# Multiclass target analysis of contaminants of emerging concern including transformation products, soil bioavailability assessment and retrospective screening as tools to evaluate risks associated with reclaimed water reuse

Vasiliki G. Beretsou[a,b], Maria-Christina Nika[c], Kyriakos Manoli[b], Costas Michael[b], Qian Sui[d,e], Lian Lundy[f], D. Michael Revitt[f], Nikolaos S. Thomaidis[c*] and Despo Fatta-Kassinos[a,b*]

[a]*Department of Civil and Environmental Engineering, School of Engineering, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus*

[b]*Nireas-International Water Research Center, School of Engineering, University of Cyprus, P.O. Box 20537, 1678 Nicosia, Cyprus*

[c]*Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771, Athens, Greece*

[d]*State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resources and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, China*

[e]*Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China*

[f]*Middlesex University, Department of Natural Sciences, School of Science and Technology, London NW4 4BT, United Kingdom*

*Corresponding authors.

E-mail addresses: dfatta@ucy.ac.cy (D. Fatta-Kassinos), ntho@chem.uoa.gr (N.S. Thomaidis)

## Abstract

The occurrence of 200 multiclass contaminants of emerging concern (CECs) encompassing 168 medicinal products and transformation products (TPs), 5 artificial sweeteners, 12 industrial chemicals, and 15 other compounds was investigated in influent and effluent wastewater samples collected during 7 consecutive days from 5 wastewater treatment plants (WWTPs) located in Cyprus. The methodology included a generic solid-phase extraction protocol using mixed-bed cartridges followed by Ultra-High Performance Liquid Chromatography coupled with Quadrupole-Time of Flight Mass Spectrometry (UHPLC-QTOF-MS) analysis. A total of 63 CECs were detected at least in one sample, with 52 and 55 out of the 200 compounds detected in influents and effluents, respectively. Ten out of the 24 families of parent compounds and associated TPs were found in the wastewater samples (influent or effluent).

Tramadol, carbamazepine, venlafaxine, citalopram, lamotrigine, sucralose, and 1-H-benzotriazole (>80% frequency of appearance in effluents) were assessed with respect to their bioavailability in soil as part of different scenarios of irrigation with reclaimed water following a qualitative approach. A high score of 12 (high probability) was predicted for 2 scenarios, a low score of 3 (rare occasions) for 2 scenarios, while the rest 28 scenarios had scores 5-8 (unlikely or limited possibility).

Retrospective screening was performed with the use of a target database of 2466 compounds and led to the detection of 158 additional compounds (medicinal products (65), medicinal products TPs (15), illicit drugs (7), illicit drugs TPs (3), industrial chemicals (11), plant protection products (25), plant protection products TPs (10), and various other compounds (22).

This work aspires to showcase how the presence of CECs in wastewater could be investigated and assessed at WWTP level, including an expert-based methodology for assessing the soil bioavailability of CECs, with the aim to develop sustainable practices and enhance reclaimed water reuse.

**Keywords:** microcontaminants, high-resolution mass spectrometry, wastewater, Cyprus

## 1. Introduction

Water plays an integral role in many aspects of our daily lives and its importance extends beyond its direct use. In the United Nations' Sustainable Development Goals (SDGs), the availability of safe water is not limited to "Goal 6 - Water and Sanitation" and addresses, often indirectly, most of the other SDGs, from poverty and hunger to climate action and inequality (United Nations, 2015). Different factors such as increasing population, climate change, intensive agricultural practices, and urbanization constitute a challenge that requires a transformation of the water industry based on the combination of innovative technologies and new management approaches, with the aim to supply, protect, and reuse water in agricultural and urban contexts in the framework of circular economy.

Based on the "think global, act local" approach, the European Green Deal provides an action plan for a systemic transformation towards a climate-neutral continent by 2050, and constitutes an integral part of the European Commission's strategy to implement the United Nations' 2030 Agenda and the SDGs (European Commission, 2019a). Since the impact of research and innovation should be reflected on policy as well, EU launched, in September 2020, a €1 billion Call for research projects that respond to the climate crisis and help to protect human health and

65 Europe's unique ecosystems and biodiversity towards sustainability and a toxic-free environment.
66 Indeed, awareness about the human exposure to a true "cocktail" of chemicals (the so-called
67 contaminants of emerging concern, CECs) is increasing (Wang et al., 2021; Brack et al., 2022).
68 Importantly, only a fraction of CECs has been identified in wastewater so far, while their
69 transformation products (TPs) are mostly overlooked. It should be mentioned that in the
70 framework of this study the term "TPs" is used to describe both metabolites, which are compounds
71 resulting from human and/or animal metabolism, and TPs resulting from biotic and/or abiotic
72 processes (Fatta-Kassinos et al., 2011a).

73 Wastewater Treatment Plants (WWTPs) can be considered as an interface between the
74 anthropogenic activity and the environment, and they have been identified as key point sources of
75 CECs to the aquatic and terrestrial environment. Due to their ubiquitous distribution, pseudo-
76 persistence, and potential adverse activity to the human and ecological health, CECs and their TPs
77 require immediate attention as stated extensively in the scientific literature (Evgenidou et al., 2015;
78 Escher et al., 2020; Ibáñez et al., 2021). The need to address challenges related to CECs is also
79 recognized by the EC communication on "European Union strategic approach to pharmaceuticals
80 in the environment" (European Commission, 2019b) and "Chemicals' strategy for sustainability
81 towards a toxic-free environment" (European Commission, 2020), and OECD report on
82 "Pharmaceutical Residues in Freshwater" (OECD, 2019). A recent development is that on the 2$^{nd}$
83 of March 2022, the 5$^{th}$ UN Environment Assembly concluded with 14 resolutions to strengthen
84 actions for nature to achieve the Sustainable Development Goals. Along with putting an end to
85 plastic pollution, a second key resolution supports the establishment of a comprehensive and
86 ambitious science-policy panel on the sound management of chemicals and waste and preventing
87 pollution.

As widely accepted, CECs are not sufficiently removed by the existing wastewater treatment processes, and therefore, the application of analytical methodologies to investigate the presence of a wide range of CECs in wastewater is of paramount importance. With the development of high-resolution mass spectrometry (HRMS), a new horizon has opened in analytical chemistry providing new opportunities for the analysis of CECs. HRMS offers a powerful and suitable alternative to former low-resolution targeted methods. The high mass accuracy and resolution, together with the extensive variety of available acquisition modes make HRMS the technique of choice for wide-scope target, suspect and non-target screening of thousands of compounds (Bletsou et al., 2015; Leendert et al., 2015; Menger et al., 2020). This allowed the identification and quantification of CECs in WWTPs in many countries worldwide (Arsand et al., 2018; Boix et al., 2016; Choi et al., 2021; Ofrydopoulou et al., 2022). Moreover, the archiving of HRMS data also allows for data to be processed retrospectively; for example, to investigate the occurrence of a newly identified compound or simply one that was not considered at the time of analysis. The capability has even led to proposals for the establishment of data repositories, similar to environmental data banks, where digital information can be safely stored for future retrospective analysis (Alygizakis et al., 2018).

The occurrence of CECs in WWTPs has received considerable attention in the scientific literature with hundreds of publications addressing this issue during the last decade. However, previous studies mostly focused on a single WWTP and on parent compounds. Limited work has been done so far on WWTPs employing different treatment processes and the determination of TPs of CECs. In the case of Cyprus, the only reported study on the wide-scope screening of CECs in wastewater was also limited to the effluent of a single WWTP (Alygizakis et al., 2020), and no

110 information on the presence of CECs in influent wastewaters and other WWTPs employing
111 different treatment processes is available in the literature.

112 Considering that the effluent of WWTPs is either discharged to the aquatic environment
113 (e.g., surface waters such as lakes and rivers) or is reused in agricultural applications (e.g., crop
114 irrigation), it is of utmost importance to identify and quantify CECs in WWTPs. In June 2020, the
115 Regulation EU 2020/741 of the European Parliament and of the Council of 25 May 2020 on
116 minimum requirements for water reuse was published (European Union, 2020), according to which
117 "the potential impact of substances of emerging concern" should be considered. With the
118 increasing drive from both policy and practice to facilitate reclaimed water reuse in agricultural
119 irrigation, there is an urgent need to identify and characterize potential risks associated with the
120 occurrence of CECs in treated wastewater (Fatta-Kassinos et al., 2011b; Revitt et al., 2021). To
121 the best of the authors' knowledge, very limited work has been done so far in relation to reclaimed
122 water reuse and assessment of the soil bioavailability of CECs (Lin et al., 2020). This is of great
123 importance for irrigation with reclaimed water considering that CECs may be implicated in various
124 adverse effects regarding human health and/or the ecosystem (López-Pacheco et al., 2019; Nilsen
125 et al., 2019).

126 The objectives of this study were the following: (a) a comprehensive quantitative target
127 analysis of 200 multiclass CECs, including medicinal products and their associated TPs, industrial
128 chemicals, artificial sweeteners, and other compounds in influent and effluent wastewater, (b) a
129 comparative assessment of the occurrence of parent compounds and their associated TPs in
130 influent and effluent wastewater, (c) an evaluation of the soil bioavailability of selected CECs
131 present in effluent wastewater samples, in order to exploit the developed data set of the occurrence
132 of CECs towards the assessment of potential risks associated with reclaimed water reuse in a real-

133 context reuse scenario, such as the case of Cyprus, and (d) a retrospective screening of the
134 wastewater samples with the use of a database of 2466 CECs (list S21 UATHTARGETS on the
135 NORMAN Suspect List Exchange, https://www.norman-network.com/nds/SLE/ and on Zenodo
136 open data repository, https://zenodo.org/record/6323651). The results of this study may be useful
137 to better understand the level of occurrence of a wide array of CECs including TPs in WWTPs
138 employing different wastewater treatment processes. The work on the assessment of the soil
139 bioavailability of CECs may guide future studies in evaluating the bioavailability of other CECs
140 detected in the effluents of WWTPs globally.

141

142 **2. Materials and methods**

143 **2.1 Study area and sample collection**

144 The sampling campaign of the 24-h composite influent and effluent wastewater samples
145 was performed in April 2018 for 7 consecutive days at the 5 largest WWTPs in Cyprus, serving 2
146 catchment areas in Nicosia, 1 in Limassol, 1 in Larnaca, and 1 in Paphos. The studied WWTPs
147 employ different wastewater treatment processes, i.e., 2 WWTPs utilize conventional activated
148 sludge (CAS) process followed by chlorination (i.e., CAS+chlorination I and CAS+chlorination
149 II), 2 WWTPs employ membrane bioreactor (MBR) technology (i.e., MBR I and MBR II), and 1
150 WWTP with MBR followed by chlorination (i.e., MBR+chlorination). The hydraulic retention
151 time (HRT), solids retention time (SRT), average population equivalent (PE) and average influent
152 flow rate of each WWTP are presented in **Table S1 of the Supplementary material**. The main
153 water quality characteristics of influent and effluent wastewater samples are presented in **Tables**
154 **S2** and **S3**, respectively.

155

156 **2.2 Chemicals and reagents**

157 The list of 200 multiclass target compounds including their molecular formulas, CAS
158 numbers, and PubChem CID is provided in **Table S4**. The list was comprised of 168 medicinal
159 products and associated TPs, 5 artificial sweeteners, 12 industrial chemicals, and 15 compounds
160 belonging to other categories. The target analytes selection was based on environmental relevance,
161 potential environmental hazards, previous detection in the water cycle, the national market as well
162 as the necessity to fill the gaps in unavailable data. Details on the used chemicals and reagents are
163 provided in **Text S1**.

164

165 **2.3 Sample preparation**

166 Influent and effluent wastewater samples of initial volume of 100 mL were extracted using
167 a slightly modified protocol from the one developed by Kern et al. (2009). Solid-phase extraction
168 (SPE) was conducted using four different SPE materials simultaneously in an in-house mixed-bed
169 cartridge to achieve sufficient enrichment for a very broad range of compounds with different
170 physicochemical properties (200 mg Oasis HLB, 150 mg Isolute ENV+, 100 mg Strata-X-AW and
171 100 mg Strata-X-CW). Details on the procedure can be found in **Text S2**.

172

173 **2.4 Instrumental analysis**

174 Analysis was carried out using a UHPLC-QTOF-MS system, equipped with a UHPLC
175 apparatus (Dionex UltiMate 3000 RSLC, Thermo Fisher Scientific, Dreieich, Germany),

consisting of a solvent rack degasser, auto-sampler, a binary pump with solvent selection valve and a column oven coupled to the QTOF-MS mass analyzer (Maxis Impact, Bruker Daltonics, Bremen, Germany). The QTOF-MS system was equipped with an electrospray ionization (ESI) source, operating in positive and negative ionization mode. An Acclaim RSLC C18 column (2.1 × 100 mm, 2.2 μm) from Thermo Fisher Scientific (Dreieich, Germany), preceded by an ACQUITY UPLC BEH C18 1.7 μm, VanGuard Pre-Column from Waters (Dublin, Ireland), and thermostated at 30 °C, was used. The instrumental analysis is described elsewhere (Gago-Ferrero et al., 2020) and further details are given in **Text S3**.

### 2.5 Quality assurance and quality control (QA/QC) parameters

A thorough experimental design was performed considering the different complexity of each matrix. For this purpose, pooled samples of each matrix and of each WWTP were spiked at two different concentration levels (i.e., 100 ng $L^{-1}$ and 500 ng $L^{-1}$) with the 200 target compounds and structure-related isotope labeled compounds (IS), processed using the above methods and analyzed together with the wastewater samples. It should be mentioned that spiked samples were prepared at two concentration levels, since the method is already validated and calibration curves in wastewater matrices were already available, and the recoveries are provided in Gago-Ferrero et al. (2020). Meanwhile, procedural (reagent) blank samples always ran in parallel and were used for the subtraction of eventual laboratory-introduced contamination.

Compound-specific Limit of Detection (LOD) and Limit of Quantification (LOQ) values were determined as the concentration of a compound in the spiked sample that corresponds to signal-to-noise ratio that equals to 3.3 and 10, respectively. The analyzed concentrations that were

198 below LOQ were substituted with LOQ/2 values (European Commission, 2009; Yu et al., 2021),
199 while those that were below LOD were substituted with LOD/2 values (Hites, 2019).

200

201 **2.6 Quantitative target analysis performance criteria**

202 Quantitative target analysis was performed with software TASQ® 2.1 and DataAnalysis®
203 5.0 (Bruker Daltonics, Bremen, Germany). The detection was based on strict screening criteria
204 (mass accuracy < 2 mDa, retention time shift ± 0.2 min, isotopic fitting < 100 mSigma (only for
205 confirmation of positive findings)), whereas the presence of fragment ions confirmed the analytes.
206 The methodology was described in detail elsewhere (Gago-Ferrero et al., 2020).

207

208 **2.7 Retrospective screening performance criteria**

209 The same criteria of quantitative target analysis were also applied for retrospective
210 screening, where a target database with the 2266 remaining compounds was built with information
211 from already available reference standards, containing information about experimental retention
212 time and MS/MS fragments that were not initially considered. Moreover, to increase comparability
213 between influent and effluent wastewater samples and to decrease matrix effect, the most stable
214 IS was used to normalize the peak areas (i.e., peak area of the detected CEC divided by the peak
215 area of the appropriate IS in each sample). More specifically, sulfadimethoxine-d4 was used for
216 positive ionization, while bisphenol A-d16 was used for negative ionization. This approach was
217 based on Nikolopoulou et al. (2022).

218

## 2.8 Assessing the soil bioavailability of selected CECs as part of a scenario of irrigation with reclaimed water

The key variables with potential to impact the fate of CECs present in treated wastewater within agricultural irrigation have been presented (NEREUS Deliverable 20, 2017) by the NEREUS COST Action ES1403 network, http://www.nereus-cost.eu/; a global network of 380 researchers working in the field of reclaimed water reuse in a variety of disciplines. These key variables include sources contributing to raw wastewater, the level of wastewater treatment, the effect of storage and transportation prior to use, the technique used for soil irrigation, the resulting CEC load in treated wastewater, the soil CEC bioavailability/bioaccesibility behavior and the adoption of biosolid/fertilizer addition to soils and ploughing practices. The data analysis that was followed herein was described by Revitt et al. (2021), parts of which are reproduced in **Text S4**.

The assessment approach combines these data and expert judgement to assess the "likelihood of occurrence" and the "magnitude of impact" of selected CECs in soil, during irrigation with reclaimed water. Multiplying the "likelihood of occurrence" score by the "magnitude of impact" score (where scores are allocated on the basis of pre-defined data ranges) provides an overall assessment score. This overall assessment score can be ranked per substance to identify relative levels of concern in relation to their introduction into soils irrigated with reclaimed water and highlight where further consideration is required about the presence of a particular scenario (i.e., substance-WWTP). The following score ranges are proposed for distinguishing between CECs and their relative potential to influence the health of the soil environment. The scoring system of the occurrence of a CEC in soil in a bioavailable form is as follows: only on very rare occasions (Score 1-4), unlikely or limited possibility (Score 5-8), possibly (Score 9-11), or with a high probability (Score 12-16).

242

243 ## 3. Results and discussion

244 ### 3.1 Quantitative target analysis of 200 multiclass CECs in influent and effluent wastewater

245 Two hundred (200) multiclass CECs, including 168 medicinal products and associated
246 TPs, 5 artificial sweeteners, 12 industrial chemicals, and 15 compounds belonging to various
247 categories, were monitored in influent and effluent wastewater samples collected during 7
248 consecutive days from 5 WWTPs located in Cyprus. Among the 200 compounds analyzed, 63
249 compounds were detected at least in one of the wastewater samples (influent or effluent
250 wastewater). **Table S5** presents the concentrations of the detected compounds in the 7 influent and
251 7 effluent samples collected from each WWTP. **Tables S6** and **S7** present the minimum,
252 maximum, mean and median concentration of the compounds and their % FoA in influent (N=35)
253 and effluent (N=35) wastewater samples collected from the 5 studied WWTPs along with the
254 respective LODs and LOQs of the compounds.

255

256 #### 3.1.1 Occurrence of CECs in influent wastewater

257 **Figure 1a** presents the mean concentration and FoA of the detected CECs in the influent
258 wastewater samples. Fifty-two (52) compounds were detected at least once in the influent
259 wastewater samples. Out of the 52 compounds detected, 35 were medicinal products, 7 were
260 medicinal products TPs, 4 were artificial sweeteners, 3 were industrial chemicals and 3 belonged
261 to various categories. Interestingly, 36 compounds, including atenolol, carbamazepine,
262 clarithromycin, diclofenac, metformin, valsartan, venlafaxine, saccharine, sucralose, and caffeine,
263 were detected in all WWTPs influents, i.e., 100% FoA. Notably, 4 out of the 36 compounds with

264 100% FoA, namely metoprolol acid (atenolol acid), 10,11-dihydro-10,11 dihydroxy
265 carbamazepine, N-acetyl sulfamethoxazole and D,LN,O-Didesmethyl venlafaxine belong to
266 pharmaceutical TPs, i.e., TP of atenolol and metoprolol, carbamazepine, sulfamethoxazole and
267 venlafaxine, respectively. Pharmaceuticals are known to interact with conserved molecular
268 receptors, which can result in biological disruption in non-target organisms. The medicinal
269 products TPs may retain the moiety responsible for their pharmacological activity, thereby
270 continuing to exhibit the targeted mechanism of the parent compound. This observation indicates
271 the importance of monitoring TPs along with the parent compounds in wastewater. Due to the fact
272 that most of the studies available in the literature focused on the parent compounds, limited
273 information is available so far regarding the presence of medicinal products TPs in wastewater
274 employing different treatment process (Ibanez et al., 2021).

275 The highest mean concentrations were observed for metformin (160845 ng $L^{-1}$) followed
276 by caffeine (76853 ng $L^{-1}$), paracetamol (32964 ng $L^{-1}$) and sucralose (30215 ng $L^{-1}$), while the
277 lowest mean concentrations were found for N-Desmethyl citalopram (7.57 ng $L^{-1}$), N-acetyl
278 sulfamethoxazole (51.0 ng $L^{-1}$) and D,LN,O-Didesmethyl venlafaxine (85.7 ng $L^{-1}$). The
279 aforementioned highest and lowest concentrations of these CECs are in line with previous studies
280 that monitored these compounds in influents of WWTPs worldwide (Han and Gin, 2017; Paíga et
281 al., 2019).

282 The 10 most abundant compounds that were detected in the influent wastewater of each
283 WWTP are presented in **Figure 2**. Interestingly, 9 of the 10 most abundant compounds, i.e.,
284 acesulfame, caffeine, cyclamic acid, metformin, paracetamol, saccharine, sucralose, theophylline
285 and valsartan, were common in all WWTPs. Diclofenac was the tenth most abundant compound
286 in 3 WWTPs, while naproxen and gemfibrozil were the tenth most abundant compounds in the

287 remaining 2 WWTPs. These similarities regarding the most abundant compounds in the influents
288 of 5 WWTPs may be explained by the fact that Cyprus is an island with population of less than 1
289 million and the consumption habits of inhabitants within the studied sewage catchment areas are
290 similar.

291 **Table S8** presents the minimum, maximum and mean concentrations in influent
292 wastewater of each WWTP. It is noteworthy that the aforementioned most abundant compounds
293 were found at considerably elevated concentrations in the influents (up to μg $L^{-1}$ levels). These
294 findings are in agreement with previously reported studies carried out in other European countries
295 (Golovko et al., 2021; Ofrydopoulou et al., 2022). For example, elevated concentrations of 56500
296 ng $L^{-1}$ for caffeine, 21000 ng $L^{-1}$ for metformin (Golovko et al., 2021), and 56226 ng $L^{-1}$ for
297 caffeine (Ofrydopoulou et al., 2022) were reported in influent wastewater in Sweden and Greece,
298 respectively. Metformin is by far the most frequently prescribed antidiabetic drug worldwide and
299 it is usually taken in relatively high doses of 0.5-2 g $day^{-1}$. It has been shown that metformin is not
300 completely metabolized in the human body (Krentz and Bailey, 2005), and it is excreted
301 unchanged and therefore, released into the environment via wastewater. As a result, and in
302 accordance with our study, metformin has one of the highest environmental emission rates among
303 commonly prescribed drugs, a fact that has been presented already by earlier studies (Briones et
304 al., 2016; Scheurer et al., 2009).

305

306 3.1.2 Occurrence of CECs in effluent wastewater

307 **Figure 1b** shows the mean concentration and FoA of the detected CECs in the effluent
308 wastewater samples. Fifty-five (55) compounds were detected at least once in the effluent

309 wastewater samples. Among the 55 compounds, 30 were medicinal products, 15 were medicinal
310 products TPs, 5 were artificial sweeteners, 3 were industrial chemicals, while the rest of them
311 belonged to other categories. Importantly, the number of TPs detected in the effluents increased
312 from 7 (in influents) to 15 compounds (in effluents), indicating the formation of 8 additional TPs,
313 i.e., carbamazepine-10,11-epoxide, citalopram amide, N-Desmethyl clarithromycin, guanylurea,
314 norlidocaine, tramadol-N-oxide, N-Desmethyl venlafaxine and venlafaxine-N-oxide. Considering
315 that these compounds were not detected in influent samples, it is presumed that their formation
316 took place during the treatment of wastewater by the various processes employed in the studied
317 WWTPs.

318 Thirty (30) compounds, including carbamazepine and carbamazepine TPs, diclofenac,
319 guanylurea, lamotrigine, valsartan, sucralose, 1-H-benzotriazole, venlafaxine and venlafaxine TPs
320 were detected in 80% of the effluent wastewater samples. A 100% FoA was observed for only 3
321 out of 30 compounds, i.e., 1-H-benzotriazole, sucralose and N-Desmethyl citalopram. On the other
322 hand, ephedrine, ketoprofen, mefenamic acid, naproxen, 2-hydroxy-benzothiazole and
323 progesterone were not detected in any of the effluent wastewater samples, despite their presence
324 in influents. This finding suggests the degradation of these compounds during wastewater
325 treatment. The highest mean concentrations were recorded for sucralose (21962 ng $L^{-1}$), diclofenac
326 (2945 ng $L^{-1}$), guanylurea (2824 ng $L^{-1}$), valsartan (1056 ng $L^{-1}$), 1-H-benzotriazole (1075 ng $L^{-1}$),
327 while the lowest concentrations were observed for venlafaxine-N-oxide (12.1 ng $L^{-1}$), tramadol-
328 N-oxide (16.1 ng $L^{-1}$), norephedrine (17.4 ng $L^{-1}$), N-Desmethyl tramadol (22.2 ng $L^{-1}$) and N-
329 Desmethyl citalopram (26.7 ng $L^{-1}$).

330 **Figure S1** presents the effluent concentration values of the 10 most abundant compounds
331 as determined in the influent of each WWTP. It should be highlighted that cyclamic acid, caffeine,

paracetamol and theophylline were below the LOD in all WWTP effluents, irrespectively of the treatment process. Caffeine and paracetamol removal accords with earlier studies, which showed, that despite their elevated concentrations in the influents, the concentration of these compounds in the effluents were below LOD (Paiga et al., 2019). Although metformin exhibited considerably lower concentrations in the effluent than influent of each WWTP, its concentration was still considerably high in the effluents with a mean concentration of 572 ng $L^{-1}$.

**Figure 3** presents the 10 most abundant compounds that were detected in the effluent wastewater of each WWTP. A general observation is that the most abundant compound in each WWTP is sucralose, suggesting that this compound could not be degraded efficiently by the treatment processes applied in the studied WWTPs., i.e., CAS+chlorination, MBR and MBR+chlorination. This finding agrees with previous studies which found that sucralose is not liable to microbial degradation due to the presence of chlorine atoms in its structure (Ferrer and Thurman, 2010). For example, no considerable removal of sucralose by the activated sludge treatment process was reported (Subedi and Kannan, 2014). Moreover, 1-H-benzotriazole, lamotrigine and sucralose are common among the most abundant compounds in the effluent of all WWTPs. This observation may be attributed to the fact that these compounds are quite hydrophilic with logKow less than 4, and as a result, they "prefer" the aqueous phase and thus, "escape" the treatment. For example, the removal rate of 1-H-benzotriazole in most WWTPs is <80% according to previous studies (Shi et al., 2019) confirming the results of our study regarding its high abundance in the effluents of the studied WWTPs. **Table S9** presents the minimum, maximum and mean concentrations of CECs in effluent wastewater of each WWTP. Overall, varying FoA or concentration patterns were observed within different WWTP effluents. This may be due to the different treatment processes employed in each WWTP, the specific physicochemical

355 characteristics of each WWTP such as HRT, SRT and microbial activity, and the physicochemical
356 characteristics of each CEC (Archer et al., 2017).

357

358 **3.2 Families of parent compounds and their associated TPs in influent and effluent**
359 **wastewater**

360 The occurrence of twenty-four (24) families of parent compounds and their associated TPs
361 (**Table S22**) was investigated in influent and effluent wastewater. In the framework of this study,
362 the term "families of parent compounds and their associated TPs" is defined either as one parent
363 compound and one associated TP or as one parent compound and two or more associated TPs.
364 Only the families with at least one associated TP detected in the influent or effluent wastewater
365 will be discussed further below. The concentrations of the detected families in the influent and
366 effluent wastewater of the studied WWTPs are shown in **Figures 4** and **5**.

367 **Carbamazepine/10,11-dihydro-10,11 dihydroxy carbamazepine and carbamazepine-**
368 **10,11-epoxide:** Carbamazepine is metabolized in the liver, generating the metabolite
369 carbamazepine-10,11-epoxide that is pharmacologically active. This compound is then hydrolyzed
370 to 10,11-dihydro-10,11-dihydroxy carbamazepine (Heye et al., 2016). Interestingly,
371 carbamazepine-10,11-epoxide was not detected in any influent wastewater sample (**Figure 4a**),
372 but it was detected in all effluent wastewaters (**Figure 4f**). In a study by García-Galán et al. (2016),
373 carbamazepine-10,11-epoxide was detected in both influent and CAS/MBR effluents. This is in
374 partial agreement with our work. It is interesting to note though that carbamazepine 10,11-dihydro-
375 10,11-dihydroxy was detected in both influent (**Figure 4a**) and effluent (**Figure 4f**) wastewater.
376 This finding is consistent with that of Gracia-Lor et al. (2014), who detected this analyte in both

377 influent and effluent matrices indicating that it escapes wastewater treatment. Since no details on
378 the treatment technology applied in that study are given (Garcia-Lor et al., 2014), no comparison
379 on the treatment processes could be made.

380 **Citalopram/N-Desmethyl citalopram, citalopram amide, citalopram carboxylic acid**
381 **and 3-oxo-citalopram:** Citalopram has been detected in all influent samples apart from the
382 influent of CAS+chlorination I (**Figure 4b**) and in all effluent samples. N-Desmethyl citalopram
383 has been detected in all effluent samples and citalopram amide only in the effluent of
384 CAS+chlorination II (**Figure 4g**). This indicates that MBR treatment did not result in the formation
385 of citalopram amide. It should be mentioned that the TPs of citalopram included in this study had
386 been previously identified both in activated sludge experiments of citalopram and in real influent
387 and CAS effluent wastewater samples through retrospective analysis (Beretsou et al., 2016).

388 **Clarithromycin/N-Desmethyl clarithromycin:** Clarithromycin was detected in all
389 influent samples but N-Desmethyl clarithromycin was not detected in any of the influent samples
390 (**Figure 4c**). Both clarithromycin and N-Desmethyl clarithromycin were only detected in the
391 effluent samples of CAS+chlorination II (**Figure 4h**). This suggests that N-demethylation is a
392 reaction of importance for the activated sludge process and/or chlorination. In a study by Rubirola
393 et al. (2019), N-Desmethyl clarithromycin has been detected in influent wastewater and also
394 generated after chlorine dioxide treatment. It is noteworthy that the compound was not fully
395 eliminated by subsequent activated carbon treatment thereby requiring the use of reverse osmosis
396 for its efficient removal (Rubirola et al., 2019). One of the issues that emerges from these findings
397 is that the occurrence and persistence of TPs of antibiotics such as N-Desmethyl clarithromycin,
398 which can retain their antimicrobial activity, could select for antibiotic-resistant bacteria and
399 potentially contribute to antibiotic resistance.

400 **Ephedrine/Norephedrine:** Both ephedrine and norephedrine were detected in the
401 influents of all studied WWTPs (**Figure 4d**). Ephedrine was not detected in any of the effluent
402 samples, indicating its degradation by both MBR- and CAS-based processes. Norephedrine was
403 detected in the effluent of CAS+chlorination I and MBR I (**Figure 4i**). Huerta-Fontela et al. (2008)
404 observed removal rates > 91% for activated sludge treatment and > 85% for trickling filter
405 treatment for ephedrine and norephedrine, respectively. Interestingly, it has been reported that
406 norephedrine was not detected in the effluents of CAS treatment followed by UV (Paiga et al.,
407 2019).

408 **Lidocaine/norlidocaine and lidocaine-N-oxide:** Lidocaine was detected in all the influent
409 samples, while norlidocaine and lidocaine-N-oxide were not detected in any of the influents
410 (**Figure 4e**). Norlidocaine was detected in the effluent of MBR I and II and in the effluent of
411 CAS+chlorination II (**Figure 4j**), while lidocaine-N-oxide has not been detected in any of the
412 effluent samples. Interestingly, Gulde et al. (2016) observed that lidocaine-N-oxide can be back-
413 transformed to the parent lidocaine and possibly this could be the reason why this compound was
414 not detected in any of the effluent samples in the present study. Moreover, our study supports what
415 Gulde et al., report on that N-demethylation which is the reaction through which norlidocaine is
416 formed is of primary important in CAS processes. Our study suggests that N-demethylation of
417 lidocaine, which is the reaction through which norlidocaine is formed, is of primary importance
418 not only for CAS processes (Gulde et al., 2016), but also for MBR processes.

419 **Metformin/guanylurea:** Metformin was detected in both influent (**Figure 5a**) and effluent
420 (**Figure 5f**) wastewater, while its concentration in effluent wastewater was lower than the influent.
421 Metformin is excreted unchanged from the human body (Gong et al., 2012), and therefore,
422 guanylurea was not expected to be detected in influent wastewater. Indeed, guanylurea was only

423 detected in all effluent wastewater samples apart from those from MBR+chlorination (**Figure 5f**),
424 indicating that metformin is being transformed to guanylurea during CAS+chlorination and MBR
425 treatment processes. Chlorination of metformin in water showed the transformation of the
426 compound to guanylurea at wastewater-relevant chlorine concentrations and contact times
427 (Scheurer et al., 2012). Moreover, Trautwein and Kümmerer (2011) have reported that metformin
428 is aerobically biodegraded to guanylurea, which is a very recalcitrant dead-end TP, being stable
429 against further photo- and biodegradation. Guanylurea had higher concentrations than metformin
430 in MBR I, MBR II, CAS+chlorination I, while it had similar concentrations with metformin in
431 CAS+chlorination II. Previous research on guanylurea has shown that it can be toxic and induce
432 stress to zebrafish embryos (Elizalde-Velázquez et al., 2021). Scheurer et al. (2012) also stated
433 that high metformin concentrations could be an index for untreated wastewaters, whereas low
434 metformin and high guanylurea concentrations could be an index for treated wastewater.

435 **Metoprolol, atenolol/metoprolol acid or atenolol acid and atenolol-desisopropyl:**
436 Metoprolol acid is both a metabolite of metoprolol and a TP formed during wastewater treatment
437 of metoprolol, and sometimes more recalcitrant than the parent compound (Rubirola et al., 2014).
438 Moreover, it has been reported as a TP of atenolol formed during the CAS process (Radjenović et
439 al., 2008). For the reasons mentioned above, the TP of metoprolol acid or atenolol acid is plotted
440 with both metoprolol and atenolol (**Figures 5b** and **5g**). Interestingly, metoprolol acid or atenolol
441 acid has the same or higher concentration than the parent compounds in influents (**Figure 5b**).
442 This was also observed in the effluents of all the studied WWTPs (**Figure 5g**). It is noteworthy,
443 that even though we cannot distinguish if the TP is coming from atenolol or metoprolol, it is a
444 compound of interest due to its elevated concentrations and its persistence during the wastewater
445 treatment processes.

446 **Sulfamethoxazole/N-acetyl sulfamethoxazole and N-hydroxy sulfamethoxazole:** Only
447 14% of ingested sulfamethoxazole is excreted by human in its original form, yielding a large
448 fraction of metabolites. The most prominent metabolite is N-acetyl sulfamethoxazole, which
449 represents 50% of the excreted administered dose. Other metabolites include sulfamethoxazole β-
450 D-glucuronide (9%), N-hydroxy sulfamethoxazole (2.2%), and minor fractions of 4-nitroso
451 sulfamethoxazole and 4-nitro sulfamethoxazole (Bonvin et al., 2013). In this study,
452 sulfamethoxazole was detected in all influent (**Figure 5c**) and effluent (**Figure 5h**) samples, while
453 N-hydroxy sulfamethoxazole was not detected in any sample. It is possible, therefore, that due to
454 its low yield during the metabolism of sulfamethoxazole in human, N-hydroxy sulfamethoxazole
455 did not reach the WWTPs and as a result was not detected in the influents. Moreover, this finding
456 suggests that hydroxylation reaction may not be a reaction of interest for sulfamethoxazole in CAS
457 and MBR processes. However, hydroxylation is a common reaction in advanced oxidation
458 processes such as ozonation which is applied at full scale as tertiary treatment and as a result, the
459 presence of N-hydroxy sulfamethoxazole should be investigated. Contrary to N-hydroxy
460 sulfamethoxazole, N-acetyl sulfamethoxazole was detected in all the influent samples (**Figure 5c**),
461 but it was detected only in the effluent of MBR I (**Figure 5h**). Göbel et al. (2005) observed that
462 N-acetyl sulfamethoxazole can be back-transformed during the activated sludge process to the
463 parent sulfamethoxazole. This might be the reason why it was not detected it in CAS+chlorination
464 effluents. It should be also highlighted that the presence of this TP in the effluents of our study is
465 of particular interest because although this derivative does not have pharmacological activity, it
466 has been reported to be ecotoxic (López-Serna et al., 2012).

467 **Tramadol/N-Desmethyl tramadol, tramadol-N-oxide, N,N-bisdesmethyl tramadol:**
468 Tramadol was detected in all influent (**Figure 5d**) and effluent (**Figure 5i**) samples apart from the

469 effluent of MBR+chlorination. N-Desmethyl tramadol was only detected in the influent and
470 effluent of CAS+chlorination I and CAS+chlorination II. This is in agreement with reported
471 studies (Kostanjevecki et al., 2019). For example, Kostanjevecki et al. (2019) have identified N-
472 Desmethyl tramadol in enriched activated sludge cultures implying that N-demethylation is one of
473 the key mechanisms of the microbial transformation of tramadol. In addition to biotic
474 transformation, N-demethylation was also reported as an important abiotic transformation
475 mechanism which was involved in the photocatalytic degradation of tramadol (Antonopoulou and
476 Konstantinou, 2016). On the other hand, tramadol-N-oxide was not detected in any influent
477 (**Figure 5d**), but it was detected in the effluent of MBR I, MBR II, CAS+chlorination I and
478 CAS+chlorination II (**Figure 5i**) indicating its formation during wastewater treatment processes.

479 **Venlafaxine/ D,LN,O-didesmethyl venlafaxine, N-Desmethyl venlafaxine,**
480 **venlafaxine-N-oxide:** Venlafaxine and D,LN,O-didesmethyl venlafaxine were detected in all
481 influent samples (**Figure 5e**). N-Desmethyl venlafaxine and venlafaxine-N-oxide were not
482 detected in any influent sample. Notably, venlafaxine and its TPs exhibited similar pattern in the
483 effluent of CAS+chlorination I and II, MBR I and II, while none of these compounds was detected
484 in the effluent of MBR+chlorination (**Figure 5j**). Gulde et al. (2016) observed that venlafaxine-
485 N-oxide can be back-transformed to the parent venlafaxine. Moreover, Gulde et al. (2016) also
486 reported that tertiary amines, such as venlafaxine, are preferably oxidized by flavin-containing
487 monooxygenases. Ammonia monooxygenase is considered responsible for the biotransformation
488 of multiple compounds (Men et al., 2017; Su et al., 2021), frequently through oxygen insertions
489 resulting in hydroxylation reactions and sometimes in dehydrogenation or reductive
490 dehalogenation (Helbling et al., 2012; Su et al., 2021).

491

492 **3.3 Assessment of the soil bioavailability of selected CECs in the framework of reclaimed**
493 **water reuse**

494 The aim of the applied assessment approach is to enable practitioners to determine which
495 CECs in reclaimed water potentially pose a threat when applied to soils through irrigation. The
496 approach considers the relative importance of several factors that can influence both the
497 "likelihood of occurrence" and the "magnitude of impact" of CECs in the soil environment. As a
498 "catch all" term, CECs exhibit a wide range of sources, abundances, bioavailability, and
499 physicochemical properties. This assessment scheme has been developed to integrate as much
500 appropriate and current information as possible, together with understandings of the influence of
501 wastewater treatment processes, use of storage prior to irrigation and the type of irrigation
502 technique to be integrated within respective stages of the risk assessment process. Therefore, this
503 methodology represents a practical and expert-based approach to assess the soil bioavailability
504 after irrigation with reclaimed water containing CECs. Tramadol, carbamazepine, venlafaxine,
505 citalopram, lamotrigine, sucralose and 1-H-benzotriazole have been chosen for the application of
506 the soil bioavailability scheme due to their high percentage of FoA >80% in the effluent
507 wastewater samples (**Figure 1b**).

508 **Table 1** presents the overall assessment scores for the selected CECs in irrigated soils using
509 treated wastewater effluents from the studied WWTPs. An integrated score of 12 was predicted
510 for two irrigation scenarios indicating that tramadol and venlafaxine in the effluents produced by
511 MBR II showed a high probability of occurring in irrigated soil in a bioavailable form. An
512 integrated score of 9 was reported for 11 scenarios indicating the possibility of assessed substances
513 occurring in soil in a bioavailable form. This is the case for tramadol in the effluents of
514 CAS+chlorination I, CAS+chlorination II and MBR I, for carbamazepine in the effluents of

515 CAS+chlorination I and CAS+chlorination II, for venlafaxine in the effluents of CAS+chlorination
516 I, CAS+chlorination II and MBR I, and for 1-H-benzotriazole in the effluents of CAS+chlorination
517 I, MBR I and MBR+chlorination. The CEC showing the lowest score is lamotrigine with its
518 presence in treated effluents from CAS+chlorination I and II and MBR+chlorination that was
519 predicted to rarely demonstrate any potential for uptake by a receptor. A similar score was
520 calculated for citalopram in the treated effluent from MBR+chlorination.

521 These results show that the one of the main limitations of the applied methodology to assess
522 the bioavailability of the selected compounds in soil is that the factors/parameters that are
523 considered contribute equally to the overall risk, i.e., the descriptors are not weighted. Moreover,
524 this methodology should be improved so it can be applied to TPs apart from parent compounds.
525 The lack of risk assessment schemes related to reclaimed water reuse in agricultural irrigation
526 constitute an important gap of knowledge for policy, practice, and research. As a result, these
527 findings constitute the first step towards our better understanding of the threats that these CECs
528 pose under such scenarios and may guide the development of risk assessment schemes.

529

530 **3.4 Retrospective screening of >2000 compounds in influent and effluent wastewater**

531 The presence of compounds not initially considered in the target list was performed through
532 retrospective screening without the need for additional analysis. The advantageous ability of
533 HRMS to acquire full-scan accurate-mass data enabled the screening to be further widened from
534 200 to more than 2000 compounds, by reprocessing only raw data and thus, exploiting further the
535 dataset. This target database of 2466 CECs has been used so far in 8 studies, while 4 of them
536 studied wastewater samples (Alygizakis et al., 2019, 2020, 2021; Gago-Ferrero et al., 2020).

537 The results obtained from the retrospective screening are shown in **Figures 6** and **7**. In
538 order to account for potential different matrix effect among different analyzed wastewaters
539 (between influent and effluent of the same WWTP and influent or effluent from different
540 WWTPs), the results are provided as normalized peak areas (i.e., peak area of the detected CEC
541 divided by the peak area of IS in each sample). In total, 158 compounds were detected at least in
542 one of the wastewater samples (influent or effluent wastewater). Among the 158 compounds, 65
543 were medicinal products, 15 were medicinal products TPs, 7 were illicit drugs, 3 were illicit drug
544 TPs, 11 were industrial chemicals, 25 were plant protection products, 10 were plant protection
545 products TPs, while 22 belonged to other categories (i.e., surfactants, personal care products,
546 coffee and tobacco related compounds, steroids, and hormones).

547 **Medicinal products:** Thirty-three (33) compounds in influents and 19 compounds in
548 effluents demonstrated 100% FoA. Among the detected analytes, high peak areas in influents were
549 observed for amisulpride, climbazole, levetiracetam and telmisartan, while in effluents, high peak
550 areas were observed for amisulpride, climbazole and telmisartan.

551 **Medicinal products TPs:** Nine (9) analytes presented 100% FoA in influents, while 2
552 compounds had 100% FoA in effluents. It should be noted that N-acetyl mesalazine was only
553 detected in the influents. This finding is in accordance with Boulard et al. (2018), while another
554 study had reported high removal rates of the parent compound mesalazine in WWTP assuming
555 that the TP had a similar fate (Kasprzyk-Hordern et al., 2009). Clopidogrel carbon acid and
556 amisulpride-N-oxide were detected only in effluents indicating that their formation is a result of
557 the treatment processes.

558 **Illicit drugs:** Seven (7) illicit drugs were detected in influents and effluents, with cocaine
559 not being detected in the effluents. It is worth noting that most of these compounds are monitored

560 in the influents of Cyprus in the framework of the SCORE network for more than 10 years (Ort et
561 al., 2014).

562 **Illicit drug TPs:** Benzoylecgonine (the metabolite of cocaine) presented high normalized
563 peak areas in influents and lower peak areas in the effluents. Due to its stability, benzoylecgonine
564 has been extensively used as a biomarker for back-calculation of cocaine in the populations serving
565 the catchment areas (Ort et al., 2014).

566 **Plant protection products:** Twenty-five (25) plant protection products including
567 insecticides (e.g., acetamiprid, DEET, imidacloprid, pirimiphos-methyl) herbicides and fungicides
568 were detected in both influents and effluents. Imazalil, a compound included in the 3rd Watch List
569 of the compounds to be monitored in surface waters, was also detected (European Commission,
570 2020b). High peak areas were observed for carboxin both in influents and effluents. The high FoA
571 and high abundance of carboxin in wastewater samples is in accordance with Quesnel and Nakhla
572 (2005) and McEachran et al. (2018).

573 **Plant protection products TPs:** Among the 10 plant protection products TPs that were
574 detected, aminobenzimidazole and methiocarb-sulfone were detected only in effluent indicating
575 their formation by the studied treatment processes. Methiocarb-sulfone is a common pesticide TP
576 which has been reported to be found in vegetables such as tomato, zucchini, eggplant and
577 cucumber due to pesticide use (Al-Nasir et al., 2020). Thus, its detection may be of particular
578 interest considering that the pesticides along with pesticides TPs may act as additional burden to
579 the potential uptake of these compounds by edible plants when reclaimed water is used for
580 irrigation.

581 **Other compounds and TPs:** Among the compounds which belong to various categories
582 such as preservatives, stimulants, and surfactants, nicotine, and theobromine (metabolite of
583 caffeine) had considerably high peak areas in the influents, which subsequently were lower in the
584 effluents. Cotinine and hydroxy cotinine, which are metabolites of nicotine and have been used as
585 human biomarkers, have also been detected, with cotinine exhibiting relatively high peak areas in
586 the influents (Senta et al., 2015).

587 Taken together, these findings indicate which microcontaminants are of emerging interest
588 at a WWTP level and may be included in future monitoring campaigns. Since the analytical method
589 is validated following a smart validation approach (Gago-Ferrero et al., 2020) and the reference
590 standards and calibration curves are available, concentration values could be calculated in future
591 studies also considering recovery and ionization efficiency. The data also advise further analysis
592 of how regional or catchment-specific characteristics might alter the relative importance of
593 different emission pathways, and further modelling of emission loads. These results also help us
594 to understand which compounds are continuously introduced in the soil compartments *via*
595 reclaimed water reuse in irrigation, their potential accumulation in soil and provide information
596 about possible uptake by plants and crops.

597

598 **4. Conclusions**

599 This study provides a comprehensive investigation of 200 multiclass CECs including TPs
600 in influent and effluent wastewater samples collected during 7 consecutive days from 5 WWTPs
601 located in Cyprus employing CAS+chlorination, MBR, and MBR+chlorination treatment
602 processes. A key finding of this work is that 10 out of the 24 families of parent compounds and

603 their associated TPs have been detected in the studied wastewater samples (influent or effluent).
604 This underlines the importance of monitoring TPs as well in addition to the parent compounds to
605 gain a better understanding of their behavior during conventional wastewater treatment processes
606 but also, to have more information on what is in the treated wastewater. The increased number of
607 TPs, from 7 in the influents to 15 in the effluents, suggests the formation of 8 additional TPs during
608 the wastewater treatment i.e., carbamazepine-10,11-epoxide (CAS+chlorination, MBR,
609 MBR+chlorination), citalopram amide (CAS+chlorination), N-Desmethyl clarithromycin
610 (CAS+chlorination), norlidocaine (CAS+chlorination, MBR), guanylurea (CAS+chlorination,
611 MBR), tramadol-N-oxide (CAS+chlorination, MBR), N-Desmethyl venlafaxine
612 (CAS+chlorination, MBR) and venlafaxine-N-oxide (CAS+chlorination, MBR). Our results show
613 that most of the TPs are formed during CAS+chlorination and MBR treatment processes.
614 Citalopram amide and N-Desmethyl clarithromycin were only formed during CAS+chlorination,
615 while carbamazepine-10,11-epoxide was the only TP formed by CAS+chlorination, MBR and
616 MBR+chlorination. This helps us understand how parent compounds are transformed during
617 CAS+chlorination, MBR and MBR+chlorination treatment processes in real-world wastewater
618 samples.

619 The retrospective screening, which was performed by analyzing the HRMS raw data,
620 widened further the analysis from 200 to more than 2000 compounds. As a result, retrospective
621 screening allowed for the detection of 158 additional compounds that were not included in the
622 initial target list. The additional compounds belonged to several classes such as medicinal products
623 (and TPs), illicit drugs (and TPs), industrial chemicals, plant protection products (and TPs),
624 personal care products, and coffee- and tobacco-related compounds. These findings revealed
625 which microcontaminants are relevant to be monitored at a WWTP level and may guide the

development of future monitoring campaigns. To obtain a deeper understanding of the presence of chemical compounds in the wastewater, it is suggested that follow-up monitoring studies should be conducted at different times throughout a year.

The findings of the study regarding soil bioavailability of selected CECs can be useful for WWTP operators and policymakers. The bioavailability of CECs in soil varies due to several factors including the quality of the influent wastewater, the treatment processes used at the WWTP, the effluent concentration of the contaminant, its physicochemical properties, and the crop irrigation technique. Tramadol and venlafaxine, in the scenario of a WWTP employing MBR process, showed high probability for soil bioavailability, but limited possibility in a scenario where the WWTP employs MBR+chlorination. The results showed that carbamazepine could be present in soil in a bioavailable form in the scenario of CAS+chlorination, while there was a limited possibility in a scenario of either MBR or MBR+chlorination. The approach applied can be useful in any WWTP for different CECs and crop irrigation techniques, providing information relevant to a specific scenario. Our study emphasizes the necessity of performing such comprehensive studies to assess the risk related to each case which may lead to solutions tailored to the specific treatment and reuse facility. Considering that the presence of CECs in soil and/or crops may have adverse effects on the environment and human health, a greater understanding of these challenges may enhance and facilitate the policy making process. Data on soil bioavailability indicate the compounds that are continuously released into the soil compartment which may be of great interest to policymakers. To establish a more comprehensive understanding of the potential impact of irrigation with reclaimed water, our study recommends the development of risk assessment schemes related to both human and environmental health. Various exposure routes, various environmental and climatic conditions, and the precautionary principle should all be taken into

649 consideration in the decision-making process related to the use of reclaimed water for agricultural
650 irrigation.

651

652 **Acknowledgments**

653 Part of this work has received funding from the European Union's Horizon 2020 research
654 and innovation programme under the Marie Skłodowska-Curie grant agreement No 675530. The
655 University of Cyprus team would like to thank the WWTP operators for the collection of the
656 wastewater samples and for providing the physicochemical characteristics of the wastewater
657 samples. Vasiliki G. Beretsou would also like to thank Iakovos C. Iakovides, Maria Dionysiou,
658 Dr. Popi Karaolia and Dr. Evroula Hapeshi for transferring the wastewater samples from the
659 WWTPs to the lab, and Xia Yu for her assistance in the visualization of selected results.

660 **Disclaimer**

661 The content of this work reflects only the authors' views, and the Research Executive
662 Agency is not responsible for any use that may be made of the information it contains.

663

664 **References**

665 Al-Nasir, F.M., Jiries, A.G., Al-Rabadi, G.J., Alu'datt, M.H., Tranchant, C.C., Al-Dalain, S.A.,
666 Alrabadi, N., Madanat, O.Y., Al-Dmour, R.S., 2020. Determination of pesticide residues in
667 selected citrus fruits and vegetables cultivated in the Jordan Valley. Lwt 123, 109005.
668 https://doi.org/10.1016/j.lwt.2019.109005

669 Alygizakis, N., Galani, A., Rousis, N.I., Aalizadeh, R., Dimopoulos, M.A., Thomaidis, N.S.,

2021. Change in the chemical content of untreated wastewater of Athens, Greece under COVID-19 pandemic. Sci. Total Environ. 799, 149230. https://doi.org/10.1016/j.scitotenv.2021.149230

Alygizakis, N.A., Besselink, H., Paulus, G.K., Oswald, P., Hornstra, L.M., Oswaldova, M., Medema, G., Thomaidis, N.S., Behnisch, P.A., Slobodnik, J., 2019. Characterization of wastewater effluents in the Danube River Basin with chemical screening, in vitro bioassays and antibiotic resistant genes analysis. Environ. Int. 127, 420–429. https://doi.org/10.1016/j.envint.2019.03.060

Alygizakis, N.A., Samanipour, S., Hollender, J., Ibáñez, M., Kaserzon, S., Kokkali, V., Van Leerdam, J.A., Mueller, J.F., Pijnappels, M., Reid, M.J., Schymanski, E.L., Slobodnik, J., Thomaidis, N.S., Thomas, K. V., 2018. Exploring the Potential of a Global Emerging Contaminant Early Warning Network through the Use of Retrospective Suspect Screening with High-Resolution Mass Spectrometry. Environ. Sci. Technol. 52, 5135–5144. https://doi.org/10.1021/acs.est.8b00365

Alygizakis, N.A., Urík, J., Beretsou, V.G., Kampouris, I., Galani, A., Oswaldova, M., Berendonk, T., Oswald, P., Thomaidis, N.S., Slobodnik, J., Vrana, B., Fatta-Kassinos, D., 2020. Evaluation of chemical and biological contaminants of emerging concern in treated wastewater intended for agricultural reuse. Environ. Int. 138, 105597. https://doi.org/10.1016/j.envint.2020.105597

Antonopoulou, M., Konstantinou, I., 2016. Photocatalytic degradation and mineralization of tramadol pharmaceutical in aqueous TiO2 suspensions: Evaluation of kinetics, mechanisms and ecotoxicity. Appl. Catal. A Gen. 515, 136–143.

692 https://doi.org/10.1016/j.apcata.2016.02.005

693 Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2017. The fate of
694 pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants
695 (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere
696 174, 437–446. https://doi.org/10.1016/j.chemosphere.2017.01.101

697 Arsand, J.B., Hoff, R.B., Jank, L., Dallegrave, A., Galeazzi, C., Barreto, F., Pizzolato, T.M.,
698 2018. Wide-Scope Determination of Pharmaceuticals and Pesticides in Water Samples :
699 Qualitative and Confirmatory Screening Method Using LC-qTOF-MS.

700 Beretsou, V.G., Psoma, A.K., Gago-Ferrero, P., Aalizadeh, R., Fenner, K., Thomaidis, N.S.,
701 2016. Identification of biotransformation products of citalopram formed in activated sludge.
702 Water Res. 103, 205–214. https://doi.org/10.1016/j.watres.2016.07.029

703 Bletsou, A.A., Jeon, J., Hollender, J., Archontaki, E., Thomaidis, N.S., 2015. Targeted and non-
704 targeted liquid chromatography-mass spectrometric workflows for identification of
705 transformation products of emerging pollutants in the aquatic environment. TrAC - Trends
706 Anal. Chem. 66, 32–44. https://doi.org/10.1016/j.trac.2014.11.009

707 Boix, C., Ibáñez, M., Sancho, J. V., Parsons, J.R., Voogt, P. de, Hernández, F., 2016.
708 Biotransformation of pharmaceuticals in surface water and during waste water treatment:
709 Identification and occurrence of transformation products. J. Hazard. Mater. 302, 175–187.
710 https://doi.org/10.1016/j.jhazmat.2015.09.053

711 Bonvin, F., Omlin, J., Rutler, R., Schweizer, W.B., Alaimo, P.J., Strathmann, T.J., McNeill, K.,
712 Kohn, T., 2013. Direct photolysis of human metabolites of the antibiotic sulfamethoxazole:
713 Evidence for abiotic back-transformation. Environ. Sci. Technol. 47, 6746–6755.

https://doi.org/10.1021/es303777k

Boulard, L., Dierkes, G., Ternes, T., 2018. Utilization of large volume zwitterionic hydrophilic interaction liquid chromatography for the analysis of polar pharmaceuticals in aqueous environmental samples: Benefits and limitations. J. Chromatogr. A 1535, 27–43. https://doi.org/10.1016/j.chroma.2017.12.023

Brack, W., Barcelo Culleres, D., Boxall, A.B.A., Budzinski, H., Castiglioni, S., Covaci, A., Dulio, V., Escher, B.I., Fantke, P., Kandie, F., Fatta-Kassinos, D., Hernández, F.J., Hilscherová, K., Hollender, J., Hollert, H., Jahnke, A., Kasprzyk-Hordern, B., Khan, S.J., Kortenkamp, A., Kümmerer, K., Lalonde, B., Lamoree, M.H., Levi, Y., Lara Martín, P.A., Montagner, C.C., Mougin, C., Msagati, T., Oehlmann, J., Posthuma, L., Reid, M., Reinhard, M., Richardson, S.D., Rostkowski, P., Schymanski, E., Schneider, F., Slobodnik, J., Shibata, Y., Snyder, S.A., Fabriz Sodré, F., Teodorovic, I., Thomas, K. V., Umbuzeiro, G.A., Viet, P.H., Yew-Hoong, K.G., Zhang, X., Zuccato, E., 2022. One planet: one health. A call to support the initiative on a global science–policy body on chemicals and waste. Environ. Sci. Eur. 34. https://doi.org/10.1186/s12302-022-00602-6

Briones, R.M., Sarmah, A.K., Padhye, L.P., 2016. A global perspective on the use, occurrence, fate and effects of anti-diabetic drug metformin in natural and engineered ecosystems. Environ. Pollut. 219, 1007–1020. https://doi.org/10.1016/j.envpol.2016.07.040

Choi, Y., Lee, J.H., Kim, K., Mun, H., Park, N., Jeon, J., 2021. Identification, quantification, and prioritization of new emerging pollutants in domestic and industrial effluents, Korea: Application of LC-HRMS based suspect and non-target screening. J. Hazard. Mater. 402, 123706. https://doi.org/10.1016/j.jhazmat.2020.123706

736 Elizalde-Velázquez, G.A., Gómez-Oliván, L.M., Islas-Flores, H., Hernández-Navarro, M.D.,
737 García-Medina, S., Galar-Martínez, M., 2021. Oxidative stress as a potential mechanism by
738 which guanylurea disrupts the embryogenesis of Danio rerio. Sci. Total Environ. 799.
739 https://doi.org/10.1016/j.scitotenv.2021.149432

740 Escher, B.I., Stapleton, H.M., Schymanski, E.L., 2020. Tracking complex mixtures of chemicals
741 in our changing environment. Science (80-. ). 367, 388–392.

742 European Commission, 2009. Technical specifications for chemical analysis and monitoring of
743 water status 8, 36–38, https://eur-lex.europa.eu/legal-
744 content/EN/ALL/?uri=CELEX:32009L0090.

745 European Commission, 2019a. The European Green Deal. COM(2019) 640 final,
746 https://ec.europa.eu/info/sites/default/files/european-green-deal-communication_en.pdf

747 European Commission, 2019b. European Union Strategic Approach to Pharmaceuticals in the
748 Environment, Communication from the Commission to the European Parliament, the
749 Council and the European Economic and social committee, COM/2019/128 final,
750 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM:2019:128:FIN

751 European Commission, 2020. Chemicals strategy for Sustainability towards a toxic-free
752 environment, Communication from the Commission to the European Parliament, the
753 Council, the European economic and social committee and the committee of the regions,
754 COM/2020/667 final, https://eur-lex.europa.eu/legal-
755 content/EN/TXT/?uri=COM:2020:667:FIN

756 European Union, 2020. Regulation (EU) 2020/741 of the European Parliament and of the
757 Council of 25 May 2020 on minimum requirements for water reuse, https://eur-

lex.europa.eu/eli/reg/2020/741/oj.

Evgenidou, E.N., Konstantinou, I.K., Lambropoulou, D.A., 2015. Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review. Sci. Total Environ. 505, 905–926. https://doi.org/10.1016/j.scitotenv.2014.10.021

Fatta-Kassinos, D., Kalavrouziotis, I.K., Koukoulakis, P.H., Vasquez, M.I., 2011a. The risks associated with wastewater reuse and xenobiotics in the agroecological environment. Sci. Total Environ. 409, 3555–3563. https://doi.org/10.1016/j.scitotenv.2010.03.036

Fatta-Kassinos, D., Vasquez, M.I., Kümmerer, K., 2011b. Transformation products of pharmaceuticals in surface waters and wastewater formed during photolysis and advanced oxidation processes - Degradation, elucidation of byproducts and assessment of their biological potency. Chemosphere 85, 693–709. https://doi.org/10.1016/j.chemosphere.2011.06.082

Ferrer, I., Thurman, E.M., 2010. Analysis of sucralose and other sweeteners in water and beverage samples by liquid chromatography/time-of-flight mass spectrometry. J. Chromatogr. A 1217, 4127–4134. https://doi.org/10.1016/j.chroma.2010.02.020

Gago-Ferrero, P., Bletsou, A.A., Damalas, D.E., Aalizadeh, R., Alygizakis, N.A., Singer, H.P., Hollender, J., Thomaidis, N.S., 2020. Wide-scope target screening of >2000 emerging contaminants in wastewater samples with UPLC-Q-ToF-HRMS/MS and smart evaluation of its performance through the validation of 195 selected representative analytes. J. Hazard. Mater. 387, 121712. https://doi.org/10.1016/j.jhazmat.2019.121712

García-Galán, M.J., Petrovic, M., Rodríguez-Mozaz, S., Barceló, D., 2016. Multiresidue trace analysis of pharmaceuticals, their human metabolites and transformation products by fully

780 automated on-line solid-phase extraction-liquid chromatography-tandem mass
781 spectrometry. Talanta 158, 330–341. https://doi.org/10.1016/j.talanta.2016.05.061

782 Göbel, A., Thomsen, A., McArdell, C.S., Joss, A., Giger, W., 2005. Occurrence and sorption
783 behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment.
784 Environ. Sci. Technol. 39, 3981–3989. https://doi.org/10.1021/es048550a

785 Golovko, O., Örn, S., Sörengård, M., Frieberg, K., Nassazzi, W., Lai, F.Y., Ahrens, L., 2021.
786 Occurrence and removal of chemicals of emerging concern in wastewater treatment plants
787 and their impact on receiving water systems. Sci. Total Environ. 754.
788 https://doi.org/10.1016/j.scitotenv.2020.142122

789 Gong, L., Goswami, S., Giacomini, K.M., Altman, R.B., Klein, T.E., 2012. Metformin pathways:
790 Pharmacokinetics and pharmacodynamics. Pharmacogenet. Genomics 22, 820–827.
791 https://doi.org/10.1097/FPC.0b013e3283559b22

792 Gracia-Lor, E., Ibáñez, M., Zamora, T., Sancho, J. V., Hernández, F., 2014. Investigation of
793 pharmaceutical metabolites in environmental waters by LC-MS/MS. Environ. Sci. Pollut.
794 Res. 21, 5496–5510. https://doi.org/10.1007/s11356-013-2464-5

795 Gulde, R., Meier, U., Schymanski, E.L., Kohler, H.P.E., Helbling, D.E., Derrer, S., Rentsch, D.,
796 Fenner, K., 2016. Systematic Exploration of Biotransformation Reactions of Amine-
797 Containing Micropollutants in Activated Sludge. Environ. Sci. Technol. 50, 2908–2920.
798 https://doi.org/10.1021/acs.est.5b05186

799 Han, N., Gin, K.Y., 2017. Science of the Total Environment Occurrence and removal of
800 pharmaceuticals , hormones , personal care products , and endocrine disrupters in a full-
801 scale water reclamation plant. Sci. Total Environ. 599–600, 1503–1516.

https://doi.org/10.1016/j.scitotenv.2017.05.097

Helbling, D.E., Johnson, D.R., Honti, M., Fenner, K., 2012. Process Parameters and Microbial Community Characteristics. Environ. Sci. Technol. 46, 10579–10588.

Hites, R.A., 2019. Correcting for Censored Environmental Measurements. Environ. Sci. Technol. 53, 11059–11060. https://doi.org/10.1021/acs.est.9b05042

Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J., Ventura, F., 2008. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. Sci. Total Environ. 397, 31–40. https://doi.org/10.1016/j.scitotenv.2008.02.057

Ibáñez, M., Bijlsma, L., Pitarch, E., López, F.J., Hernández, F., 2021. Occurrence of pharmaceutical metabolites and transformation products in the aquatic environment of the Mediterranean area. Trends Environ. Anal. Chem. 29. https://doi.org/10.1016/j.teac.2021.e00118

Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. Water Res. 43, 363–380. https://doi.org/10.1016/j.watres.2008.10.047

Kern, S., Fenner, K., Singer, H.P., Schwarzenbach, R.P., Hollender, J., 2009. Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. Environ. Sci. Technol. 43, 7039–7046. https://doi.org/10.1021/es901979h

Krentz, A.J., Bailey, C.J., 2005. Oral Antidiabetic Agents. Drugs 65, 385–411.

https://doi.org/10.2165/00003495-200565030-00005

Kostanjevecki, P., Petric, I., Loncar, J., Smital, T., Ahel, M., Terzic, S., 2019. Aerobic biodegradation of tramadol by pre-adapted activated sludge culture: Cometabolic transformations and bacterial community changes during enrichment. Sci. Total Environ. 687, 858–866. https://doi.org/10.1016/j.scitotenv.2019.06.118

Leendert, V., Van Langenhove, H., Demeestere, K., 2015. Trends in liquid chromatography coupled to high-resolution mass spectrometry for multi-residue analysis of organic micropollutants in aquatic environments. TrAC - Trends Anal. Chem. 67, 192–208. https://doi.org/10.1016/j.trac.2015.01.010

Lin, X., Xu, J., Keller, A.A., He, L., Gu, Y., Zheng, W., Sun, D., Lu, Z., Huang, J., Huang, X., Li, G., 2020. Occurrence and risk assessment of emerging contaminants in a water reclamation and ecological reuse project. Sci. Total Environ. 744. https://doi.org/10.1016/j.scitotenv.2020.140977

López-Pacheco, I.Y., Silva-Núñez, A., Salinas-Salazar, C., Arévalo-Gallegos, A., Lizarazo-Holguin, L.A., Barceló, D., Iqbal, H.M.N., Parra-Saldívar, R., 2019. Anthropogenic contaminants of high concern: Existence in water resources and their adverse effects. Sci. Total Environ. 690, 1068–1088. https://doi.org/10.1016/j.scitotenv.2019.07.052

López-Serna, R., Petrović, M., Barceló, D., 2012. Direct analysis of pharmaceuticals, their metabolites and transformation products in environmental waters using on-line TurboFlow™ chromatography-liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1252, 115–129. https://doi.org/10.1016/j.chroma.2012.06.078

McEachran, A.D., Hedgespeth, M.L., Newton, S.R., McMahen, R., Strynar, M., Shea, D.,

845 Nichols, E.G., 2018. Comparison of emerging contaminants in receiving waters
846 downstream of a conventional wastewater treatment plant and a forest-water reuse system.
847 Environ. Sci. Pollut. Res. 25, 12451–12463. https://doi.org/10.1007/s11356-018-1505-5

848 Men, Y., Achermann, S., Helbling, D.E., Johnson, D.R., Fenner, K., 2017. Relative contribution
849 of ammonia oxidizing bacteria and other members of nitrifying activated sludge
850 communities to micropollutant biotransformation. Water Res. 109, 217–226.
851 https://doi.org/10.1016/j.watres.2016.11.048

852 Menger, F., Gago-ferrero, P., Wiberg, K., Ahrens, L., 2020. Trends in Environmental Analytical
853 Chemistry Wide-scope screening of polar contaminants of concern in water : A critical
854 review of liquid chromatography-high resolution mass spectrometry-based strategies.
855 Biochem. Pharmacol. 28, e00102. https://doi.org/10.1016/j.teac.2020.e00102

856 NEREUS COST ACTION ES 1403, 2017. A list of parameters to be taken into account for a
857 qualitative risk assessment framework 1–87, http://www.nereus-cost.eu/wp-
858 content/uploads/2020/06/D20.pdf.

859 Nikolopoulou, V., Aalizadeh, R., Nika, M.C., Thomaidis, N.S., 2022. TrendProbe: Time profile
860 analysis of emerging contaminants by LC-HRMS non-target screening and deep learning
861 convolutional neural network. J. Hazard. Mater. 428, 128194.
862 https://doi.org/10.1016/j.jhazmat.2021.128194

863 Nilsen, E., Smalling, K.L., Ahrens, L., Gros, M., Miglioranza, K.S.B., Picó, Y., Schoenfuss,
864 H.L., 2019. Critical review: Grand challenges in assessing the adverse effects of
865 contaminants of emerging concern on aquatic food webs. Environ. Toxicol. Chem. 38, 46–
866 60. https://doi.org/10.1002/etc.4290

867 OECD, 2019. Pharmaceutical Residues in Freshwater: Hazards and Policy Responses, OECD
868 Studies on Water. Paris. https://doi.org/https://doi.org/10.1787/c936f42d-en

869 Ofrydopoulou, A., Nannou, C., Evgenidou, E., Christodoulou, A., Lambropoulou, D., 2022.
870 Assessment of a wide array of organic micropollutants of emerging concern in wastewater
871 treatment plants in Greece: Occurrence, removals, mass loading and potential risks. Sci.
872 Total Environ. 802, 149860. https://doi.org/10.1016/j.scitotenv.2021.149860

873 Ort, C., van Nuijs, A.L.N., Berset, J.D., Bijlsma, L., Castiglioni, S., Covaci, A., de Voogt, P.,
874 Emke, E., Fatta-Kassinos, D., Griffiths, P., Hernández, F., González-Mariño, I., Grabic, R.,
875 Kasprzyk-Hordern, B., Mastroianni, N., Meierjohann, A., Nefau, T., Östman, M., Pico, Y.,
876 Racamonde, I., Reid, M., Slobodnik, J., Terzic, S., Thomaidis, N., Thomas, K. V., 2014.
877 Spatial differences and temporal changes in illicit drug use in Europe quantified by
878 wastewater analysis. Addiction 109, 1338–1352. https://doi.org/10.1111/add.12570

879 Paíga, P., Correia, M., Fernandes, M.J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J.G.,
880 Freire, C., Delerue-Matos, C., 2019. Assessment of 83 pharmaceuticals in WWTP influent
881 and effluent samples by UHPLC-MS/MS: Hourly variation. Sci. Total Environ. 648, 582–
882 600. https://doi.org/10.1016/j.scitotenv.2018.08.129

883 Quesnel, D., Nakhla, G., 2005. Characterization and treatability of aerobic bacterial
884 thermophilically treated wastewater by a conventional activated sludge and granular
885 activated carbon. Water Res. 39, 677–687. https://doi.org/10.1016/j.watres.2004.11.010

886 Radjenović, J., Pérez, S., Petrović, M., Barceló, D., 2008. Identification and structural
887 characterization of biodegradation products of atenolol and glibenclamide by liquid
888 chromatography coupled to hybrid quadrupole time-of-flight and quadrupole ion trap mass

889 spectrometry. J. Chromatogr. A 1210, 142–153.
890 https://doi.org/10.1016/j.chroma.2008.09.060

891 Revitt, D.M., Lundy, L., Fatta-Kassinos, D., 2021. Development of a qualitative approach to
892 assessing risks associated with the use of treated wastewater in agricultural irrigation. J.
893 Hazard. Mater. 406, 124286. https://doi.org/10.1016/j.jhazmat.2020.124286

894 Rubirola, A., Boleda, M. [a]R, Galceran, M. [a]T, Moyano, E., 2019. Formation of new disinfection
895 by-products of priority substances (Directive 2013/39/UE and Watch List) in drinking water
896 treatment. Environ. Sci. Pollut. Res. 26, 28270–28283. https://doi.org/10.1007/s11356-019-
897 06018-9

898 Rubirola, A., Llorca, M., Rodriguez-Mozaz, S., Casas, N., Rodriguez-Roda, I., Barceló, D.,
899 Buttiglieri, G., 2014. Characterization of metoprolol biodegradation and its transformation
900 products generated in activated sludge batch experiments and in full scale WWTPs. Water
901 Res. 63, 21–32. https://doi.org/10.1016/j.watres.2014.05.031

902 Scheurer, M., Sacher, F., Brauch, H.J., 2009. Occurrence of the antidiabetic drug metformin in
903 sewage and surface waters in Germany. J. Environ. Monit. 11, 1608–1613.
904 https://doi.org/10.1039/b909311g

905 Scheurer, M., Michel, A., Brauch, H.J., Ruck, W., Sacher, F., 2012. Occurrence and fate of the
906 antidiabetic drug metformin and its metabolite guanylurea in the environment and during
907 drinking water treatment. Water Res. 46, 4790–4802.
908 https://doi.org/10.1016/j.watres.2012.06.019

909 Senta, I., Gracia-Lor, E., Borsotti, A., Zuccato, E., Castiglioni, S., 2015. Wastewater analysis to
910 monitor use of caffeine and nicotine and evaluation of their metabolites as biomarkers for

911 population size assessment. Water Res. 74, 23–33.
912 https://doi.org/10.1016/j.watres.2015.02.002

913 Shi, Z.Q., Liu, Y.S., Xiong, Q., Cai, W.W., Ying, G.G., 2019. Occurrence, toxicity and
914 transformation of six typical benzotriazoles in the environment: A review. Sci. Total
915 Environ. 661, 407–421. https://doi.org/10.1016/j.scitotenv.2019.01.138

916 Su, Q., Schittich, A.R., Jensen, M.M., Ng, H., Smets, B.F., 2021. Role of ammonia oxidation in
917 organic micropollutant transformation during wastewater treatment: Insights from
918 molecular, cellular, and community level observations. Environ. Sci. Technol. 55, 2173–
919 2188. https://doi.org/10.1021/acs.est.0c06466

920 Subedi, B., Kannan, K., 2014. Fate of Arti fi cial Sweeteners in Wastewater Treatment Plants in
921 New York State, U.S.A. https://doi.org/10.1021/es504769c

922 Trautwein, C., Kümmerer, K., 2011. Incomplete aerobic degradation of the antidiabetic drug
923 Metformin and identification of the bacterial dead-end transformation product Guanylurea.
924 Chemosphere 85, 765–773. https://doi.org/10.1016/j.chemosphere.2011.06.057

925 United Nations, 2015. Transforming our world: The 2030 agenda for sustainable development,
926 https://sdgs.un.org/sites/default/files/publications/21252030%20Agenda%20for%20Sustain
927 able%20Development%20web.pdf

928 Wang, Z., Altenburger, R., Backhaus, T., Covaci, A., Diamond, M.L., Grimalt, J.O., Lohmann,
929 R., Schäffer, A., Scheringer, M., Selin, H., Soehl, A., Suzuki, N., 2021. We need a global
930 science-policy body on chemicals and waste. Science (80-. ). 371, 774–776.
931 https://doi.org/10.1126/science.abe9090

932 Yu, X., Sui, Q., Lyu, S., Zhao, W., Wu, D., Yu, G., Barcelo, D., 2021. Rainfall Influences
933 Occurrence of Pharmaceutical and Personal Care Products in Landfill Leachates: Evidence
934 from Seasonal Variations and Extreme Rainfall Episodes. Environ. Sci. Technol. 55, 4822–
935 4830. https://doi.org/10.1021/acs.est.0c07588