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Environmental impact assessment of COVID-19 therapeutic solutions. A prospective analysis

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HIGHLIGHTS

• Covid-19 pandemic is increasing the use and emission of antivirals and other drugs.
• We have adapted models/scenarios for predicting levels in aquatic environments.
• Environmental concerns are identified for ivermectin and azithromycin.
• An innovative pharmacodynamic-based read-across covers antiviral’s ecotoxicity data gap.
• Concerns on fish sublethal effects are observed at local and regional levels.

GRAPHICAL ABSTRACT

Several medicinal products for human use are currently under consideration as potential treatment for COVID-19 pandemic. As proposals cover also prophylactic use, the treatment could be massive, resulting in unprecedented levels of antiviral emissions to the aquatic environment. We have adapted previous models and used available information for predicting the environmental impact of representative medicinal products, covering the main groups under consideration: multitarget antiparasitic (chloroquines and ivermectin), glucocorticoids, macrolide antibiotics and antiviral drugs including their pharmacokinetic boosters. The retrieved information has been sufficient for conducting a conventional environmental risk assessment for the group of miscellaneous medicines; results suggest low concern for the chloroquines and dexamethasone while very high impact for ivermectin and azithromycin, even at use levels well below the default value of 1% of the population. The information on the ecotoxicity of the antiviral medicines is very scarce, thus we have explored an innovative pharmacodynamic-based approach, combining read-across, quantitative structure-activity relationship (QSAR), US EPA’s Toxicity Forecaster (ToxCast) in vitro data, pharmacological modes of action, and the observed adverse effects. The results highlight fish sublethal effects as the most sensitive target and identify possible concerns. These results offer guidance for minimizing the environmental risk of treatment medication for COVID-19.

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1. Introduction

Human pharmaceuticals are considered a group of emerging pollutants requiring environmental risk assessment (Ågerström et al., 2015; Backhaus, 2016). In addition to direct effects on aquatic organisms, there are also concerns regarding human exposure via drinking water, the consumption of contaminated organisms (Almeida et al., 2020), and emergence of antibiotic resistance (Stanton et al., 2020). In the last decade, the environmental risk of human medicines has driven a significant number of scientific studies and systematic reviews (Ebele et al., 2017; Klatte et al., 2017; Madzikizela et al., 2020). The available information includes monitoring data (Deshiobles et al., 2018; Yang et al., 2019) as well as predictive tools, including exposure scenarios and risk assessment approaches for supporting the decision making (EMA, 2018). The environmental emission of human pharmaceuticals or their metabolites is widespread and triggered by the percentage of the human population treated with medication in the area. The use of antiviral drugs and related therapeutic agents increases exponentially during pandemics. Considering the worldwide dissemination, assessing the environmental impact of treatment medications for COVID-19 is highly relevant.

Vaccines are demanded not only to prevent COVID-19 spread but also to restore social and economic activities by generating mass immunization (Chung et al., 2020). Candidate vaccines must induce the production of a high level of neutralizing antibodies and specific T cell-mediated immune response in almost all participants in the clinical trial (Bakadia et al., 2021). Current vaccine candidates can be grouped into six different platforms: (i) protein subunit vaccines, (ii) viral vector vaccines, (iii) nucleic acid vaccines (mRNA and DNA vaccines), (iv) inactivated viruses, (v) live attenuated viruses, and (vi) virus-like particle (VLP) vaccines (Won and Lee, 2020; Ghorbani et al., 2020). The environmental concern focuses on genetically modified live vaccines (Tell et al., 2020).

Parallel to vaccination, several therapeutic options using antiviral and related medicines are under evaluation (WHO, 2020). Since the beginning of this pandemic, several classes of drugs indicated for other uses (repositioned drugs) and/or experimentally assayed were used to treat the infected patients. Few of the repositioned drugs for SARS-CoV-2 Infection have evidenced clinical efficacy and safety. Clinical trials are confirming the efficacy of some drugs for COVID-19 clinical indications, while for other medicinal products, efficacy and safety have not yet been corroborated.

No effective drug treatment is available for fighting this COVID-19. However several drugs including antiviral agents to treat other virus (e.g., remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon β-1b), the anthelmintic ivermectin, and the antimalarial hydroxychloroquine, have been widely used (Calv et al., 2020; Liu et al., 2020; Shehan et al., 2020). Different immunomodulatory and anti-inflammatory drugs such as tocilizumab (antirheumatic drug), chloroquine/hydroxychloroquine, and dexamethasone have been proposed to decrease or suppress the release/production of pro-inflammatory cytokines with the objective to minimize the cytokine storm associated with SARS-CoV-2 (Jamilloux et al., 2020; Zhao, 2020). Additionally, bacteriostatic macrolide antibiotics such as azithromycin, used against many Gram-positive and intracellular bacteria causing respiratory infections, demonstrate also immunomodulatory and anti-inflammatory effects (Bhavana et al., 2020; Pagliano et al., 2021). The prothrombotic effects of SARS-CoV-2 often manifested hypercoagulability and responsible of pulmonary thromboembolism, can be prevented by the prophylactic use of heparin (Pagliano et al., 2021).

Besides, monoclonal antibodies against the S protein (e.g., gimsilumab, leeronlimab) have been utilized in the treatment of COVID-19. By this mechanism, monoclonal antibodies reduce the cytokine storm in the pulmonary airway pathways and provides clinical symptomatic relief to patients from COVID-19 (Bhavana et al., 2020). Finally, convalescent plasma is a promising plasma antibody treatment that could help patients whose bodies cannot produce enough antibodies against COVID-19 to cure the disease (Won and Lee, 2020).

Lack of ecotoxicological studies is usually an issue for assessing the environmental risk of human drugs (Oelkers, 2020), requiring the use of in silico methods (Gunnarsson et al., 2019). Regarding the exposure assessment, although some scientific studies cover environmental emissions during local pandemic episodes (Singer et al., 2011; Azuma et al., 2015b), the methods and assessments do not cover the magnitude expected regarding treatment medication for COVID-19. We have updated the available exposure models for addressing massive/bulky treatment, developing a set of complementary scenarios that can be adapted to different areas worldwide. Our environmental assessment covers both, possible effects on the ecosystems, as well as the environmental impact on humans health, exposed indirectly to the human medicines and their metabolites following the contamination of sources used for drinking water extraction.

2. Materials and methods

The database https://www.nih.gov/coronavirus and information from regulatory agencies have been used for selecting illustrative medicinal products for human use under current assessment and extracting the proposed dosage regimen. This an area under continuous development and we have selected representative drugs covering the main therapeutic groups.

For the exposure assessment, EMA (European Medicines Agency) and ECHA (European Chemicals Agency) exposure models have been adapted and refined with excretion values and half-lives compiled from pharmacokinetic studies following a peer-review literature search (Web of Science www.webofknowledge.com and PubMed https://pubmed.ncbi.nlm.nih.gov/) and extraction from databases (PUBChem and Drugbank https://pubchem.ncbi.nlm.nih.gov/; https://www.drugbank.ca/). The details and selected references are provided in the Supporting information. These estimations were complemented with a regional model, based on population and river flow variability, using Madrid (Spain) metropolitan area as realistic worst case conditions region, with quantifications based on maximum and minimum water flow data extracted from the river basin authority http://www.chtajo.es/Paginas/default.aspx. Searches and data extractions were conducted between April 2020 and January 2021.

The ecotoxicity assessment was based on a targeted peer-review literature search conducted in Web of Science and PubMed. The extracted information was sufficient for deriving Predicted No Effect Concentrations (PNECs) for chloroquine, ivermectin, and azithromycin, and for selecting the Ecotoxicity Reference Point for dexamethasone. It was complemented with quantitative structure-activity relationship (QSARs) for supporting the read-across of chloroquine to hydroxychloroquine. QSAR were performed between 1 and 11 May 2020 with the Vega platform (https://www.vegahub.eu/) using the SMILES indicated in the supplementary information.

For the selected antiviral drugs the literature search only retrieved information on oseltamivir sold under the brand name Tamiflu. Additional data were generated using the QSAR (Vega platform) as described above, but were of low reliability. As the results were insufficient for offering reliable ecotoxicological estimations, the assessment was extended with an innovative approach. A broader search on experimental ecotoxicity data on antiviral drugs provided information on 5 additional antiviral drugs that were used as source group for a pharmacodynamic-based read-across. The ecotoxicological information retrieved for the antiviral drugs was compiled in a metanalysis, deriving the distribution of tentative PNEC values for each aquatic taxonomic group. A tentative PNEC value was calculated for each retrieved experimental ecotoxicity data, according to the following procedure: An standard interspecies variability factor of 10 was applied to the chronic No Observed Effect Concentrations (NOECs) and Effective Concentrations producing a 10% response (EC10) measuring population relevant effects (reproduction or population...
growth rate). Additional assessment factors (also named extrapolation, uncertainty or safety factors) (Tarazona, 1997) were applied to other ecotoxicological endpoints, as follows. An assessment factor of 10 was used for extrapolations related to exposure duration (i.e. from short-term to long-term exposures); an assessment factor of 10 for extrapolations from lethality to population relevant sublethal effects, an assessment factor of 2 for the extrapolation between NOEC or EC10 and EC50 measurements for the same ecotoxicological endpoint and exposure duration, and an assessment factor of 1 for effects relevant at individual level but with moderate impact at population level. The innovative pharmacodynamic-based read-across was designed to compare the characteristics of the antiviral drugs included in the source group with those of the target group. The selected characteristics included the antiretroviral mode of action, the metabolic pathways, the in vitro toxicity profiles from the US ToxCast (https://comptox.epa.gov/dashboard, data extracted between 1 and 11 May 2020), and the assessment of adverse effects, complementing previous reviews (Arab-Alameddine et al., 2011; Pau and George, 2014), with database searchers (https://pubchem.ncbi.nlm.nih.gov; https://www.drugbank.ca/) and a targeted peer-review literature search. Details on the findings and selected peer-review scientific publications are presented in the supplementary information. Following the assessment of similarities and gaps, the expertise of the authors (expert judgment) was used to develop a grouping approach for the environmental assessment of antiviral drugs based on the results of the meta-analysis of experimental ecotoxicity data, and the uncertainties related to the extension of this grouping approach to the target group.

3. Results

3.1. Selected groups of medicinal product for human use

There is a large number of medicinal products under study; our focus has been on medicinal products in phase III trials for COVID-19 treatment, and those already authorised for other clinical infectious diseases. The selection includes antiviral drugs, boosters and a group of miscellaneous drugs approved for other uses. The selected antiviral drugs are remdesivir (a nucleoside analogue prodrug), oseltamivir (a potent and selective inhibitor of influenza A and B neuraminidase, also known as Tamiflu), lopinavir (a second-generation protease inhibitor), darunavir (a second generation protease inhibitor) and umifenovir (a dual-acting direct antiviral and host-multitargeting agent). The pharmacologically active substances used as their pharmacokinetic boosters or enhancers are cobicistat and ritonavir. The miscellaneous group includes multitarget antiparasitic drugs approved for human treatment such as antimalarial (hydroxychloroquine, chloroquine) and anthelmintic (ivermectin) substances, the glucocorticoid dexamethasone and the macrolide antibiotic azithromycin. Monoclonal antibodies (tocilizumab and sarilumab) or adhesion blocking agents are assumed of low environmental concern as are expected to follow the metabolic and biodegradation processes of proteins and were excluded from this environmental risk assessment.

3.2. Exposure scenarios, models and predictions

The European Medicines Agency (EMA) scenario (EMA/CHMP, 2006; EMA, 2018) was based on very simplified assessment using default values and daily dose, and is currently under update. The default is 1% of the human population treated, which is insufficient for a therapeutic control of the pandemic; thus, additional levels of treatment medications were also considered. As in most cases the environmental emissions are expected to be treated by a Wastewater Treatment Plant (WWTP), the EMA scenario was complemented with a module covering the WWTP process. This module was based on the SimpleTreat 3.0 model described in the European Chemical Agency (ECHA) guidance document and also recommended in the draft EMA guidance. The log octanol/water partition coefficient (Pow) value triggers the proportion to water and to sludge was not relevant for any of the selected medicinal products. For the antiviral drugs, several studies have indicated negligible or highly variable degradation in WWTP (Leknes et al., 2012; Singer et al., 2014; Nannou et al., 2020), thus the SimpleTreat model seems to be inadequate for predicting the WWTP fate for this group, and the estimations should be considered with caution. Table 1 summarises the main source, intermediate and final data for the selected medicinal products. Surface water is frequently used for drinking water extraction, and conventional treatment does not remove antiviral drugs such as oseltamivir (Simazaki et al., 2015), thus human indirect exposure from drinking water should be also considered.

For medicinal products with faster elimination (half-life of 1 day or less), the environmental emission concurs with the day of administration. However, when the elimination half-life is longer, the environmental emission is distributed through a number of days, and consequently the maximum Predicted Environmental Concentration (PEC) value depends on the actual elimination half-life and the dosage regimen (dose, interval and treatment duration). An additional module, considering the prescription conditions and time for elimination of the administered dose, was developed for increasing the realism when using the model for assessing environmental emissions during pandemic massive uses. Most proposed dosage regimen have a duration of about 14 days, Fig. 1A presents the influence of the elimination half-life on the temporal distribution of environmental emissions following 14 days of treatments. Chloroquine and its metabolite hydroxychloroquine have longer elimination half-lives. The temporal environmental emission distributions for a single patient according to

### Table 1

| Drug                | Daily dose mg | % elimination | % WWTP to water | % WWTP to sludge | PECin WWTP | PECout WWTP | PEC water (mg/L) |
|---------------------|---------------|---------------|-----------------|------------------|------------|-------------|-----------------|
| Hydroxychloroquine  | 400           | 75            | 74.78           | 25.22            | 1.50       | 1.12        | 0.12            |
| Chloroquine         | 400           | 60            | 53.27           | 46.73            | 1.20       | 0.64        | 0.06            |
| Ivermectin          | 12            | 100           | 24.97           | 75.03            | 0.06       | 0.015       | 0.0015          |
| Dexamethasone       | 6             | 100           | 99.00           | 1.00             | 0.03       | 0.03        | 0.003           |
| Azithromycin        | 500           | 10            | 70.53           | 29.47            | 1.72       | 1.21        | 0.12            |
| Remdesivir          | 100           | 100           | 98.00           | 2.00             | 0.50       | 0.49        | 0.05            |
| Opinavir            | 800           | 93            | 50.44           | 49.56            | 3.72       | 1.88        | 0.37–0.19       |
| Ritonavir           | 200           | 98            | 67.14           | 32.86            | 0.98       | 0.66        | 0.10–0.607      |
| Oseltamivir         | 75            | 99            | 100.00          | 0.00             | 0.37       | 0.37        | 0.04            |
| Darunavir           | 1200          | 93.5          | 96.00           | 4.00             | 5.61       | 5.39        | 0.54            |
| Cobicistat          | 150           | 100           | 55.34           | 44.67            | 0.71       | 0.39        | 0.07–0.03       |
| Umifenovir          | 600           | 100           | 43.65           | 56.35            | 3.00       | 1.31        | 0.30–0.13       |

WWTP: Wastewater Treatment Plant.

* These values have a high uncertainty as, for antivirals, the modelled fate in the WWTP is not in line with measured data.
the therapeutic recommendations for hydroxychloroquine are presented in Fig. 1B.

This refined model was used to estimate the consequences on environmental emissions and final predicted concentrations in water for different therapeutic regimes. Fig. 2 compares the results for simultaneous treatment of the entire human population, consecutive treatments in three or four groups, and for non-consecutive groups. The data results confirm that a proper selection of the dose regimen may reduce significantly the aquatic environmental concentrations of human drugs, and therefore could be considered as a possible risk mitigation option when needed.

The data results for the regional model based on population and river flow using the Madrid cosmopolitan region (Spain) as realistic worst-case confirms that the dilution factor of the receiving water plays a key role in the final predicted aquatic concentrations. As example, Table 2 provides the maximum PEC value obtained for the different models and scenarios for the metabolite hydroxychloroquine.

### 3.3. Risk assessment for multitarget antiparasitic drugs, dexamethasone and azithromycin

The antimalarial mode of action of chloroquines targets a very specific process in the biology of the parasite. Chloroquine concentrates in erythrocytes parasitized with malaria, where they bind with high affinity to hemoglobin degradation, which is not informative for the environmental assessment. The available ecotoxicological information on chloroquine is limited but covers acute oral toxicity on fish, cladocerans (*Daphnia magna*), algae and bacteria, chronic toxicity on algae, and sublethal effects on fish and mussels (Zurita et al., 2005; Moore et al., 2007; Rendal et al., 2011; Ramesh et al., 2017). Considering the data and the similarities observed among taxonomic groups, the application of an assessment factor of 100 to the algae EC$_{10}$, resulting in a predicted no-effect concentration (PNEC) value of 0.12 mg/L, seems appropriate and covers the sublethal effects observed in fish such as erratic swimming and loss of equilibrium, among others. There is no information on hydroxychloroquine (the less toxic metabolite of chloroquine), but QSAR estimations with moderate reliability predict that hydroxychloroquine is slightly less toxic than chloroquine for aquatic organisms; supporting the extrapolation of the chloroquine PNEC value to hydroxychloroquine. Most models predict maximum concentrations below the PNEC value, suggesting low concern except when the default dilution factor of 10, between wastewater and receiving system flows, cannot be achieved.

Opposite to the chloroquines, the anthelmintic mode of action of ivermectin is highly informative for targeting the environmental assessment. Ivermectin is an agonist of several types of ligand-gated chloride ion channel receptors; increases the activity of γ-aminobutyric acid (GABA) receptors or glutamate-gated chloride ion channels (Glu-Cl), which blockades the signal between neuron and muscle of parasite; the blood-brain barrier (BBB) secures vertebrate’s GABA-sensitive neurons, resulting in a high sensitivity for invertebrates (Juarez et al., 2018). This is confirmed by available risk assessments, that confirm moderate toxicity for fish and algae and extremely high toxicity for invertebrates, with a PNEC value of 0.03 μg/L based on chronic toxicity to *Daphnia magna* (Liebig et al., 2010). The estimations based on the currently approved dosage as human antiparasitic drugs indicate a very high risk for aquatic invertebrates. A significant retention in the WWTP sludge is also expected, which will trigger a complementary risk for agricultural soils in case the of sludge reclamation as soil fertilizer considering the high sensitivity of soil invertebrates (Liebig et al., 2010).

### Table 2

Comparison of exposure models and scenarios results for hydroxychloroquine

| Comparison of exposure models and scenarios results for hydroxychloroquine | PEC (mg/L) |
|---|---|
| EMA default estimation (1% population treated) | 0.002 |
| EMA default estimation (100% population treated) | 0.2 |
| Corrected by WWTP fate | 0.11 |
| Corrected by WWTP fate and real toxicokinetics (simultaneous treatment) | 0.086 |
| Corrected by WWTP fate and real toxicokinetics (3 consecutive groups) | 0.036 |
| Corrected by WWTP fate and real toxicokinetics (3 non-consecutive groups) | 0.033 |
| Corrected by WWTP fate and real toxicokinetics (4 consecutive groups) | 0.028 |
| Simplified regional estimation based on emissions/river-flow for the Tagus river downstream Madrid, Spain | 0.013–0.42 |

WWTP: Wastewater Treatment Plant.
For dexamethasone, a class of steroid hormone, the available information includes ecotoxicity data on algae, aquatic invertebrates, amphibians and fish species. The NOEC and LOEC (Lower Observed Effect Concentration) values for standard relevant endpoints are in the mg/L range, however sublethal effects have been reported at much lower concentrations, including effects on vertebrate grown, suitable for a PNEC derivation, with effects observed at 40–50 μg/L (Lorenz et al., 2009; LaLone et al., 2012). The reported NOEC values for these studies are 4 and 1 μg/L, with LOEC/NOEL ratios of 10 and 50 respectively. These ratios are suboptimal for establishing a PNEC; and the margin of exposure (MoE) approach is more suitable for the risk characterisation. Using 40 μg/L as the Ecotoxicity Reference Point, a margin of 13.3 is observed for the maximum predicted concentration (Table 1) assuming 100% treatment of the population and similar toxicity for the parent and the metabolites. Considering that there is a wide coverage of taxonomic groups and that the use of dexamethasone is restricted to hospitalized patients requiring supplemental oxygen or mechanical ventilation (Horby et al., 2021), this margin is sufficient for considering that the specific use of dexamethasone in COVID-19 patients does not have specific environmental concerns.

Azithromycin, a semisynthetic derivative of erythromycin, is included in the EU monitoring program under the Water Framework Directive, with measured median and maximum concentrations of 0.02 and 5 μg/L respectively (Loos et al., 2018) and measured levels in WWTP effluents between 0.05 and 0.6 μg/L (Rodríguez-Mozaz et al., 2020). As expected, is particularly toxic to cyanobacteria with a proposed PNEC of 0.02 μg/L (Loos et al., 2018; Rodríguez-Mozaz et al., 2020), which is considered protective for minimizing the development of antibiotic resistance (Stanton et al., 2020). According to our estimations, a potential environmental risk should be expected if the use rate is higher than 0.016% of the human population. In addition, it should be considered that the use in COVID-19 patients should be added to environmental emissions from current uses, which already represent an environmental concern in certain areas. COVID-19 treatments covering between 0.004% and 0.05% of the human population would duplicate current WWTP emissions.

### 3.4. Risk assessment for antiviral drugs and pharmacokinetic boosters or enhancers

These medicinal products for human use have rapid and almost total (90–100%) excretion; metabolism is variable and the parent pharmacologically active substances represent between 10 and 100% of excreted dose. The maximum predicted WWTP inflow concentrations ranged from 0.5 to 5.61 mg/L, representing maximum concentrations in the receiving waters between 0.05 and 0.56 mg/L assuming a minimum dilution factor of 10. These estimations are around three orders of magnitude higher than currently measured concentrations, but in line with previous estimations for influenza pandemics when corrected by the penetration factor as a maximum of 30–50% of the human population treated was assumed for influenza pandemics (Straub, 2009). According to a recent report (Nannou et al., 2020), antiviral measured environmental concentrations are usually below 100 ng/L, and only occasionally exceed 500 ng/L. These estimations indicate that a massive worldwide treatment for COVID-19 may produce a bulky increase in current concentrations, well above those reported for previous pandemic situations.

The available ecotoxicological information on these medicinal products is very limited with the remarkable exception of oseltamivir used to treat and prevent influenza A and influenza B (flu), orally administered prodrug that must be activated by ester hydrolysis. As experimental ecotoxicity data are not available for the other antivirals, we tried to fill the gaps using QSARs, and approach used in other assessments, but the reliability of the estimations was very poor in terms of accuracy. Therefore, in this study we have used a different approach, combining a metaanalysis of all available data on antiviral drugs with a pharmacodynamic-based read-across assessment.

The results summarised in Fig. 3 suggests general taxonomic differences in toxicity, with fish particularly sensitive to sublethal and reproductive effects, observed with the three antivirals: efavirenz and nevirapine (non-nuclease reverse transcriptase inhibitors (NNRTIs)) and glancilovir (a deoxyguanosine analog nucleoside reverse transcriptase inhibitor (NRTI)). Table 3 summarises additional information on the antivirals with ecotoxicity data (source group) and those to be assessed in this scientific study (target group), covering the mode of action (MoA), physico-chemical properties, metabolic pathway, in vitro cytotoxicity extracted from ToxCast, and reported drug adverse effects (Pau and George, 2014).

Both groups show large variability. The pharmacodynamic information confirms that all MoAs are target specific viral processes, and cannot be associated to presumed susceptibility to specific taxa or ecological traits. The main gap regarding MoA is observed for protease inhibition, and the metabolic abnormalities associated to this class, that includes dyslipidemia (primarily triglycerides), insulin resistance, hyperglycemia, and lipodystrophy. Similarities are observed for some adverse effects, not only for the apical effects reported in the table but also for cellular mediated effects, although linked to different pathways.

### Table 3

Comparison of key properties of source (with available ecotoxicity data) and target (selected for this study) antivirals. See Supporting information for details.

| Antivirals | Number (common) | MoA | Pow range | Metabolic pathways | Cytotoxicity range (μM) | Adverse effects<sup>a,b</sup> |
|-----------|----------------|-----|-----------|--------------------|------------------------|-----------------------------|
| Source group | 7 (1) | NRTI, NNRTI, Ni | -1.52 to 4.11 | CYP3A4 CYP2A6 CYP2B6 | 6.94–1000 | Gl, Res, CNS, Dev, Hep, Skin |
| Target group | 6 (1) | NRTI, Ni, PtI, Dual | -1.71 to 4.73 | CYP3A4 CYP3A5 CYP2D6 | 4.22–1000 | Gl, Hep, Skin, Met. |

<sup>a</sup> Mode of Action (MoA) acronyms. NRTI. Nucleoside reverse transcriptase inhibitor. NNRTI. Non-nucleoside reverse transcriptase inhibitor. Ni. Neuraminase inhibitor. PtI. Protease inhibitor. Dual. Direct viridical and host-targeting effects.

<sup>b</sup> Adverse effects acronyms. GI. Gastrointestinal effects. Res. Respiratory effects. CNS. Central nervous system. Dev. Developmental effects. Hep. Hepatotoxicity. Skin. Skin effects (rash). Met. Metabolic abnormalities.
Fig. 4. Comparison of in vitro activities (as AC₅₀) for the Tox21 assays with shared activity by the three antivirals.
(Bertrand et al., 2019). Source and target groups share a main metabolic pathway (Arab-Alameddine et al., 2011), CYP3A4, which is the metabolic pathway linked to the activity of ritonavir (HIV protease inhibitor) and coibicitat (a selective, mechanism-based inhibitor of CYP3A enzymes) for modifying the pharmacokinetic boosters of the HIV-1 protease inhibitor antivirals (only approved as a fixed-dose combination product with coibicitat). The ToxCast in vitro data base includes sufficient number of in vitro results only for efavirenz in the source group and lopinavir/ritonavir (clinical trials have shown that lopinavir/ritonavir is highly effective as a component of highly active antiretroviral therapy) for the target group. Fig. 4 summarises the data, the AC50 value reflects the concentration at which 50% of the maximum response of the in vitro activity is achieved. Most endpoints have AC50s in the range of 20–40 μM supporting the read-across.

The pharmacokinetic boosters ritonavir and coibicitat inhibit the cytochrome P450 3A (CYP3A) drug-metabolizing enzyme. The role of hepatic cytochrome P450 (CYP) 3A family has been associated to xenobiotics’ metabolism in fish, but interspecies differences in metabolism pattern between fish and mammals should be expected (Verbuken et al., 2017). As the antiviral drug environmental emissions through municipal and hospital effluents will concur with many other xenobiotics, this MoA requires particular attention. Despite the uncertainties in the extrapolation of the ecotoxicity metadata, the information suggests that the predicted concentrations for the antiviral and pharmacokinetic boosters are in the range of the generic PNEC values for antivirals, and that specific attention is required for sublethal effects on fish. Pending the development of specific PNECs, the median and 25th percentiles of the PNEC values for fish are proposed as realistic and conservative predictions respectively. The risk for the aquatic community can be expressed as the PEC/PNEC ratio, values higher than 1 indicate a potential risk. The actual risk will depend on the level of use of the antiviral drugs by the human population discharging the wastewater in each aquatic system. Table 4 presents estimations for percentages of the population (penetration factors) between 10 and 0.01%. In the realistic case (median PNEC value) PEC/PNEC ratios exceed the value of 1 when around 10% of the human population in the area is treated. In the conservative (25th percentile of the PNEC distribution), this is already achieved for treatments corresponding to 0.1% of the population in the area.

4. Discussion

Several authors have previously reported the seasonal increase of antiviral medicinal products in WWTP and receiving waters during influenza pandemics (Hutchinson et al., 2009; Straub, 2009; Ellis, 2010; Leknes et al., 2012; Singer et al., 2014; Azuma et al., 2015b). Most studies have focused on oseltamivir and its metabolite, oseltamivir carboxylate, with predicted (Azuma et al., 2015b) and measured (Singer et al., 2014) concentrations in WWTP reaching 1 μg/L and measured concentrations in the receiving waters up to 0.2 μg/L. These estimations and measurements correspond to levels of treatment up to 40% of the human population, much lower than those that may be needed for the coronavirus pandemic. Concentrations of several antiviral drugs increase during the same season (Azuma et al., 2015a) indicating that several medicinal products are used during the influenza pandemic. In addition, the measurements are limited to the pharmacologically active substances and few, if any, selected metabolites; while our predictions are based on total emissions. The combination of these factors indicates that our predictions are in line with the expectations regarding concentrations in WWTP inflows for extensive treatments of the coronavirus pandemic. As expected and confirmed by the regional model, the dilution capacity of the receiving water plays a key role in the final concentrations in the aquatic systems (Prasse et al., 2010).

Low environmental concern is expected for chloroquines even under pandemic situations, when the models are refined for the elimination rate. Additional mitigation is possible by programming the therapeutic regimen medical treatment. In contrast, a high risk is expected for ivermectin, despite the significant removal by the WWTP. Even at the WHO Defined Daily Dose (DDD) as human antiparasitic, the use by 0.05% of the human population discharging the wastewater in a particular aquatic environment could represent an environmental impact. Federal agencies such as the US FDA have alerted on the risks of unspecified self-treatment with ivermectin for Covid-19 treatment, triggered by “fake news” following some results in preclinical research. Our analysis and estimations suggest that this illegal use in the coronavirus pandemic may also result in significant environmental impacts. Low environmental concern is estimated for dexamethasone if use is limited to the treatment with of hospital patients with severe clinical symptoms. Regarding the antibiotic azithromycin, a generalised use for treating COVID-19 patient would increase current environmental emissions that already represents a potential risk in certain areas (Loos et al., 2018; Grill et al., 2016; Rodriguez-Mozaz et al., 2020).

The combination of a metanalysis of ecotoxicity data, QSAR predictions and in vitro ToxCast results, with pharmacodynamic information on the MoA, metabolic pathway and adverse effects suggest that fish sublethal effects are the key effect to be assessed for antiviral drugs. The distribution of PNEC values can be used for probabilistic risk estimations and the lower end allows conservative deterministic assumptions. As there is an overlap of the PEC and PNEC distributions concerns for the predicted concentrations cannot be excluded. Predictions, as those presented in Table 4, may be used for identifying levels of concern regarding environmental impacts. The predictions are based on the level of treatment of the human population in the area, and can be applied to local, regional and even global scenarios. PEC/PNEC ratios above 1 are used in the risk assessment as threshold of concerns, when this value is exceeded, the size of the affected area and the effects used for setting the PNEC offer information on the magnitude of the potential environmental impact. The PNEC selected for the realistic scenario is based on reproductive effects on fish, and exceedances are expected to impact fish populations. Considering the heterogeneous distribution of the COVID-19 disease, levels of treatment reaching 10% of the population may be expected under local conditions in areas with very high incidence. The PNEC selected for the conservative scenario is based on sublethal individual effects, which may or may not have consequences at population level. Cumulative disease incidences up to 1% have been already achieved in certain regions, thus in the conservative scenario, the possibility of risk has been identified not only for local conditions but also for larger regions. As a large-scale treatment would result in unprecedented environmental emissions, additional information is needed for refining the environmental risk assessment, focusing on chronic sublethal effects on fish, combined effects of co-exposure to several antivirals and pharmacokinetic boosters, and understanding the observed variability in the WWTP fate for antivirals (Singer et al., 2014; Nannou et al., 2020). Regarding drinking water, the estimated concentrations are low and will be reduced in case of specific treatment

| Table 4: Estimated PEC/PNEC values for different levels of population treatment (from 10 to 0.01% of the population in the area) for each antiviral. Results for two generic antiviral PNECs, the median and the 25th percentile, are presented. |
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| Population treated (%) | 10 | 1 | 0.1 | 0.01 |
| PNEC value | Median | 25th per. | Median | 25th per. | Median | 25th per. | Median | 25th per. |
| PEC/PNEC value of each antiviral | Remeclavir | 0.24 | 31.63 | 0.02 | 3.16 | 0.00 | 0.32 | 0.00 | 0.03 |
| Lopinavir | 0.91 | 121.12 | 0.09 | 12.11 | 0.01 | 1.21 | 0.00 | 0.12 |
| Ritonavir | 0.32 | 42.47 | 0.03 | 4.25 | 0.00 | 0.42 | 0.00 | 0.04 |
| Oseltamivir | 0.18 | 23.96 | 0.02 | 2.40 | 0.00 | 0.24 | 0.00 | 0.02 |
| Darunavir | 2.60 | 347.64 | 0.26 | 34.76 | 0.03 | 3.48 | 0.00 | 0.35 |
| Coibicitat | 0.19 | 25.33 | 0.02 | 2.53 | 0.00 | 0.25 | 0.00 | 0.03 |
| Umifenovir | 0.63 | 84.53 | 0.06 | 8.45 | 0.01 | 0.85 | 0.00 | 0.08 |
(Simazaki et al., 2015); no specific concerns are identified regarding toxicity, however, the pharmacokinetic boosters are very potent CYP3A inhibitors with covalent binding stoichiometry close to one (Roch et al., 2014), suggesting that the effect of exposure through drinking water, even at low concentrations, on CYP3A inhibition should be further assessed.

5. Conclusions

Simplified models based on available information are suitable for the identification of the potential environmental impact of treatment medication for COVID-19. Although human health is the main priority, the concepts of “One Health” and “Sustainability”, requires the inclusion of environmental considerations in the decision making for Public Health at regional, national and global levels, using these estimations for selecting possible risk mitigation approaches.

For the group of miscellaneous drugs, risk management should consider the expected increase in the medication use, which can be easily monitored in countries with registered sales data. We have selected as a proof of concept drugs with different characteristics; covering different conceptualisations in the integration of the results. Although not supported by scientific evidence, massive medication use of chloroquine and ivermectin has been suggested as preventive treatment, our results confirm high environmental concern for the second. Regarding the treatment of hospitalized patients with severe clinical symptoms, no specific concerns is identified for dexamethasone, while additional environmental emissions would increase current concerns for azithromycin, a second-generation macrolide antibiotic. Considering the similitude in the mode of action, the individual assessment presented here should be expanded with group assessments covering all glucocorticoids and all macrolide class antibiotics.

The lack of information was particularly relevant for the antiviral drugs. An innovative approach has been developed based on grouping and situations of potential concern. Further experimental research studies on antivirals are required for confirming the approach and reduce the assessment uncertainty. In the meantime a group assessment as presented could be used for supporting prioritisation and the identification of areas and situations of potential concern.

CRediT authorship contribution statement

All authors have contributed.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146257.

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