE2S	C20H42O6S1	409.2629	15.0	*n.d.*	*Yes*	*No*	*n.e.*	YES
C16-AE3S	C22H46O7S1	453.2891	15.0	*n.d.*	*Yes*	*No*	*n.e.*	YES
C16-AE4S	C24H50O8S1	497.3154	15.0	*n.d.*	*Yes*	*No*	*n.e.*	YES
NPEO1-SO4	C17H28O5S1	343.1585	7.7	*n.d.*	*No*	*No*	*n.e.*	NO
NPEO1-SO4	C17H28O5S1	343.1585	12.5	1.19	*Yes*	*No*	*n.e.*	YES
NPEO2-SO4	C19H32O6S1	387.1847	12.7	1.26	*Yes*	*No*	*n.e.*	YES
NPEO3-SO4	C21H36O7S1	431.2109	12.9	1.32	*Yes*	*No*	*n.e.*	YES
NPEO4-SO4	C23H40O8S1	475.2371	12.9	1.35	*Yes*	*No*	*n.e.*	YES
NPEO5-SO4	C25H44O9S1	519.2633	13.0	*n.d.*	*Yes*	*No*	*n.e.*	YES
NPEO6-SO4	C27H48O10S1	563.2895	13.0	*n.d.*	*Yes*	*No*	*n.e.*	YES
SPA-2DC	C10H10O7S1	273.0074	5.7	*n.d.*	*No*	*No*	*n.e.*	NO
SPA-5DC	C13H16O7S1	315.0544	1.3	7.40	*Yes*	*No*	*n.e.**	YES
SPA-6DC	C14H18O7S1	329.0700	1.7	7.31	*Yes*	*No*	*n.e.*	YES
SPA-7DC	C15H20O7S1	343.0857	5.4	*n.d.*	*No*	*No*	*n.e.*	NO
SPA-7DC	C15H20O7S1	343.0857	1.6	7.26	*Yes*	*Yes*	*Yes*	YES
SPA-8DC	C16H22O7S1	357.1013	6.0	7.20	*No*	*No*	*n.e.*	NO
SPA-8DC	C16H22O7S1	357.1013	3.0	7.20	*Yes*	*No*	*n.e.*	YES
SPA-10DC	C18H26O7S1	385.1326	7.6	*n.d.*	*No*	*No*	*n.e.*	NO

Name	Neutral formula	Monoisotopic mass	RP t_R (min)	HILIC t_R (min)	Plausible t_R among the analogue series	MS/MS data available	MS/MS plausibility	Tentatively identified
SPA-12DC	C20H30O7S1	413.1639	6.7	*n.d.*	*No*	*No*	*n.e.*	NO
STA-1C	C12H14O5S1	269.0489	3.0	*n.d.*	*Yes*	*No*	*n.e.*	YES
STA-2C	C13H16O5S	283.0646	4.7	*n.d.*	*Yes*	*No*	*n.e.*	YES
STA-3C	C14H18O5S1	297.0802	5.5	*n.d.*	*Yes*	*No*	*n.e.*	YES
STA-4C	C15H20O5S1	311.0959	6.0	*n.d.*	*Yes*	*Yes*	*Yes**	YES
STA-5C	C16H22O5S1	325.1115	6.8	*n.d.*	*Yes*	*Yes*	*Yes*	YES
STA-6C	C17H24O5S1	339.1272	7.8	*n.d.*	*Yes*	*No*	*n.e.*	YES
STA-7C	C18H26O5S1	353.1428	8.0	*n.d.*	*No*	*No*	*n.e.*	NO
STA-8C	C19H28O5S1	367.1585	12.0	*n.d.*	*No*	*Yes*	*No*	NO
STA-9C	C20H30O5S1	381.1741	9.6	*n.d.*	*Yes*	*Yes*	*No*	NO
STA-9C	C20H30O5S1	381.1741	10.6	*n.d.*	*Yes*	*Yes*	*No*	NO
C14-SAS	C14H30O3S1	277.1843	13.3	1.72	*n.e.*	*No*	*n.e.*	NO
PEG-7EO	C14H30O8	325.1868	13.6	*n.d.*	*n.e.*	*Yes*	*n.e.*	NO
NPEO8-SO4	C31H56O12S1	651.3420	5.8	*n.d.*	*n.e.*	*No*	*n.e.*	NO
benzenesulfonate	C6H6O3S1	156.9965	2.6	1.87	*Yes*	*Yes*	*Yes*	YES
naphthalene-1-sulfonate	C10H8O3S1	207.0121	5.8	1.85	*Yes*	*Yes*	*Yes**	YES
2-chloro-4-amiNotoluene-5-sulfonate	C7H8Cl1N1O3S1	219.9841	5.9	*n.d.*	*n.e.*	*No*	*n.e.*	NO
2-chloro-5-amiNotoluene-4-sulfonate	C7H8Cl1N1O3S1	219.9841	5.9	*n.d.*	*n.e.*	*No*	*n.e.*	NO

*n.d. not detected, n.e. not evaluated, * spectra available in MassBank*

Suspect surfactants results - Positive ESI mode :

Name	Neutral formula	Monoisotopic mass	RP t_R (min)	HILIC t_R (min)	Plausible t_R among the analogue series	MS/MS data available	MS/MS plausibility	Tentatively identified
C10-AEO-2	C14H30O3	247.2268	13.2	1.40	*Yes*	*Yes*	*Yes*	YES
C10-AEO-3	C16H34O4	291.2530	13.3	1.41	*Yes*	*Yes*	*Yes*	YES
C10-AEO-4	C18H38O5	335.2792	13.3	1.43	*Yes*	*Yes*	*Yes*	YES
C10-AEO-4	C18H38O5	335.2792	9.0	*n.d.*	*No*	*No*	*n.e.*	NO
C10-AEO-5	C20H42O6	379.3054	13.4	1.45	*Yes*	*No*	*Yes*	YES
C11-AEO-2	C15H32O3	261.2424	13.8	1.40	*Yes*	*Yes*	*Yes*	YES
C11-AEO-3	C17H36O4	305.2686	13.85	1.40	*Yes*	*Yes*	*Yes*	YES
C11-AEO-4	C19H40O5	349.2949	13.9	1.41	*Yes*	*No*	*n.e.*	YES
C12-AEO-2	C16H34O3	275.2581	14.3	1.38	*Yes*	*No*	*n.e.*	YES
C12-AEO-3	C18H38O4	319.2843	14.3	1.39	*Yes*	*No*	*n.e.*	YES
C12-AEO-4	C20H42O5	363.3105	14.3	1.40	*Yes*	*No*	*n.e.*	YES
C13-AEO-2	C17H36O3	289.2737	14.4	1.38	*Yes*	*No*	*n.e.*	YES
C13-AEO-3	C19H40O4	333.2999	14.6	1.39	*Yes*	*No*	*n.e.*	YES
C13-AEO-4	C21H44O5	377.3262	14.7	1.40	*Yes*	*No*	*n.e.*	YES
C7DEA	C12H25N1O3	232.1907	9.1	1.71	*Yes*	*Yes*	*Yes**	YES
C9DEA	C14H29N1O3	260.2220	11.2	1.65	*Yes*	*Yes*	*Yes**	YES
C11DEA	C16H33N1O3	288.2533	12.7	1.60	*Yes*	*Yes*	*Yes**	YES
SPA-12DC	C20H30O7S1	415.1785	5.6	2.52	*n.e.*	*No*	*n.e.*	NO
STA-15DC	C27H42O7S1	511.2724	5.8	3.44	*n.e.*	*No*	*n.e.*	NO
C11-DATS	C17H26O3S1	311.1675	1.9	*n.d.*	*n.e.*	*No*	*n.e.*	NO
C11-SPC	C17H26O5S1	343.1574	10.6	*n.d.*	*n.e.*	*No*	*n.e.*	NO

*n.d. not detected, n.e. not evaluated, * spectra available in MassBank*

Results in sludge

Target Analysis

Screening of the sludge samples was performed. The method and the database for the evaluation of the data was the same as in the wastewater samples. The compounds detected at least once in the sludge samples of 2014 and 2015 are presented in Table 7.

Table 7. Screening results of sludge samples

	Compound name
1	1 3 5(10)-estratrien-3 7?-diol/ 17a-ESTRADIOL (E2)
2	2-Aminobenzimidazole
3	2-OH-Benzothiazole
4	2-phenethylamine
5	4-androsten-3 17-dione (A4)
6	4-Me-Benzotriazole /5-Me-Benzotriazole
7	4-nonylphenol (4-NP)
8	4-nonylphenol-mono ethoxylate
9	4-Nonylphenoxy acid
10	4-tert-octylphenol (4-t-OP)
11	8-OH-Mirtazapine
12	Aceclidine
13	Acetochlor-OXA / Alachlor-OXA
14	Adenine
15	Adenosine
16	Aliskiren
17	Allopurinol
18	Altretamine
19	Amantadine
20	Amisulpride
21	amitryptyline
22	Atazanavir
23	Atenolol acid (Metoprolol acid)
24	Azoxystrobin
25	Azoxystrobin acid
26	benzododecinium (benzyl-dimethyl dodecyl ammonium
27	Benzotriazole (BTR)
28	Benzyl butyl phthalate
29	Bethanidine
30	Bis-(2-ethylhexyl) phthalate
31	Bisphenol A (BPA)
32	Buspirone
33	Carbachol
34	Carbamazepine

35	Celiprolol
36	Cetirizin
37	Cimetidine
38	Cinnarizine
39	Ciprofloxacin
40	Citalopram
41	Citalopram amide
42	Clarithromycin
43	Climbazol
44	Cortisol
45	Cotinine
46	Cyproconazole
47	D L-N O-Didesmethylvenlafaxine
48	D617_metabolite_verapamil
49	DADMAC C10:C10
50	deacetyl diltiazem
51	Desvenlafaxine (O-Desmethylvenlafaxine)
52	Diethyl phthalate
53	Difenoconazole
54	Di-n-butyl phthalate
55	Di-n-octyl phthalate
56	Dioxacarb
57	Diphenhydramine
58	DMPEA (HMA)
59	EDDP
60	Eprosartan
61	Esmolol
62	Fludioxonil
63	Flufenamic acid
64	Fluometuron
65	Fluoxetine
66	Formetanate
67	Fuberidazole
68	Gabapentin
69	Galaxolide
70	Galaxolidone
71	Harman
72	Harmine
73	Histamine
74	Irbesartan
75	Isoconazole
76	Lacosamide
77	Lamotrigin
78	Levofloxacin
79	MDA
80	Mefenamic acid

81	Memantine
82	Metazachlor-ESA
83	Methprene
84	Metoprolol
85	Mirtazapine
86	Morantel
87	Morphine
88	N-Desmethyl Citalopram
89	N-Desmethylvenlafaxine
90	Nicotinamide
91	Niflumic acid
92	Norcitalopram
93	Norethisterone acetate
94	Norfenefrine
95	Norfloxacin
96	Normirtazapine
97	Normorphine
98	Nortriptyline
99	Octocrylene
100	Ofloxacin
101	Orphenadrine
102	Penconazole
103	PFOS
104	Phenoxybenzamide
105	Phentermine
106	Pioglitazone
107	Piperonylbutoxide
108	Pregabalin
109	Progesterone
110	Propafenone
111	Propham
112	Propranolol
113	Pymetrozine
114	Ranitidine
115	Roxithromycin
116	Salicylic acid
117	Seneciphylline-N-oxide
118	Sertraline
119	Sotalol
120	Telmisartan
121	Temephos
122	Thebacon
123	Thiabendazole
124	Tralkoxydim
125	Tramadol
126	Tributylamin

127	Triclocarban
128	Triclosan
129	Triethylphosphate
130	Triphenylphosphate
131	Tyramine
132	Ursodeoxycholic acid
133	Valsartan
134	Venlafaxine
135	Verapamil

2nd Study – Batch experiment and identification of transformation products (TPs)

Emerging pollutants, once released in the environment, they reach wastewater treatment plants, where they are subject to various processes for their degradation. These processes can be either biological or chemical and they end up to transformation products, which have different physicochemical properties and different toxicity than the parent compounds.

It is important to study the occurrence and toxicity of the transformation products formed during the wastewater treatment procedure, since they are released in the environment.

The biological degradation is the main technique for the elimination of the emerging pollutants. During the disinfection procedure of the treatment (tertiary treatment), chlorination is the main technique.

Chlorination experiments

Although the primary purpose of chlorination is the elimination of micropollutants via oxidation, several investigations have shown that chlorine reacts with micropollutants leading in the production of undesired by-products. Recently, the identification and the evaluation of chlorination by-products has been a topic of growing interest, since these by-products may pose an environmental or human health risk, even higher than their parent compounds do.

Initially, experiments were conducted in ultrapure water and effluent samples from the main WWTP in Greece, Psyttaleia, in order to investigate the degradation of benzotriazoles and benzothiazoles in different chlorination conditions [applied molar ratio of NaOCl and the target contaminant (m.r.), reaction's contact time, pH value of the reaction's solution and the influence of total suspended solids (TSS) presence]. Thereafter, UHPLC High Resolution (HR) MS technique was used in order to look into the formation of possible by-products in the ultrapure water chlorinated samples and then verify their occurrence in spiked wastewater samples treated with chlorine. Information on the experimental protocol of the chlorination batch

experiment can be found by Nika et al. (2015) and the results of the chlorinated by-products are presented in Table 8.

Table 8. Transformation products, along with their parent compound, which were detected and structurally elucidated in chlorination batch experiments.

Parent Compound	Transformation product name	Proposed Structure	Molecular formula
5,6-dimethyl-1H-benzotriazole (XTRi) $C_8H_9N_3$	4-chloro-XTRi or 7-chloro-XTRi		$C_8H_8ClN_3$
	4-hydroxy-XTRi or 7-hydroxy-XTRi		$C_8H_9N_3O$
	4,7-di-hydro-XTRi		$C_8H_9N_3O_2$
	6-methyl-BTR-5-carbaldehyde or 5-methyl-BTR-6-carbaldehyde		$C_8H_7N_3O$
2-aminbenzothiazole (2-amino-BTH) $C_7H_6N_2S$	2-amino-6-chloro-BTH		$C_7H_5ClN_2S$
	2-amino-5,6-di-chloro-BTH		$C_7H_4Cl_2N_2S$
	2-amino-5,6-di-hydroxy-BTH		$C_7H_6N_2O_2S$
	2-amino-6-chloro-5-hydroxy-BTH		$C_7H_5ClN_2OS$
	2-[2-(BTH-2-yl)hydrazin-1-yl]-6-chloro-BTH		$C_{14}H_9ClN_4S_2$

Biotransformation experiments

Since wastewater treatment is mainly concerned with biodegradation, the detection and identification of biotransformation TPs is a necessary but at the same time challenging task which requires the use of modern high-resolution mass spectrometry (HR-MS) systems and appropriate analysis strategies.

Formed TPs differ to some extent in their environmental behavior and ecotoxicological profile to their related parent compounds. Despite low concentrations, the effects of these substances in the environment and human health cannot be excluded.

In table 9, the biotransformation products of emerging pollutants found in influent wastewater samples are presented. The batch experiments are presented by Beretsou et al. (2015).

Table 9. Transformation products, along with their parent compound, which were detected and structurally elucidated in biotransformation experiments.

Parent Compound	Transformation product name	Proposed Structure	Molecular formula
Lidocaine (LD) $C_{14}H_{22}N_2O$	LDC 207		$C_{12}H_{18}N_2O$
	LDC 251		$C_{14}H_{22}N_2O_2$
	LDC 219		$C_{13}H_{18}N_2O$
	LDC 233		$C_{13}H_{16}N_2O_2$
	LDC 265	n.p.*	$C_{14}H_{20}N_2O_3$
Ranitidine (RAN) $C_{13}H_{22}N_4O_3S$	RAN 286 (three chromatographic peaks)	n.p.	$C_{11}H_{15}N_3O_4S$
	RAN 301 a		$C_{12}H_{20}N_4O_3S$
	RAN 301 b		
	RAN 302 (two	n.p.	$C_{11}H_{15}N_3O_5S$

	chromatographic peaks)		
	RAN 316 (three chromatographic peaks)	n.p.	$C_{13}H_{21}N_3O_4S$
	RAN 317 (two chromatographic peaks)	n.p.	$C_{12}H_{20}N_4O_4S$
	RAN 331 a (S-oxide)		$C_{13}H_{22}N_4O_4S$
	RAN 331 b (N-oxide)		
Metformin $C_4H_{11}N_5O$	Guanylurea		$C_2H_6N_4O$
Atorvastatin (ATR)	ATR 515		$C_{31}H_{31}FN_2O_4$
	ATR 471		$C_{29}H_{27}FN_2O_3$
$C_{33}H_{35}FN_2O_4$	ATR 487 (two chromatographic peaks, two isomers)		$C_{29}H_{27}FN_2O_4$
Citalopram (CTR)	CTR 311 (DesmethylCTR)		$C_{19}H_{19}FN_2O$
	CTR 329 (DesmethylCTR amide)		$C_{19}H_{21}FN_2O_2$
	CTR 330 (DesmethylCTR carb. acid)		$C_{19}H_{20}FNO_3$
$C_{20}H_{21}FN_2O$	CTR 339A (3-oxo-CTR)		$C_{20}H_{19}FN_2O_2$
	CTR 339B	n.p.	$C_{21}H_{23}FN_2O$

	CTR 341 (CTR-N-oxide)		$C_{20}H_{21}FN_2O_2$
	CTR 343 (CTR amide)		$C_{20}H_{23}FN_2O_2$
	CTR 344 (CTR carb. acid)		$C_{20}H_{22}FNO_3$
	CTR 355		$C_{20}H_{19}FN_2O_3$
	CTR 357		$C_{20}H_{21}FN_2O_3$
	CTR 359A		$C_{20}H_{23}FN_2O_3$
	CTR 359B (Amide of CTR-N-oxide)		
	CTR 360A (Carb. acid of CTR-N-oxide)		$C_{20}H_{22}FNO_4$
	CTR 360B		

n.p.: no structure could be proposed

Retrospective suspect screening of citalopram and its bio-TPs in wastewater samples

Retrospective suspect screening was performed in order to evaluate the occurrence of the identified TPs during the biotransformation experiments in real wastewater samples.

The parent compound CTR along with its primary metabolite N-desmethyl CTR (CTR 311) was found in all evaluated wastewater samples. 3-oxo-CTR (CTR 339A) was the second most frequently detected compound in influent and effluent wastewater samples. CTR-N-oxide (CTR 341) was also detected in all effluents and in 7 out of 16 the influents. This fact can be easily explained since this

compound is also a human metabolite. Finally, the compounds CTR amide (CTR 343) and CTR carboxylic acid (CTR 344) were only found in 3 influent and 1 effluent samples, respectively, as a result of biotransformation of CTR in activated sludge.

The presence of TPs of CTR in wastewater samples can be thus attributed to a double contribution: (1) the direct input from human excreted metabolites in IWW and (2) the action of microorganisms during wastewater treatment, biodegrading CTR in known and unknown TPs.

Risk Assessment

In order to assess the environmental impact of these projects' findings, toxicity calculations with ECOSAR software (Ecological Structure Activity Relationship, http://www.epa.gov/oppt/newchems/tools/21ecosar.htm, v. 1.11, last accessed October 2015) were performed, so as to obtain an insight on the acute toxicity of the identified transformation products (TPs) on three classes of aquatic organisms; fish, algae and daphnia magna. This program provides the probable toxicity of a compound according to its octanol/water partition coefficient (K_{ow}) value and its structure similarity with other compounds whose toxicity in aquatic environment has been previously estimated.

Pharmaceuticals, drugs of abuse and related metabolites

For target compounds detected at least once toxicity data (EC50 or LC50) for three different trophic levels (algae, daphnids and fish) has been collected either through literature search or from ECOSAR program which is used from the US EPA. In either case the lowest short period toxicity values were collected in order to take into consideration the worst case scenario. According to the Technical Guidance Document of the European Commission, the risk quotient (RQ) is calculated as the maximum Measured Environmental Concentration (MEC) divided to the Predicted No Effect Concentration (PNEC), which is EC50 or LC50 value divided to 1000 in case short-term toxicity data is used.

Table 10 summarizes the toxicity data, the maximum measured concentration and the risk quotients. Risk quotients above 1 indicate potential environmental risk.

Table 10. Estimation of Risk Quotients, RQ (MEC/PNEC) for the emerging organic contaminants contained in treated wastewater.

Emerging contaminants	RQ values		
	Fish	Daphnia magna	Algae
Pharmaceuticals			
Amoxicillin	<1	<1	44
Atorvastatin	NA	<1	NA
Azithromycin	<1	<1	15
Caffeine	<1	<1	927
Clarithromycin	<1	<1	31
Clofibric acid	<1	1.9	<1
Diclofenac	1.3	<1	<1
Fluoxetine	<1	<1	1.2
Gemfibrozil	1.9	<1	<1
Ofloxacin	<1	<1	9.8
Pentobarbital	<1	<1	39
Phenobarbital	<1	<1	18
Sertraline	<1	<1	2.4
Sulfamethoxazole	<1	<1	3.5
Theophylline	<1	<1	38
Tramadol	7.5	13	1
Tylosin	NA	<1	1.2
Valsartan	<1	<1	2.4
Venlafaxine	<1	<1	1.1
Endocrine disrupters			
4-t-octylphenol	1.4	<1	<1
Bisphenol A	7	<1	1.1
Nonylphenol	835	<1	30
Nonylphenol diethoxylate	54	<1	31
Nonylphenol monoethoxylate	32	<1	22
Triclosan	27	<1	4914
Benzotriazoles			
Tolytriazole	<1	<1	1.5
Artificial sweeteners			
Sucralose	<1	<1	113
Siloxanes			
Octamethylcyclotetrasilane	20	NA	NA
Decamethylcyclopentasilane	NA	2076	NA
Dodecamethylcyclohexasilane	NA	NA	30

*NA, not available

Chlorination experiments

The prediction of the acute toxicity of the proposed chlorination by-products of 5,6-dimethyl-1H-benzotriazole (XTRi) and 2-aminobenzothiazole (2-amino-BTH) was realized by ECOSAR software and the calculated values, LC50 (median lethal concentration) for fish and EC50 (median effective concentration) for algae, are listed in Table 11. XTRi and its by-products were considered as benzotriazoles structure-alike chemicals, while 2-amino-BTH and its produced chlorination by-products were considered as unhindered anilines-structure alike chemicals. As it can be concluded from the results, the chlorinated derivatives of both XTRi and 2-amino-BTH are more toxic than their parent compounds while the hydroxylated derivatives were proved to be less hazardous. The toxicity effect of 2-[2-(BTH-2-yl)hydrazin-1-yl]-6-chloro-BTH is not presented because this chemical may not be soluble enough to measure the predicted values. Although these results are not coming from real toxicity experiments, they can be interpreted as an indication of the potential environmental risk of the identified by-products.

Table 11. ECOSAR results for a) 2-amino-BTH, b) XTRi and their identified chlorination by-products respectively.

a)

Organism (96 h)	**Predicted mg/L** (EC 50 for fish and LC 50 for algae)				
	XTRi	**4-chloro-XTRi or7-chloro-XTRi**	**4-hydroxy-XTRi or 7-hydroxy-XTRi**	**4,7-di-hydro-XTRi**	**6-methyl-BTR-5-carbaldehyde or 5-methyl-BTR-6-carbaldehyde**
Fish	9.376	5.238	18.524	36.246	27.872
Green Algae	2.484	1.626	4.418	7.782	6.157

b)

Organism (96 h)	**Predicted mg/L** (EC 50 for fish and LC 50 for algae)				
	2-amino-BTH	**2-amino-6-chloro-BTH**	**2-amino-5,6-di-chloro-BTH**	**2-amino-5,6-di-hydroxy-BTH**	**2-amino-6-chloro-5-hydroxy-BTH**
Fish	21.349	9.336	3.940	120.828	21.913
Green Algae	1.707	1.474	1.229	3.505	2.084

Biotransformation experiments

To estimate the ecotoxicological risk for the aquatic biota due to citalopram (CTR) and its TPs, risk quotients (RQ) were calculated for the Measured Environmental Concentrations (MEC). Predicted No Effect Concentration (PNEC) was also calculated by dividing the lowest short-term L(E)C50 available in the literature or as it was estimated by ECOSAR program, by an appropriate assessment factor (AF) of 1000, since no long-term toxicity data were available. For CTR, EC50 values for daphnia magna and algae were obtained from an already published study (Christensen et al. 2007). For RQ values greater than 1, the ecotoxicological risk for the aquatic environment is raised and further research is needed whereas, in cases where RQ is lower than 1, no ecotoxicological risk is expected for the aquatic biota.

Semi-quantitation of the identified TPs was performed in an effluent wastewater sample, collected in March 2015 from Psytallia, in order to obtain the MEC values. The concentrations of CTR and its TPs, whose structure was confirmed either through the analysis of a reference standard or by library matching, in the effluent sample, PNEC values (along with the used assessment factors) and RQ deemed for each analyte are presented in Table 12. According to these results, both CTR and its TPs have RQ lower than 1 and therefore no risk is expected. However, it should be noted that, given the mixture of these compounds with the same pharmacological mechanisms, additive or even synergistic effects could be expected, posing real environmental threat higher than the calculated one.

Table 12: Risk assessment data for CTR and its confirmed TPs.

Compound	**MEC** ($\mu g\ L^{-1}$)	**PNEC** ($\mu g\ L^{-1}$) **Algae**	**RQ Algae**	**PNEC** ($\mu g\ L^{-1}$) ***Daphnia Magna***	**RQ *Daphnia Magna***	**PNEC** ($\mu g\ L^{-1}$) **Fish**	**RQ Fish**
CTR	1.010	1.60[a]	0.630	20[a]	0.05	4.47	*0.226*
CTR 311	0.329	0.486	0.677	0.838	0.393	5.88	0.056
CTR 339 a	0.023	1.92	0.012	02.77	0.008	21.3	0.001
CTR 341	0.048	1.32	0.036	0.200	0.024	15.0	0.003
CTR 343	0.010	1.40	0.007	2.11	0.005	15.9	0.001
CTR 344	0.008	2.21	0.004	4.33	0.002	28.5	0.001

[a]Values retrieved from the literature (Christensen et al. 2007)

Conclusion and Future Research

An RQ based approach was applied to evaluate the potential threat due to the existence of emerging contaminants in treated wastewater. This method is simple in use, it is applied to micropollutants that are known to be present in the aquatic environment and it is based on actual measurements. It could be used as starting point for organizations just beginning to monitor emerging contaminants and as a screening diagnostic tool for assessing specific sites. According to the results for Greece, EDCs presented the highest risk of all emerging pollutants in both wastewater and rivers. Triclosan (in algae) and nonylphenol (in fish) had the highest RQs among EDCs, while caffeine (in algae) had the highest RQ of all studied pharmaceuticals. The class of emerging contaminants that had the highest contribution to the mixture toxicity, in both wastewater and rivers, was EDCs. Despite the fact that the rivers with low flows presented the highest ecological threat due to the presence of micropollutants, a possible ecological threat cannot be excluded even for rivers with high dilution factors (up to 2388). In the near future, researchers should deepen on assessment of single and mixture toxicity of emerging contaminants and their metabolites to different aquatic organisms. Environmental engineers should think again STPs design aiming to micropollutants removal, while authorities should update relevant legislation, setting limit values for specific emerging contaminants (Thomaidi et al., 2015).

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