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## **Ecotoxicological risk assessment of contaminants of emerging concern identified by “suspect screening” from urban wastewater treatment plant effluents at a territorial scale.**

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### Abstract

Urban Wastewater Treatment Plants (WWTP) are a major vector of highly ecotoxic Contaminant of Emerging Concern (CECs) for urban and sub-urban streams. Ecotoxicological Risk Assessments (ERAs) provide essential information to public environmental authorities. Nevertheless, ERAs are mainly performed at very local scale (one or few WWTPs) and on pre-selected list of CECs. To cope with these limits, the present study aims to develop a territorial-scale ERA on CECs previously identified by a “suspect screening” analytical approach (LC-QToF-MS) and quantified in the effluents of 10 WWTPs of a highly urbanized territory during three periods of the year. Among CECs, this work focused on pharmaceutical residue and pesticides. ERA was conducted following two complementary methods: (1) a single substance approach, based on the calculation for each CEC of Risk Quotients (RQs) by the ratio of Predicted Environmental Concentration (PEC) and Predicted No Effect Concentration (PNEC), and (2) mixture risk assessment (“cocktail effect”) based on a Concentration Addition model (CA), summing individual RQs. Chemical results led to an ERA for 41 CEC (37 pharmaceuticals and 4 pesticides) detected in treated effluents. Single substance ERA identified 19 CECs implicated in at least one significant risk for streams, with significant risks for citalopram, DEET, diclofenac, lidocaïne, atenolol, terbutryn, atorvastatin, methocarbamol, and venlafaxine (RQs reaching 22.85, 39.84, 62.10, 125.58, 179.11, 348.24, 509.27, 1509.71 and 3097.37, respectively). Mixture ERA

allowed for the identification of 9 of the 10 WWTPs a risk ( $RQ_{mix} > 1$ ). It was also remarked that CECs leading individually to a negligible risk could imply a significant risk in a mixture. Finally, the territorial ERA showed a diversity of risk situations, with the highest concerns for 3 WWTPs: the 2 biggest of the territory discharging into a large French river, the Rhône, and for the smallest WWTP that releases into a small intermittent stream.

### Key-words

Suspect-screening; Contaminants of Emerging Concern; Pharmaceutical; Ecotoxicological risk assessment; Cocktail effect; Urban wastewater

## 1. Introduction

In recent decades, the synthesis, consumption, and use of chemicals, such as plasticizers, flame retardants, detergents, pharmaceutical residues, or pesticides have been growing (Escher et al., 2020; Hider-Mlynarz et al., 2018; Liu et al. 2015). As a result, thousands of these are continuously discharged into urban wastewaters (Wang et al., 2019). Due to the ecotoxic properties (e.g. endocrine disruption) of many of them (Gosset et al., 2017; Johnson et al., 2020; Orias et al., 2013), it has been logically demonstrated that urban wastewaters present an ecotoxic character to many aquatic organisms (Laquaz et al., 2017; Smital et al., 2011).

Urban wastewater treatment plants (WWTPs) have been implemented to reduce the high levels of nitrogen, phosphorus, and organic matter; however, many studies have already shown that the abatement of Contaminants of Emerging Concern (CECs) remains very poor (Gurke et al., 2015). These pollutants are thus continuously released in trace amounts (typically from ng/L to µg/L) into receiving watercourses. Knowledge of their environmental hazards and the associated risks to the environment has greatly increased in recent decades (Johnson et al., 2020). For example, an in-depth study of ecological risk assessment (ERA) was carried out by Pereira et al. (2015) on the occurrence of pharmaceuticals in Portuguese wastewater treatment plants.

Yet, ERA studies are most often performed on isolated wastewater treatment plants. Whatever the context of the pollution, it is necessary to consider ecotoxicological risks at a territorial scale in order to provide decision support tools and useful information to help public authorities design planning

policies (Gosset et al., 2020; Loiseau et al., 2012). This is why spatialized ERA methods, which are now essential, have emerged in recent years (Brus and Perrodin, 2017; Grifero et al., 2019; Lei et al., 2019).

Besides, most ERA studies have focused on searching for a few key model molecules in WWTP effluents, pre-selected for their toxicity, bioaccumulation potential, or high consumption, and often based on well-known, high-performance liquid chromatography techniques (Guo et al., 2019; Pinasseau et al., 2019; Singer et al., 2016). The “bandwagon” effect or the cost of large experiments and the lack of chemical standards can also explain this (Freeling et al., 2019; Johnson et al., 2020). The degradation products (metabolites) are not extensively studied either (Deeb et al., 2017), awhile in some cases can be more ecotoxic than the parent compounds and lead to higher risks (Bertanza et al., 2013; Stalter et al., 2010). To overcome these limitations and have a more comprehensive view of the pollution in environmental complex samples, suspect screening analyses (SSA) have recently been deployed (Freeling et al., 2019). SSA are based on the comparison of compounds (e.g. accurate mass, retention time, or isotopic pattern) detected by high-resolution mass spectrometry (HRMS) coupled with either gas or liquid chromatography, with databases of thousands molecules, to identify present compounds (Sobus et al., 2018; Phillips et al., 2018; Escher et al., 2020). This method has been recently applied to various contaminant families (e.g. plasticizers, hydrocarbons, pharmaceuticals, pesticides, surfactants, industrial chemicals) and aqueous matrices such as runoffs (Pinasseau et al., 2019), surface drinking and ground waters (Sjerps et al., 2016) or wastewater (Assress et al., 2019; Freeling et al., 2019; Hug et al., 2014; Singer et al., 2016; Wang et al., 2019). Due to this method’s novelty and the critical nature of the problem, the number of studies aiming to carry out "suspect-screening" on wastewater samples has rapidly increased in recent years and yet still remains limited (see Supplementary Figure S1). Moreover, a suspect-screening analysis combined with a targeted quantification of identified pollutants would permit a more exhaustive and quantitative assessment of the environmental risk associated with discharge. Freeling et al. (2019) recently applied this method to better identify surfactants and associated metabolites in German WWTP effluents and evaluate the final risk for watercourses. Nevertheless, the joint implementation of these two analytical methods in the context of ERA remains unexplored.

Finally, the present study aimed to develop an innovative ERA framework applied to contaminants of emerging concern identified by suspect screening and released from urban WWTP

effluents into freshwater watercourses in a highly anthropized territory (the second biggest city in France, the Grand Lyon area). Results from a suspect screening of pharmaceutical residue and pesticides (around 2000 substances) in urban effluents of ten WWTPs (Wiest et al., submitted) were used to evaluate the associated ecotoxicological risk following two complementary methods. The first method corresponded to a single substance ERA, assessing the risk of each pollutant alone and based on the comparison of Predicted Environmental Concentrations (PEC) and Predicted No Effect Concentration (PNEC) values, following European guidelines (ECB, 2003). The second one considered the "cocktail effect" due to the mixture of CECs in WWTP effluents in the territory, using a procedure recently described by Backhaus and Faust (2012), and based on a Concentration addition (CA) model. This approach has recently shown its usefulness in detecting hidden risks (Gosset et al., 2020).

## 2. Materials and methods

### 2.1. Studied area and sampling procedure

In this study, the territory identified to assess the ecotoxicological risk of WWTP CECs on receiving watercourses is located in the urbanized area of the city of Lyon, France ("Grand Lyon", with around 1,400,000 inhabitants and with an area of 540 km<sup>2</sup>). Ten WWTPs of this territory were monitored in the current study and are located as presented in Figure 1. In function of the plant, hospital, industrial and/or domestic wastewater is collected in varying proportions. All the WWTPs are equipped with classical pretreatment, primary, and secondary treatment processes. WWTP No. 10 is equipped with a tertiary treatment process. WWTP No. 3 only collects wastewater from an industrial area. WWTP No. 9 and 10 collect 95 % of the effluents of the Grand Lyon area. More details on treatment plant sites and design are provided in Wiest et al. (submitted). Five WWTPs (No. 4, 5, 8, 9 and 10) discharge their effluents into the Rhône River, one of the longest rivers in Europe (810 km length). Four WWTPs (No. 2, 3, 6 and 7) dump into the Saône River (480 km length), the main tributary of the Rhône River. Finally, WWTP No. 1 emits its treated wastewaters into a small stream (Gorges stream, 1.7 km length). All of the main WWTP and receiving watercourse characteristics are summarized in Table 1.

Effluents from the 10 WWTPs were collected during three sampling campaigns, from May to December 2019: The first one in May (Campaign C1), the second in October/November (C2) and the

last in December 2019 (C3) at the outlet of the secondary or tertiary treatment. Thus, this sampling procedure allowed for an account of the spatiotemporal pollutant concentration variability in the territory. In short, 24-h composite effluent samples were collected according to the flow rate using refrigerated automatic samplers with high-density polyethylene containers, transferred into brown glass bottles, transported to the lab in coolers, and finally treated within the sampling day for analyses. In sum, a total of 30 effluents were sampled and analyzed. More details about sampling conditions and procedures are available in Wiest et al. (submitted).

## 2.2. Conventional parameters and metallic trace element analyses

Conventional physico-chemical parameters (pH, conductivity, Total Suspended Solids (TSS), Dissolved Organic Carbon (DOC)), anion ( $\text{Cl}^-$ ,  $\text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ) and cation concentrations ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ) were measured employing the methods described by Perrodin et al. (2016), following the European standards. Moreover, major non-essential metallic trace elements (MTE) (Pb, Cr, Ni, Cd, Cu, and Zn) were analyzed by ICP-AES (PinAAcle 900T Perkin Elmer) after acidification with  $\text{HNO}_3$  permitting mineralization of the MTE trapped in the very fine suspended matter. All ion and MTE analyses were carried out on samples filtered at  $0.45\ \mu\text{m}$ . Other analyses were performed on the whole sample. All the results are provided in supplementary data.

## 2.3. Sample preparation, extraction, LC-QToF MS suspect screening and quantification of contaminants of emerging concern

All the detailed extraction and analytical procedure (LC-QToF MS suspect-screening, quantification, quality assurance and quality controls) have been previously developed and described in Wiest et al. (submitted). In short, sample preparation, extraction, and analyses were conducted as follows: after filtration, effluent samples were passed through an automated Solid Phase Extraction (SPE) system. Samples of May 2019 (Campaign C1) were used to carry out a suspect-screening analysis to identify and confirm the presence of 41 CECs in the effluents using Ultra High Performance Liquid Chromatography (UHPLC) system (Thermo Scientific® Ultimate 3000) coupled with a Quadrupole Time of Flight (Q-ToF) Mass Spectrometer (Bruker Daltonics® Maxis Plus.). Quality controls, solvent

and procedural blanks were injected in every sequences in order to check sensibility and retention time variability and to evaluate background signals, respectively. Two databases (PesticideScreener 2.1 and ToxScreener 2.1 (Bruker Daltonics®)) were used to compare all detected signals, couples of exact mass and retention time (m/z; RT). Exact masses, retention times, isotope patterns and fragments of about 2000 pesticides and pharmaceutical substances are compiled in these two databases respectively. To perform the suspect screening, tolerances on identification criteria (exact mass, retention time, isotope pattern and fragments) were as follow: exact mass precision  $\Delta m/z < 10$  ppm, retention time of the parent  $< \pm 0.5$ min, isotope pattern of the parent :  $m_{\text{sigma}} < 30$  ( $m_{\text{sigma}}$  represent the correlation between the isotope pattern of the expected spectra and the experimental spectra); Signal to Noise (S/N)  $> 3$  of a minimum of half of the fragments with tolerance on exact mass precision  $\Delta m/z < 20$  ppm. Substances identified by the software were then further sorted according to a filtering strategy described in Pinasseau et al. 2019. It is based on the calculation of a T-score for each identified substance. Only molecules with high T-scores were considered, leading to a list of 41 compounds. Presence of the suspected molecules was confirmed using samples spiked with authentic analytical standards. Finally, these substances were quantified in all samples from campaigns C1, C2, and C3 by internal standard calibration.

#### 2.4. Ecotoxicological risk assessment (ERA) for receiving watercourses

For each WWTP, an Ecotoxicological Risk Assessment (ERA) was performed for each substance in isolation, but also for the mixture. ERA are classically based on the comparison of a Predicted Environmental Concentration (PEC) of a substance (mixture) in watercourses and a Predicted No Effect Concentration (PNEC) data reflecting the ecotoxicity of a molecule on the aquatic (in our case, freshwater) ecosystems (Perrodin et al., 2011).

##### 2.4.1 Predicted Environmental Concentration (PEC) determination

The PEC's calculation of each identified CEC in the receiving watercourses was based on: the WWTP and watercourse flow rates and their concentration quantified by LC-QToF MS in effluents using the following equation (1):

$$PEC_y = DR \times Conc_y \quad (1)$$

With  $PEC_y$ : Predicted Environmental Concentration the pollutant  $y$  for the selected WWTP in  $\mu\text{g/L}$ ;  
DR: the dilution rate, calculated respectively using WWTP and receiving watercourse flow rates; and  
 $Conc_y$ : The quantified concentration of the pollutant  $y$  in the selected urban WWTP effluent, in  $\mu\text{g/L}$ .

Pollution data for PEC calculation were only considered for CECs found in wastewater samples at concentrations above their limits of quantification (LOQ) (Riva et al., 2019). In the aim of modelling the worst-case scenario (precaution principle), the 5-year lowest water flow rate (source: hydro.eaufrance.fr; <https://www.rdbmrc.com>; Greater Lyon) was considered for the receiving watercourses. Nonetheless, similar low flow rates have been observed for the various streams during the three annual sampling periods. It is also important to note that during the driest period of the year the WWTP 1 discharges into a small dried-up watercourse, leading to a flow composed of 100% of treated wastewater.

#### 2.4.1 Predicted No Effect Concentration (PNEC) collection and calculation

The PNEC values of the 41 identified CECs were obtained using the following procedure:

- They were first identified in nation-wide databases (INERIS (2019), AGRITOX (2019), FASS.se (2019)), which refer to data validated by French and European experts and extracted from scientific literature, or marketing authorization applications submitted by manufacturers.

- When no PNEC data was available in the databases, PNEC values were extracted from the scientific literature.

- When no clear PNEC values were found in the literature, PNECs were constructed using ecotoxicological scientific studies and framework derived from Orias and Perrodin (2013), Gosset et al. (2017), and the Technical Guidance Document (TGD) from the European Union (ECB, 2003). In short, bibliographical research about acute and chronic ecotoxicity of each concerned pollutant was performed. The EPA Ecotox database was preferentially employed to search in scientific studies. Using all found data, an Extrapolation Factor (EF) was applied (by division) to the lowest toxicity values to establish the PNEC.



- Finally, when no PNEC and experimental ecotoxicity data were available in the international databases and scientific literature, PNEC values were calculated using ecotoxicological data obtained by a QSAR (ECOSAR) modelling, from literature or using EPI<sup>®</sup> Suite software (Becker et al., 2020; Dong et al., 2013; Sanderson et al., 2003).

#### 2.4.3 ERA of individual substances

The ecotoxicological risk for watercourses linked to each pollutant in isolation was calculated taking into account its PNEC and PEC values, according to the equation (2):

$$RQ_y = \frac{PEC_y}{PNEC_y} \quad (2)$$

With  $RQ_y$ : Risk Quotient of the pollutant  $y$  for the selected WWTP;  $PEC_y$ : Predicted Environmental Concentration of the pollutant  $y$  for the selected WWTP in  $\mu\text{g/L}$  and  $PNEC_y$ : Predicted No Effect Concentration of the pollutant  $y$  in  $\mu\text{g/L}$ .

When the  $RQ$  value was below 1, it was considered that no ecotoxicological risk existed, and when it was above 1, an ecotoxicological risk for watercourse was considered as existent, with a risk ranking depending on the  $RQ$  value and classified as presented in Table 2.

#### 2.4.4 ERA of the mixture

A cumulative risk evaluation of the mixture of detected pollutants in watercourses was attempted for each WWTP. These pollutants do not affect ecosystems in an isolated manner. It has already been demonstrated that aquatic organisms exposed to a mixture of pollutants such as pharmaceuticals or biocides at individual concentrations that are not expected to provoke impacts (below the NOEC – Not Observed Effect Concentration) can lead to toxic effects (Backhaus and Karlsson, 2014; Escher et al., 2020; Heys et al., 2016; Kortenkamp, 2007). Ignoring the risk associated with their mixing would therefore lead to an underestimation of the real risk to ecosystems. Thus, in this study, the micropollutant mixture ERA method implemented aimed to assess the risk of the whole mixture of the detected pollutants using a procedure recently described by Backhaus and Faust (2012), and based on a Concentration addition (CA) model. It supposes that all pollutants have a similar action and same site

of action on the aquatic organisms, and it can consequently lead to an overstatement of the mixture risk (as a conservative method), but it can also be considered a good preliminary mixture risk assessment method for worst case scenario ERA studies (Palma et al., 2014). This tiered approach has already been used in recent studies and in some cases permitted the observation of a significant chemical mixture risk related to pollutants such as surfactants, pesticides, alkylphenols and pharmaceuticals in contaminated surface waters, as opposed to those associated with these substances alone (Gosset et al., 2020; Palma et al., 2014; Riva et al., 2019). Finally, the cumulative risk of the mixture was calculated for each WWTP using the equation (3):

$$RQ_{\text{Mix}} = \sum_{y=1}^n RQ_y \quad (3)$$

With  $RQ_{\text{Mix}}$  : Risk Quotient of the mixture of pollutants for the selected WWTP ;  $RQ_y$ : Risk Quotient of the pollutant  $y$  for the selected WWTP;

Similarly, to the risk quotients calculated for each substance alone,  $RQ_{\text{Mix}}$  were compared to 1, and ranked following the graduation described in Table 2.

### 3. Results and discussion

The classification employed to categorize the CEC is presented in Table 3. Among them, pharmaceutical residue was presented and discussed following Anatomic Therapeutic and Chemical (ATC) classifications proposed by the World Health Organization (WHO) (Orias and Perrodin, 2014).

#### 3.1 Predicted CECs concentrations in the rivers and stream

Two approaches exist to determine exposure concentrations of aquatic organisms in substance-based environmental risk assessment studies: direct chemical analysis of receiving streams, leading to the determination of measured environmental concentrations (MEC) (Riva et al, 2019), or an indirect approach through the calculation of a predicted environmental concentration (PEC), based for example on the analysis of WWTP effluents and dilution factors of these treated wastewaters in watercourses (Brus and Perrodin, 2017; Celle-Jeanton et al., 2014). The consideration of PEC values overcomes the problem of analytical chemistry instruments that are sometimes not sensitive enough to detect some of

the CECs present in environmental matrices (Celle-Jeanton et al., 2014). It also ensures that in the current ERA the observed risk for watercourses comes from treated wastewater treatment plant discharge CECs and not from other potential (sub-)urban sources (Castiglioni et al., 2018). For this reason, the calculated PEC data were assumed to be a good proxy of the CEC concentrations in the receiving watercourses and released from WWTPs.

Conventional physico-chemical parameters (e.g. Cond., COD) and metal concentrations from wastewater treatment plant effluents are provided in Supplementary Data. They are consistent with values observed in the literature (e.g. Wiest et al., 2018; Brus and Perrodin, 2017), which argues for considering studied effluents in conventional pollution situations and not in critical cases. Thus, PEC values of the 41 identified CECs are reported in Figure 2. For the majority of the CECs, concentrations in treated effluents were above the LOQs, with the exception of gabapentin and fluopyram, allowing a PEC calculation. CECs were predicted in receiving streams at concentrations ranging from few pg/L to several µg/L. Highest PECs were obtained for gabapentin (median: 7.71 ng/L), sitagliptin (1.39 ng/L), irbesartan (1.16 ng/L), valsartan (1.15 ng/L) and diclofenac (1.02 ng/L). On the contrary, lowest PECs were calculated for terbutryn (median: 0.016 ng/L). 27 of the 41 CECs presented median PEC values between 0.1 and 1 ng/L.

The present results are generally in accordance with previous studies that measured or predicted CECs in worldwide streams (Chiffre et al., 2016; Gosset et al., 2020; Kasprzyk-Hordern et al., 2008 ; Maasz et al., 2019; Miège et al., 2006; Perrodin et al., 2013; Riva et al., 2019; Vergeynst et al., 2015; Vulliet and Cren-Olivé, 2011; Yan et al., 2014; Zhou et al., 2014), validating the relevance of the present methodology. It is interesting to observe that only one study was found to measure tiapride (N) concentration in surface water (Hungarian river), with higher concentrations (0.1-566.1 ng/L depending of the period of the year) (Maasz et al., 2019) than those in this study (0.0038-3.99 ng/L). To our knowledge, this original work (coupling suspect-screening, quantification, and ERA) provided data about predicted contamination of some CECs (e.g. trospium (G) or milnacipran (N)) in surface water for the first time. No comparison for these compounds was then possible.

Nonetheless, current PEC values tend to be in the lower part of the range of values commonly measured/predicted in surface waters. There may be four reasons for this: (1) a greater dilution ratio of

the WWTPs in receiving watercourses compared to other studies (e.g. in Chiffre et al., 2016; Veyrgnest et al., 2015), (2) as expressed before the non-consideration of other sources of CECs in surface waters, such as combined sewer overflows (Gosset et al., 2017; Kay et al., 2017), stormwater runoffs (Pinasseau et al., 2019) or agriculture (Spycher et al., 2018), (3) the presence of many WWTPs releasing effluents into the same streams (Chiffre, 2016; Maasz et al., 2019), (4) a sampling at the WWTP outlet, where the plume and the watercourse are not well mixed and homogenized (Burns et al., 2018).

The special case of WWTP No. 1 needs to be specified. As no dilution existed because of the dried-up situation of the Gorges streams, PECs of CECs are thus equal to the concentration measured in the WWTP effluents, which is in accordance to current values observed and reviewed in the literature (e.g. in Verlicchi et al., 2012).

### 3.2 Ecotoxicity of the CECs - Predicted No Effect Concentrations (PNEC)

The ecotoxicity (PNEC) of the 41 identified CECs in the present study is presented in Tables 4 and 5. A wide range of PNEC values can be observed, from 0.019 ng/L (terbutryn, a pesticide (Pe)) to 704 µg/L (irbesartan, an antihypertensive drug (ATC class C)). The terbutryn ecotoxicity is directly linked to its algaecidal properties, leading to significant algal (*Craticula accomoda*) growth inhibition at very low concentration (Larras et al., 2013). The other most ecotoxic identified pollutants are 3 pharmaceuticals: methocarbamol (ATC class M; PNEC = 0.23 ng/L), atorvastatin (C; PNEC = 0.26 ng/L) and venlafaxine (N; PNEC = 0.313 ng/L), with PNEC values below 1 ng/L. Among the pollutants, 14 can be classified as most ecotoxic (PNEC < 0.1 µg/L), 7 presenting an intermediate ecotoxicity (0.1 µg/L < PNEC < 1 µg/L), and 20 as least ecotoxic (PNEC > 1 µg/L) molecules (Orias and Perrodin, 2014). Drugs dedicated to the nervous system (N) were the most represented (5/14) pharmaceuticals in the most ecotoxic pollutants. Moreover, 2 of the 4 (50 %) identified pesticides (Pe) are included in this category. In another way, drugs for the cardio-vascular system (C) are in the majority (7/20) among the less ecotoxic pollutants, followed by drugs dedicated to nervous system (N - 4/20).

Some ecotoxicity data have to be considered with caution. Several PNEC values were calculated with little experimental data (5/41 – See Table 4) and these PNEC would need to be refined. Some others (12/41 – See Table 5), and in particular 6 drugs in ATC class N, were obtained from ECOSAR (a QSAR

model) modelling, as no experimental data was available. QSAR modelling can sometimes lead to significant deviations in toxicity values compared to experimental data (Sangion and Grammatica, 2016). Moreover, modelling can also lead to an overestimation of the PNECs because it often requires the use of large extrapolation factors (1000) to calculate them (Zhou et al., 2019). Among the modeled PNEC, it is interesting to note that few substances such as lidocaine and gabapentin have been extensively studied in surface and wastewaters, although their ecotoxicity remains very poorly studied. It could partially be due to their selection based on high human consumption, excretion, low degradation by WWTPs, well-known high-performance liquid chromatography techniques, or a “bandwagon” effect (Johnson et al., 2020). Some others, such as trospium or milnacipran are both currently poorly studied, both analytically and ecotoxicologically, in aqueous matrices.

### 3.3 ERA of each contaminant of emerging concern in isolation

The Risk Quotients (RQ) calculated for each WWTP, sampling period and CEC can be found represented and reported in Figure 3 and Supplementary data. Three of the ten WWTPs never constituted a risk to receiving watercourses. It can be observed that for WWTP No.1, 19 of the 41 CECs led at least once to a RQ value above 1, implying a significant ecotoxicological risk. Highest RQ values were obtained for the antidepressant drug venlafaxine (N; RQ = 3097,37; WWTP No.1 – Campaign C1), the muscle relaxant methocarbamol (M; RQ = 1509,71; N°1 – C1), the statin atorvastatin (C; RQ = 509.27; No.1 – C3) and the pesticide terbutryn (Pe; RQ = 348.24; No.1 – C2). These four molecules led to frequent RQ above 1 (respectively 53.33, 56.67, 23.33 and 33.33 % of the samples). These results can be explained by the very high toxicity of these molecules (e.g. venlafaxine leading to toxic impact on freshwater snails at ng/L scale (Fong and Hoy, 2012)), as all the WWTP had very different dilution ratios. With the exception of the WWTP No.1, another unique significant risk was also observed for anesthetic lidocaine (N; RQ = 3.66) for WWTP No.10 (C2). Otherwise, the CECs leading to the lowest RQ values were 3 antihypertensive drugs (C): acebutolol, bisoprolol and irbesartan, one anti-allergy (R): cetirizine, two antiarrhythmic drugs (C): disopyramide and flecaidine, and one antidepressant drug (N): milnacipran. Significant variations in risk for the same molecule and WWTP were observed throughout year (e.g. sulfamethoxazole: RQs of 31.17, 0.94 and 8.35 for WWTP No.1 and campaigns C1, 2 and 3

respectively). Finally, it should be also specified that 4 CECs have RQ values between 0.1 and 1 (negligible risk): atenolol (C), diclofenac (M), DEET (Pe) and citalopram (N). For these molecules, risk cannot be excluded, as in some studies the threshold of 0.1 has been set as an environmental risk limit (e.g. Yan et al., 2014).

These results are in accordance with previous ERA studies performed with ECs PEC determination (e.g. Martín et al., 2012), or directly quantifying them into receiving streams (e.g. Riva et al., 2019; Yan et al., 2014). For most of the detected ECs, no risks existed. Nevertheless, Vergeynst et al. (2015) realized an ERA for local streams on 8 pharmaceutical residues (and among them carbamazepine, sulfamethoxazole, diclofenac, and venlafaxine) investigated in Belgian WWTPs. Authors reported PEC/PNEC ratios above 1 only for the two last ones, with higher values than ours, mainly due to the lower dilution of the effluents. Thomaidi et al. (2015) also calculated RQ above 1 for atorvastatin released from urban effluents into 25 Greek streams, validating the current results. For several CECs (e.g. flecainide, methocarbamol, milnacipran, trospium or tiapride), no comparison with other studies was possible as no published risk data are available to our knowledge.

To conclude, this study showed the utility of the present approach, coupling innovative analytical chemistry and ERA, which allowed identification of various CECs with an ecotoxicological risk ( $RQ > 1$ ) for (sub-)urban streams of the Greater Lyon territory. Thus, these pollutants should be the subject of more in-depth studies (e.g. methocarbamol, whose PNEC is still uncertain) and potentially integrated within the Watching List (EU, 2018) drawn up by the European Union in under the Water Framework Directive (WFD). It may allow to obtain large-scale monitoring data about potential risk they pose and to establish, if required, some environmental quality standards.

### 3.4 ERA of the mixture of CECs – cocktail effect approach

The present paper also aimed to consider the ecotoxicological risk linked to the whole mixture of the previously identified pollutants, as it is now recognized that classic single ERA is not sufficient to evaluate accurately the risk related to a complex mixture of pollutants that are non-independently affecting aquatic biota. The mixture risk quotients ( $RQ_{mix}$ ) for watercourses was calculated for each WWTP and sampling period, and are respectively represented and reported in Figure 4 and

Supplementary data. To our knowledge, the present work is the first to investigate the risk of the mixture of CECs from urban WWTPs not previously selected following pre-established criteria, but identified following a more exhaustive and objective way (by suspect-screening). According to Riva et al. (2019), the biggest circles in Figure 4 represent the mixture risk related to all CECs whatever the individual RQ value, and the smallest circles represent the mixture risk associated with the pollutants with individual RQ below 1.

Considering the  $RQ_{mix}$  related to all the CECs (Figure 4), values were above 1 for 9 of the 10 WWTPs and 2 (C1 and C2) of the 3 sampling periods. These treatment plants represented a wide variety of risks (low, moderate or high). Maximum  $RQ_{mix}$  (5307.12 – C1) was obtained for WWTP No. 1. For campaign C3, only 7 WWTPs exceeded the threshold value. According to the RQ of each CEC alone (section 3.3) and the CA additive model used to evaluate the global risk, median  $RQ_{mix}$  (calculated based on all WWTP and campaign data) was mainly driven by the following CECs (Figure 5): methocarbamol (median RQ representing 32.03 % of the median  $RQ_{mix}$ ), venlafaxine (31.75 %), terbutryn (22.90 %), atorvastatin (7.02 %), lidocaine (1.49 %), atenolol (1.37 %) and diclofenac (1.36 %). For all the other CECs, the contribution was less than 1% of the total risk (see Supplementary data).

~~This mixture approach remains rarely used at present. Nevertheless,~~ The present results are in accordance to some recent experimental and theoretical studies based on a review of literature (Thomaidi et al., 2015; Gosset et al., 2020) which determined the cumulative risk of sets of micropollutants (207 and 55, respectively) for 25 and 33 streams receiving urban effluents. The calculated  $RQ_{mix}$  values (obtained for median and worst-case scenario) were in the same range of values ( $RQ_{mix}$  reaching values above 1 recursively) as present ones. The differences in values can however be explained by the difference in the list of molecules observed, as well as observed/reviewed concentrations and PNEC (more or less protective depending of the study).

Looking for the  $RQ_{mix}$  of pollutants with individual RQ below 1, 8 of the 10 WWTPs led to at least one significant risk depending on the measurement campaigns ( $1 < RQ_{mix} < 10$ ): WWTPs 3 and 8 never exceeded the threshold. This result demonstrates the interest of the mixture approach, revealing that individually “safe” CECs can contribute to a significant risk in whole effluents. Several recent studies reached similar conclusions about various mixtures of micropollutants in surface waters. Riva et

al. (2019) observed similar results ( $0.89 < RQ_{mix} < 2.9$ ) when performing the same approach for 47 CECs (synthetic hormones, illicit drugs, disinfectants, personal care products, alkylphenols and plasticizers, perfluorinated compounds, and anthropogenic markers) quantified into stream samples of the highly urbanized area of Milan, Italy. Moreover, Yan et al. (2014) also observed a  $RQ_{mix}$  value of 2.02 considering algal compartment alone for a mixture of 21 individually “safe” pharmaceuticals (antibiotics, analgesics, antiepileptics, antilipidemics and antihypersensitives) studied in the Yangtze river downstream from 2 important urban WWTPs near Chongqing. Finally, Freeling et al. (2019) also recently quantified a surface water mixture risk of surfactants and transformation products, identified by LC-QToF MS and then quantified by targeted analysis, from 33 German WWTP effluents. They also observed in one case a mixture risk above 1, whereas this risk was absent for all of these compounds considered alone.

Thus, the present results demonstrated the interest in considering the mixture ERA. There is a consensus concerning the need for developing tiered approaches for chemical mixtures in ERA (Beyer et al., 2014). As observed above, all CECs can contribute to the final ecotoxicity of a mixture, even if they are under their own effect limit (LOEC/NOEC) and/or under analytical limits (Escher et al., 2020; Johnson et al. 2020) because they do not affect aquatic organisms in an isolated way. The present study was based on a simple concentration addition model (Backhaus and Faust, 2012) tiered approach, supposing that pollutants have a similar mode of toxic action and same sites of action on aquatic organisms. Nevertheless, other models exist, such as independent action (IA), which implies that different modes of action of pollutants have an identical toxic effect (Beyer et al., 2014; Riva et al., 2019). Escher et al. (2020) assumed that at low ecotoxic and environmental concentrations, dose-response curves are linear, and the CA and IA models lead to similar results. Nevertheless, CA and IA models present the same limitation: the non-consideration of the potential interaction of pollutants with each other (e.g. synergy) during toxic action on organisms. Some studies have expressed some concern about their use, as additive predictions depend on the concentration-response curves' shapes for each pollutant of a mixture due to their specific mode of action, leading to severe limitations when the concentration-response curves present different shapes (Berthoud, 2013; Godoy and Kummrow, 2017). However, pollutant interactions, such as synergy, generally appear with the mixture of components at



high concentrations with a limited increase of effects. These interactions remain irrelevant for low doses, especially considering that at low environmental concentrations the ecotoxic responses of pollutants lie on the linear section of the dose-response curves (Boobis et al., 2011; Cedergreen, 2014; Escher et al., 2020). It can therefore be considered more relevant to use CA or IA models when studying the risk of bodies of water contaminated by WWTP effluents potentially containing hundreds to thousands of CECs.

### 3.5 ERA evaluation at territorial scale and risk prioritization

Considering the territorial risk assessment of the WWTPs, results highlighted a variability of risky situations, depending on the exposure of the stream to the effluents. Results showed that the riskiest situations were reached for two distinctive situations: (1) for the WWTP No.1, the smallest WWTP (Flow rate = 235 m<sup>3</sup>/day) of the territory, discharging into a small stream (Gorges stream), which is dry for part of the year, leading to an absence of dilution and thus a stream flow composed of 100% wastewater, (2) for the WWTPs No. 9 and 10, the two biggest (250 000 > flow rate (m<sup>3</sup>/day) > 100 000) of the territory, releasing effluents into one of the major European river, the Rhône River. On the contrary, the lowest risk situation was obtained for WWTP No.3. This result is not surprising, because this WWTP collects wastewaters composed of a 100 % industrial sewershed that is not supposed to emit important loads of pharmaceuticals and pesticides. An intermediate risk (single substance or mixture) was observed for the other WWTPs. Finally, based on mean RQ<sub>mix</sub>, the risk ranking for the WWTPs of this territory is the following: WWTP No.1 > 10 > 9 > 7 > 8 > 6 > 4 > 2 > 5 > 3 (Figure 4). One of the main explanations of this ranking is the difference of dilution ratios, for if CECs concentrations in the effluents varied from one WWTP to another, these variations were not sufficient to explain these risk differences, except for WWTP No.3. Thus, linear regression performed between log-transformed RQ<sub>mix</sub> and dilution factors values showed a strong ( $R^2 = 0.8482$ ) significantly positive relationship (p-value < 0.0001 - Figure 6). Similar dilution-based results have been previously observed on Greek WWTPs (Thomaidi et al., 2015). Finally, the risk ranking results are also in accordance with previous theoretical studies carried out both on the same and neighboring territories as the current experimental

study (Brus and Perrodin, 2017; Gosset al., 2020). The difference in RQ values was only explained by the fact that the list of studied micropollutants was not all the same.

### 3.6 Implications for further researches and environmental policy managers

As already expressed above, the present study pointed out the lack of ecotoxicological data for a significant part of the identified CECs (17/41), and among them, some are implicated in the risk of the WWTP effluents for receiving streams (e.g. methocarbamol). There is therefore a critical need for data on the acute and chronic ecotoxicity of different trophic levels to achieve a more robust ERA. In addition, if the present work considered the mixture risk of the detected CECs, there is a critical need to improve the ERA method, considering some crucial organism exposure parameters such as bioaccumulation/biomagnification factors through the trophic webs, or the persistence/(bio)degradation of pollutants in streams. For example, some pharmaceuticals are subject to degradation and adsorption on particles, which could decrease the exposure concentrations and result in a lower risk (Chen et al., 2015). Some criteria have been developed to consider these factors, such as PBT (Persistence – Bioaccumulation – Toxicity) indices and applied to prioritize risky CECs through the calculation of scores (e.g. Pharmaceuticals) (Blum et al., 2017; Li et al., 2020), but do not currently improve the quantification of the whole risk represented by effluent discharges.

Furthermore, this work highlights once again that a part of present CECs (e.g. pharmaceutical residues) should be considered as priority pollutants list in the national/international programs, based on their PBT criteria, but also based on their final risk for ecosystems and the frequency of such a risk. Moreover, a wide range of other CEC families can potentially be found in effluents, such as surfactants and their metabolites recently identified following a similar method (Freeling et al., 2019). The present framework should be extended to other CEC families to increase considerably the “real” risk evaluation linked to the municipal discharges and better select the CECs that should be regulated in priority. Besides, the scale of the study should be increased, to confirm the current result obtained for this territory at a national scale (each territory having specific pollution characteristics), to carry out national public policies to reduce pollution coherently.

Finally, three strategies currently exist to manage identified risky CECs:

- The first would be to optimize current secondary treatment and to generalize tertiary treatment in WWTPs. If their removal performance is not perfect, several methods, such as ozonation, peroxonation, peroxidation, or activated carbon, significantly abate the residual pollution of secondary effluents (Kårelid et al., 2017; Wang et al., 2018).
- The second complementary strategy would be to reduce wastewater pollution through upstream source management (Jackson and Sutton, 2008). The pollution of wastewaters by CECs is associated with many factors, such as population density, sales, practices, consumption patterns, economic structure, water use rates, and other socio-economic factors (Dickenson et al., 2011; Vystavna et al. 2013). Thus, levers for action such as socio-economic and regulatory policies, and incentives on economic actors, could be implemented to reduce the wastewater pollution load. However, a more precise comprehension of the factors influencing the different loads of CECs in wastewater is needed.
- In the case of the implementation of a new WWTP, special attention should be paid to the dilution factor of the effluents into surface waters, as it appears to be a key factor in the final risk for ecosystems. At the present stage, this factor is often disregarded during WWTP effluent routine monitoring and its influence may increase in the future in a context of climate change and disturbance of streamflows all over the Rhône valley (Ruiz-Villanueva et al., 2015).

#### 4. Conclusion

In this study, an ~~innovative~~ ERA was carried out for 10 WWTPs located in one highly urbanized territory. Advanced chemical analyses (suspect-screening coupled with a targeted quantification) were combined with an ERA methodology to study CECs in isolation and in mixtures. For the first time, this work demonstrates the interest of this procedure to identify and quantify more exhaustively the ecotoxicological risks related to two important families of CECs (pharmaceuticals residues and pesticides) into urban WWTP effluents. In this way, it has been possible to increase knowledge related to the ecotoxicological risks represented by these discharges by monitoring the risk of CECs that are currently poorly or un-studied (e.g. trospium, tiapride or milnacipran) in surface and urban wastewater.

An important part of CECs (19/41) exhibited at least one time a significant risk alone ( $RQ > 1$ ), even though they all contributed to the mixture risks observed following a CA model. A few CECs mostly contributed to the observed risks (methocarbamol, venlafaxine, terbutryn, atorvastatin, lidocaine, atenolol, and diclofenac). This study may thus help update the Watch List of Water Framework Directive drawn up by the European Commission about pesticides and pharmaceutical residues. Finally, this work has made it possible to highlight a diversity of risks linked to various WWTPs in one territory. It has enabled the prioritization of higher risk situations (e.g. small WWTPs discharging into a stream in a very small dried-up streams), which will help public and private actors target WWTPs for priority action (e.g. improving treatment).

Nevertheless, the present approach contains some shortcomings that must be overcome in the future to improve its efficiency. For example, exposure parameters for organisms such as bioaccumulation/magnification factors and (bio)degradation of CECs are currently not taken into account in the ERA methodology to quantify the final risk to aquatic ecosystems (only for the prioritization of pollutants, based on modelled data due to a lack of experimental data). In addition, the study focused on two families of CECs but should be extended in further studies to other families of pollutants potentially present in wastewater in order to have a more global view of the risk associated with this discharges.

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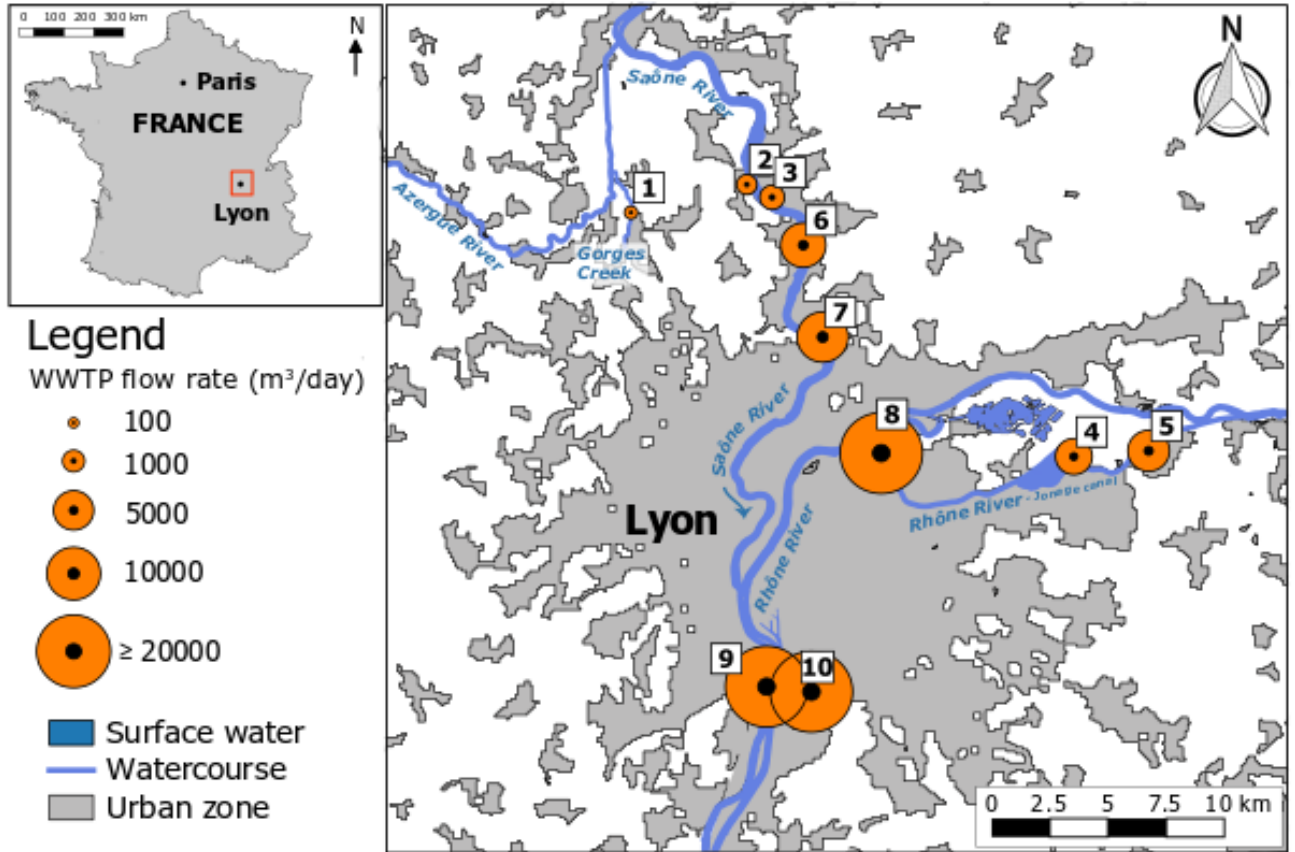


Figure 1: Localization of the 10 studied WWTPs and receiving watercourses on the Grand Lyon (France) urbanized territory.



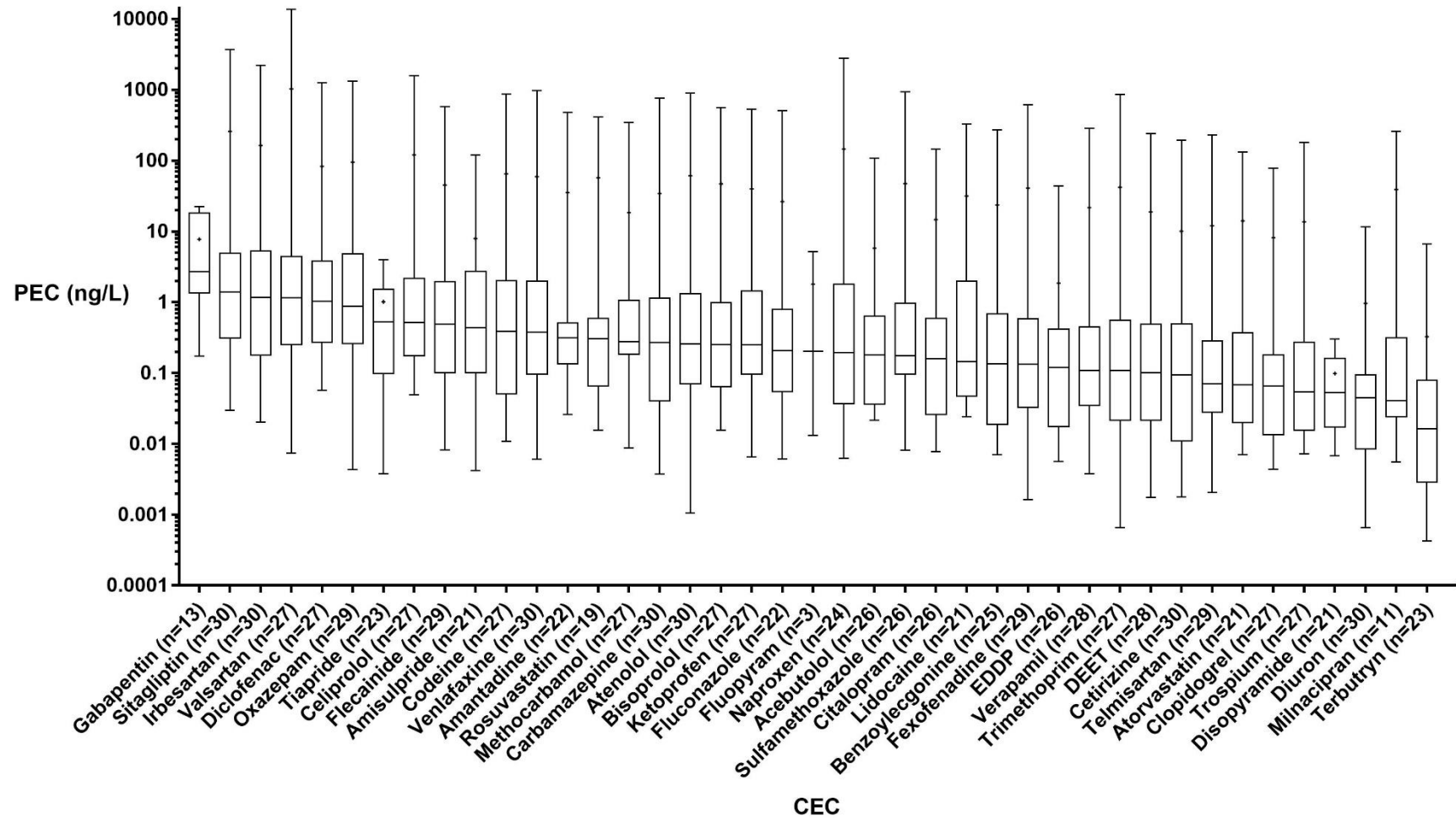


Figure 2: Representation of the PEC (median, min, max, quartiles) values for each CEC (41), considering all WWTP and sampling campaign. Mean values are represented by a cross. Number of data above LOQs are in the brackets. For raw values, please refer to Supplementary data.

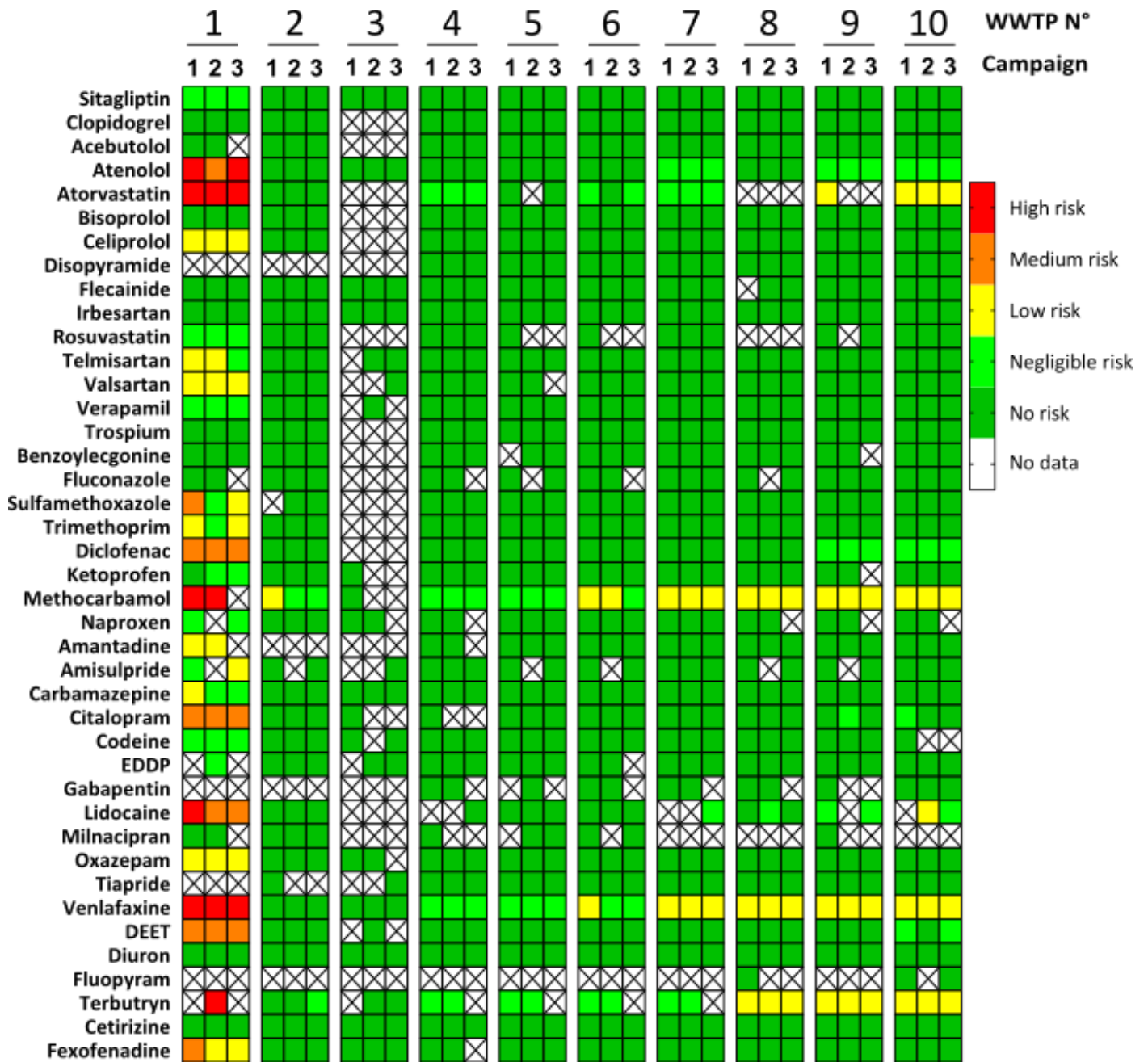
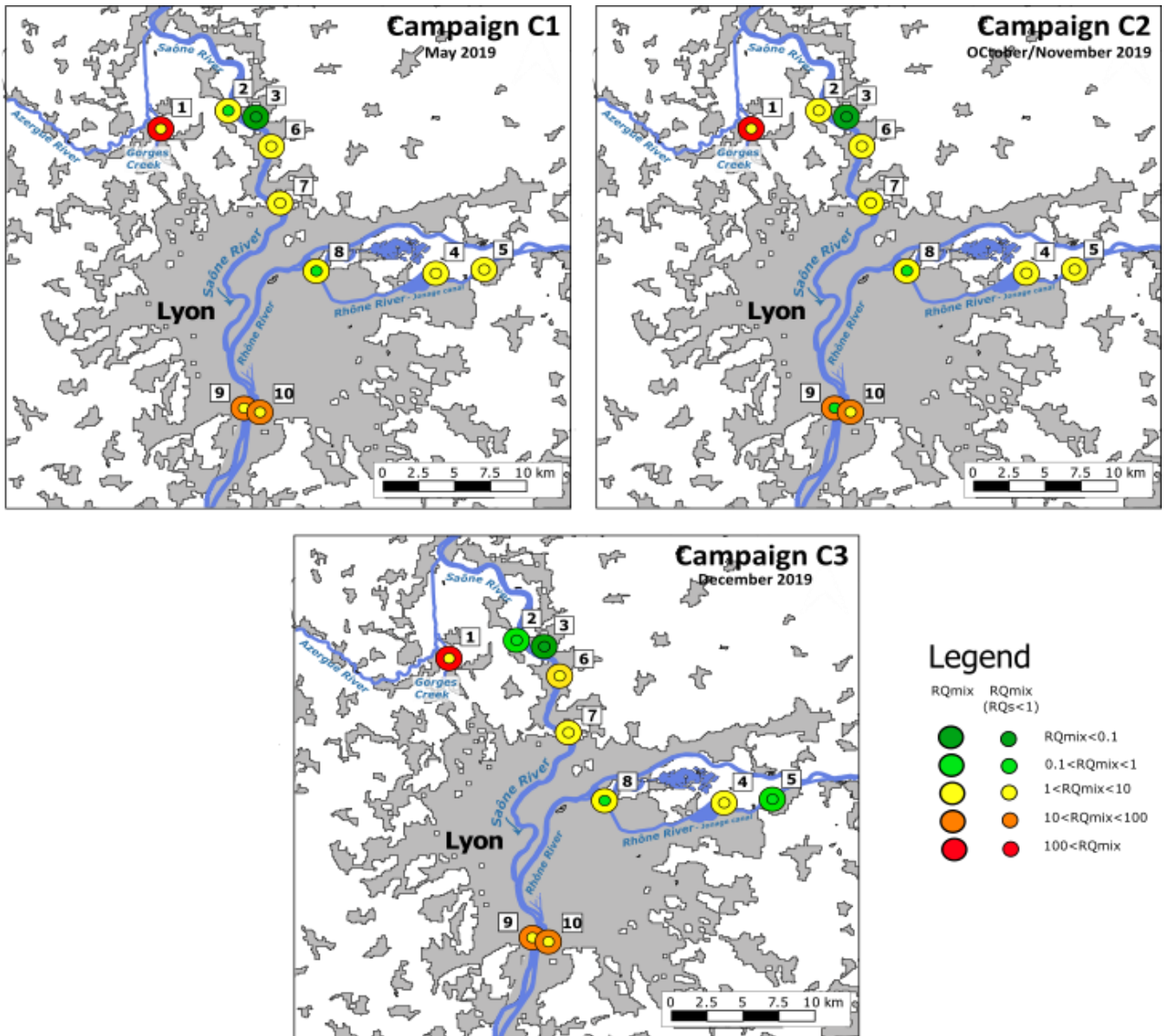


Figure 3: Representation of the risk quotients calculated for each CEC (41), WWTP (10) and sampling campaign (3), following the ranking described in Table 2. For raw values, please refer to Supplementary data.



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Figure 4: Representation of the risk quotients (RQ<sub>mix</sub>) calculated for the mixture of the 41 CECs, each WWTP (10) and sampling campaign (3), following the ranking described in Table 2. For raw values, please refer to Supplementary data. The biggest circles represent the RQ<sub>mix</sub> linked to all CECs whatever the individual RQ value, and the smallest circles represent the RQ<sub>mix</sub> associated to the pollutants with individual RQ below 1.

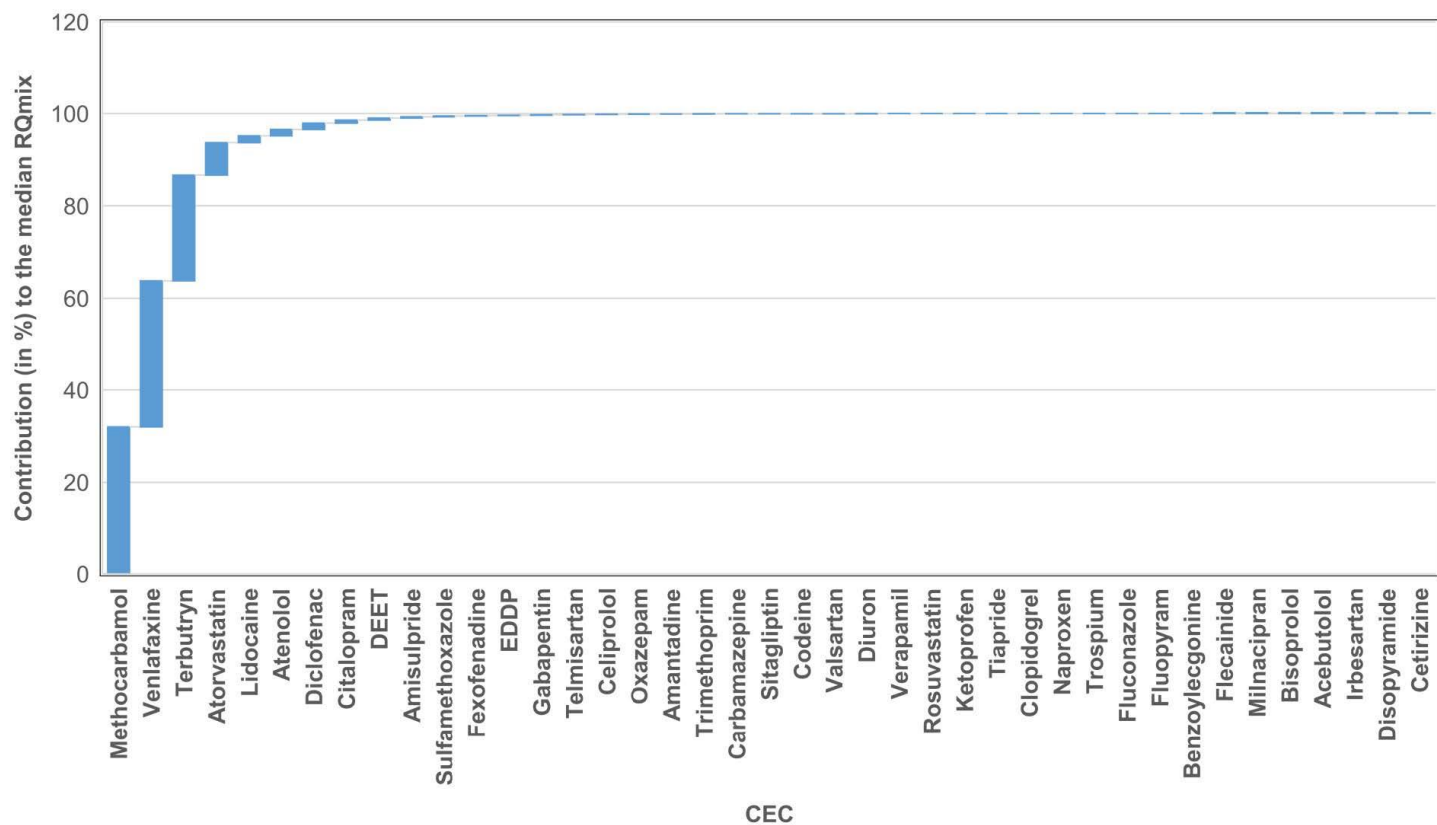


Figure 5: Cumulative contribution (in %) of each CEC to the median RQmix calculated considering all WWTPs and sampling campaigns. For raw values, please refer to Supplementary data.

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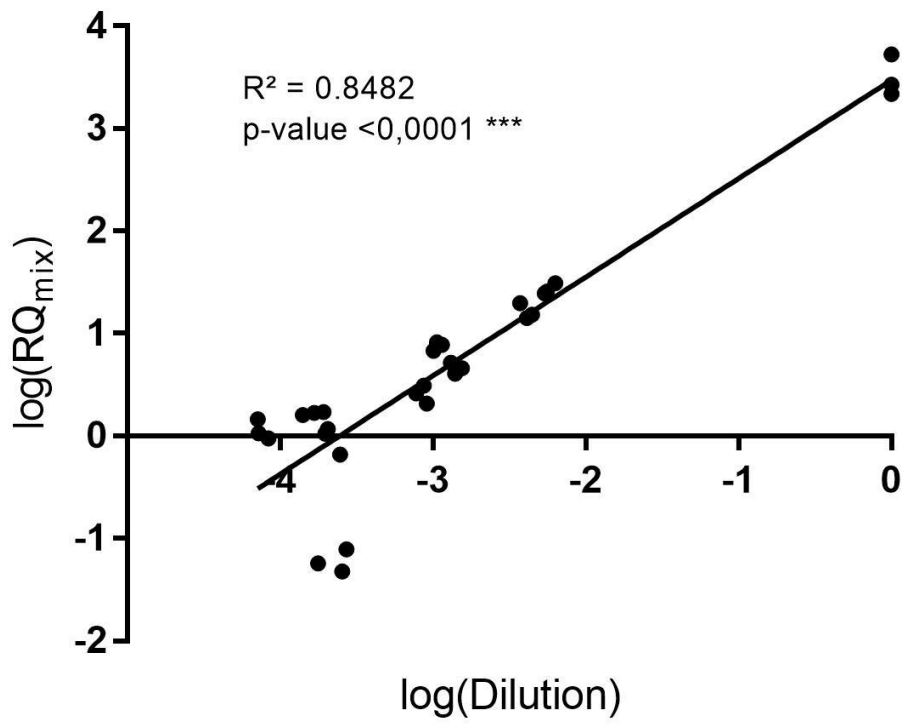


Figure 6: Linear regression plot of the log-transformed RQ<sub>mix</sub> calculated for each WWTP and sampling campaign against the respective log-transformed dilution ratios. P-value corresponds to the value obtained following a linear regression statistical test.

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32 Table 1: Characteristics of the WWTP studied in the Lyon (France) urbanized territory.

No.	Coordinates of the WWTP (Latitude / Longitude)		Number of drained municipal ities	Incoming load in Population Equivalent (PE))	Annual mean incoming flow rate of the WWTP (m <sup>3</sup> /day)	Secondary treatment process	Name of the receiving watercourse
1	45° 87' 54.371"	4° 74' 22.744"	1	3025	235	Activated sludges	Gorges stream
2	45° 53' 22.628"	4° 48' 18.451"	2	2843	679	Activated sludges	Saône River
3	45° 88' 52.795"	4° 81' 96.091"	1	9150	1300	Radial flow fluidized filter	Saône River
4	45° 47' 28.366"	4° 59' 44.352"	2	25732	4016	Biofilter	Rhône River – Jonage canal
5	45° 47' 43.464"	5° 2' 12.133"	7	21800	5544	Activated sludges	Rhône River – Jonage canal
6	45° 52' 2.215"	4° 50' 18.915"	4	16165	6745	Activated sludges	Saône River
7	45° 49' 53.882"	4° 51' 9.84"	12	44087	8980	Biofilter	Saône River
8	45° 47' 15.042"	4° 53' 21.753"	10	179772	38188	Activated sludges	Rhône River
9	45° 41' 33.04"	4° 50' 5.364"	34	524325	156962	Activated sludges	Rhône River
10	45° 41' 28.235"	4° 50' 55.267"	20	622800	215092	Activated sludges	Rhône River

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45 Table 2: Single substance and mixture ecotoxicological risk ranking based on the risk quotient (RQ).

Risk Quotient (RQ)	Ranking categories
RQ < 0.1	No risk
0.1 < RQ < 1	Negligible risk
1 < RQ < 10	Low risk
10 < RQ < 100	Medium risk
100 < RQ	(Very) High risk

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65 Table 3: Classification employed for the identified contaminants of emerging concern.

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Classification of the contaminants of emerging concern	Abbreviation
<i>Pharmaceuticals (ATC (Anatomic Therapeutic Chemical) classification)</i>	
Cardio-vascular system	Ph CVS
Nervous system	Ph NS
Anti-infective for systemic use	Ph AI
Musculoskeletal system	Ph MS
Blood and blood forming organs	Ph BBFO
Dermatologicals	Ph D
Alimentary tract and metabolism	Ph ATM
Genito-urinary system and sex hormones	Ph GUS
<i>Others</i>	
Pesticide	P
(Illegal) Drugs	ID

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75 Table 4: Inventory of the ecotoxicological data available for contaminants from urban wastewaters with no PNEC data in scientific database or literature - PNEC  
 76 calculation. (D: Decomposer; P: Primary producer; 1C: Primary consumer; 2C: Secondary consumer).

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No. CAS	Compound	Family	ECOSAR (mg/L) (EPI suite; Sanderson et al., 2003 ; Dong et al., 2013)			Experimental ecotoxicity								Experimental data used for PNEC calculation				EF	PNEC (µg/L)	Ref.	
						Test species	Trophic level				Chronic test				Specie	Parameter	Endpoint				Value (µg/L)
			Algae	Daphnia	Fish		D	P	1C	2C	D	P	1C	2C							
37517-30-9	Acebutolol	Ph-CVS	94.126	61.477	131.821	1	0	0	1	0	0	0	0	0	<i>Ceriodaphnia dubia</i>	Immobilization	EC50	5.09E+04	1000	5.09E+01	Fraysse and Garric (2005)
83799-24-0	Fenofexadine	Ph-NS	18.619	34.111	79.356	5 (+ C)	1	1	2	2	1	1	0	1	Microbial (fungi + bacteria) community	Microbial activity	LOEC	2.00E+00	50	4.00E-02	Fass.se ; Jonsson et al. (2014, 2015)
54143-55-4	Flecainide	Ph-CVS	6.405	4.08	6	1	0	0	0	1	0	0	0	1	<i>Danio rerio</i>	Motility defect	LOEC	4.14E+04	100	4.08E+01	Richards et al. (2008) ; Wen et al. (2012)
60142-96-3	Gabapentin	Ph-NS	229.686	1611.06	32040.607	1	0	1	1	1	0	0	0	1	<i>Danio rerio</i>	Malformation	LOEC	1.00E+02	100	1.00E+00	Li et al. (2018) ; He et al. (2019) ; Minguéz et al. (2016)
532-03-6	Methocarbamol	Ph-MS	961.708	1769.544	643.219	1	0	0	0	1	0	0	0	1	<i>Pimephales promelas</i>	Hepato-toxicity	LOEC	2.30E-02	100	2.30E-04	Schoenfuss and al. (2016)

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83 Table 5: Ecotoxicity (Predicted No Effect Concentration (PNEC)) of the identified contaminants of  
 84 emerging concern.

<b>Chemical class</b>	<b>CAS RN</b>	<b>Molecule</b>	<b>PNEC (µg/L)</b>	<b>Source</b>
P	886-50-0	Terbutryn	0.000019	Gosset et al. (2017)
Ph-MS	532-03-6	Methocarbamol	0.00023	See Table 4
Ph-CVS	134523-00-5	Atorvastatin	0.00026	Zhou et al. (2019)
Ph-NS	93413-69-5	Venlafaxine	0.000313	Vergeynst et al. (2015)
Ph-NS	137-58-6	Lidocaine	0.0026	(Orias and Perrodin, 2013 - ECOSAR)
Ph-CVS	29122-68-7	Atenolol	0.005	Zhou et al. (2019)
P	134-62-3	DEET (Diethyltoluamide)	0.006	Gosset et al. (2017)
Ph-NS	59729-33-8	Citalopram	0.00635	Orias and Perrodin (2013)
Ph-MS	15307-86-5	Diclofenac	0.02	Orias and Perrodin (2013)
Ph-CVS	144701-48-4	Telmisartan	0.026	Zhou et al. (2019)
Ph-AI	723-46-6	Sulfamethoxazole	0.03	Rivera-Jaimes et al. (2018)
Ph-NS	71675-85-9	Amisulpride	0.04	Helwig et al. (2016)
Ph-NS	83799-24-0	Fexofenadine	0.04	See Table 4
ID / Ph-NS	30223-73-5	EDDP	0.044	ECOSAR
Ph-AI	738-70-5	Trimethoprim	0.16	Rivera-Jaimes et al. (2018)
P	330-54-1	Diuron	0.2	Gosset et al. (2017)
Ph-NS	768-94-5	Amantadine	0.256	Chen et al. (2015)
Ph-CVS	56980-93-9	Celiprolol	0.265	ECOSAR
Ph-NS	298-46-4	Carbamazepine	0.42	Helwig et al. (2016)
Ph-NS	604-75-1	Oxazepam	0.481	Bouissou-Schurtz et al. (2014)
Ph-CVS	52-53-9	Verapamil	0.600	Orias and Perrodin (2013)
Ph-NS	60142-96-3	Gabapentin	1	See Table 4
Ph-NS	76-57-3	Codeine	1.28	Zhou et al. (2014)
Ph-BBFO	113665-84-2	Clopidogrel	1.6	Helwig et al. (2016)
Ph-CVS	287714-41-4	Rosuvastatin	1.8	Helwig et al. (2016)
Ph-GUS	10405-02-4	Tropium	1.822	ECOSAR
Ph-MS	22071-15-4	Ketoprofen	2	Orias and Perrodin (2013)
Ph-CVS	137862-53-4	Valsartan	3.865	Zhou et al. (2019)
Ph-ATM	486460-32-6	Sitagliptin	3.9	Vestel et al. (2016)
Ph-NS	92623-85-3	Milnacipran	4.048	ECOSAR
Ph-NS	510012-32-9	Tiapride	5.081	ECOSAR
Ph-MS	22204-53-1	Naproxen	6.6	Orias and Perrodin (2013)
Ph-D	86386-73-4	Fluconazole	9.46	Coors et al. (2018)
ID / Ph-MS	519-09-5	Benzoylcgonine	10	Fernandez-Rubio et al. (2019)
P	658066-35-4	Fluopyram	13.5	AGRITOX (2019)
Ph-CVS	66722-44-9	Bisoprolol	35.6	Helwig et al. (2016)
Ph-CVS	37517-30-9	Acebutolol	50.9	See Table 4

Ph-CVS	54143-55-4	Flecainide	40.8	See Table 4
Ph-CVS	3737-(0)9-5	Disopyramide	63	Komori et al. (2013)
Ph-NS	83881-51-0	Cetirizine	278	Helwig et al. (2016)
Ph-CVS	138402-11-6	Irbesartan	704	FASS.se (2019)

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