Contaminants of Emerging Concern in the Basque coast (N Spain): occurrence and risk assessment for a better monitoring and management decisions

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ABSTRACT

The study of the presence in the aquatic environment of certain substances considered as contaminants of emerging concern (CEC) is a preliminary step to the analysis of the possible harmful effects on aquatic ecosystems and the establishment of the corresponding environmental quality standards. In order to monitor the occurrence of CECs in the aquatic environment, the European Commission established in 2015 and 2018 two watch-list of substances for Union-wide monitoring in the field of water policy (Decision (EU) 2015/495 and Decision (EU) 2018/840). In the coast of the Basque Country, southeast of the Bay of Biscay, 19 of these watch list substances were monitored quarterly from May 2017 to March 2019. Water samples were collected at the effluent of three wastewater treatment plants and five control points associated with receiving waters (transitional and coastal water bodies). The most frequently quantified substances were azithromycin (91%), imidacloprid (82%), clarithromycin (80%), diclofenac (78%) and erythromycin (73%), with frequencies of quantification higher in wastewaters (83-100%) than in receiving waters (70-85%). In general, concentrations in wastewater were also higher than in receiving waters, indicating a dilution effect in the environment. In receiving waters, six out of the nineteen substances monitored exceeded their respective Predicted No-Effect Concentrations: azithromycin (34%), imidacloprid (9%), 17β-estradiol (E2) (9%), clarithromycin (7%), ciprofloxacin (7%), and diclofenac (5%); and therefore, their levels could pose an environmental risk.

KEYWORDS

EU Watch List; Water Framework Directive; Priority Substances Directive; Wastewater; Receiving water.

1. INTRODUCTION

In the last decades, huge management efforts have been undertaken, in developed and developing countries, to reduce the discharge of 'traditional' contaminants (i.e., nutrients, metals, persistent organic compounds) into the aquatic systems, including rivers, estuaries and coasts (WWAP, 2017). This has resulted in some areas in a significant reduction of the concentration of some contaminants, such as nutrients (Andersen et al., 2017; Harding et al., 2015), metals (Bowman et al., 2020; Pinedo-González et al., 2018; Schøyen et al., 2019) and organic compounds (Melwani et al., 2014; Miège et al., 2012; Munaron et al., 2012; Robinson et al., 2019; Sericano et al., 2014), especially in developed countries. However, at the same time, contaminants of emerging concern (CECs; i.e., pharmaceuticals, personal care products, polar pesticides, micro- and nano-plastics, etc.) have gained importance, because of their potential harmful effects in the environment and human health (Borja et al., 2020).

CECs can be defined as natural or anthropogenic substances (i) recently introduced into the environment; (ii) known to be present in the environment for a long time, but not recognized as potentially dangerous to ecosystems and/or humans; or (iii) recently detected using novel analytical techniques (Barbosa et al., 2016; Sousa et al., 2018). These substances are neither currently regulated nor included in routine monitoring programs (Apeti et al. 2018), and there is often a lack of comprehensive information regarding their sources (i.e., industrial wastewater; runoff from agriculture, livestock and aquaculture; landfill leachates; and domestic and hospital effluents) (Barbosa et al., 2016), distribution, persistence, and potential risks to humans and/or ecological systems (Sauvé and Desrosiers, 2014). In addition, the continuous but non-detectable effects of these substances may gradually accumulate, and the co-occurrence of different compounds may lead to synergistic interactions that may cause unexpected adverse effects in exposed organisms (Sousa et al., 2018).

In Europe, the main instrument to phase out pollution in aquatic systems is the Water Framework Directive (WFD; EC, 2000), which commits Member States to achieve good chemical status of their surface waters (i.e., all monitored chemical substances should be below established Environmental Quality Standards (EQS) by 2021 or 2027). This Directive was further developed to set the substances to be monitored (the so-called 'priority substances') and the EQS to be achieved (EC, 2013). After various reporting and status outlooks (EEA, 2019), some CECs were in the spotlight for further monitoring and eventual regulation in the near future (EC, 2015, 2018). This is called the 'Watch List' and includes hormones, antibiotics, anti-inflammatories, pesticides, antioxidants, and UV-filters (Tornero and Hanke, 2016, 2017).

To date, only a few studies on the monitoring and occurrence of these Watch List CECs have been published (e.g., Barbosa et al., 2016; Sousa et al., 2018, 2019, 2020), since most countries do not have yet appropriate legislation or monitoring programs for their routinely analysis and assessment (Sousa et al., 2019). However, to take informed decisions on management measures, it is necessary to monitor and assess the risk that these CECs can cause to the environment and human well-being, especially because it is well known that conventional wastewater treatment plants (WWTPs) are not originally designed to remove organic micropollutants (Archer et al., 2017). In the case of the Basque Country (Northern Spain; Figure 1), the Basque Water Agency (URA) has a long record of monitoring estuarine and coastal waters for chemical status assessment (Menchaca et al., 2014; Solaun et al., 2013; Tueros et al., 2009), and thus, has supported the implementation of the WFD implementation at its earliest stages (Borja et al., 2004). In fact, the Basque Country has a large population, especially around the cities of San Sebastián and Bilbao, and a long industrial history, which contributed to estuarine and coastal contamination and degradation in the past (Borja et al., 2016). This region has substantially recovered from industrial pollution and thus, can serve as a good test area to determine the risk of CECs.

In this context, the main objectives of this research were to investigate the occurrence and fate of the Watch List substances, following European legislative decisions, in the estuarine and coastal waters of the Basque Country, and to assess their origin and their potential environmental risk. This finding of this study will be useful to take management decisions in the near future regarding CECs monitoring, to minimize the environmental risks posed by this category of substances as required by the WFD, provided that the local conditions investigated can be also representative of similar scenarios. This is important because with the aim of reducing monitoring effort, the design of efficient monitoring frameworks will become a priority in Europe (Borja and Elliott, 2013).

2. MATERIALS AND METHODS

2.1. Study area and sample collection

The study was carried out in the Basque coast, in the southeast of the Bay of Biscay. Focused on two highly populated estuaries (Nerbioi and Oiartzun), a rural estuarine area (Oka), and one coastal area between San Sebastian and Pasaia (Figure 1), the two most populated areas, and the associated WWTPs as emission points, were selected: Galindo, in the Ibaizabal estuary, which serves Bilbao city and all towns around the estuary, an agglomeration of 1.2 million population equivalents; and Loiola -emission point in Ulia-, in the Oiartzun estuary and its coastal area, serving the San Sebastián area, an agglomeration of 0.55 million population equivalents. In addition, the WWTP of Gernika, in the Oka estuary, serving an agglomeration of 18,600 population equivalents, was also selected as an example of a protected area where the sanitation is still not complete. The receiving waters corresponding to these emission points are routinely monitored for the ecological and chemical status assessment by URA (Borja et al., 2019).

The Watch List CECs were analysed in the three WWTPs (emission sampling points: Galindo, Gernika, and Ulia) and in five sampling stations related to the receiving waters of their discharges (E-N15, E-N17, E-OK5, E-OI15, and L-UR20) (Figure 1 and Table S1, in Supplementary Material, for details).

Water samples were collected quarterly (spring, summer, autumn, winter) from May 2017 to March 2019. Grab water samples were collected at the outlet of the WWTP (by the WWTP personnel), and in receiving water bodies. Surface waters were collected using 5 L Niskin bottles, at low tide for the estuarine locations and independent of the tide level at the coastal location. Hydrographic data in receiving waters (temperature, salinity, pH and dissolved oxygen) were obtained *in situ* using a CTD-Seabird 25 multiprobe. The concentration of suspended solids was measured following Clesceri et al. (1989), after filtration of the water through Whatman GF/C filters. Once in the laboratory, the water samples, in preparation for the analysis of the Watch List compounds, were allowed to settle for a few minutes at room temperature, then a 500 mL-aliquot was transferred to a volumetric flask containing the mixture of the surrogate compounds (see section 2.2.), subsequently filtrated through 0.7 μm glass fiber filters and stored in amber polyethylene terephthalate (PET) bottles at 20 °C.

All CECs of the first Watch List (Decision (EU) 2015/495; EC, 2015; Table 1) were analysed in the water samples collected from May 2017 to February 2018, while all CECs of the second Watch List (Decision (EU) 2018/840; EC, 2018; Table 1), except amoxicillin, were monitored in the water samples collected from May 2018 to March 2019.

2.2. Chemicals and reagents

The CECs investigated, included in the first and second Watch Lists, comprise the compounds listed in Table 1, and include the anti-inflammatory diclofenac, the estrogenic compounds 17β-estradiol (E2), 17α-ethinylestradiol (EE2) and Estrone (E1), the anti-oxidant 2,6-ditert-butyl-4-

methylphenol (BTH), the UV filter 2-ethylhexyl 4-methoxycinnamate (EHMC), the antibiotics erythromycin, clarithromycin, azithromycin, amoxicillin and ciprofloxacin, and the pesticides methiocarb, imidacloprid, thiacloprid, thiamethoxam, clothianidin, acetamiprid, oxadiazon, triallate and metaflumizone. Analytical standards of these substances and isotopically labelled analogues used as surrogate standards (SS) in the quantification process were provided by Sigma Aldrich (Madrid, Spain) and Toronto Research Chemicals (North York, Canada). Individual stock solutions were prepared in MeOH at a concentration of 1 $mg \cdot mL^{-1}$. These solutions were used to prepare working standard mixtures at different concentrations by appropriate dilution in methanol. These standard mixtures were then used to freshly prepare the calibration solutions containing the target compounds in the range 0.035-1000 $ng \cdot L^{-1}$ and the isotopically labelled compounds at a fixed concentration of 5 $ng \cdot L^{-1}$ in salted HPLC water (1.75% NaCl). A methanolic solution containing only the isotopically labelled standards was also prepared to fortify the water samples immediately after collection at a concentration of 10 ng/L. All standard solutions were stored in the dark at – 20 ºC until use.

All solvents used (HPLC-grade) were supplied by Merck (Darmstadt, Germany), as well as formic acid (> 98%).

Whatman® glass fiber filters (GF/F, 0.7 µm pore size) were supplied by Merck (Barcelona, Spain). On-line solid-phase extraction cartridges CHROspe PLRP-s (styrene/divinylbenzene polymer, 10 mm x 2 mm i.d., 15-20 µm particle size) were purchased at Spark Holland (Emmen, The Netherlands) (currently available at Axel Semrau GmbH & Co. KG, Srockhövel, Germany).

2.3. Analysis of Watch List CECs

Due to the wide spectrum of the physical-chemical properties of the target compounds, three different analytical methods had to be developed and implemented to comply with the sensitivity requirements set in the European decisions (EC, 2015, 2018).

BTH and EHMC were analysed with a method based on liquid-liquid extraction (LLE) and gas chromatography coupled to tandem mass spectrometry (GC-MS/MS). Fifty mL of sample was fortified with the surrogated standard mixture, acidified to pH 3 with acetic acid and extracted with n-pentane (3x10 mL). The extract was water-dried using an ISOLUTE® Sodium Sulfate Drying Cartridge from Biotage (Uppsala, Sweden), concentrated to dryness under a gentle stream of nitrogen, and finally reconstituted with 0.5 mL hexane. GC-MS/MS analysis of the extract was conducted with a 7890B gas chromatograph coupled to a 7000C mass spectrometer (Agilent Technologies), using electron ionization. MS acquisition was done in the selected reaction monitoring (SRM) mode. Further details on the GC-MS/MS experimental conditions used have been provided in Supplementary Material (Table S2).

The remaining compounds were analysed by on-line solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry (on-line SPE-LC-MS/MS), using a Prospekt-2 automated extraction system (Spark Holland, Emmen, The Netherlands) connected in series with a 1525 binary HPLC pump (Waters, Milford, MA, USA) and a triple quadrupole mass spectrometer Xevo TQ (Waters) operated in the SRM mode. Chromatographic separation was achieved with a Purospher STAR RP-18 column (100 mm x 2 mm, 5 µm, from Merck, Darmstadt, Germany) and a mobile phase consisting of water and acetonitrile in the case of the compounds detected in the negative electrospray mode (ESI-), i.e. E2, E1, EE2 and diclofenac, and water and acetonitrile both acidified with formic acid (0.1%) in the case of the remaining compounds, detected in the positive electrospray (ESI+) mode, i.e., azithromycin, clarithromycin, erythromycin, methiocarb, acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam, oxadiazon, triallate, ciprofloxacine, and metaflumizone. In both cases, preconcentration of the samples (50 mL for the analysis of compounds amenable to ESI(-) and 30 mL for the analysis of compounds detected under ESI(+), diluted with HPLC-grade water, 1:1, v/v) was performed with PLRP-s cartridges.

Quantification of all analytes was performed using the isotope dilution method, which ensures the reliability of the results regardless of eventual matrix effects and/or variabilities in the instrument performance. Method detection limits (MDLs) achieved were lower than the maximum acceptable MDLs (Table 1) set in the regulation, except for EE2 (from August 2018 to March 2019) and methiocarb (in May 2018). Amoxicillin, initially included within the group of compounds analysed by on-line SPE-LC-ES(+)-MS/MS, was finally discarded from the analysis due to poor performance of the analytical method.

Quality controls, i.e., an aqueous standard solution containing the compounds and corresponding surrogate standards at concentrations of 50 $ng \cdot L^{-1}$ and 5 $ng \cdot L^{-1}$, respectively, were analysed every 6 samples to check the correct operation of the instrument. MS signals for the target analytes were absent in solvent blanks (HPLC-grade water injected every 3 samples), discarding analyte carryover between injections. Also MS signals for all target analytes, but ciprofloxacin, azithromycin, clarithromycin and erythromycin, were absent in method blanks (HPLC-grade water processed following the same treatment protocol as samples and thus fortified with the surrogate standard mixture at a concentration of 5 $ng \cdot L^{-1}$), discarding cross-contamination during sample preparation. In the case of ciprofloxacin, azithromycin, clarithromycin and erythromycin, the MS signal present in the method blanks, likely coming from their presence as impurities in the internal standard solution, was considered (subtracted) during the quantification of the positive samples.

2.4. Data analysis

Taking into account that concentrations below MDL (corresponding to the 52% of the measured concentrations) are considered as not quantifiable by water managers, in all statistical analysis, non-detected compounds (<MDL) were considered as below the method quantification limit (<MQL). In accordance with the Commission Directive 2009/90/EC (EC, 2009), chemical concentrations <MQL were set to half of the value of the limit of quantification concerned (i.e.,

206 MQL/2) for the calculation of mean values. Since quantification limits changed throughout the
207 studied period, as they depend on the status of the analytical instrumentation and the samples
208 themselves, where a calculated mean value was below the maximum MQL, the value was
209 referred to as the maximum MQL.

210 Compound ubiquity was assessed through the calculation of the frequency of quantification (F
211 (%)), which corresponds to the percentage of cases above the MQL compared to the total
212 number of cases analysed:

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214 Since there are no EQS defined for the studied CECs, some authors propose to use risk quotients
215 (RQ) for assessing the intensity of local impacts (Sousa et al., 2018; von der Ohe et al., 2011). To
216 estimate the impact of these CECs on the receiving water bodies, RQs were calculated as the
217 ratio between the measured concentrations (MC) and the predicted no-effect concentration
218 (PNEC) values (Table 1), according to the European technical guidance document on risk
219 assessment (EC, 2003):

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221 Regarded as a concentration below which an unacceptable effect will most likely not occur, the
222 PNEC can be derived using an assessment factor approach or, when sufficient data is available,
223 using statistical extrapolation methods (EC, 2003). The PNEC values used in the present study
224 were initially calculated by Carvalho et al. (2015) and have been recently updated by Loos et al.
225 (2018) (Table 1).

226 The RQs are classified into three risk levels: (i) RQ<0.1 indicate a low risk; (ii) 0.1≤RQ≤1 indicate
227 a medium risk; and (iii) RQ>1 reveal a high risk (Gusmaroli et al., 2019; Sousa et al., 2018). In this
228 study, the risk was considered as low for compounds present at concentrations <MQL, except in

229 those cases where the MQL was above the corresponding PNEC (as occurred in some samples
230 for EE2, E2 and methiocarb) and RQs were therefore not calculated.

231 Paired samples t-tests (at $\alpha = 0.05$) were carried out to compare the mean concentrations at the
232 receiving water sites with those of the closest emission points (these tests were carried out only
233 for those substances with frequencies of quantification above 65%).

234 ## 3. RESULTS

235 ### 3.1. Physical-chemical parameters

236 The ranges of physical-chemical parameters measured in the receiving waters were 8.1-24.1 ºC
237 (temperature), 0.1-35.1 (salinity), 4.5-12.6 $mg \cdot L^{-1}$ (dissolved oxygen), 54-119% (oxygen
238 saturation), 7.7-9.7 (pH), and 1.6-60.6 $mg \cdot L^{-1}$ (suspended solids) (see Table S3 in Supplementary
239 Material, for details).

240 ### 3.2. Occurrence of Watch List compounds

241 Throughout the considered time period (spring 2017-winter 2019), 14 out of the total 19
242 compounds analysed were quantified (>MQL) in at least one emission sampling point, and 16
243 compounds in at least one receiving water sample (Table 2 and Table S4, in Supplementary
244 Material). 17α-ethinylestradiol (EE2), methiocarb, and metaflumizone showed concentrations
245 <MQL in all sampling stations, being all concentrations of EE2 also <MDL. Azithromycin,
246 however, was quantified in all water samples collected in wastewater stations and in 85% of the
247 receiving water samples. Imidacloprid, clarithromycin, diclofenac and erythromycin also showed
248 quantification frequencies higher than 83% in WWTP effluents, and between 70 and 75% in
249 receiving waters. Conversely, E2, E1, clothianidin, oxadiazon, and triallate were quantified more
250 frequently in receiving waters than in wastewater (Table 2). Galindo showed up to six
251 compounds (diclofenac, erythromycin, clarithromycin, azithromycin, ciprofloxacin and

252 imidacloprid) above the MQL in all samples, while other four (E1, E2, EE2 and metaflumizone)
253 were neither quantified nor detected in samples collected in this sampling point (Figure 2).

254 In general, mean concentrations of the target CECs were higher in WWTP effluents than in
255 receiving waters, except for oxadiazon (Table 2). Galindo showed the highest mean
256 concentration for seven of these substances, namely diclofenac, clarithromycin, azithromycin,
257 ciprofloxacin, imidacloprid, thiamethoxam and acetamiprid; Gernika showed the highest mean
258 concentrations for erythromycin, and Ulia for BTH (Table S4 in Supplementary Material). The
259 concentrations of azithromycin, clarithromycin, acetamiprid, diclofenac, and erythromycin were
260 significantly lower at some sampling sites located in receiving waters, in comparison to the
261 closest wastewater site (Table 3). The other studied substances did not fulfil the requirements
262 to carry out the paired samples t-test (see section 2.4 for further details).

263 ### 3.3. Temporal variation

264 Considering the 12 CECs that are included in both Watch Lists and that were therefore analysed
265 in both studied time periods (from May 2017 to February 2018, and from May 2018 to March
266 2019), higher mean concentrations of clarithromycin, azithromycin, thiamethoxam, and
267 acetamiprid were observed in the first time period then in the second one. Conversely, higher
268 mean concentrations of erythromycin, imidacloprid and thiacloprid were observed in the second
269 time period as compared to the first one (Table 4).

270 There was a generalized decrease in the sum of concentrations of these 12 CECs from the first
271 period (up to 1283 $ng \cdot L^{-1}$ of total CECs, observed in Galindo in August 2017) to the second period
272 (up to 725 $ng \cdot L^{-1}$ of total CECs, observed in Galindo in August 2018), mainly due to the decrease
273 in the concentrations of macrolide antibiotics in wastewater effluent samples (Galindo, Gernika
274 and Ulia), in E-OI15 and in E-N17. Conversely, in the other receiving water stations (E-N15, E-

OK5 and L-UR20) the total CEC concentrations were similar or higher in the second period as compared to the first one (Figure S1 to Figure S13, in Supplementary Material).

3.4. RQ assessment in receiving waters

In order to assess the intensity of the local impact of WWTPs emissions in the aquatic environment, RQs were determined for all substances in the receiving waters (sampling stations: E-N15, E-N17, E-OK5, E-OI15 and L-UR20) (Figure 3). In 34% of the receiving water samples, azithromycin presented high risk (RQ≥1), with the highest frequency in the Oka estuary (E-OK5; up to 57%).

Other substances showing high risk were imidacloprid (in 9% of receiving waters), ciprofloxacin (7%), clarithromycin (6%), and diclofenac (5%), with RQ values up to 5.7, 1.9, 1.5 and 2.1, respectively. Ciprofloxacin only showed high risk at L-UR20 point and diclofenac at E-OI15. On the other hand, E2, not detected in the WWTP effluent samples, showed a high risk in three receiving water sampling stations (E-OK5, E-OI15 and L-UR20), with RQ values from 1.1 to 2.9.

4. DISCUSSION

The presence and spatial-temporal distribution of CECs, included those considered in the Watch lists adopted in Decisions (EU) 2015/495 and 2018/840, depend on many factors, such as the extent of product usage by the local human populations (Meador et al., 2016), seasonality (precipitation, temperature, river flow), coastal dynamics (currents, waves, tides), physical-chemical properties of the water in the receiving environment, biodegradation and photodegradation processes (Sousa et al., 2018) and also the physical-chemical properties of the CECs.

296 **4.1. Occurrence of Watch List substances and comparison with previous studies**

297 The Watch Lists comprise six pharmaceuticals, namely, the non-steroidal anti-inflammatory drug
298 (NSAID) diclofenac (only considered in the first Watch List); the macrolide antibiotics
299 azithromycin, clarithromycin and erythromycin; the quinolone antibiotic ciprofloxacin (only
300 considered in the second Watch List) and the synthetic estrogen 17α-ethinylestradiol (EE2), as
301 well as the two natural estrogens estrone (E1) and 17β-estradiol (E2). Pharmaceuticals and
302 hormones, widely used in human and veterinary medicine, as well as in aquaculture, are
303 intended to have a biological effect on their consumers, and therefore, organisms living in
304 aquatic ecosystems may be adversely affected by their release into the environment (Sousa et
305 al., 2018). These compounds are generally in the range of $ng \cdot \mu g \cdot L^{-1}$ in raw wastewaters, and they
306 are partially removed during wastewater treatment, especially when using secondary treatment
307 processes such as those applied in the study area. Therefore, they are likely to be detected in
308 receiving waters (Barbosa et al., 2016; Tiedeken et al., 2017; Verlicchi et al., 2012).

309 Among the pharmaceuticals and estrogens analysed in the present study in wastewater
310 effluents and receiving waters of the Basque coast, azithromycin showed the highest
311 quantification frequencies (100% of wastewater samples and 85% of receiving water samples),
312 followed by clarithromycin, diclofenac and erythromycin (quantified in more than 83% of
313 wastewater samples and in 70-75% of receiving water samples). These substances have also
314 been found in Spain, in the Ebro delta main WWTP effluents, with detection frequencies
315 between 83 and 100% (Gusmaroli et al., 2019). Similar frequency values have also been reported
316 for diclofenac in effluents from three Basque WWTPs (Mijangos et al., 2018), five English WWTPs
317 (Ashton et al., 2004), and in an EU-wide study of WWTPs (Loos et al., 2012). In coastal and
318 estuarine receiving waters, diclofenac has shown detection frequencies between 24 and 79% in
319 Basque estuaries studied by Mijangos et al. (2018), the Gulf of Cadiz and the Cadiz Bay (Spain)

(Biel-Maeso et al., 2018), the Saronikos Gulf (Greece) (Alygizakis et al., 2016), or the Humber Estuary (United Kingdom) (Letsinger et al., 2019).

Conversely, the hormones E2, EE2 and E1 were not quantified in WWTP effluents sampled in the present study. EE2 was neither detected in the WWTP effluents of the Ebro delta (Gusmaroli et al., 2019) nor in Japan (Isobe et al., 2003). However, in receiving waters, E2 and E1 were found at measurable levels in the Basque coast (quantified in 8% and 3% of the samples, respectively); similarly, E1 was measured in some sampling stations in the Scheldt estuary (The Netherlands-Belgium) (Noppe et al., 2007) or Douro estuary (Ribeiro et al., 2009), for example, but not in the Puget Sound (Washington; Meador et al., 2016).

Considering the concentrations of pharmaceuticals and estrogens measured in the WWTP effluent samples collected in the present work, they were, in general, higher than in receiving water samples. This could be probably related to the dilution effect of the WWTP effluents when discharged into the receiving waters, although other sources (i.e. discharge of untreated waters from the non-connected households or overflow of WWTPs during heavy rain events) should be also considered. In fact, for azithromycin, clarithromycin, diclofenac, erythromycin, and acetamiprid, concentrations at some sampling sites located in receiving waters were significantly lower than concentrations in the corresponding WWTP effluent. A recent study carried out in three Basque estuaries and the WWTP effluents of each estuary has also reported higher levels of diclofenac in WWTP effluents than in the receiving aquatic environment (Mijangos et al., 2018).

The range of concentrations for diclofenac in the present study (<3.3-139 $ng \cdot L^{-1}$) is similar to those observed in estuaries of the United Kingdom (Thomas and Hilton, 2004), and in effluents of WWTP in South Korea (Kim et al., 2007) or Taiwan (Fang et al., 2012), but lower than those detected in the Basque coast by Mijangos et al. (2018) and in effluents of WWTPs in United Kingdom (Ashton et al., 2004). The minimum value quantified (3.4 $ng \cdot L^{-1}$) in the present study is

345 comparable to the maximum values observed in the Gulf of Cadiz (Spain; Biel-Maeso et al., 2018)
346 or in the waters of the Gola de Ter beach (in Girona, Spain; Gros et al., 2012).

347 Among the macrolide antibiotics, azithromycin showed the highest concentrations, up to 672
348 and 649 ng·L^{-1} in wastewater effluent samples and receiving waters, respectively. These
349 concentrations are higher than those previously reported for other coastal areas of Spain (like
350 Cadiz, Ebro delta, Mar Menor, or Gola de Ter in Girona), China, Portugal, and the USA, or
351 wastewaters from Greece and the USA. Higher concentrations than those determined in the
352 present study have been, however, reported in wastewater from China and the Ebro delta area
353 (see Tables S5 and S6, in Supplementary Material). Concentrations of erythromycin, ranging
354 from <0.08 to 62.2 ng·L^{-1}, were lower than those observed in the effluents of WWTP in the
355 United Kingdom (Ashton et al., 2004), China (Ben et al., 2018), South Korea (Kim et al., 2007) or
356 the United States (Meador et al., 2016), but similar to those found in the Beibu Gulf (China;
357 Zheng et al., 2012), China Sea (Li et al., 2020), Mar Menor (Spain; Moreno-González et al., 2015)
358 or effluents of WWTPs in the Ebro delta (Spain; Gusmaroli et al., 2019). Clarithromycin, with
359 concentrations between <0.1 and 535 ng·L^{-1}, showed similar values to those observed in
360 effluents of WWTPs in the Ebro delta (Spain; Gusmaroli et al., 2019), but higher than
361 concentrations in effluents of WWTP in China (Ben et al., 2018), Girona (Spain; Gros et al., 2012)
362 or Washington (Meador et al., 2016), or in estuarine and coastal environments of the Cadiz Bay
363 (Spain; Biel-Maeso et al., 2018), Mar Menor (Spain; Moreno-González et al., 2015) and
364 Chesapeake Bay (USA; He et al., 2019) (Table S5 and Table S6, in Supplementary Material).

365 Ciprofloxacin concentrations were also higher in wastewater than in receiving waters. Similarly,
366 Mijangos et al. (2018) observed higher concentrations in WWTP effluents studied in the Basque
367 coast (up to 4719 ng·L^{-1} in Gernika WWTP) than in estuarine waters (up to 540 ng·L^{-1}), values
368 that are also higher than the concentrations measured in wastewater effluents in this study (<1-
369 802 ng·L^{-1}). Concentrations in receiving waters (<1-236 ng·L^{-1}) are similar to those observed in

370 the Cadiz Bay (Biel-Maeso et al., 2018), while in the Ibaizabal (E-N15 and E-N17 sampling points)
371 and Oka (E-OK5 sampling point) estuaries concentrations are lower and higher, respectively,
372 than those measured previously by Mijangos et al. (2018) in the same area.

373 The first Watch List also included the antioxidant 2,6-di-tert-butyl-4-methylphenol (BTH) and the
374 organic UV filter 2-Ethylhexyl 4-methoxycinnamate (EHMC). BHT, a highly active antioxidant
375 applied as food and cosmetic additive, has been detected in the aquatic environment (Bendz et
376 al., 2005; Fries and Püttmann, 2002, 2004; Gusmaroli et al., 2019). The environmental risk
377 associated to this compound is caused by its biodegradation product 3,5-di-tert-butyl-
378 4hydroxybenzaldehyde, BHT-CHO (Fries and Püttmann, 2002, 2004). EHMC is an organic UV
379 filter often used as ingredient in sunscreens, and in polymer-based products and paints to
380 prevent their photodegradation (Kameda et al., 2011; Tsui et al., 2014b). These substances are
381 poorly removed during conventional wastewater treatment processes (Tsui et al., 2014a) and
382 therefore, can enter the aquatic environment indirectly from wastewater (Giokas et al., 2007).
383 UV filters are lipophilic, and consequently, they may bioaccumulate in organisms (Gackowska et
384 al., 2016). Furthermore, they were reported to pose various ecological risks to marine
385 ecosystems (Tsui et al., 2014b).

386 Concentrations of BTH showed a high variability (<180-3000 $ng \cdot L^{-1}$) in the study area, with
387 higher values than in WWTP effluents of Galicia (Spain; Rodil et al., 2010), Sweden (Bendz et
388 al., 2005; Remberger et al., 2004) or USA (Wang and Kannan, 2018) (Table S6, in
389 Supplementary Material).

390 As for EHMC, concentrations in the Basque coast (<270-1520 $ng \cdot L^{-1}$) were higher than those
391 observed in the Canary Islands (Spain; Sánchez Rodríguez et al., 2015) and the South China Sea
392 (Tsui et al., 2019).

393 The compounds listed in the Watch Lists also include five insecticides from the neonicotinoid
394 family (imidacloprid, thiacloprid, thiamethoxam, clothianidin, and acetamiprid), two herbicides

(oxadiazon and triallate), the carbamate pesticide methiocarb, and the insecticide metaflumizone. The last two compounds were not quantified in either WWTP effluents or receiving waters sampled in the present study. In the WWTP effluents of the Ebro delta (Gusmaroli et al., 2019) and in the Arade Estuary (Portugal; Gonzalez-Rey et al., 2015) methiocarb was not detected either.

The neonicotinoid insecticides and the two herbicides showed concentrations below the quantification limits or close to them, except imidacloprid, quantified in 96% of the samples, with concentrations in wastewaters (<0.17-172 $ng \cdot L^{-1}$) similar to the values observed in the WWTP effluents of the Ebro delta (Spain; Gusmaroli et al., 2019). However, imidacloprid concentrations in receiving waters (<0.17-46.9 $ng \cdot L^{-1}$; quantified in 73% of the samples) were lower than those reported in the Ebro delta (Spain; Gusmaroli et al., 2019), likely due to a higher dilution factor in the study area, and higher than those measured in the Belgian zone of the North Sea (Vanryckeghem et al., 2019) and the Arade Estuary (Portugal; Gonzalez-Rey et al., 2015) (Table S5 and Table S6, in Supplementary Material).

Thiamethoxam, acetamiprid and triallate showed also higher concentrations in wastewater effluent samples than in receiving waters, with quantification frequencies up to 79% and 55%, respectively. These compounds were not detected in the WWTP effluents of the Ebro delta, and concentrations observed in the receiving waters (Gusmaroli et al., 2019) were lower than in the present study. However, thiamethoxam concentrations measured in the Belgian coast of the North Sea (Vanryckeghem et al., 2019) are even higher than in receiving waters of the Basque coast.

Contrarily, concentrations of thiacloprid, clothianidin and oxadiazon were higher in receiving waters than in wastewater. While the first two compounds showed similar concentrations in this study to those observed in the Ebro delta (Gusmaroli et al., 2019), for oxadiazon values were lower in the Basque coast than in the Ebro delta.

Considering the total 19 compounds analysed, five of them (E2, E1, clothianidin, oxadiazon and triallate) were more frequently quantified in samples collected in receiving waters than in wastewater samples, where, as mentioned above, E1 and E2 were not quantified. In addition, three out of these five (E2, E1 and oxadiazon), along with EHMC and thiacloprid, showed the maximum concentration in receiving water samples. Therefore, their main source in the study area is likely other than WWPT effluents. They could be generated in urban and rural areas, and enter the aquatic environment through leaching or run-off, especially in the case of pesticides.

4.2. Spatial and temporal distribution

In general, the highest concentrations of the Watch Lists compounds were observed in the WWTP effluents of Galindo (diclofenac, clarithromycin, ciprofloxacin, imidacloprid, thiamethoxam and acetamiprid), Gernika (erythromycin), and Ulia (BTH, azithromycin and triallate). Although WWTPs can reduce the emission of these compounds (Krzeminski et al., 2019), some of them remain in the final effluent, ending up in the receiving waters. The maximum average removals found in literature for pharmaceuticals and hormones in WWTPs with only secondary treatment by conventional activated sludge, such as those considered in the present study, vary between 62 and 99% for macrolide antibiotics and hormones, respectively (Verlicchi et al., 2012). In the Galindo WWTP, more than 100 emerging pollutants were analysed in 2010 and 2011, and removal efficiencies in secondary treatment effluents were estimated to be 25-75% for antibiotics (including ciprofloxacin, 71%), and higher than 90% for anti-inflammatories, with the exception of diclofenac (1%; although it was totally removed when tertiary treatment was considered) (González et al., 2018).

Hospital wastewater effluents are an important source of drugs to the sanitation network (González et al., 2018). Although this kind of discharges represented only 0.56% of the water treated in Galindo, they may contribute between 4 and 20% on average to some of the compounds detected in the influent reaching the WWTP. Therefore, instead of implementing a

445 treatment for the elimination of certain emerging pollutants in the effluent of Galindo, which
446 treats 106 million m^3 per year, a previous purification treatment at the source (hospitals),
447 together with the responsible use of medicines by the population, was proposed to mitigate
448 their arrival at WWTPs.

449 Nitrogen elimination processes during secondary treatment can reach removal efficiencies for
450 estrogenic compounds up to 90% (Andersen et al., 2003). In the present study, all wastewater
451 samples showed concentrations of E1, E2 and EE2 below the quantification limits, even in
452 Gernika and Ulia, the emission point of Loiola WWTP, that do not perform nitrogen removal.

453 However, E2 and EHMC showed maximum concentrations in coastal receiving waters (sampling
454 station L-UR20), E1 in the Ibaizabal estuary (station E-N17), oxadiazon in the Oka estuary (station
455 E-OK5), and thiacloprid and clothianidin pesticides in the Oiartzun estuary (station E-OI15). Since
456 higher values were observed in receiving waters than in WWTP effluents, they could be
457 generated in urban and rural areas, being leaching or run-off their main entrance to the
458 estuarine and coastal areas.

459 Concerning temporal distribution, there was a generalize decrease in the sum of concentrations
460 of CECs from the first period (from May 2017 to February 2018) to the second one (from May
461 2018 to March 2019), mainly due to the decrease in the concentrations of macrolide antibiotics
462 in WWTPs (Galindo, Gernika and Ulia), in E-OI15 and E-N17. The reduction in the consumption
463 or usage of these antibiotics in the study area could explain this temporal decrease in
464 concentrations. However, there is no evidence available to support this assumption.

465 Conversely, in the other receiving water stations (E-N15, E-OK5 and L-UR20) the total
466 concentrations were similar or higher in the second period.

4.3. Risk assessment

In the present study the RQs of each compound were calculated in all receiving water samples. However, the risk assessment of methiocarb was not considered since the observed concentrations were lower than the MQL (2-16.5 $ng \cdot L^{-1}$), being the PNEC value of 2 $ng \cdot L^{-1}$.

Although imidacloprid, ciprofloxacin, diclofenac, and clarithromycin could represent a high risk in the receiving environment, azithromycin was the substance that showed the highest potential to pose a risk to organisms, with 34% of the receiving water samples showing high risk (RQ≥1) due to its presence. Similarly, high risk by azithromycin was found both in Portuguese rivers (Sousa et al., 2019) and in the Ebro Delta (Gusmaroli et al., 2019), although concentrations observed in the Basque coast were slightly lower compared to those reported in the aforementioned studies.

Concerning the PNEC values used to calculate the RQs, it should be mentioned that they were derived mainly using freshwater species (*Daphnia magna*, green algae, fish) and therefore, they are more indicated for assessing the environmental risk in freshwater than in estuarine/marine waters. In the present study, these PNEC values have been used as a reference to estimate the risk of Watch List substances in estuarine and coastal waters of the Basque coast. Therefore, the risk estimations should be interpreted with care.

To estimate the pressure of Watch List compounds discharged by WWTPs into the receiving waters, the mass loads of the five substances quantified in the wastewater stations (Galindo, Gernika and Ulia) that could pose a risk in receiving waters, were analysed. The estimated mass load was calculated by multiplying the mean concentration of each compound found in wastewater effluents by the WWTP flow rate in 2019, provided by the WWTPs (Table S7, in Supplementary material), since daily flows for each sampling data were not available. The mass loads of these substances in Galindo were the highest (from 4.22 $kg \cdot year^{-1}$ for imidacloprid to 31.05 $kg \cdot year^{-1}$ for azithromycin), followed by Ulia (from 0.18 $kg \cdot year^{-1}$ for ciprofloxacin to 7.60

kg·year^{-1} for azithromycin) and Gernika (from 0.03 kg·year^{-1} for imidacloprid to 0.40 kg·year^{-1} for azithromycin). When mass loadings of each compound were normalized by the population equivalent (p.e.) served by each WWTP (Table S7, in Supplementary material), Galindo also showed the highest values (from 3.48 mg·year^{-1}·p.e.$^{-1}$ for imidacloprid to 25.63 mg·year^{-1}·p.e.$^{-1}$ for azithromycin), followed by Gernika (from 1.67 mg·year^{-1}·p.e.$^{-1}$ for imidacloprid to 21.74 mg·year^{-1}·p.e.$^{-1}$ for azithromycin) and by Ulia (from 0.33 mg·year^{-1}·p.e.$^{-1}$ for ciprofloxacin to 13.74 mg·year^{-1}·p.e.$^{-1}$, for azithromycin). Overall, this represents an important input of CECs, and especially of the antibiotics azithromycin, ciprofloxacin and clarithromycin, to estuarine and coastal waters, with the consequent environmental risk that this represents, in terms not only of toxicity but also of antibiotic resistance development. These figures also show a distinctly higher concentration of all 5 substances in the Galindo WWTP effluent, which would denote a comparatively higher use of all them in the Ibaizabal estuary area due to the higher number of inhabitants.

5. CONCLUSIONS

In the monitoring of the substances included in the EU Watch Lists undertook in the Basque Coast, the most frequently quantified substances were azithromycin, imidacloprid, clarithromycin, diclofenac and erythromycin, with frequencies of quantification between 71 and 100% in wastewater effluents (Galindo, Gernika and Ulia) and between 25 and 100% in receiving waters (E-N15, E-N17, E-OK5, E-OI15 and L-UR20).

In general, the concentrations in wastewater were higher than in receiving waters. Results also showed a higher contaminated pattern (concentrations in both wastewater and receiving waters) and estimated pressure (total and normalised mass loads) in the area of the Ibaizabal estuary as compared to the others, in line with its higher population density. There was not a clear increasing or decreasing pattern in the occurrence of the investigated substances in either waste or surface waters throughout the studied period.

Six of the studied substances (diclofenac, 17β-estradiol (E2), clarithromycin, azithromycin, ciprofloxacin and imidacloprid) exceeded their corresponding PNEC in receiving waters, indicating possible risk to the environment. In fact, the level of risk (RQ) of these substances was evaluated as high in 5% to 34% of the cases. Among the investigated substances, the antibiotics azithromycin, ciprofloxacin and clarithromycin rise the highest concern on the basis of their occurrence and potential toxicity, including the development of antibiotic resistances, and their consumption should be controlled and, as far as possible, limited and/or substituted with other alternative drugs. From the environmental point of view, the occurrence of imidacloprid and estradiol in some points is also of concern.

Taking this into account, the current limitations (i.e., due to budget restrictions) in monitoring in many countries (and likely in the near future) (Borja and Elliott, 2013), and what is described in the article 8ter of the Directive 2013/39, our recommendation for this region is to focus in the most populated area (Bilbao), both in the WWTP and the receiving waters, to assess the CECs status. At least the six substances exceeding the PNEC should be monitored, whilst those never exceeding the detection limits can be removed from the monitoring and eventually, upon consideration of the results obtained in other European studies, from the Watch List.

ACKNOWLEDGEMENTS

This work was supported by the Basque Water Agency (URA) through the project "Network for monitoring the ecological and chemical status of transitional and coastal waters of the Basque coast", and partly by the Generalitat de Catalunya (Consolidate Research Group 2017-SGR-14) and the Ministry of Science and Innovation (Project CEX2018-000794-S). Biotage is acknowledged for the gift of ISOLUTE® Na_2SO_4 drying cartridges. The authors acknowledge the help of the entities in charge of the management of the three studied WWTPs (Consorcio de Aguas Bilbao Bizkaia, Consorcio de Aguas de Busturialdea and Aguas del Añarbe), as well as the

work of all sampling and laboratory technicians. This is contribution number XXX from the Marine Research of AZTI-BRTA (Basque Research and Technology Alliance).

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Table 1. Watch List substances, their class, Chemical Abstract Service (CAS) number, maximum acceptable method detection limits (MDL) established by the European Commission (EC, 2015, 2018), and the analytical method detection (MDL) and quantification limits (MQL). Predicted No-Effect Concentration (PNEC), according to Loos et al. (2018).

Class	Substance	CAS number	Maximum acceptable MDL ($ng \cdot L^{-1}$)		MDL	MQL	PNEC
			EC (2015)	EC (2018)	($ng \cdot L^{-1}$)	($ng \cdot L^{-1}$)	($ng \cdot L^{-1}$)
Anti-inflammatory	Diclofenac	15307-79-6	10	-	1	3.3	50 [3, 5]
Estrogens	17β-estradiol (E2)	50-82-2	0.4	0.4	0.035-0.4	0.116-1.0	0.4 [1]
	17α-ethinylestradiol (EE2)	57-63-6	0.035	0.035	0.035-0.5	0.116-1.0	0.035 [1]
	Estrone (E1)	53-16-7	0.4	0.4	0.035	0.116	3.6 [1]
Anti-oxidant	2,6-Ditert-butyl-4-methylphenol (BTH)	128-37-0	3160	-	60	180	3160 [2]
UV filter	2-Ethylhexyl 4-methoxycinnamate (EHMC)	5466-77-3	6000	-	100	270	6000 [2]
Antibiotics	Erythromycin	114-07-8	90	19	0.024-0.035	0.08-5.0	200 [2]
	Clarithromycin	81103-11-9	90	19	0.05	0.17-2.5	120 [4]
	Azithromycin	83905-01-5	90	19	0.05	0.17-15	19 [4]
	Amoxicillin	26787-78-0	-	78	n/a	n/a	78 [7]
	Ciprofloxacin	85721-33-1	-	89	0.5	1-1.67	89 [4]
Pesticides and herbicides	Methiocarb	2032-65-7	10	2	0.5-5	2.0-16.5	2 [3, 6]
	Imidacloprid	105827-78-9/ 138261-41-3	9	8.3	0.05	0.17	8.3 [3]
	Thiacloprid	111988-49-9	9	8.3	0.05	0.17	10 [3]
	Thiamethoxam	153719-23-4	9	8.3	0.035	0.116	42 [4]
	Clothianidin	210880-92-5	9	8.3	0.05-1	0.17-5.0	130 [2]
	Acetamiprid	135410-20-7/ 160430-64-8	9	8.3	0.015-0.035	0.05-0.1	500 [2]
	Oxadiazon	19666-30-9	88	-	0.05	0.17	88 [2]
	Triallate	2303-17-5	670	-	0.035	0.17	410 [3]
	Metaflumizone	139968-49-3	-	65	1	3.3	65.4 [7]

n/a: not analyzed.

PNEC taken from:

(1) EU, 2012.
(2) Carvalho et al., 2015.
(3) Carvalho et al., 2016.
(4) Oekotoxzentrum Centre Ecotox, 2016.
(5) UBA, 2017.
(6) Ctgb, 2010.
(7) Loos et al. (2018).

Table 2. Number of samples, minimum, maximum and mean concentrations ($ng \cdot L^{-1}$), and quantification frequency, F (%), of Watch List substances determined in the Basque coast wastewater and receiving waters.

Substance	PNEC	Wastewater					Receiving waters				
		n	Minimum	Maximum	Mean	F	n	Minimum	Maximum	Mean	F
Diclofenac	50	12	<3.3	139	66.03	83	20	<3.3	103	14.78	75
E2	0.4	24	<0.116	<1	<1	0	40	<0.116	1.15	<1	8
EE2	0.035	24	<0.116	<1	<1	0	40	<0.116	<1	<1	0
E1	3.6	24	<0.116	<0.116	<0.116	0	40	<0.116	0.242	<0.116	3
BTH	3160	12	<180	3000	463.33	33	20	<180	2370	359.00	25
EHMC	6000	12	<270	350	<270	25	20	<270	1520	<270	15
Erythromycin	200	24	<0.08	62.2	22.22	83	40	<0.08	32.7	<5	70
Clarithromycin	120	24	2.5	535	102.01	92	40	<0.1	176	14.63	75
Azithromycin	19	24	19.4	672	253.26	100	40	0.5	649	37.55	85
Ciprofloxacin	89	12	<1	802	148.79	58	20	1.67	236	39.53	40
Methiocarb	2	24	<2	<16.5	<16.5	0	40	<2	<16.5	<16.5	0
Imidacloprid	8.3	24	<0.17	172	28.25	96	40	<0.17	46.9	4.40	73
Thiacloprid	10	24	<0.17	2	0.25	21	40	<0.17	2.4	0.23	10
Thiamethoxam	42	24	<0.116	5.4	0.83	54	40	<0.116	1.6	0.18	18
Clothianidin	130	24	<0.17	<5	<5	8	40	<0.17	<5	<5	13
Acetamiprid	500	24	0.06	314	18.59	79	40	<0.05	9.1	0.57	48
Oxadiazon	88	12	<0.17	0.79	0.24	33	20	<0.17	3.1	0.34	35
Triallate	410	12	<0.17	16.3	2.31	50	20	<0.17	11.1	1.50	55
Metaflumizone	65.4	12	<3.3	<3.3	<3.3	0	20	<3.3	<3.3	<3.3	0

Table 3. P-values of the paired samples t-tests carried out to compare the mean concentration at each sampling site at the receiving waters site versus the closest emission point. n.s: not significant.

Station	Azithromycin	Clarithromycin	Acetamiprid	Diclofenac	Erythromycin
L-UR20	n.s.	<0.001	<0.05	n.s.	<0.05
E-OK5	<0.05	n.s.	n.s.	n.s.	<0.05
E-N17	<0.001	<0.05	n.s.	<0.001	<0.001
E-N15	<0.001	<0.05	n.s.	<0.001	<0.001

Table 4. Number of analysis, minimum, maximum and mean concentrations ($ng \cdot L^{-1}$), and detection frequency, F (%), of Watch List substances determined in the Basque coast for the two considered periods (spring 2017-winter 2018 and spring 2018-winter 2019).

Substance	PNEC	Spring 2017-Winter 2018					Spring 2018-Winter 2019				
		n	Minimum	Maximum	Mean	F	n	Minimum	Maximum	Mean	F
E2	0.4	32	<0.116	1.15	<0.116	6	32	<0.116	<1	<1	3
EE2	0.035	32	<0.116	<0.116	<0.116	0	32	<0.116	<1	<1	0
E1	3.6	32	<0.116	0.242	<0.116	3	32	<0.116	<0.116	<0.116	0
Erythromycin	200	32	<0.08	62.2	8.73	94	32	<0.08	50	11.53	56
Clarithromycin	120	32	0.24	535	71.54	100	32	<0.1	106	23.25	63
Azithromycin	19	32	0.8	672	141.75	100	32	0.5	424	95.14	81
Methiocarb	2	32	<16.5	<16.5	<16.5	0	32	<2	<16.5	<16.5	0
Imidacloprid	8.3	32	<0.17	58.8	8.49	88	32	<0.17	172	18.19	75
Thiacloprid	10	32	<0.17	2	0.19	13	32	<0.17	2.4	0.29	16
Thiamethoxam	42	32	<0.116	5.4	0.70	53	32	<0.116	1.4	0.15	9
Clothianidin	130	32	<0.17	3.6	0.23	13	32	<0.17	<5	<5	9
Acetamiprid	500	32	<0.05	314	14.02	75	32	<0.05	3.6	0.63	44

Figure captions

Figure 1. Sampling stations in the study area. In yellow, sampling points related to wastewater (Galindo, Gernika and Ulia) and in green, sampling points in receiving waters (E-N15, E-N17, E-OK5, E-OI15 and L-UR20).

Figure 2. Range of concentrations (ng L-1) of Watch List substances determined in the Basque coast, by sampling station. The numbers represent frequencies of quantification (%). Note: For representation of the floating bars, the values below the limit of quantification have been considered as zero. PNEC: Predicted No-Effect Concentration. EE2, methiocarb and metaflumizone are not represented since their concentrations were in all cases below the quantification limits.

Figure 3. Frequency of low, medium and high risk for the Watch List substances determined in the Basque coast receiving waters by sampling station.

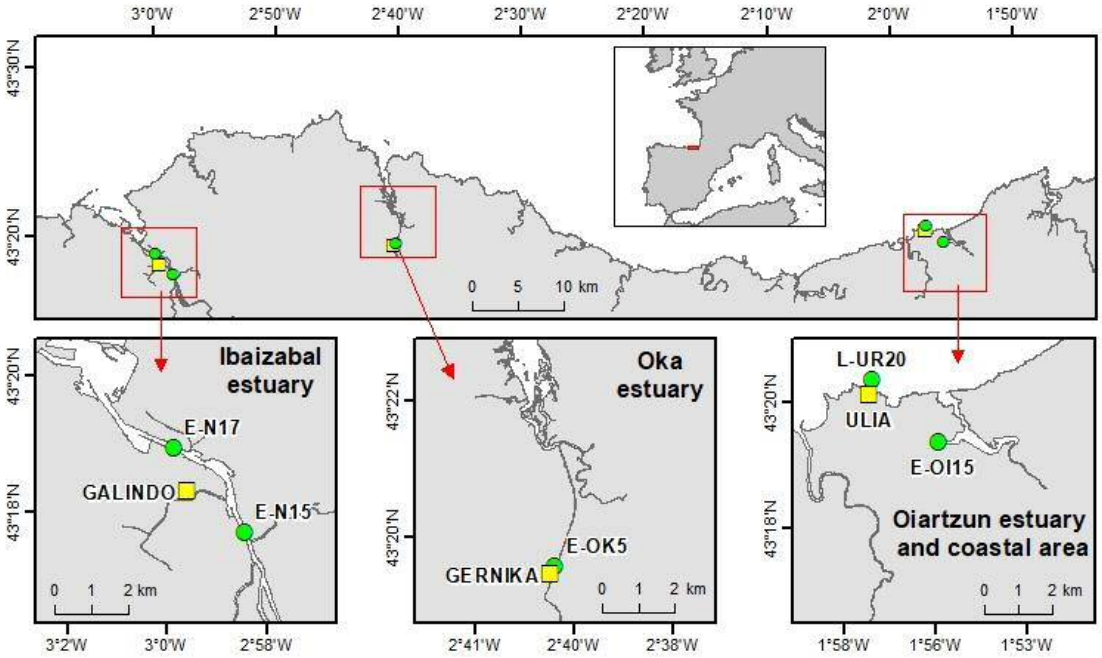
3°0'W
2°50'W
2°40'W
2°30'W
2°20'W
2°10'W
2°0'W
1°50'W
43°30'N
43°20'N
0 5 10 km
Ibaizabal estuary
E-N17
GALINDO
E-N15
0 1 2 km
3°2'W
3°0'W
2°58'W
43°22'N
43°20'N
Oka estuary
E-OK5
GERNIKA
0 1 2 km
2°41'W
2°40'W
2°38'W
L-UR20
ULIA
E-OI15
Oiartzun estuary and coastal area
0 1 2 km
43°18'N
1°58'W
1°56'W
1°53'W

Figure 1.

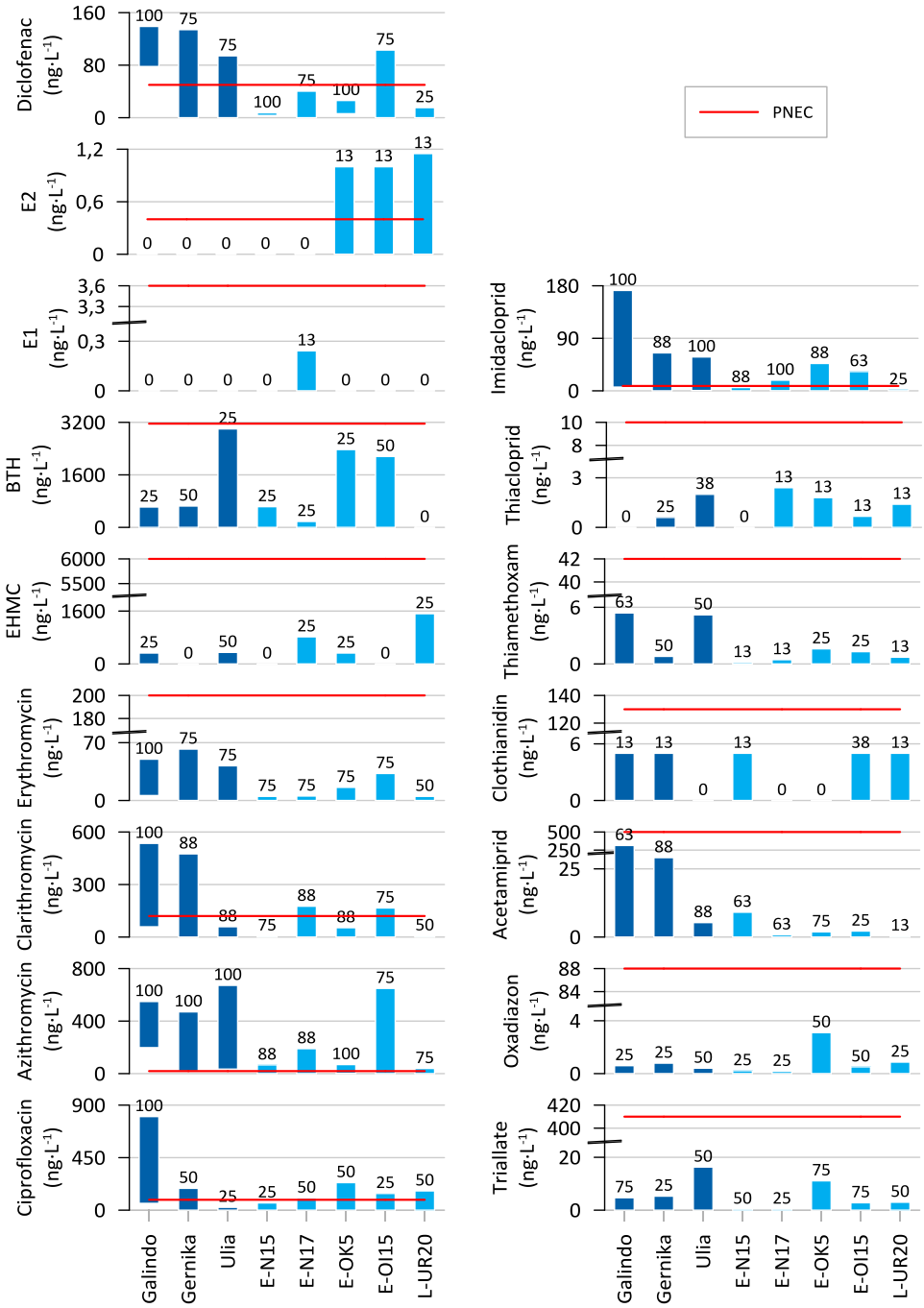
Diclofenac (ng·L-1)
E2 (ng·L-1)
E1 (ng·L-1)
BTH (ng·L-1)
EHMC (ng·L-1)
Erythromycin (ng·L-1)
Clarithromycin (ng·L-1)
Azithromycin (ng·L-1)
Ciprofloxacin (ng·L-1)
Imidacloprid (ng·L-1)
Thiacloprid (ng·L-1)
Thiamethoxam (ng·L-1)
Clothianidin (ng·L-1)
Acetamiprid (ng·L-1)
Oxadiazon (ng·L-1)
Triallate (ng·L-1)
PNEC
Galindo
Gernika
Ulia
E-N15
E-N17
E-OK5
E-OI15
L-UR20

Figure 2.

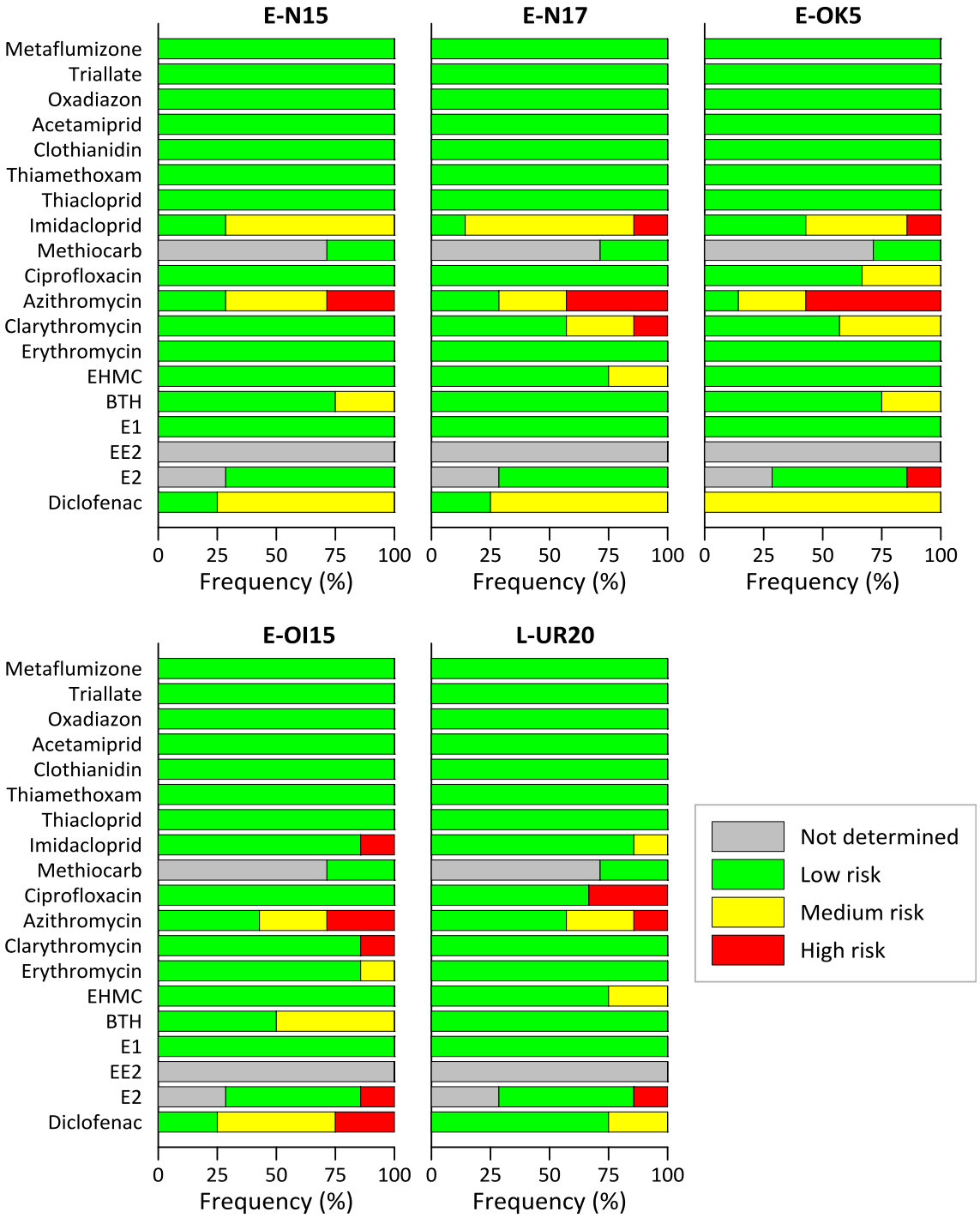
E-N15
E-N17
E-OK5
E-OI15
L-UR20
Metaflumizone
Triallate
Oxadiazon
Acetamiprid
Clothianidin
Thiamethoxam
Thiacloprid
Imidacloprid
Methiocarb
Ciprofloxacin
Azithromycin
Clarythromycin
Erythromycin
EHMC
BTH
E1
EE2
E2
Diclofenac
0
25
50
75
100
Frequency (%)
Not determined
Low risk
Medium risk
High risk

Figure 3.